Mechanisms of Action and Persistent Neuroplasticity by Drugs of Abuse

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ABBREVIATIONS: AC, adenylyl cyclase; ADHD, attention deficit hyperactivity disorder; 2-AG, 2-arachidonoylglycerol; ALDH, aldehyde dehydrogenase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPH, amphetamine; BDNF, brain-derived neurotrophic factor; BLA, basolateral complex of the amygdala; BNST, bed nucleus of stria terminalis; BOLD, blood oxygen level dependent; BZ, benzodiazepine; CAM, cell adhesion molecule; CaMKII, Ca²⁺/calmodulin kinase II; CCK, cholecystokinin; CeA, central nucleus of the amygdala; CNS, central nervous system; CPP, conditioned place preference; CREB, cAMP response element-binding protein; CRF, corticotropin-releasing factor; D₁R, dopamine-1 receptor; D₂R, dopamine-2 receptor; DA, dopamine; DARPP-32, DA- and cAMP-regulated phosphoprotein; DAT, dopamine transporter; DBI, diazepem binding inhibitor; DGL, diacylglycerol lipase; DOI, 2,5-dimethoxy-4-iodoamphetamine; DSE, depolarization-induced suppression of excitation; DSI, depolarization-induced suppression of inhibition; eCB, endocannabinoid; ECM, extracellular matrix; EEG, electroencephalography; EPSC, excitatory postsynaptic current; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ETC, electron transport chain; FC, frontal cortex; fMRI, functional magnetic resonance imaging; GABA, γ-aminobutyric acid; GHB, γ-hydroxybutyrate; GPCR, G protein-coupled receptor; HDAC, histone deacetylase; HFS, high-frequency stimulation; HPPD, hallucinogen persisting perception disorder; 5-HT, 5-hydroxytryptamine, serotonin; ICSS, intracranial self-stimulation; iGluR, ionotropic glutamate receptor; IL, infralimbic cortical region; IP₃, inositol 1, 4,5-trisphosphate; IPN, interpeduncular nucleus; IPSC, inhibitory postsynaptic current; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinases; Kal-7, postsynaptic density-localized kalirin-7; KCC, potassium-chloride cotransporter; KCNQ1, potassium inwardly rectifying channel subunit 1; LC, locus ceruleus; LDTg, laterodorsal tegmental nucleus; LDX, lisdexamfetamine; LFS, low-frequency stimulation; LHB, lateral habenula; LSD, lysergic acid diethylamide; LTD, long-term depression; LTP, long-term potentiation; MAO, monoamine oxidase; MAPK, mitogen-activated protein kinase; MDMA, 3,4-methylenedioxymethamphetamine (ecstasy); MDMC, methylone; METH, methamphetamine; mGlu, metabotropic glutamate receptor; Mhb, medial habenula; MK-801, dizocilpine; 4-MMC, mephedrone; MPH, methylenediphendylidene; MSN, medium spiny neuron; nNOS, neuronal nitric oxide synthase; NAc, nucleus accumbens; nAChR, nicotinic acetylcholine receptors; NE, norepinephrine; NET, norepinephrine transporter; NPS, nuclear factor kappa-B; NMDA, N-methyl-D-aspartate; NO, nitric oxide; nOPl, norepinephrine transporter; O/FQ, norepinephrine/orphin FQ; OBF, orbitofrontal cortex; OR, odds ratio; ox, ox; ORX, orin; PAG, periaqueductal gray area; PBR, peripheral benzodiazepine receptor; PCP, phencyclidine; PET, positron emission tomography; PFC, prefrontal cortex; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKMζeta, atypical PKC; PLC, phospholipase C; PNN, perineuronal nets; PrL, prelimbic cortical region; PAG, periaqueductal grey; PV, parvalbumin; R, receptor; RGS, regulator of G protein signaling; rMg, rostromedial tegmental nucleus; ROS, reactive oxygen species; SA, self-administration; SEBT, serotonin transporter; SN, substantia nigra; TA, trace amine-associated receptor 1; TH, tyrosine hydroxylase; THC, tetrahydrocannabinol; TLR4, toll-like receptor 4; TPH, tryptophan hydroxylase; TRkB, tropomyosin receptor kinase B; TSPO, mitochondrial translocator protein; VGCC, voltage-gated calcium channel; vHCC, ventral hippocampus; VMAT-2, vesicular monoamine transporter 2; VTA, ventral tegmental area.
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Abstract—Adaptation of the nervous system to different chemical and physiologic conditions is important for the homeostasis of brain processes and for learning and remembering appropriate responses to challenges. Although processes such as tolerance and dependence to various drugs of abuse have been known for a long time, it was recently discovered that even a single pharmacologically relevant dose of various drugs of abuse induces neuroplasticity in selected neuronal populations, such as the dopamine neurons of the ventral tegmental area, which persist long after the drug has been excreted. Prolonged (self-) administration of drugs induces gene expression, neurochemical, neurophysiological, and structural changes in many brain cell populations. These region-specific changes correlate with addiction, drug intake, and conditioned drugs effects, such as cue- or stress-induced reinstatement of drug seeking. In rodents, adolescent drug exposure often causes significantly more behavioral changes later in adulthood than a corresponding exposure in adults. Clinically the most impairing and devastating effects on the brain are produced by alcohol during fetal development. In adult recreational drug users or in medicated patients, it has been difficult to find persistent functional or behavioral changes, suggesting that heavy exposure to drugs of abuse is needed for neurotoxicity and for persistent emotional and cognitive alterations. This review describes recent advances in this important area of research, which harbors the aim of translating this knowledge to better treatments for addictions and related neuropsychiatric illnesses.

I. Introduction

Brain diseases are associated with an enormous cost to affected individuals, their families, and the society. In Europe, it has been estimated that the total yearly cost of brain diseases in 2010 was close to 800 billion euros (Olesen et al., 2012), of which addictions are responsible for as much as anxiety disorders, with only dementia and mood illnesses costing more (DiLuca and Olesen, 2014). Drug abuse produces both direct and indirect costs to the society, although many of the drugs are also clinically used to treat various patient groups. The purpose of this review is to present up-to-date knowledge of the mechanisms of action of the main drugs of abuse and to reveal the possible long-term alterations in the nervous system associated with the use and abuse of various drugs acting on the brain, also paying attention to the trajectory of brain development.

Addiction is a complex phenomenon, which is not only dependent on pharmacological mechanisms, but also has a societal/cultural dimension. This is reflected in the proportion of drug addiction among different age and ethnic groups studied in the United States. Individuals with alcohol or illicit drug abuse or dependence in the past year constituted 5% of the adolescents aged between 12 and 17 and more than 8% of all individuals aged 12 or older (SAMSHA, 2014a). Of the racial/ethnic groups studied among those aged 12 and older, Asians had the lowest proportion of binge alcohol drinkers and past-month illicit drug users, likely partly due to societal/cultural traditions. These findings on rather widespread drug and alcohol exposures at young ages are very alarming, because brain development continues well past the age of 17 years and because the exposure to several different drugs, such as illicit drugs, cigarettes, and alcohol, appears to concentrate on the same individuals (SAMSHA, 2014b). Therefore, it is possible that harmful effects from early drug use will prevail later in life, because drugs of abuse induce different modulations in brain circuitries (adaptation, plasticity, learning, and memory) due to their pharmacological actions and due to behavioral/social effects associated with their use and settings. Thus, we will

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explore the data on different drugs and how they persistently affect brain functions if the drug exposure occurs during adolescence.

The review first gives a general basic introduction to neuroplasticity. Then sections on different drugs follow and they have different foci, because the various drugs do not invoke exactly similar mechanisms or adaptations in the nervous system. The review will end with a short general summary.

Certain mechanisms seem to be common for many drugs, for example, activation of the extracellular signal-regulated kinase (ERK) pathway in specific brain structures is necessary for effects of and tolerance to cocaine, nicotine, MDMA, phencyclidine, alcohol, and cannabinoids after both acute and chronic treatments in rodents (Kyossева et al., 2001; Salzmann et al., 2003; Valjent et al., 2004; Rubino et al., 2005; Tonini et al., 2006; Schroeder et al., 2008). One human postmortem brain study suggested that cocaine, cannabis, and/or phencyclidine abuse all decrease transcription of calmodulin-related genes and increase transcription of genes related to lipid/cholesterol and Golgi/endoplasmic reticulum (ER) function in the anterior prefrontal cortex (PFC), which may underlie changes in synaptic function and plasticity (Lehrmann et al., 2006). However, postmortem brain regional gene expression profiling in alcohol-dependent patients has indicated widely differing sets of affected genes between different brain regions (Flatscher-Bader et al., 2005, 2006), with alcoholics nevertheless being easily separable from non-alcoholic controls and smokers (Flatscher-Bader et al., 2010). The same situation was found for other drugs of abuse (Albertson et al., 2004, 2006). Significant alterations in glutamate and GABA receptor mRNAs were found in postmortem brains of alcohol-dependent subjects, but the changes differed from one brain region to another (Jin et al., 2011, 2014a,b; Bhandage et al., 2014). For these obvious reasons, we reviewed the literature for each drug according to the specific brain regions where alterations have been observed most commonly in preclinical experiments. These brain areas and pathways, which serve for positive and negative reinforcing behavioral and emotional effects and for goal-directed and habitual drug seeking, are illustrated in Fig. 1. However, it will be necessary in the future to study the plasticity and mechanisms more precisely at the level of different neuronal populations and subpopulations.

As is usual in addiction-related reviews and research, the dopamine (DA) mechanisms are center stage. The reader is referred to recent reviews on various aspects of DA as a neurotransmitter and a regulator of motor, cognitive, and motivated behavior (Bjorklund and Dunnett, 2007; Beaulieu and Gainetdinov, 2011; Salamone and Correa, 2012). DA is important for a wide number of brain functions, and it will be impossible to cover each and every aspect of the DA mechanisms in drug actions and adaptations. The focus in this review will be more on the neuroplasticity of the glutamate synapses on DA neurons.

Pharmacological actions of drugs are mediated by specific receptors. On the other hand, the effects of the drugs of abuse are often modulated by experimental settings and expectancies in both preclinical studies and human experiments. Drug intake in rodents is dependent, for example, on cage conditions, with the effects differing between opioids and stimulants (Badiani et al., 2011). Voluntary self-administration (SA) (self-stimulation or cocaine) induces different brain regional activations (Porrino et al., 1984), or more prolonged synaptic molecular adaptations, than experimenter-given stimulation or drug injections (Chen et al., 2008). Placebo/nocebo effects are real in human studies, and the expectancy of strong or uncertain drug effects are known to affect human brain imaging results in response to acute drugs (Volkow et al., 2010). For example, in smokers, positive and negative beliefs on nicotine content of cigarettes influenced functional magnetic resonance imaging (fMRI)-scanned striatal responses to value and reward prediction errors during an investment task (Gu et al., 2015), indicating that beliefs can affect cognitive performance also under the drug-associated states. These issues indicate that, in addition to direct pharmacological actions, abused drugs may also change basic learning and memory processes, for example, by conditioning and environmental factors.

A. Different Forms of Neuroplasticity

The ability of the brain to remodel its connections functionally and structurally in response to individual experience has been described by the concept of neuroplasticity. Neuroplasticity occurs on a variety of levels ranging from molecular changes in synapses to large-scale changes involved in neurocircuitry remapping. Synaptic plasticity refers to adaptive changes in the strength of synaptic connections. On the basis of its time frame, synaptic plasticity has been classified as short-term (acts on a timescale of milliseconds to minutes) and long-term (hours to days) plasticity. Short-term plasticity is achieved through transient changes, such as facilitation or depression of a synaptic connection, which then quickly return to their initial state. However, repeated stimulation causes a persistent change in the connection to achieve long-term plasticity.

Hebbian or activity-dependent plasticity is the most studied form of long-term plasticity. It occurs when presynaptic stimulation coincides with postsynaptic depolarization (Hebb, 1949; Bi and Poo, 2001). The best-known example of Hebbian plasticity is N-methyl-D-aspartate receptor (NMDAR)-mediated long-term potentiation (LTP). Importantly, this form of LTP occurs only in synapses that actively contribute to the induction process, so it is input and synapse specific. The term “anti-Hebbian” plasticity currently describes
either long-term depression (LTD) (Nelson, 2004) or LTP that occurs when presynaptic activation coincides with postsynaptic inactivity (Kullmann and Lamsa, 2007).

In rodents, the neuronal organization remains immature at birth (see below). The process that describes changes in neuronal organization during development as a result of environmental interactions and experience/learning-induced neural changes is known as developmental plasticity. To maintain the balance between neuronal excitation and inhibition, homeostatic plasticity regulates the overall activity of complex circuits by specifically regulating the destabilizing effects of developmental and learning processes. Otherwise, activity-dependent forms of plasticity could drive neural activity toward runaway excitation or quiescence (Miller, 1996; Turrigiano, 1999; Turrigiano and Nelson, 2004).

Metaplasticity refers to “plasticity of synaptic plasticity,” which describes changes in the ability to induce further synaptic plasticity (Abraham and Bear, 1996). For example, prolonged exposure to cocaine induces a population of silent glutamatergic synapses in the nucleus accumbens (NAc) that form sites for future plasticity (reviewed in Lee and Dong, 2011).
To help appreciate the effects of abused drugs on synaptic plasticity, in the following paragraphs we will briefly introduce mechanisms of the most common presynaptic and postsynaptic forms of synaptic plasticity.

B. Short-term Neuroplasticity

Short-term plasticity can appear as a transient facilitation, depression, or augmentation and posttetanic potentiation in synaptic strength that lasts for up to a few minutes (reviewed in Fioravante and Regehr, 2011). Despite the variety in synaptic neurotransmitters, all forms of short-term plasticity are primarily governed by presynaptic mechanisms associated with fluctuations of presynaptic residual Ca^{2+}, which acts on one or more molecular targets, resulting in the changes in neurotransmitter release (Fig. 2). Enhancement of Ca^{2+} channel activity and increases in the probability of Ca^{2+} influx, altered vesicle pool properties, local depletion of Ca^{2+} buffers, and increases in quantal size of neurotransmitter release contribute to short-term facilitation (Fioravante and Regehr, 2011). On the other hand, vesicle depletion, inactivation of neurotransmitter release sites, and Ca^{2+} channels contribute to short-term synaptic depression (Neher and Sakaba, 2008). In addition, glial-neuronal interactions impact on short-term synaptic plasticity by controlling the speed and extent of neurotransmitter clearance from the synaptic cleft (Bergles et al., 1999) as well as by astroglial release of substances that can affect synaptic efficacy (Araque et al., 2001). Most importantly, there is retrograde communication between post- and presynaptic terminals: both endocannabinoids (reviewed in Wilson and Nicoll, 2002; Kano et al., 2009) and nitric oxide

![Fig. 2. Typical induction protocols and main factors regulating mechanisms of short-term plasticity. (A) Brief paired-pulse (PP) stimulation induces short-term facilitation of neurotransmission by transiently increasing the Ca^{2+}-dependent readily releasable pool (RRP) of synaptic vesicles. (B) Short-term depression of neurotransmission can be induced by frequent tetanic stimulation, which transiently depletes synaptic vesicles. (C) Depolarization-induced suppression of inhibition (DSI) or similarly that of excitation (DSE; not shown) is a locally induced transient depression of neurotransmission that is dependent on retrograde eCB signaling. Depolarization-induced suppression of inhibition/excitation begins with stimulation of excitatory neuronal connections inducing eCB synthesis, and 2-AG in particular then moves to activate presynaptic CB1 receptors on surrounding neurons. This induces local, transient depression of inhibitory or excitatory (not shown) neurotransmission.](image-url)
(NO)-guanylyl cyclase signaling (Sammut et al., 2010) contribute in a general transient “local” process that tunes neurotransmitter release and different aspects of synaptic dynamics both in inhibitory and excitatory synapses (discussed in more detail in sections II.G and II.H). Short depolarization of a neuron may cause a transient suppression of excitation or inhibition of that and neighboring neurons, called depolarization-induced suppression of excitation or inhibition (DSE and DSI), which was found to be dependent on retrograde endocannabinoid (eCB) signaling (see section II.H on cannabinoids).

C. Main Forms of Long-term Neuroplasticity: Long-term Potentiation and Long-term Depression

Long-term forms of synaptic plasticity can appear as potentiation (long-term potentiation, LTP) or depression (long-term depression, LTD) in synaptic strength.
that last for hours to weeks. In contrast to short-term plasticity, the nature of long-term forms of plasticity involves both pre- and postsynaptic alterations (Fig. 3).

1. Presynaptic Forms of Long-term Plasticity.

Presynaptic LTP has been best studied at hippocampal CA3 mossy fiber synapses (Weisskopf et al., 1994; Nicoll and Schmitz, 2005), but similar forms of LTP have been found in multiple brain areas (Salin et al., 1996; Castro-Alamancos and Calcagnotto, 1999; Lopez de Armentia and Sah, 2007). This presynaptic LTP appears at both excitatory and inhibitory synapses and does not require postsynaptic NMDARs (but see Yeckel et al., 1999). Instead, presynaptic LTP appears to be induced by an activity-dependent rise of presynaptic residual calcium that results in a rise in cAMP and subsequent activation of protein kinase A (PKA). This, in turn, modifies the functions of proteins that act to coordinate synaptic vesicle interactions with the presynaptic active zone, leading to a long-lasting increase in neurotransmitter release (Castillo, 2012).

Endocannabinoid-mediated long-term depression (eCB-LTD) is a widely expressed form of long-term plasticity at both excitatory and inhibitory synapses. Brief robust neuronal stimulation triggers the synthesis of eCBs, lipophilic molecules that travel retrogradely across the synapse to activate the presynaptic CB1 cannabinoid receptors (see section II.H), which suppresses neurotransmitter release via a wide range of effector molecules, including voltage-dependent calcium channels (VGCC), potassium channels, PKA, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinases (JNK) (reviewed in Howlett et al., 2002). Importantly, CB1 receptors activation per se is not sufficient for eCB-LTD induction. Rather, the presynaptic terminal integrates multiple signals to generate eCB-LTD (Heifets and Castillo, 2009). CB1 receptors also mediate short-term plasticity of DSI and DSE (Fig. 2). Interestingly, some hippocampal inhibitory synapses can undergo both short- and long-term forms of eCB-mediated plasticity, where the time frame of depression (short-term versus long-term manner) depends on the downstream signaling pathways (Heifets and Castillo, 2009). Particularly, cAMP/PKA-dependent signaling has been shown to be necessary only for eCB-LTD but not for DSI (Chevaleyre and Castillo, 2003).

2. Postsynaptic Forms of Long-term Plasticity.

NMDAR-dependent LTP and LTD are the best understood forms of long-lasting synaptic plasticity (Bliss and Lomo, 1973; Hayashi et al., 2000). Early phases of LTP and LTD are mediated by a redistribution of both α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid receptors (AMPAR) and NMDARs at the postsynaptic membrane and/or by changes in presynaptic transmitter release. With time, such changes are consolidated by structural alterations, which require synthesis of new proteins (Kasai et al., 2010).

In vitro experiments on acute brain slices, LTP and LTD can be induced by distinct patterns of activity (reviewed in Holscher, 1999; Luscher and Malenka, 2012) (Fig. 3). High-frequency stimulation (HFS) of the presynaptic cell (tetanic pulses at 50–100 Hz) is commonly used to induce LTP. This stimulation protocol causes a strong postsynaptic depolarization that removes the Mg2+ block of NMDARs, allowing timed Ca2+ influx. This triggers the downstream molecular cascades inducing LTP. In contrast, low-frequency stimulation (LFS, at 0.1–5 Hz) often induces LTD. Typically, it causes only a weak postsynaptic depolarization that results in a modest but prolonged Ca2+ influx triggering the downstream molecular cascades driving to LTD.

Since the original discovery of LTP in the hippocampus (HC) (Bliss and Lomo, 1973), LTP and LTD have been observed in a variety of other brain regions including the ventral tegmental area (VTA), NAc, PFC, and amygdala ex vivo (Luscher and Malenka, 2011) and in vivo (Canals et al., 2009; Zhang et al., 2015b). Different brain regions appear to exhibit different forms of LTP and LTD, and therefore, synapses recruit different signaling pathways to accomplish their functions. The most studied forms of neuroplasticity are NMDAR-dependent LTP and LTD, with the NMDARs providing the major pathway for Ca2+ influx (Huang and Kandel, 1996). There is also NMDAR-independent LTP and LTD, in which VGCC (Kato et al., 2009) or GluA2 subunit-lacking AMPARs (Lamsa et al., 2007) provide the Ca2+ influx triggering induction.

In the early phases of LTP, elevated Ca2+ triggers persistent activation of protein kinases including PKA, Ca2+/calmodulin kinase II (CaMKII), and protein kinase C (PKC). A striking feature of CaMKII is its capacity for autophosphorylation at threonine residue Thr286, which keeps this kinase activated even in the absence of Ca2+ (Giese et al., 1998). During this stage, atypical protein kinase C (PKMζ) may also become autonomously active (Ling et al., 2006; Sacktor, 2008). Autonomously active and other protein kinases use phosphorylation to carry out the two major mechanisms underlying the expression of LTP: first, they phosphorylate existing AMPARs and NMDARs to increase their activity, and second, they mediate the insertion of new receptors into the postsynaptic membrane (see below for more detailed description).

3. α-Amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid and N-methyl-d-aspartate Receptor Phosphorylation.

Huganir and coworkers made detailed experiments on the regulation of AMPAR trafficking and function by phosphorylation during LTP/LTD (reviewed in Shepherd and Huganir, 2007). During LTP, PKA and CaMKII are recruited to phosphorylate serine residues in the GluA1 subunit at Ser831 (Mammen et al., 1997) and Ser845 (Roche et al., 1996) and the GluA2 subunit at Ser880 (Chung et al., 2000), promoting receptor insertion and synaptic potentiation (Malenka and Bear,
Inhibiting of PKA and CaMKII or removal of the above-mentioned phosphorylation sites from AMPAR subunits can impair LTP (Lee et al., 2003; Malenka and Bear, 2004). Importantly, knock-in mutant mice that lack both Ser831 and Ser845 phosphorylation sites on GluA1 subunits demonstrated impaired memory in behavioral tests (Olivito et al., 2014).

Conversely, during LTD, phosphatases calcineurin and protein phosphatases 1 and 2 are recruited to dephosphorylate AMPARs, promoting their removal from the synapse (Malenka and Bear, 2004; Huganir and Nicoll, 2013).

Phosphorylation of NMDARs is also essential during LTP/LTD expression because it contributes to stabilization of the receptors at the synapse by forming more stable binding to postsynaptic density (PSD) proteins. In fact, Fyn and Src kinases phosphorylate GluN2B subunit at Tyr1472, which prevents the clathrin adapter protein AP-2 from binding to the GluN2B subunit, thus blocking endocytosis (Zhang et al., 2008).

4. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-d-aspartate Receptor Trafficking. During LTP, new GluA2 subunit-lacking AMPARs are rapidly transported to the cell surface under the influence of protein kinases (Malinow and Malenka, 2002; Luscher and Malenka, 2012). Importantly, these new AMPARs exhibit some unique properties: first, they have greater single-channel conductance and, second, greater permeability to Ca2+ ions, which facilitates Ca2+-dependent signaling events. Noteworthy, this early insertion of new Ca2+-permeable non-GluA2-containing AMPARs to the synapse is independent of protein synthesis. This is achieved by having a non-synaptic pool of AMPARs adjacent to the postsynaptic membrane (Kauer and Malenka, 2007). Moreover, NMDARs are also actively replaced during LTP/LTD (Barria and Malinow, 2002). However, the data on NMDARs are still puzzling due to wide variability in expression, kinetics, and regulation sites (reviewed in Shipton and Paulsen, 2014). Particularly interesting for LTP expression are the GluN2B subunit-containing NMDARs because of their special association with CaMKII. This interaction maintains CaMKII close to its substrates, such as AMPARs, to initiate the phosphorylation events that support synaptic strengthening.

Postsynaptic LTD is associated with receptor withdrawal from the synapses (Hayashi et al., 2000; Bellone and Luscher, 2006). LTD can also be generated by activation of VGCCs (Christie et al., 1997) or metabotropic glutamate receptors (mGlu) (Bellone and Luscher, 2006) and does not require NMDAR activation. Although the above cellular model of LTP/LTD presented above is explained entirely by its postsynaptic mechanisms, additional events may also occur presynaptically, for example, via alteration in probability of glutamate vesicle release by different retrograde signaling mechanisms (reviewed in Luscher and Malenka, 2012).

With time, the insertion/removal of additional receptors is likely to result in the rearrangement of the PSD with different scaffolding proteins, spine ultrastructure, or even spine density that requires gene transcription and protein synthesis. In fact, spines associated with synapses that underwent LTP were found to be enlarged (Matsuzaki et al., 2004; Holtmaat and Svoboda, 2009; Kasai et al., 2010), whereas spines that underwent LTD were shrunken or even disappeared (Nagerl et al., 2004; Kasai et al., 2010). Although these structural changes are not absolutely required for neuroplasticity, the stabilization and maintenance of existing synapses needs activity of several kinases such as PKA, CaMKIV, PKM2, and ERK that induce protein synthesis either locally in the dendrites from prefabricated mRNAs or by nuclear transcription (Sacktor, 2008). These mechanisms are also involved in synaptic tagging, an activity-driven molecular labeling of synapses to be strengthened (Frey and Morris, 1997; Sajikumar and Frey, 2004; Sajikumar and Korte, 2011).

D. Forms of Long-term Plasticity at Inhibitory Synapses

Several forms of NMDA receptor-dependent neuroplasticity (both LTP and LTD) have been described at inhibitory GABAergic synapses (reviewed in Castillo et al., 2011). Depending on interneuron subtype and brain region, inhibitory synaptic plasticity results in changes either in presynaptic GABA release or in postsynaptic γ-aminobutyric acid A (GABA_A) receptor responsiveness. Interneurons also show glutamatergic synapse plasticity, which has characteristics that are partly different from those of principal neurons (reviewed in Kullmann and Lamsa, 2011).

Presynaptic inhibitory plasticity is mediated via retrograde messengers produced in an activity-dependent manner (by afferent stimulation of excitatory inputs) in the postsynaptic neuron and transferred back across the synapse to modulate presynaptic GABA release. The most studied presynaptic form of the plasticity is the eCB-mediated inhibitory LTD, in which released eCBs inhibit presynaptic GABA release by acting on CB1 cannabinoid receptors (Chevaleyre et al., 2006; Adermark et al., 2009) (Fig. 3). Another form of inhibitory plasticity, NO-mediated inhibitory LTP, requires NO as a retrograde messenger to stimulate presynaptic guanylyl cyclase, resulting in the potentiation of presynaptic GABA release (Nugent and Kauer, 2008). Brain-derived neurotrophic factor (BDNF)-tropomyosin receptor kinase B (TrkB)-mediated inhibitory LTP recruits BDNF that activates its presynaptic receptor TrkB and potentiates GABA release (Xu et al., 2010). It is evident that GABAergic synapses from interneuron populations are very plastic and actively participate in brain circuit refinement, learning, and memory formation (Chen et al., 2015).
Postsynaptic inhibitory plasticity is also mediated by diverse mechanisms in different synapses. Similar to glutamatergic receptors, phosphorylation of postsynaptic GABA<sub>A</sub> receptors by protein kinases, including PKA, PKC, CaMKII, and Src, leads to changes in the integral anion channel function (reviewed in Nakamura et al., 2015). The effects of phosphorylation are dynamic (increases and decreases in receptor activity) and dependent on the subunit composition of the GABA<sub>A</sub> receptor in a fashion that is not fully known at present. Moreover, phosphorylation of cation-chloride cotransporters such as Na<sup>+</sup>-Cl<sup>-</sup> cotransporters, Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporters, and K<sup>+</sup>-Cl<sup>-</sup> cotransporters (KCCs) affects their activity and consequently the amplitude of GABA<sub>A</sub> receptor-mediated responses by altering the transmembrane anion gradients (reviewed in Kaila et al., 2014). The function of GABA<sub>A</sub> receptors is further dynamically regulated by constitutive exo- and endocytosis of receptors at GABAergic synapses (reviewed in Michels and Moss, 2007).

E. Developmental Maturation of the Brain in Rodents and Humans

Brain development is a delicate and complicated process that can be adversely affected by drugs during pregnancy, leading to teratogenic effects (reviewed in Manent et al., 2008; Jutras-Aswad et al., 2009; Holbrook and Rayburn, 2014; Ross et al., 2015). In the present review, there will be only short notes on drug actions on fetal brain development, such as fetal alcohol effects during pregnancy or early postnatal period in rodents (Valenzuela et al., 2012). A thorough comparative analysis of early brain maturation in different species, including rodents and human, recently appeared (Workman et al., 2013). It is possible that vulnerability for drug abuse may be greater the younger the exposure/use of drugs is started.

Developmental neuroplasticity is based on genetic programs in addition to activity-dependent processes, and after the maturation, the neuroplasticity is expected to be more homeostatic, dependent on molecular interactions in brain cells, neurites, spines, and synapses within a relatively rigid extracellular milieu. The relevant question to the review of drug-induced neuroplasticity is the vulnerability of the brain to drug effects during periods of childhood, adolescence, and adulthood. These periods are often defined in humans as adolescence, starting at 10 to 12 years of age and ending at 18 to 20 years. Childhood precedes it and adulthood comes after it, and these periods can be further subdivided into shorter periods by using behavioral and brain maturation indexes (Bossong and Niesink, 2010; Pressler and Auvin, 2013; Semple et al., 2013). For the present review an important aspect to consider is how rodent models at specific postnatal ages compare with human childhood, adolescence, and adulthood. Figure 4 gives a rough estimation of how to relate differently timed rodent experiments to human brain developmental stages. The figure depicts various brain cell developmental processes, emergence of selective behavioral phenotypes, and the development of the behavioral modulation of brain processes by external stimuli in humans and rodents.

Several precautions have to be kept in mind, though. First, there is a large variation between the developmental program timescale for different brain regions, with the cortical regions and the cerebellum developing last (Huttenlocher, 1979; 1990; Huttenlocher and Darbholkar, 1997). Second, there are genetic, sex, and disease vulnerability differences between the phases of brain development (Thompson et al., 2001; Vasileiadis et al., 2009; Asato et al., 2010; Brex et al., 2013; Dennis and Thompson, 2013). Third, various neurotransmitter systems develop at different timelines.
in different brain regions, with the DAergic and GABAergic systems of the cortex developing late (Tseng et al., 2007; Hashimoto et al., 2009; Kilb, 2012; Manit et al., 2013; Ouellet and de Villers-Sidani, 2014). Fourth, various neurotrophic factors and their receptors have differential expression profiles in various brain regions (Perovic et al., 2013). Thus, the prolonged effects of drugs of abuse are also dependent on the developmental time of the exposure to drugs (Stanwood and Levitt, 2004). Furthermore, activating and stress-relieving effects including neonatal handling, maternal care, juvenile play, enriched environment, social contacts or lack thereof, and lesioning effects on the brain also have long-term effects and their critical times (Meaney et al., 1988; Liu et al., 1997; Caldji et al., 1998; Bouwmeester et al., 2002; Sale et al., 2007; Tseng et al., 2009; Foscarin et al., 2012). A good example is the differential activation of the caudate-putamen, NAc, and PFC of rats at different ages by a stress-inducing dose of the GABA_A receptor benzodiazepine-site inverse agonist FG-7142 (Lysy et al., 1999) known to induce a stress-like activation of the mesocortical DA system in adult rats. The DA system has a delayed maturation in the cortex, becoming mature by postnatal day 60 (Kalsbeek et al., 1988). At postnatal day 10, only the NAc is strongly activated by FG-7142, at day 18 all regions start to be activated, and finally at 45 and 100 days of age, the PFC is the brain region that is activated the most.

Potential for neuroplasticity is greater in young than adult rodents (Hensch, 2005; Bavelier et al., 2010; Hensch and Bilimoria, 2012), as is demonstrated, for example, by easier induction of the NMDAR-dependent LTP using tetanic stimulation in the NAc neurons of 3-week-old than adult mice (Schramm et al., 2002). Furthermore, the brain growth-promoting and limiting responses to injury are blunted and dysregulated in elderly (22–24 months) versus adult (2–4 months) rats (Li and Carmichael, 2006; Li et al., 2010b).

Perhaps an extreme illustration of the prolonged neurodevelopmental process and normal potential for neuroplasticity in humans may be the profiles of layer IIIc and V spine densities in normal PFC (Petanjek et al., 2011), which is illustrated in Fig. 4A. The spine densities were determined using Golgi staining of postmortem brain sections at various ages. The peak density of dendritic spines is seen at the age of 5–12 years, after which the density slowly reduces to stable levels after 30 years of age. This extended spine-pruning period is consistent with the results of human functional magnetic resonance imaging and connectivity studies that have indicated prolonged developmental changes in the cortical areas (Thompson et al., 2005; Knickmeyer et al., 2008; Dosenbach et al., 2010). A corresponding delayed development of the structural connectivity between basolateral amygdala and mPFC has been observed in the rat (Cunningham et al., 2002), with a wide range of different experiences, including the effects of various drugs of abuse, known to induce long-lasting plasticity in the PFC also in the adulthood (Kolb and Gibb, 2015).

F. Developmental Neuroplasticity: Critical Periods, Reopening of Plasticity in Adults

The development and critical period plasticity of the sensory systems, particularly the visual cortex, have been well described. In rats and mice, critical periods of plasticity in the visual, somatosensory barrel, and auditory cortices take place early in the development of these sensory systems. Importantly, some of the associated plasticity mechanisms are reused or reopened in adult neuronal plasticity. Drugs inhibiting or stimulating the GABA_A receptors can promote or delay the critical periods, respectively (Hensch, 2005). Synaptic pruning eliminates extra nonfunctional connections formed during initial overproduction of synaptic connections, while strengthening functionally important ones (Changeux and Danchin, 1976). In the normal adult brain, neural plasticity is needed for maintaining neuronal network excitability, large-scale regulation of cortical and subcortical circuits, and fine-scale experience-dependent refinement and maintenance of local circuits (Griffen et al., 2012).

The extracellular matrix (ECM) network is an active participant in brain function and neuroplasticity (reviewed in Pavlov et al., 2004). The ECM consists of various molecules (glycoproteins and proteoglycans) secreted by all cells that are assembled inside this matrix (Bosman and Stamenkovic, 2003). ECM network regulates synaptogenesis, consolidation, strengthening, and maintenance of synapses (Fields and Itoh, 1996). During development, ECM participates in neuronal differentiation, neuronal movement, guidance for growing axons, and synaptogenesis (Pavlov et al., 2004). In the mature brain, ECM is crucial not only for anchoring of neurons and organization of brain regions (structural function) but also for transducing a wide range of signals to the neurons (Thalhammer and Cingolani, 2014). Interaction between the ECM and cell is facilitated by cell adhesion molecules (CAMs), particularly by integrins. Integrins are heterodimeric transmembrane glycoprotein receptors that mediate ECM-cell interactions via binding ECM proteins and cellular transmembrane proteins (e.g., ion channels and growth factor receptors). ECM plays an important role in regulating actin polymerization, spine morphology, and GluA2 subunit trafficking in culture (Wu and Reddy, 2012). In regard to this review, suppression of the synthesis of integrin-linked kinase increases the level of Ser845 phosphorylated GluA1 subunit-containing receptors and spine density in the NAc and blocks cocaine-induced sensitization (Chen et al., 2010). Moreover, disruption of integrin expression in the NAc interfered with trafficking of GluA2 subunit-containing receptors, which affected cocaine-induced conditioned place preference (CPP) and reinstated drug seeking (Wiggins et al., 2011).
Perineuronal nets (PNN), first described by Camillo Golgi (reviewed in Celio et al., 1998), develop around specific neuronal populations to protect highly active neurons and to preserve their structure in an experience-dependent manner during pruning of extra connections in the visual cortex and many other parts of the central nervous system (CNS) (Kwok et al., 2011; Soleman et al., 2013; Ye and Miao, 2013). PNNs are primarily composed of chondroitin sulfate proteoglycans (e.g., aggrecan, versican, neurocan, brevican, and phosphacan), hyaluronan, link proteins such as cartilage link protein 1 Crtl1 or Hapln1 (Carulli et al., 2010), and tenascins. In the cortex, PNNs selectively surround the parvalbumin (Pv)-containing fast-spiking GABAergic interneurons (Ye and Miao, 2013), starting after postnatal day 10 (P10) and achieving adult levels by P42. Thus, the PNNs develop during the critical period, and after closing, the critical period for plasticity can be reopened by degradation of the PNNs (Pizzorusso et al., 2002). Although the negatively charged components of the PNN can have multiple molecular interactions and functions, for the maturation of Pv-interneurons and regulation of critical periods, the PNN provides a high-affinity binding site for the lysine-arginine containing domain of the transcription factor Otx2 (orthodenticle homeobox 2) (Beurdeley et al., 2012). Once Otx2 reaches the Pv-interneurons of the visual cortex, being synthesized in the retina or the lateral geniculate of the visual tract (Sugiyama et al., 2008), it induces expression of Pv, GAD65 (glutamate decarboxylase 65 kDa), GABA_A α1 subunit, and K,3.1b K+ channels specifically in these target neurons (Sugiyama et al., 2008). This process is experience dependent, that is it cannot begin before eye opening, after which the critical period for setting ocular dominance and visual acuity is started (Fagiolini and Hensch, 2000; Sugiyama et al., 2009). Pv-interneurons in the visual cortex mature in connections (e.g., forming perisomatic synapses on principal neurons) and intrinsic firing properties (e.g., fast spiking) by the end of the critical period, thus allowing the firm functional cortical connections for binocular visual acuity to be formed (Kuhlman et al., 2010). PNNs interact with receptors such as NgR1 (NoGo receptor) and leukocyte common antigen- and phosphatase (Abbk et al., 2013; Ye and Miao, 2013).

PNN formation in the basolateral amygdala (BLA) stabilizes fear memories in adults, unlike in the developing brain when they can be readily extinguished (Gogolla et al., 2009). Degradation of PNN by the enzyme chondroitinase ABC in the BLA, left the fear conditioning intact, but after extinction the animals failed to spontaneously recover the fear and to express freezing under the fear-related context, indicating erasure of the memory by degradation of the PNN. Two recent papers indicate that PNNs affect drug- and experience-induced memory in rodents. First, Karpova et al. (2011) showed that fluoxetine treatment during extinction training in fear-conditioned adult mice led to erasure of fear memory, associated with a reduction in the proportion of Pv-containing interneurons with PNN expression in the BLA, HC, and infralimbic cortex (IL). In the BLA, fluoxetine treatment also increased the expression of polysialyated neuronal cell adhesion molecule (PSA-NCAM) and decreased KCC2 levels in the BLA and HC, with all these data suggesting that fluoxetine treatment with extinction training induced a juvenile-like state in the interneurons of the fear circuitry. Second, infusions of chondroitinase ABC into the BLA or the central nucleus of the amygdala (CeA) of adult rats during extinction of morphine- and cocaine-induced CPP prevented priming-induced reinstatement (Xue et al., 2014). This effect was associated with increased levels of AMPAR GluA1 and GluA2, but not GluA3, and increased BDNF levels in the BLA. Thus, the extracellular matrix and its restructuring seem to be involved in the modulation of specific memories that can also be associated with persistent drug effects. Perhaps, changes in the matrix may be developed into future therapies for enhancing or reversing plasticity (Castren et al., 2012). However, we should learn more about how the extracellular matrix induces or restricts plasticity and when would be the right time to increase or decrease the matrix components in appropriate neuronal circuits.

One of the major anionic extracellular components around mature interneurons is formed by PSA-NCAM molecules with antiadhesive properties. Expression of PSA-NCAM has been described in a subpopulation of GABAergic calbindin-positive and somatostatin-positive neurons in the adult rat and mouse cerebral cortex, without expression in the principal neurons or other interneuron types (Gomez-Climent et al., 2011). These PSA-NCAM-positive interneurons had reduced synaptic connections as judged from confocal immunohistochemical counting of synaptophysin-positive perisomatic and peridendritic puncta in close apposition to GAD67-expressing cells. This suggests that expression of PSA restricts the synaptic connections of the interneurons in mature brain. Interestingly, DAergic agonists increase (Castillo-Gomez et al., 2008) and also serotonergic drugs increase (or downregulate stress-elevated) (Wedzony et al., 2013) production of PSA in the mPFC. NCAM is needed for hippocampal LTP that is deficient in NCAM-KO mice (Caroni et al., 2012; Stoenica et al., 2006). Intraventral mPFC infusion of endonectumaminidase, which cleaves PSA from NCAM to mice that were trained to extinguish operant alcohol-seeking behavior, strongly attenuated extinction (Barker et al., 2012), suggesting that reduced levels of PSA-NCAM restricts the plasticity needed for extinction. Similarly to PNNs, extracellular NCAM and PSA-NCAM mechanisms also participate in retaining the mature structures, although they are also needed for novel connections and pathways of plasticity. They do offer a number of possible, but still deficiently characterized, targets for drug action, and they may be important for understanding impairments of interneuron function.
in depression and schizophrenia (Nacher et al., 2013; Wedzony et al., 2013).

**G. Neurotrophins as Regulators of Neuroplasticity: Brain-Derived Neurotrophic Factor as an Example**

BDNF is an important trophic factor for nerve cells in the CNS (Park and Poo, 2013), and it serves here as an example of the multiple roles that the neurotrophins are playing in orchestrating development, maintenance, and plasticity of neurogenesis and neural connections. It is secreted from various cells activity dependently and exerts effects on local synaptic plasticity.

In short, mature BDNF is a 14-kDa protein that is cleaved from prepro-BDNF expressed both in neurons and glial cells in the CNS. BDNF gene has IX promoters and at least the promoter IV is activity dependent with Ca²⁺ and cAMP response elements (Park and Poo, 2013). BDNF preferentially acts via activation of tropomyosin receptor kinase B (TrkB) (Minichiello, 2009). Like many of the trophic factor receptors, TrkB belongs to the family of receptor tyrosine kinases. Signaling of BDNF via TrkB leads to activation of Ras-ERK, phosphatidyl inositol 3-kinase (PI3K) and phospholipase C, gamma (PLCγ) pathways, which serve for survival, differentiation, and synaptic plasticity phenomena in neurons (Minichiello, 2009). Pro-BDNF activates preferentially P75 neurotrophin receptor and often has opposite or different effects than mature BDNF (Lu et al., 2005).

BDNF has important interactions with the GABA system. It is needed for the GABA system to become inhibitory during development (Huang et al., 1999), but its activation in the mature nervous system may lead to reduction of KCC2 expression and subsequent change to GABAergic excitation. This has been described during development of neuropathic pain in the spinal cord, with activated microglial cells releasing BDNF, which makes the circuitry abnormally sensitive to stimuli due to downregulation of KCC2 expression (Coulil et al., 2005) and due to LTP induction via GluN2B upregulation (Ding et al., 2014). In similar preclinical models, certain benzodiazepine-site agonists, the efficacy of which is supposed to be dependent on KCC2-driven Cl⁻ gradients, surprisingly show strong analgesic effects by ablating neuropathic pain symptoms (Knabl et al., 2008; Paul et al., 2014a), stressing that multiple parallel pathways are involved (Zeilhofer et al., 2012). The KCC2 downregulation and GABAergic excitation also appear to switch rewarding responses in the CPP test to intra-VTA GABA_A agonist muscimol from DA-sensitive to DA-insensitive (or vice versa for the antagonist bicuculline) in opioid-dependent rats (Laviolette et al., 2004). This condition could be mimicked by intra-VTA infusion of BDNF (Vargas-Perez et al., 2009). Furthermore, more recently it was shown that in stressed animals, specific hypothalamic neurons are excited by GABA due to stress-induced phosphorylation and endocytosis of KCC2 (Sarkar et al., 2011). These striking results appear to link chronic drug exposure, stress, and aversive pain stimuli to pathologic neuroadaptation in BDNF and GABA systems and indicate that boosting the chloride gradients might be an attractive drug development target (Gagnon et al., 2013).

Although GABA_A receptor-mediated excitation or depolarization in principal neurons is known to be important in early neuronal development (Ben-Ari et al., 2007; Blaesse et al., 2009) and perhaps in specific pathologic conditions in the mature nervous system (Medina et al., 2014), to our knowledge there is still little evidence that drugs acting on GABA_A receptors would induce tolerance via reversed Cl⁻ gradients during in vivo treatments. In addition, acute behavioral effects of drugs with preferential action on extrasynaptic GABA_A receptors, including gaboxadol, were not affected in mice hypomorphic to KCC2, although the responses to the synaptic GABA_A receptor agonist diazepam were strongly blunted (Tornberg et al., 2007). Interneurons express KCC2 already early in development (Batista-Brito et al., 2008), consistent with shunting inhibition in the interneurons already at postnatal days <10 (Banke and McBain, 2006). The stabilized role of specific interneuron populations, such as Pv-interneurons, may become even more important at the end of the critical periods (Hensch, 2014).

7,8-Dihydroxyflavone, a specific small molecule agonist of TrkB receptors, has a wide activity in various preclinical tests for cognitive and emotional behaviors (Baker-Andresen et al., 2013), but to our knowledge, it has not yet been tested for modulation of drug-induced plasticity. Perhaps only together with a systemically active antagonist of TrkB receptors, the roles of BDNF-TrkB signaling in various phases of drug-induced plasticity would become better established. However, as mentioned above, in the developing CNS during the critical periods of cortical circuitry formation BDNF signaling is indispensable for maturation of GABAergic inhibition (Huang et al., 1999) (reviewed in Kuczewski et al., 2010).

As discussed above, inhibitory interneurons have an important role in neuroplasticity, and their diversity, development, and functions were recently characterized in the cortical regions (Klausberger and Somogyi, 2008; Ascoli et al., 2008; Lapray et al., 2012), but only initially in the midbrain (Olson and Nestler, 2007; Nair-Roberts et al., 2008). Because the midbrain GABAergic interneurons even have different embryonic origins to the cortical interneurons, likely subpopulations and characteristics of midbrain interneurons will be an interesting target for research and drug effects.

**H. Animal Models of Drug Reinforcement and Addiction**

Animal models are critical for understanding the neurobiological basis of drug actions and drug addiction.
Because addiction is a human phenomenon, there are no complete animal models for it. However, some characteristics of this syndrome can be satisfactorily modeled in laboratory animals. It is generally agreed that when viewed as experimental preparations for studying the human syndrome, animal models exhibit good construct and predictive validity, even if their face validity can be disputed (for a comprehensive review, see Sanchis-Segura and Spanagel, 2006).

Conceptualizations of the development of addiction hold that drugs are initially taken voluntarily because of their positive reinforcing or “rewarding” effects, and therefore models have been constructed for evaluating the ability of drugs to act as reinforcers or conditioned reinforcers, including a variety of animal models of drug SA. Behaviorally, they can be classified as operant models based on operant conditioning and nonoperant models that are restricted to various oral SA procedures used particularly for measuring ethanol consumption. Traditionally, these models were based on a two-bottle choice between water and ethanol solution, but it was subsequently found that manipulation of the temporal access to ethanol can increase the consumed ethanol during drinking bouts, leading to development of binge-drinking models (Wise, 1973; Rhodes et al., 2005; Simms et al., 2008).

Operant models are based on the delivery of a reinforcer contingently to the completion of the required response that is determined by the reinforcement schedule. Reinforcement schedules exert powerful control over behavior and can be used for evaluating the nonspecific and motivational drug effects. For example, progressive ratio schedules can measure the reinforcing efficacy of a drug, whereas the second-order schedules are useful for studying the role of conditioned reinforcers in maintaining behavior. Various routes of SA can be used, e.g., intravenous, intracranial, intragastric, or oral. In particular, the intravenous drug SA animal model is considered to be predictive of drug abuse potential in humans.

In conditioned preference paradigms, drug’s effects (unconditioned stimulus) are paired repeatedly with a previous neutral stimulus. Through Pavlovian conditioning, the neutral stimulus acquires the ability to act as a conditioned stimulus. Most commonly, conditioned preference is studied using CPP procedures. These procedures use an apparatus with two or more separate distinctive compartments. Access to one compartment is paired with drug injections, whereas the other compartment is accessible after vehicle injections. After repeated pairings of the drug and the vehicle with their compartments, the animal is allowed to move freely in all compartments during the test session. Increase in the time spent in the drug-associated compartment is taken as a measure of CPP.

Animals will perform tasks to self-administer electrical trains of stimulation to many different brain areas, particularly along the medial forebrain bundle, leading to the idea that direct activation of brain reveals the circuitry involved in reinforcement from natural rewards. Various intracranial self-stimulation (ICSS) procedures have been used for mapping the neural circuitry mediating drug reinforcement and pharmacological suppression thereof. Many abused drugs acutely decrease the ICSS reward threshold, whereas increased thresholds have been seen during withdrawal after induction of dependence.

Compulsive drug seeking and relapse to drug use are considered to be the hallmarks of addiction, and therefore attempts have been made to model these aspects of addiction in animal models. Particularly for modeling drug-seeking behavior, reinstatement procedures are now being commonly used. These procedures consist of the initial phase of operant drug SA, the extinction phase during which operant responses have no programmable consequences, and the reinstatement phase during which the extinguished responding is reinstated either with a priming dose of the drug, conditioned stimuli associated with the drug, stressful footshocks, or pharmacological stressors. Drug seeking induced by re-exposure to drug-associated cues has been shown to progressively increase over several weeks after withdrawal from drug SA, a phenomenon that has been called “incubation of drug craving” (Lu et al., 2004), which is reminiscent of the alcohol deprivation effect found earlier in rats after a period of abstinence (Sinclair, 1972). The compulsive features of drug taking, such as resistance to adverse consequences and escalated intake, are accentuated in models in which the daily access to a drug is extended (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004).

Psychomotor or behavioral sensitization refers to the progressive increase in locomotion when abused drugs are administered repeatedly. This phenomenon is in essence nonassociative, but the context of drug administration has been demonstrated to have a role in the induction and expression of sensitization. It has been theorized that psychomotor sensitization also entails increased incentive salience of stimuli associated with drug effects, and therefore sensitization is implicated in transition to compulsive drug seeking and taking (Robinson and Berridge, 1993).

### II. Actions and Persistent Effects of Specific Drugs of Abuse

#### A. Cocaine, a Stimulant and Local Anesthetic

Cocaine, derived from the leaves of the coca plant, is a very potent psychostimulant. Cocaine and its derivatives, can be inhaled, injected into the bloodstream, or smoked, with the duration and intensity of effects depending on the route of administration. Its effects include euphoria (which might eventually be replaced by anxiety), hyperactivity, suppression of appetite, local anesthesia, and possible sudden death due to cardiac arrest that makes it considerably more
dangerous than other psychostimulants (for comprehensive review, see Grabowski, 1984). In addition, cocaine has been associated with a variety of cardiovascular disorders, including myocardial infarction, heart failure, cardiomyopathies, arrhythmias, aortic dissection, and endocarditis (Schwartz et al., 2010).

Cocaine is a DA, norepinephrine (NE), and serotonin reuptake inhibitor (Fig. 5; Table 1). It also blocks voltage-dependent sodium channels, which produces local anesthesia, and also has been shown to cause locomotor depression rather than stimulation (Reith et al., 1985). Cocaine increases plasma catecholamine levels; therefore, it exhibits sympathomimetic properties. As a consequence, it is a potent vasoconstrictor of blood vessels in the brain (Kaufman et al., 1998; Volkow et al., 1996; Wallace et al., 1996). The addictive potential of cocaine is widely considered to be mainly because of its block of DA reuptake (Sulzer, 2011). The vast majority of studies have focused on elucidating the mechanisms via which DA reuptake suppression leads to addictive behaviors.

In this regard, the holy grail of the field has been to understand lasting changes induced in the brain due to this reuptake inhibition, which outlasts the presence of the drug in the system. The main rationale for this approach is that addictive behaviors persist well beyond the presence of the drug in the system. One idea that has gained considerable experimental support in the past decade or so is that the normal learning processes in the brain (involving the mesocorticolimbic DA system) are "hijacked" in addiction to reinforce the acquisition of the addictive drug. As is discussed in this review, experimental results have lent considerable support to this notion; however, recently emerging evidence indicates this is only part of the picture (Nutt et al., 2015). Furthermore, emerging evidence indicates that other structures play a role in addiction as well. In this review, the effects of cocaine on lasting changes in the brain are discussed in a brain area-dependent fashion, because the evidence indicates different brain regions might play different roles in addiction (Thomas et al., 2001; Ungless et al., 2001; Conrad et al., 2008; Kalivas, 2009; Koob and Volkow, 2010; Luscher and Malenka, 2011; Pascoli et al., 2012, 2014).

1. Persistent Ventral Tegmental Area Neuroplasticity after a Single Dose. The VTA, along with the adjacent substantia nigra (SN), forms the principle source of DAergic projection neurons in the brain. Located in the midbrain, it projects to the many forebrain regions including the ventral striatum (NAc), amygdala, lateral habenula (LHb), mPFC, ventral hippocampus (vHC), and bed nucleus of stria terminalis (BNST) (see Fig. 1). Although the majority of its projections are DAergic in nature, significant populations of GABAergic and glutamatergic projection neurons have been observed as well (Nair-Roberts et al., 2008; Stuber et al., 2010; Hnasko and Edwards, 2012). It is believed that the VTA plays a role in assigning "salience" to rewarding or punishing events, and the more salient (or surprising) an event is, the greater the chance that it is learned. DAergic neurons in the VTA drastically increase firing in response to unexpected rewards or punishments and the cues associated with them (Mirenowicz and Schultz, 1996; Ungless et al., 2004; Brischoux et al., 2009). Therefore, a drug that evokes a persistent increase in DA neuronal firing or leads to a long-lasting potentiation of excitatory inputs to DA neurons—possibly by inducing LTP—could in theory drive motivational behaviors such as are observed toward salient events (Wolf, 2002).

Initial evidence indicated that a single, noncontingent (experimenter administered) dose of cocaine led to
Furthermore, functionally overriding mGlu1 receptors in the VTA removed these NMDARs (Yuan et al., 2013). Therefore, removing the GluN3A and GluN2B subunits. It has been hypothesized that GluN3A-containing NMDARs might play a role in cocaine-evoked AMPAR plasticity as well (Yuan et al., 2013). Therefore, removing the GluN3A-containing NMDARs might reduce cocaine-evoked AMPAR plasticity and mitigate its effects on downstream targets. Activation of mGlu1 receptors in the VTA removed these NMDARs (Yuan et al., 2013).

The increase in AMPAR/NMDAR ratio is due to increased AMPAR currents, which is mainly due to insertion of Ca²⁺-permeable GluA2 subunit-lacking AMPAR receptors (Fig. 6) (Mameli et al., 2011). However, NMDAR-dependent currents are reduced as well (reviewed in Luscher, 2013). This reduction is due to insertion of quasi-Ca²⁺-impermeable NMDARs containing GluN3A and GluN2B subunits. It has been hypothesized that GluN3A-containing NMDARs might play a role in cocaine-evoked AMPAR plasticity as well (Yuan et al., 2013). Therefore, removing the GluN3A-containing NMDARs might reduce cocaine-evoked AMPAR plasticity and mitigate its effects on downstream targets. Activation of mGlu1 receptors in the VTA persistent (that is, it did not return to normal after 7 days) (Mameli et al., 2009). This suggests mGlu1
signaling plays an important role in regulating plasticity in the VTA, and manipulating its activity could potentially provide a means of reducing downstream changes induced by VTA activation in the initial vulnerable stages of usage of drugs. In addition to redistribution of receptors, another attractive model for plasticity in the CNS is dendritic reorganization and dendritic spine remodeling (which influences dendritic input to the neuron). In this regard, spine density in a subset of neurons (MSNs) from the NAc that strongly project to VTA DA neurons and potentiated NMDAR currents on the other hand, increased release of glutamate onto DAergic neurons observed in the VTA (Hahn et al., 2009), whereas an OX1 receptor antagonist simultaneously blocked locomotor sensitization as well. Activation of OX2 receptors, on the other hand, increased release of glutamate on to VTA DA neurons and potentiated NMDAR currents (Borgland et al., 2008). Nonetheless, these neuropeptides actively modulate cocaine-induced changes in VTA DA neurotransmission (the role of CRF and OX in VTA-mediated reinstatement of drug seeking is further discussed below), emphasizing the importance of the interaction of the environment with the drug of abuse.

The majority of the studies have focused on the role of the VTA in initial drug taking, which might predispose the individual to later compulsive drug seeking. However, there is a preponderance of evidence that indicates the VTA plays a role in drug relapse as well. A variety of factors contribute to reinstatement of cocaine seeking after drug-seeking behaviors have been extinguished.

![Diagram of neuronal plasticity](image)

**Fig. 6. Neuronal plasticity such as long-term potentiation (LTP) or long-term depression (LTD) after exposure to acute and subchronic stimulant drugs may occur via several distinct mechanisms.** Acute cocaine exposure induces postsynaptic LTP that may occur when novel Ca\(^{2+}\)-permeable non-GluA2 subunit-containing AMPA receptors (AMPAR) are inserted into the postsynaptic membrane. This process is mediated by Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) after its activation by Ca\(^{2+}\) and is also influenced by signaling from G protein-coupled orexin and corticotropin-releasing factor (CRF) receptors and by recruitment of GluN2B and GluN3A subunit-containing NMDA receptors (NMDAR). Synaptic LTD occurs due to internalization of membrane-expressed AMPARs in response to elevated Ca\(^{2+}\) levels or activation of metabotropic glutamate receptors (mGlu). Another type of LTD is due to on-demand synthesis of endocannabinoids (eCB) such as 2-arachidonoylglycerol and anandamide (shown in green) that activate presynaptic cannabinoid CB\(_1\) receptors, resulting in inhibition of presynaptic neurotransmitter release (not shown here; please, see Fig. 12). Subchronic cocaine treatment may generate silent synapses mediated by selective recruitment of GluN2B subunit-containing NMDARs into new synaptic locations. These sites might then mature into functional synapses after synaptic targeting of new AMPARs.
These include stress, cues related to the drug, the context the drug was administered in, and a "priming" injection of the drug. In this regard, footshock—a stressor—reinstates cocaine seeking by releasing CRF, which in turn leads to an activation of VTA DA neurons via the release of glutamate (Wang et al., 2005). CRF neurotransmission, via CRF$_1$ and CRF$_2$ receptors, in the VTA promotes reinstatement, in long-access and short-access SA protocols, respectively (Wang et al., 2007; Blacktop et al., 2011; reviewed in Koob and Zorrilla, 2010; Wise and Morales, 2010). Interestingly, activation of OX$_1$ receptors increases reinstatement of cocaine seeking as well, in an independent manner from the effects of CRF (Borgland et al., 2010; Baimel and Borgland, 2012). Therefore, neuropeptides not only modulate initial cocaine intake but play a major role in relapse as well.

The studies discussed thus far involve administration of cocaine by the experimenter to the animal in a noncontingent manner (nondependent on the behavior of the animal). Studies have remarkably shown that the effects of noncontingent and contingent administrations on the brain are different. In the VTA, SA of cocaine leads to a persistent synaptic potentiation, which lasts for up to 3 months of abstinence (Chen et al., 2008). Therefore, further studies are required to elucidate the mechanisms underlying these disparate changes in the VTA.

2. Changes in the Nucleus Accumbens Mediate Cocaine Addiction and Relapse. The NAc, principally owing to its connectivity, is believed to play a role in initiation of movements based on emotional salience of encountered events. The major excitatory inputs to the NAc are from the mPFC, BLA, vHC, and thalamus. Furthermore, the NAc receives dense projections from the VTA, mostly DA-releasing neurons. Anatomic studies reveal a repetitive "loop-like" mesostriatal circuitry (Haber et al., 2000). Medial aspects of the VTA project to the shell of the NAc, projecting back to more lateral aspects of the VTA that in turn sends projections to the NAc core. The core then projects to the SN that projects more dorsally to the striatum and so on. Indeed, this loop-like circuitry has been hypothesized to be critically involved in addictive behaviors, by virtue of its role in reinforcement learning and eventual habit formation (for discussion, see Everitt and Robbins, 2005). The majority of ventral and dorsal striatal neurons are projecting MSNs, whose activity is mostly regulated by excitatory inputs from cortical and limbic areas (Sesack and Grace, 2010).

Unlike in the VTA, multiple noncontingent doses of cocaine administration are required to elicit synaptic plasticity in excitatory synapses in the NAc (Thomas et al., 2001), consistent with the hypothesis that the NAc is affected due to the action of cocaine on the VTA and its projections. Interestingly, cocaine is intracranially self-administered only into the NAc or mPFC, not into the VTA (Wise, 1996). The direction of the synaptic plasticity induced due to repeated cocaine administration was a huge controversy in the field that was recently resolved. Earlier work (Thomas et al., 2001; Beurrier and Malenka, 2002), showed a reduction in the AMPAR/NMDAR current ratios 10–14 days after repeated cocaine exposure on administration of a challenge dose of cocaine. In line with these findings, because mPFC-NAc synapses (a major excitatory input) on MSNs exhibited a reduced AMPAR/NMDAR ratio (indicative of an LTD state), further LTD could not be elicited via electrical means and the amplitude of miniature EPSCs was reduced. Activation of D$_1$Rs by DA led to an enhanced reduction in the AMPAR-mediated synaptic transmission, but not NMDAR-mediated synaptic transmission in the NAc shell (Beurrier and Malenka, 2002). On the other hand, if a challenge dose of cocaine was not administered, a potentiation of NAc excitatory synapses is observed (Kourrich et al., 2007). Therefore, these results together indicate that NAc excitatory synaptic transmission is potentiated gradually on withdrawal from repeated cocaine exposure and becomes depressed on administration of a cocaine challenge dose (the ‘transient’ depression returns to normal levels in 7 days). The mechanisms underlying the withdrawal-induced increase in AMPAR/NMDAR ratio are still under active investigation. Using commounoprecipitation studies and dissociated NAc-mPFC cultures, it was earlier shown that an increased number of AMPARs are recruited to the cell membrane (reviewed in Wolf and Ferrario, 2010), with no detectable change in the subunit composition of the AMPARs. However, longer withdrawal periods were recently shown to lead to expression of GluA2 subunit-lacking AMPARs (which are Ca$^{2+}$-permeable). Furthermore, the expression of these receptors was correlated with incubation of cocaine craving (defined as the progressive increase of cue-induced cocaine seeking with time of withdrawal and abstinence). Pharmacological inactivation of GluA2 subunit-lacking AMPARs blocked the incubation of cocaine craving as well (Conrad et al., 2008). This indicates that Ca$^{2+}$-permeable AMPARs in the NAc are involved in increased vulnerability of relapse with longer withdrawal times. Moreover, the increased expression of GluA2-lacking AMPARs is preceded by a reduction in mGlu$_1$ receptor expression. Indeed, systemic mGlu$_1$ activation reverses cue-induced cocaine craving by resetting AMPAR-mediated neurotransmission to control levels (McCutcheon et al., 2011a; Loweth et al., 2014).

Of note here are the parallelisms between the VTA and NAc, including the increased expression of GluA2-lacking AMPARs in response to a single cocaine dose and extended withdrawal from SA, respectively, and their reversal by mGlu$_1$ activation (Fig. 6). These results taken together suggest that mGlu receptor modulators have therapeutic potential for curbing
addiction. However, it is important to note that the expression of GluA2-lacking AMPARs in the NAc is only observed after long-access cocaine SA protocols and not after short-access or noncontingent protocols (McCutcheon et al., 2011b; Purgianto et al., 2013). In theory, an increase in AMPAR/NMDAR ratio could be also mediated by a decrease in the number or function of NMDAR receptors as well. In this regard, Ferrario et al. (2012) found that the percentage of spines mediating NMDAR currents is reduced, but not other characteristics of NMDAR function, during the abstinence from cocaine as well.

The importance of dissecting the specific roles of inputs to the NAc is increasingly being realized. However, intriguingly, Stuber et al. (2011) and Britt et al. (2012) demonstrated that three major inputs to the NAc from the vHC, mPFC, and BLA induce self-stimulation behavior when animals are set for specific optogenetic stimulation of these pathways. These results suggest that intensity of excitatory input and not the origin are important for motivated behavior, which, remarkably, is dependent on a very small subset of neurons (an ensemble consisting of 2–3% of neurons in the NAc), which

Consistent with the earlier findings, they observed an increase in silent synapses in the BLA-NAc shell projections during early withdrawal from cocaine SA regimens (Lee et al., 2013b) by comparing synaptic failure rates in NAc MSNs in response to selective optogenetic “minimal” stimulation. Furthermore, they discovered a similar time course for reduction in silent synapses and emergence of GluA2-lacking AMPARs (critical for cue-induced cocaine craving as discussed before). Inhibiting GluA2-lacking AMPARs increased the number of silent synapses after extended withdrawal, suggesting that the silent synapses generated in early withdrawal will express GluA2-lacking AMPARs later. Interestingly, an optical LTD protocol on BLA-NAc axons internalized the GluA2-lacking AMPARs and reduced the increase in cocaine seeking during withdrawal.

Ma et al. (2014) studied the role of silent synapses in the other major excitatory projections to the NAc from the mPFC. Silent synapses are generated in both prelimbic subregion of the mPFC (PrL)-NAc core and infralimbic subregion of the mPFC (IL)-NAc shell projections during early withdrawal. Extended withdrawal led to a recruitment of GluA2-lacking AMPARs in IL-NAc synapses (showing more rectification) but to a recruitment of GluA2-containing AMPARs in PrL-NAc synapses in NAc slices (showing less rectification). Behaviorally, internalization of these receptors in IL-NAc and PrL-NAc synapses, by selective optogenetic LTD stimulation protocols, led rats to show an increase and decrease in enhanced cocaine craving during abstinence, respectively (further discussed in the PFC section). Of note here are the parallels between the similar effect of PrL and BLA manipulations on initial cocaine seeking discussed earlier (Britt et al., 2012; Stuber et al., 2011) and on incubation of cocaine craving (Lee et al., 2013b; Ma et al., 2014); that is, motivated behaviors require an excitatory drive to the NAc, which can be from either the BLA or the PrL. Similarly, cocaine withdrawal leads to an increase in BLA-NAc and PrL-NAc silent synapses, reversal of which reduces the enhancement of cocaine seeking during withdrawal/abstinence.

These results taken together indicate that silent synapses generated in the NAc due to chronic cocaine exposure might eventually express AMPARs, which owing to their higher conductivity, make the NAc neurons more excitable, leading to increased cocaine craving with time.

The role of silent synapses in the NAc might not be limited to enhancement of cocaine craving during withdrawal. A series of studies conducted by Hope and colleagues (Koya et al., 2009, 2012) showed that induction of context-specific psychomotor sensitization on noncontingent repeated administration of cocaine is dependent on a very small subset of neurons (an ensemble consisting of 2–3% of neurons in the NAc), which
can be visualized in c-fos-lacZ transgenic rats. Selective inactivation of these neurons by β-galactosidase-induced formation of a cell toxin daunorubicin impaired psychomotor sensitization. These highly active neurons produced higher levels of silent synapses on cocaine sensitization than the general neuron population, which unlike the whole NAc persisted up to 6–11 days after sensitization. However, the precise role of these silent synapses is yet to be determined.

Although many earlier reports have studied the role of NAc in cocaine addiction without considering the heterogeneity in the NAc, it is increasingly being realized that the NAc has distinct subcircuits. The main population of neurons in the NAc is the MSNs. These constitute 95% of NAc neurons and are the major class of projection neurons from the NAc. MSNs have been further subdivided depending on whether they express D1R or dopamine-2 receptors (D2R). Akin to the much-studied dorsal striatum, these two classes of MSNs form distinct projection pathways. D1R-expressing MSNs constitute the "direct pathway" that projects directly to the midbrain, whereas D2R-expressing MSNs form the "indirect pathway" that projects to the midbrain via the ventral pallidum. Moreover, these classes of MSNs have different electrophysiological and neurochemical characteristics (Cepeda et al., 2008; Planert et al., 2013). Hence, a host of recent studies have discovered differing roles subserved by these MSNs in addictive behaviors. Nonspecific optogenetic inhibition of NAc core neurons reduces cocaine-primed cocaine reinstatement (Stefanik et al., 2013b); however, this effect is only replicated by inhibiting the pallidal projections from the NAc (indirect pathway) and not the nigral projections (direct pathway) (Stefanik et al., 2013a). However, another study found that optogenetic activation of channel rhodopsin-2 expressing D2R-MSNs in the NAc suppressed responding for cocaine, whereas their inhibition using a chemogenetic approach (designer receptor exclusively activated by a designer drug-mediated protocol) enhanced the motivation (higher breakpoints of responding) to work for cocaine (Bock et al., 2013).

The differences observed in these results might be due to the different stimulation protocols employed and to different behavioral tests. Stefanik et al. (2013a) stimulated NAc-pallidial axons, whereas Bock et al. (2013) manipulated D2R-MSNs directly. Alternatively, D2R-MSNs might only be involved in compulsive cocaine seeking. Nonetheless, further studies are required to clarify the precise roles of these subpopulations of NAc MSNs in addictive behaviors.

In concert with the changes in AMPAR expression in the NAc, withdrawal from chronic noncontingent cocaine exposure results in structural changes in MSN dendrites. A short withdrawal period (2 days) results in increased dendritic spine formation—mediated by a variety of transcription factors and epigenetic mechanisms as discussed later—in both D1R- and D2R-MSNs, whereas this increase is only sustained in D1R-MSNs after prolonged withdrawal of 30 days (Lee et al., 2006). In addition, both D1R- and D2R-MSNs exhibit dendritic remodeling on chronic cocaine exposure, which can be mediated by D1R activation (Li et al., 2012; Zhang et al., 2012). Furthermore, spine structure varies considerably as well. Withdrawal from repeated noncontingent cocaine exposure results in increased spine diameter. Subsequent cocaine priming elicits a biphasic change in spine diameter: transiently further increasing the size of the spine (45 minute after injection) and then reducing it when observed 24 hours later. This change in spine diameter is paralleled by changes in AMPAR expression, which has been shown to transiently increase (30 minutes after administration) on cocaine reexposure before decreasing (24 hours later); and then subsequently increasing again as the duration of withdrawal is prolonged (Boudreau et al., 2007; Kourrich et al., 2007; Anderson et al., 2008).

Reorganization of the cytoskeletal architecture of the neuron, or lack thereof, is critical for synaptic plasticity and stability. Likewise, the structural changes in dendrites of MSNs are associated with changes in the cytoskeletal architecture as well. Withdrawal from
repeated cocaine administration produces lasting elevation in polymerized filamentous actin in the NAc, due to suppression of actin cycling by reducing the active form of Rac1, a Rho GTPase that controls actin remodeling (Dietz et al., 2012). Reducing actin polymerization or increasing depolymerization results in reduced dendritic spine density and explains the transient increase in spine head diameter on cocaine priming and behavioral sensitization (Toda et al., 2010; Dietz et al., 2012). Indeed, knockout of Rac1 is sufficient to increase the density of immature dendritic spines in the NAc. Hence, these studies establish a mechanistic link between the behavioral responses, changes in spine morphology, and actin cycling.

In agreement with these findings, PSD-localized kalirin-7 (Kal-7; a brain-specific guanine nucleotide exchange factor) and its downstream effectors Rac1 and p21-activated kinase PAK are increased in the NAc, alongside with AMPAR surface expression and spine density on withdrawal from a repeated cocaine administration regimen (Wang et al., 2013d). Single-hairpin RNA (shRNA)-mediated silencing of Kal-7 in the NAc before cocaine exposure eliminates all these changes (increased AMPAR expression and spine density). This Kal-7 silencing, however, failed to prevent locomotor sensitization indicating the involvement of other mechanisms in eliciting this behavior. Incentive sensitization, the ability to rapidly learn to self-administer cocaine, was also impaired. Interestingly, constitutive Kal-7 knockout enhanced cocaine SA (Kiraly et al., 2013), which might be due to compensations by other kalirin isoforms.

In addition to intracellular biochemical pathways associated with the cytoskeletal architecture, extracellular CAMs are affected by cocaine as well. In this regard, integrins are CAMs in the ECM that are associated with the cytoskeletal architecture, extracellular glutamate receptor trafficking. Mirroring the regard, integrins are CAMs in the ECM that are associated with the cytoskeletal architecture, extracellular glutamate receptor trafficking. Kal-7 knockout enhanced cocaine SA (Kiraly et al., 2013), which might be due to compensations by other kalirin isoforms.

In addition to intracellular biochemical pathways associated with the cytoskeletal architecture, extracellular CAMs are affected by cocaine as well. In this regard, integrins are CAMs in the ECM that are involved in the regulation of synaptic plasticity, including glutamate receptor trafficking. This is evident in the NAc, where the β3 integrin subunit is reduced in the NAc core 24 hours after a SA cocaine protocol. However, after 3 weeks of abstinence and extinction, the levels are elevated (Wiggins et al., 2011). Moreover, cocaine priming further regulates the levels of β3 integrin. Daily NAc microinfusions of a peptide ligand that mimics a protein binding to integrins before SA or reinstatement, inhibited cocaine-induced drug seeking and normalizes the surface expression of AMPARs.

Repeated cocaine exposure and the subsequent withdrawal results in other changes in MSNs synaptic and extrasynaptic neurotransmission, in addition to alterations in AMPAR expression, dendritic arborization, silent synapses, spine head length, and spine density. In general, D1-R-MSNs exhibit decreased membrane excitability on withdrawal from repeated cocaine exposure, whereas D2-R-MSNs show no change in membrane excitability (Kim et al., 2011). Furthermore, cocaine disrupts the normal glutamate homeostasis necessary for maintenance of appropriate levels of glutamate in the synaptic and extrasynaptic (a much larger pool) spaces. In this regard, the extracellular glutamate in the NAc is regulated by cysteine/glutamate exchanger xCT and glutamate transporter GLT-1 in glia (Baker et al., 2003; Knackstedt et al., 2010). Withdrawal from repeated cocaine injections or SA substantially reduces extracellular glutamate, glutamate uptake, and the expression of these transporters (Baker et al., 2003; Knackstedt et al., 2010). Extrasynaptic glutamate normally binds to presynaptic mGlu_{2/3} receptors and reduces synaptic glutamate release. Therefore, chronic cocaine exposure results in an increase in the synthetically releasable pool of glutamate (reviewed in Kalivas, 2009; Wolf and Ferrario, 2010). Importantly, N-acetylcysteine, a well-tolerated produg for increasing cysteine levels and used to replenish liver glutathione levels in paracetamol (acetaminophen) intoxication, has been shown to enhance extrasynaptic glutamate levels and to reduce relapse to cocaine in rodents and in early studies on cocaine addicts (Baker et al., 2003; Schmaal et al., 2012; LaRowe et al., 2013). Hence, cocaine abuse results in a host of changes in MSNs in the NAc; indeed, it has been suggested that these changes in glutamate transmission and membrane excitability are linked to AMPAR regulation (Wolf and Ferrario, 2010) to mediate the behavioral phenotype.

3. Altered Gene Expression in the Nucleus Accumbens in Cocaine Seeking and Relapse. Not all individuals have an equal propensity to develop addiction. In this regard, approximately half of a person’s risk for addiction is genetic (Goldstein, 2001; Wang et al., 2012a). Therefore, long-lasting alterations in gene regulation upon cocaine exposure, via transcriptional or epigenetic mechanisms, are likely to play a pivotal role in addiction (reviewed in Robison and Nestler, 2011). Transcription factors are proteins that regulate gene expression in response to intracellular signaling pathways by binding to certain sites on the DNA and subsequently altering the expression of the genes. Genetic material in the nucleus is packed into chromatin complexes (DNA, histones, and other proteins), generally split into two classes: euchromatin (loosely packed DNA around histones) and heterochromatin (densely packed, hence less active), depending on the activity states of the chromatin. Epigenetic changes are considered alterations in this packing and thus alter activity of different regions of DNA. Regulation of genetic activity via transcriptional and epigenetic mechanisms involve a myriad of processes; the field has focused mainly on a few mechanisms that will be discussed here, including notable work on transcription factors like ΔFosB, CREB, NFKB, and MEF2, and epigenetic histone acetylation and methylation. Because the majority of these alterations in levels of transcription factors and epigenetic regulation are observed on repeated exposure to cocaine, most of the studies have focused on the
Induction of 1996; Kelz et al., 1999; reviewed in Nestler et al., 2001). However, only in the NAc is increased transiently on acute cocaine exposure. This increased induction might be due to accumulation in the NAc was enhanced by cocaine re-administration, demonstrated that after prolonged withdrawal from a repeated cocaine administration regimen (Lee et al., 2006).

Intriguingly, overexpression of ΔFosB in D1R-MSNs decreased the AMPAR/NMDAR ratio and increased silent synapses on these neurons in the NAc shell and core, whereas overexpression in D2R-MSNs resulted in increased excitatory synaptic strength as assessed by the AMPAR/NMDAR ratio and in decreased silent synapses in the NAc shell only. Furthermore, density of immature spines in D1R- but not D2R-MSNs increased as well on respective overexpression of ΔFosB in these neuronal populations (Grueter et al., 2013). Taken together, these findings indicate that ΔFosB plays a critical role in mediating the rewarding and locomotion-inducing properties of cocaine.

Alterations in induction of ΔFosB in the NAc might play a role in relapse as well. Damez-Werno et al. (2012) demonstrated that after prolonged withdrawal from repeated cocaine administration, ΔFosB induction and accumulation in the NAc was enhanced by cocaine re-exposure. This increased induction might be due to sustained elevation of ΔFosB, and increased dendritic spine density as well, in D1R-MSNs after prolonged withdrawal. D2R-MSNs, however, only exhibited increased ΔFosB and dendritic spine density after short withdrawal periods (2 days) on cessation of a repeated cocaine administration regimen (Lee et al., 2006).

CREB, another transcription factor, seems to increase in both major subtypes of NAc MSNs. CREB, unlike ΔFosB, reduces the sensitivity to the rewarding effects of cocaine (tolerance) and increases SA and relapse via negative reinforcement. For instance, the κ-opioid peptide dynorphin, regulated by CREB, suppresses DA transmission to the NAc via the activation of κ-opioid receptors, thus impairing rewarding behaviors (Carlezon et al., 1998; Larson et al., 2011; Muschamp et al., 2011; Ehrich et al., 2014). Furthermore, the induction of ΔFosB in the NAc is dependent on CREB and serum response factor (Vialou et al., 2012). Deletion of CREB and serum response factor, but not either one alone, eliminates induction of ΔFosB on cocaine exposure in the NAc, reduces the rewarding properties of moderate doses of cocaine as assessed by CPP, and blocks locomotor sensitization at higher doses of cocaine. Interestingly, deletion of CREB alone has the opposite effect, that is, an increase in behavioral sensitization and CPP in response to cocaine (Carlezon et al., 1998; Walters and Blendy, 2001; McClung and Nestler, 2003). The sustained expression of ΔFosB in the NAc and the accompanied increase in dendritic spines and behavioral responses, requires CaMKIIα, which is increased on chronic cocaine exposure as well (Robison et al., 2013).

Other transcription factors have been implicated in cocaine addiction as well. For instance, reduction of myocyte enhancer factor 2 activity in the NAc is required for cocaine-induced increased spine density and possibly is linked to behavioral sensitization as well (Pulipparacharuvil et al., 2008). Similarly, nuclear factor kappa-B (NFκB), involved in maintaining cell structure, is induced by chronic cocaine exposure. Elevation and reduction of NFκB signaling in the NAc increased and decreased (and also prevented cocaine-induced increase in) the number of dendritic spines on NAc neurons, respectively. Furthermore, inhibition of NFκB makes cocaine less rewarding (Russo et al., 2009).

The N-terminus of the histone (the histone tail) is particularly susceptible to modifications. Relevant histone tail modifications involve the enzymes histone acetyltransferases, whereas histone deacetylases (HDACs) catalyze the removal of acetyl groups. Similarly, histone methyltransferases and histone demethylases catalyze the addition and removal of methyl groups, respectively. Generally, acetylation is associated with increased activity and methylation with reduced gene activity.

Earlier studies found conflicting results on cocaine-induced behavioral responses with inhibition of HDAC. Two studies found that systemic treatment with HDAC inhibitors, trichostatin A and phenylbutyrate, reduced the motivation for SA of cocaine and cocaine-plus-cued reinstatement of drug seeking (Romieu et al., 2008, 2011), whereas a third one (Kumar et al., 2005) reported increased rewarding effects of cocaine as assessed by CPP and stimulation of cocaine-induced locomotion. Furthermore, chronic cocaine exposure increased ΔFosB, cdk5, and BDNF in the NAc (as discussed earlier). This increase is mirrored by an increase in H3 acetylation at promoters of cdk5 (regulated by ΔFosB) and BDNF (Kumar et al., 2005). Although these studies indicated a link between histone modifications and transcription factors in cocaine addiction, their role was not clear because of the conflicting results.

However, the use of specific experimental manipulations in more recent studies has resolved this controversy. Kennedy et al. (2013) found that only prolonged
knockdown of HDAC1, not HDAC2 or HDAC3, in the NAc reduced cocaine-induced behavioral plasticity. Overexpression of HDAC3, 4, or 5 in the NAc, on the other hand, suppressed cocaine-induced acquisition of CPP; that is, the cocaine-context association took longer to form (Rogge et al., 2013). Furthermore, chronic cocaine exposure or stress decreases HDAC5 function in the NAc; conversely, loss of HDAC5 in the NAc increases the responsiveness to chronic cocaine or stress as assessed by the CPP paradigm (Renthal et al., 2007).

The role of methylation, and the associated enzymes, in cocaine addiction has been studied as well. Repeated cocaine exposure reduces overall levels of histone 3 lysine 9 dimethylation and lysine dimethyltransferase G9a in the NAc as well (Maze et al., 2010). ΔFosB downregulates G9a, which results in reduced histone 3 lysine 9 dimethylation. Experimental reduction of G9a increases dendritic spine plasticity in the NAc and rewarding properties of cocaine as assessed by CPP. This reduction in G9a is observed in both D1R- and D2R-MSNs. However, the effects of experimental manipulation of G9a in the MSN subpopulations are different. Similar to a nonspecific downregulation of G9a in MSNs (Maze et al., 2010), knockout of G9a in D2R-MSNs increased cocaine reward and locomotor sensitization, whereas knockout in D1-R-MSNs had the opposite effects (Maze et al., 2014). Intriguingly, knockout of G9a in D2-R-MSNs resulted in these neurons having a phenotype similar to D1-R-MSNs.

On another note, experimental reduction of G9a in the NAc also results in a depression-like phenotype. This phenotype emerges on chronic cocaine exposure as well. Indeed, increasing G9a activity in the NAc after chronic cocaine exposure rescues the depression-like phenotype (Covington et al., 2011). It has been suggested that G9a reduction by chronic cocaine exposure might result in development of stress-related illness in addicts. Further studies are warranted to determine if this is indeed the case.


A popular hypothesis for addiction proposes that addiction initially depends on the ventral striatum (including the NAc), but progressively engages and hijacks the circuitry in the dorsal striatum, which is normally required for habitual learning. It is suggested that this shift might mirror the change from voluntary to involuntary (or habitual) drug intake in addiction (Robbins and Everitt, 1999; Everitt and Robbins, 2005, 2013). This hypothesis fits well with the human literature on cocaine addiction, which implicates the dorsal striatum in cocaine addiction. Because a substantial amount of human imaging studies compare cocaine addicts to nonaddicted individuals, assuming this hypothesis were true, it is not surprising that altered activity in the dorsal striatum is observed. Consistent with this hypothesis (and the human literature), Ito et al. (2000, 2002) observed increased DA release in the dorsal striatum, but not NAc, on presentation of cocaine-associated cues after prolonged exposure to cocaine. Similarly, blocking (or transiently inactivating) DA receptors of dorsal striatum, but not those of NAc, reduced the cocaine-seeking behaviors after protracted cocaine exposure (Vanderschuren et al., 2005; Zapata et al., 2010). On the basis of these findings, the authors postulated that initial dependence on the VTA-NAc pathway might be replaced by an increasing involvement of the nigrostriatal pathway as drug seeking becomes more habitual (that is, on prolonged exposure to the drug). It is well known that midbrain DAergic projections to the striatum and backprojections to the midbrain form a “spiraling” cascade: medial VTA projects to the NAc, which backprojects to lateral VTA, and so on (progressively involving the dorsal striatum and SN as well, Fig. 1)(Haber et al., 2000). To test whether this circuitry might be involved in cocaine addiction, an elegant study disconnected various parts of this serial circuitry using alpha-fluphenixtol (an antipsychotic with strong antagonism at D1, D2, D3, and histamine H1 receptors) and found a selective reduction in habitual drug seeking (Belin and Everitt, 2008).

Although the ventral striatum is critical for voluntary behavior, a host of studies also implicate the dorsal striatum in goal-directed (action-outcome) and habitual (stimulus-response) behaviors. More specifically, the dorsomedial striatum is involved in goal-directed behavior, whereas the dorsolateral striatum is involved in habitual behavior (Yin et al., 2004, 2005a,b; Corbit and Janak, 2010; Corbit et al., 2012) (Fig. 1). In agreement with these findings, repeated cocaine exposure has been shown to perturb the electrophysiological properties of neurons in the dorsomedial striatum and to reduce goal-directed behavior as well. Furthermore, administration of N-acetylcysteine reversed the behavioral deficits and the associated changes in properties of dorsomedial striatal neurons (Corbit et al., 2014) (see also discussion in section II.D on alcohol).

5. Changes in Lateral Habenula and Rostromedial Tegmental Nucleus Contribute to Aversive Symptoms of Cocaine Withdrawal. Although "withdrawal symptoms" from cocaine are not as pronounced as from some other drugs of abuse, a depression-like phenotype is observed, which might result in increased drug intake (Barr et al., 2002; Koob and Le Moal, 2008). Thus, given the role of the lateral habenula (LHb) in depressive disorders, it is not surprising that an interest in its role in drug addiction has recently emerged (Li et al., 2011; Maroteaux and Mameli, 2012; Zuo et al., 2013; Lecca et al., 2014). In this regard, earlier studies demonstrated that the Lhb inhibits the VTA via its projections to the GABAergic rostromedial tegmental nucleus (rmTg), promoting avoidance behaviors (Jhoo et al., 2009b; Kauffing et al., 2009; Balcita-Pedicono et al., 2011; Stamatakis and Stuber, 2012) (Fig. 1). By using an ex vivo preparation similar to earlier studies in
the VTA and NAc (discussed earlier), it was recently shown that two prior in vivo, noncontingent doses of cocaine selectively enhance glutamatergic transmission onto LHb neurons projecting to the rmTg (as well as increasing excitability of these neurons by reducing K+ currents) (Meye et al., 2013, 2015). These changes persisted for 1 week, whereas a chronic cocaine regimen (5 noncontingent doses) results in similar persistent changes for 2 weeks (at which point, depressive-like "withdrawal" behaviors were observed). Furthermore, the changes were dependent on GluA1 subunit-containing AMPAR trafficking in these neurons. Its inhibition by virally expressed GluA1 C-terminal peptide resulted in a reduction in depression-like behaviors on withdrawal and in persistent neuronal changes in the LHb (Meye et al., 2015). Therefore, these studies implicate the LHb-rmTg-VTA axis in the negative symptoms associated with cocaine withdrawal. Interestingly, initially acute cocaine results in a reduction of activity of a subpopulation of LHb neurons, followed by increased activity, perhaps in putative rmTg-projecting neurons (Lecca et al., 2014), suggesting that these neurons might disinhibit VTA DA neurons during the early pleasant experience on cocaine intake, but might later reverse roles, leading to withdrawal symptoms. Intriguingly, stimulation of the midbrain DAergic structures modulates activity of pain-associated LHb neurons as well, indicating that there are functionally relevant connections from the VTA to LHb as well (Shen et al., 2012). Obviously, further studies are required to determine the roles of various LHb pathways in cocaine addiction. In addition, to the aforementioned role of LHb in cocaine addiction, stimulants have been shown to induce strong excitation of LHb efferents to the rmTg (as well as other regions) are less excitable after an extended SA protocol. This compulsive cocaine seeking is increased or decreased by optogenetic inhibition and activation of PrL pyramidal neurons, respectively (Chen et al., 2013a). It is intriguing to speculate that in these compulsive cocaine-seeking rats, VTA-PrL synaptic excitability, which should convey aversive values of encountered stimuli (Lammel et al., 2011), is downregulated as well. This would reduce aversion and hence enable cocaine seeking even at the cost of "aversive consequences." However, further studies are needed to determine if this indeed is the case. Another possible explanation of these findings might be that these rodents, due to reduced activity in the PrL, exhibit reduced flexibility in switching behaviors (a function subserved by the mPFC)—or perseveration—on introduction of aversive consequences. Indeed, Allen and Leri (2014) found that lesions of the PrL (which might have similar functional consequences as hypoactivity of PrL pyramidal neurons) resulted in increased perseveration after chronic cocaine exposure.

6. Complex Alterations in the PFC. The role of the PFC in cocaine addiction is more complex. A single dose of cocaine fails to elicit increased glutamate receptor neuroplasticity in those VTA DA neurons that project the mPFC (Lammel et al., 2011), although the same treatment induces a prolonged increase (at least 21 days) in AMPAR/NMDAR current ratios of those VTA DA neurons that project to NAc medial shell. The mesocortical DA neurons might be more responsive to aversive stimuli (Lammel et al., 2011). These findings indicate that the PFC might not be involved in initial cocaine-seeking behaviors.

DA neurons in the VTA responsive to aversive stimuli project to the mPFC (Lammel et al., 2012). Interestingly, there is considerable evidence from early studies implicating excitatory neurotransmission from the mPFC to the NAc in cue-induced reinstatement (for discussion, see Kalivas, 2009). However, the role of mPFC inputs might be subtler. Optogenetic inhibition of PrL-NAc core projections reduces cocaine-primed reinstatement of cocaine seeking, and activation of the PrL pathways is required for the cue-associated transient increase of dendritic size and synaptic strength in the NAc (Gipson et al., 2013; Stefanik et al., 2013b; Shen et al., 2014). Furthermore, as discussed earlier, Pascoli et al. (2014) found that optogenetic reversal of cocaine-evoked plasticity in mPFC-NAc shell D1R-MSN synapses attenuates response discrimination in cue-induced cocaine reinstatement. In addition, Ma et al. (2014) present evidence that PrL-NAc inputs promote, whereas IL-NAc inputs reduce, the enhancement of cocaine craving during prolonged PrL abstinence. PrL neurons show enhanced responsiveness to cocaine-associated cues, unlike the IL neurons (West et al., 2014). BDNF in the IL promotes extinction of CPP (Otis et al., 2014). Taken together, these findings indicate that PrL-NAc activation promotes cue-induced cocaine seeking, whereas IL-NAc activation promotes extinction of cocaine seeking.

On the other hand, pyramidal neurons in the PrL of the mPFC in "compulsive" cocaine-seeking rats (a subset of rodents that will seek cocaine despite negative consequences; more truly representative of addictive individuals) are less excitable after an extended SA protocol. This compulsive cocaine seeking is increased or decreased by optogenetic inhibition and activation of PrL pyramidal neurons, respectively (Chen et al., 2013a). It is intriguing to speculate that in these compulsive cocaine-seeking rats, VTA-PrL synaptic excitability, which should convey aversive values of encountered stimuli (Lammel et al., 2011), is downregulated as well. This would reduce aversion and hence enable cocaine seeking even at the cost of "aversive consequences." However, further studies are needed to determine if this indeed is the case. Another possible explanation of these findings might be that these rodents, due to reduced activity in the PrL, exhibit reduced flexibility in switching behaviors (a function subserved by the mPFC)—or perseveration—on introduction of aversive consequences. Indeed, Allen and Leri (2014) found that lesions of the PrL (which might have similar functional consequences as hypoactivity of PrL pyramidal neurons) resulted in increased perseveration after chronic cocaine exposure.

Similar to the hypoaactivity observed in the PrL, cocaine SA also leads to a hypoactivity of pyramidal neurons in the orbitofrontal cortex (OFC; although the underlying mechanisms are different), a locus of decision-making and insight in the brain (Lucantonio et al., 2014). Furthermore, this correlated with a ‘lack of insight’ in the animals (lack of expectation of a larger reward summation, when two different cues were presented at the same time, as shown by unchanged responding), which was normalized by optogenetic stimulation of the OFC neurons. Moreover, the VTA-OFC-BlA forms a functional circuit for context-induced drug reinstatement. Specifically, contextual reinstatement of cocaine seeking in extinguished rats is dependent on OFC-BlA activity and D1R signaling in the OFC (Lasseter et al., 2011, 2014), as demonstrated by local inactivation with microinjections of GABA<sub>A</sub>/GABA<sub>B</sub> agonist cocktail and D1R antagonist.
7. Hippocampal Functional Changes. Although the HC is one of the principal regions in the brain involved in spatial and contextual memory processing, not much attention has been paid to the role of the HC in cocaine addiction. An earlier study (Vorel et al., 2001) observed reinstatement of cocaine-seeking behaviors after theta burst electrical stimulation of the ventral subiculum (part of the vHC). This reinstatement was mediated by HC glutamatergic projections to the VTA, as it could be mimicked by intra-VTA infusion of NMDA and blocked by infusion of an NMDAR antagonist. Similarly, a tri-synaptic pathway originating from the CA3 of the dorsal HC to the VTA (via the lateral septum), which excites DA neurons, is required for reinstatement of cocaine seeking on exposure to the context (Luo et al., 2011). Optogenetically inducing LTD in vHC-NAc D1R-MSN synapses reduces the number of responses on cue-associated reinstatement of cocaine seeking, in contrast to induction of LTD on mPFC-NAc D1R-MSN synapses, which diminishes response discrimination (Pascoli et al., 2014). Taken together, these findings indicate that the HC plays a role in reinstatement of cocaine-seeking behaviors. However, further studies are required to discern the precise roles of specific subregions in the HC in cocaine addiction.

Like in other neuropsychiatric disorders, dysfunctional adult neurogenesis has been observed to play a role in cocaine addiction as well. Reducing hippocampal neurogenesis increases cocaine SA. Furthermore, neurogenesis is also negatively correlated to chronic cocaine intake and seeking behaviors (Noonan et al., 2008, 2010). Cocaine SA reduces dentate gyrus neurogenesis, which returns back to baseline during extinction (Deschaux et al., 2014). LFS of the dorsal or ventral HC after extinction session prevented the increase in neurogenesis and actually enhanced cocaine seeking after a priming dose. Further studies are required to elucidate the precise mechanisms underlying these effects.

Selective reactivation of the dorsal dentate gyrus neurons originally involved in encoding a rewarding memory in an aversive environment reverses the valence of that rewarding memory (its ability to evoke appetitive behaviors as assessed by CPP) (Redondo et al., 2014). A similar reactivation of the neurons originally active in the BLA did not reverse the valence of the memory (Redondo et al., 2014). However, selective ablation (CREB-Cre in iDT mice expressing cell-specific diphtheria toxin receptor after cre-recombinase induction) or silencing (chemogenetic silencing with CREB-hM4Di designer receptor exclusively activated by a designer drug receptors) of "cocaine-memory-trace"-activated neurons in the lateral nucleus of amygdala (LA; about 10% of the neurons that increased expression of CREB in response to cocaine administration) disrupted the expression of a previously acquired cocaine-context-related memory, because the animals failed to show CPP (Hsiang et al., 2014). Interestingly, when these animals were taken through extinction session and tested later for cocaine-induced reinstatement, the small number of previously activated LA neurons was needed also for this kind of drug memory. Taken together, these findings indicate that in these HC and amygdala structures, a small subset of neurons are required for reward processing and are possibly hijacked during cocaine-seeking behaviors.

The hippocampal formation plays a role in spatial navigation and memory consolidation. In this regard, HC "place cells" fire in a specific location in an environment (O'Keefe and Dostrovsky, 1971; Colgin et al., 2008). When the animal traverses space, sequential HC place cell firing is observed, encoding the animal's trajectory. These sequences are replayed during subsequent sleep and quiet wakefulness, which are proposed to consolidate this memory (Skaggs and McNaughton, 1996; Davidson et al., 2009). However, it was recently demonstrated that replay or reactivation of HC activity does not simply depend on experience, but might be modulated by the behavioral state of the animal, including inputs from the VTA, and the environment (Gupta et al., 2010; McNamara et al., 2014). Such "off-line" reactivation of neurons associated with rewarding behaviors is observed in the NAc as well (Lansink et al., 2008), possibly providing consolidating memories with a motivational component. Interestingly, using multineuron recordings, reactivation of HC place cells leads to activation of striatal reward site-related neurons as ensembles in sleep, followed shortly after HC sharp wave-ripple complexes, suggesting that the HC plays a pivotal role in consolidation of reward-related memories (Lansink et al., 2009). Furthermore, contextual fear conditioning remaps HC cells (Moita et al., 2004). These studies indicate that the HC is critical for forming associations between the context and appetitive or aversive behaviors. This raises the intriguing possibility that cocaine might alter hippocampal ensemble activity, and thus spatial memory processing of the cocaine-associated cues and context.

8. Bed Nucleus of Stria Terminalis and Amygdala are Involved in Stress-Induced Reinstatement of Cocaine Seeking. The BNST is required for stress-, cue-, or yohimbine-associated reinstatement of cocaine seeking (Buffalari and See, 2011), based on experimental inactivation experiments of the BNST with locally infused GABA_A and GABA_B agonists. CRF in the BNST, possibly via projections from the CeA, induces reinstatement (Erb and Stewart, 1999; Erb et al., 2001a,b). Furthermore, noradrenergic neurotransmission in the BNST or CeA is required for stress-induced but not cocaine-induced reinstatement (Leri et al., 2002). Finally, stress-induced reinstatement of cocaine seeking requires activity in a ventral BNST-VTA pathway, which releases CRF in the VTA. This release of CRF is modulated by β2 adrenergic receptors in the BNST (Vranjkovic et al., 2014). Therefore, the CeA-BNST-VTA system forms a functional circuit involved in
stress-induced reinstatement of cocaine seeking. The connections are complicated, as illustrated by reciprocal connections between the BNST and VTA, with both GABA and glutamate neurons from the BNST targeting VTA GABA neurons. When selectively activated by optogenetics, BNST-VTA GABA projection disinhibits the VTA DA neurons, whereas BNST-VTA glutamate projection indirectly inhibits the VTA DA neurons, producing rewarding and aversive/anxiety-like responding, respectively (Jennings et al., 2013).

9. Effects of Adolescent Cocaine Exposure. Adolescents are more prone to develop cocaine addiction than adults; they administer cocaine more readily, are more sensitive to lower doses, show increased activation of VTA DA neurons, and show greater escalation of cocaine intake (reviewed in Gulley and Juraska, 2013). Indeed, a significant proportion of studies (specifically, the majority of ex vivo electrophysiology studies) discussed in this review were performed in adolescent animals, because the experiments are less tedious and the results more pronounced. However, the effect of adolescent exposure to cocaine on adult behavior has not been widely studied.

Of note are two studies, which employed a novel escalating "binging" cocaine administration protocol in adolescent rats (12 days between P35 to P46) and its effects on subsequent behavior during adulthood. This binging protocol was aimed at recapitulating human adolescent drug use. Black et al. (2006) demonstrated that these rats displayed increased responsiveness to the stimulus effects of cocaine in adulthood. Furthermore, they exhibited "abnormally" rapid shifts in attention on the attentional set-shifting task, implicating the PFC in this dysfunction. A similar cocaine-binging protocol in adolescents subsequently decreased anxiety and acquisition of contextual fear responses in adulthood (Sillivan et al., 2011). Furthermore, this protocol perturbed dendritic arborization and synapses, which persisted in adulthood. Taken together, these studies provide the first insights into the effects of adolescent cocaine exposure on subsequent behavior in adulthood. However, because no adult controls (Black et al., 2006; Sillivan et al., 2011) with binge administration of cocaine were used, the possibility that these changes might be due to binge administration of cocaine per se and not due to exposure in adolescence cannot be ruled out. Nonetheless, further studies are required to elucidate these changes.

10. Human Imaging Studies. Imaging studies attempting to elucidate the neural mechanisms underlying cocaine addiction in humans have been of limited scope due to a variety of reasons, including poor resolution, difficulty in quantifying the cocaine abuse history of the participants, and problems with determining causality. However, human studies have the distinct advantage over animal models of providing information about the subjective effects of the drugs. These subjective effects, nonetheless, are prone to a variety of problems. For instance, it was recently argued that researchers studying the neural correlates of "craving" in drug addicts might be inadvertently studying a low-level urge (Moeller et al., 2015; Wilson and Sayette, 2015). Moreover, acute as well as chronic cocaine intake (even after a 10-day withdrawal period) reduces cerebral blood flow (possibly due to vasoconstriction, because it is a sympathomimetic), which might make the interpretation of experiments studying glucose metabolism and blood oxygenation (considered correlates of neural activity) more difficult (Volkow et al., 1988; London et al., 1990; Wallace et al., 1996). However, Gollub et al. (1998) argue that regional changes in neuronal activity using the aforementioned techniques can be reliably determined despite the global reduction in blood flow. Nonetheless, one has to be careful while drawing comparisons across studies. Another important caveat in human brain imaging studies is the effect of expectancy. For example, the apparent DA release in the human NAc is significantly enhanced [reduced binding potential of raclopride in positron emission tomography (PET) scanning] by expectation of receiving caffeine (Kaasinen et al., 2004). Notwithstanding these limitations, significant insights into the mechanisms underlying cocaine addiction have been achieved using neuroimaging and neurophysiological techniques including fMRI and PET. In cocaine-dependent individuals, intravenous infusion of cocaine induces increased fMRI signals in the reward network of the brain, correlating either with self-reported "rush, high, low, or craving" (Breiter et al., 1997). Importantly, repeated sessions reproduced the findings, but activations also emerged in some saline infusions, which were interpreted as expectations. The effects were registered within 100 seconds, but still the dependent individuals might have differentiated the active drug from saline, which might have altered the responses. However, the pharmacological effects of cocaine in the reward areas might not be altered by expectation (Kufahl et al., 2008).

A seminal PET study revealed that the DAT occupancy by cocaine correlates with the subjective feeling of the "high" in cocaine addicts (Volkow et al., 1997a). This drug-induced high is negatively correlated to activity in the NAc, OFC, and anterior cingulate cortex (Riser et al., 2005). Cocaine addicts have lower levels of DA receptors in the striatum than controls, when assessed using PET after 1 week of abstinence. However, 1 month of abstinence is sufficient to return the receptors to normal levels (Volkow et al., 1990). Furthermore, acute exposure to methylphenidate, a DAT blocker like cocaine (and a drug that is reported to produce a similar high as cocaine in addicts), demonstrates a reduced release of DA in the striatum in cocaine addicts, even after extended withdrawal (Volkow et al., 1997b; Martinez et al., 2004). Moreover, Gu et al. (2010), using fMRI, found that resting state functional connectivity between the VTA and NAc was negatively correlated to years of cocaine use, further implicating a downregulation in
DAergic signaling in cocaine addicts. Studies in macaques extend these findings by demonstrating that chronic cocaine SA per se reduces D_{2}Rs, which might contribute to increased drug use (reviewed in Nader and Czoty, 2005; Nader et al., 2006). In human cocaine addicts, D_{2}Rs are reduced in several regions of the frontal cortex (FC, particularly the OFC and cingulate gyri), which is apparent even after 3–4 months of abstinence (Volkow et al., 1993). Similarly, the anterior cingulate cortex, along with the OFC and dorsolateral PFC, is hypoactive in response to rewarded drug cue-reactivity task (Goldstein et al., 2009). This deficit is reversed by oral methylphenidate and is not a consequence of reduced motivation or impaired behavioral responses, because these were controlled for in the experiments (Goldstein et al., 2009, 2010). Intriguingly, acute exposure to methylphenidate, presumably by raising DA levels, increases metabolism in the OFC and mPFC of cocaine addicts but not controls (Volkow et al., 2005). Nonetheless, cocaine abusers (active or abstinent) exhibit reduced signaling via D_{2}/3 receptors compared with controls on exposure to methylphenidate (Volkow et al., 2014). Taken together, these findings indicate that cocaine abuse results in persistently reduced DA release, reduced DA receptor signaling, and hypoactive striatal and frontal circuits.

Strikingly, although chronic cocaine users exhibit reduced cerebral blood flow on withdrawal as discussed (Volkow et al., 1988), after 1 week of withdrawal, increased global brain glucose metabolism and also regional increases in the basal ganglia and OFC are observed, which subside in 2–4 weeks (Volkow et al., 1991). Further studies are required to understand these differences.

As highlighted earlier, recent work has challenged the construct validity of experiments studying craving in addiction (Moeller et al., 2015; Wilson and Sayette, 2015). Nonetheless, a study employing PET reported increased cerebral blood flow to limbic regions (including the amygdala and anterior cingulate gyrus) and decreased blood flow to the basal ganglia on cue-induced cocaine craving compared with controls (Childress et al., 1999). These findings were corroborated by fMRI studies, which demonstrate increased cingulate and low frontal lobe activation in cocaine addicts on exposure to cocaine-associated cues (Maas et al., 1998; Wexler et al., 2001). Risinger et al. (2005) studied cocaine SA by nontreatment-seeking cocaine addicts. Simultaneously with SA-associated behavioral ratings they also had the subjects scanned with fMRI. Craving was positively correlated with activity in the NAc, OFC, and anterior cingulate cortices, whereas cocaine-induced high was negatively correlated with the activity in these regions, stressing the altered states of the reward circuitry in cocaine-related subjective states.

11. Limited Evidence of Cocaine Neurotoxicity. In humans, PET studies have reported decreased blood flow and frontal cortical glucose metabolism as well as decreased D_{2} receptor availability in cocaine users compared with control subjects. These changes were sustained up to 4 months of abstinence (Volkow et al., 1988, 1992, 1993). Cocaine users also displayed an attenuation of the increase in extracellular striatal DA levels but a greater increase in thalamic DA levels compared with control subjects in response to a methylphenidate challenge (Volkow et al., 1997b). Studies employing SPECT and MRI have reported changes in regional cerebral blood flow and blood oxygen level dependent (BOLD) signal intensity in cocaine users while performing cognitive tests measuring memory and executive functions. Alterations have been observed in cortical regions, the globus pallidus, putamen, cingulate, hippocampus, and amygdala, but are not always associated with decreased task performance (Ernst et al., 2000; Bolla et al., 2003; Hester and Garavan, 2004; Tomasi et al., 2007; Moeller et al., 2010). Importantly, these studies are subject to the same limitations (discussed in more detail in section II. B on amphetamines) that limit the conclusions that can be drawn from them.

Unlike amphetamines (see section II.B), the evidence of cocaine neurotoxicity in animal models is more limited. In rodents, cocaine does not produce any long-term depletion of DA, 5-HT, or their metabolites in brain regions normally affected by amphetamines, such as the striatum, cortex, hypothalamus, and hippocampus (Klever et al., 1988; Yeh and De Souza, 1991). Other markers of monoamine system integrity, such as monoamine transporter levels, are likewise unaffected by binge cocaine treatments. In studies employing silver staining, even very high-dose, multiday treatments with cocaine up to 450 mg/kg per day failed to produce any evidence of axonal degeneration in the striatum, cortex, or other regions (Ryan et al., 1988; Benmansour et al., 1992; Goodman and Sloviter, 1993). Even treatment at high ambient temperatures, an exacerbating factor in amphetamine toxicity, did not result in any cocaine neurotoxicity (Cappon et al., 1998).

Although widespread toxicity is generally not observed, axonal degeneration after cocaine binge treatments has been reported in one specific region extending from the Hb toward the VTA (Ellison, 1992). The neurodegeneration in this region is not specific to cocaine, however, and has been reported across a wide range of stimulant drugs and also nicotine. Interestingly, this degeneration has been implicated to play a role in the loss of forebrain control circuitry and development of behaviors related to binge drug use and relapse (Carlson et al., 2000; Ellison, 2002). The activation of LHB activates the "tail" region of the VTA, which is the rostromedial tegmental nucleus (rmTg) (Jhou et al., 2009a). Screening of the effects of many pharmacological agents on the GABAergic cells of the rmTg has been carried out by monitoring the induction of c-Fos or ΔFosB expression in acute
The robust activation of these neurons was only shared by stimulant drugs, including cocaine, amphetamines, methylphenidate, and caffeine, and by a DAT blocker, but not by SERT or NET blockers, indicating that strong DAergic activity was needed to activate the network that involves the LHb. Many other groups of drugs of abuse do not strongly activate the neurons in the rmTg (Fig. 1), including morphine, ethanol, diazepam, Δ²-tetrahydrocannabinol, ketamine, and phencyclidine (Perrotti et al., 2005; Kauffling et al., 2010). Nicotine preferentially activates, and degenerates at high doses, the medial habenula-interpeduncular (MHB-IPN) pathway after subchronic administration (Carlson et al., 2001; Ciani et al., 2005) (see Fig. 10), but it also acutely excites the rmTg neurons in anesthetized rats (Lecca et al., 2011).

12. Potential Mechanisms in Cocaine Neurotoxicity and Neuroprotection. Some data suggest that, under some circumstances, cocaine can function as a stressor or as a neuroprotective agent. In cultured fetal mesencephalic DA neurons, a 5-day cocaine treatment (10⁻⁵ and 10⁻⁴ M) produced no effect on levels of TH-positive cells or high-affinity [³H]DA uptake (Bennett et al., 1993). However, other studies employing PC12 cells found that cocaine treatment produced DNA fragmentation, cytotoxicity, decreases in Bcl-2, and increases in caspase-3 levels (Gramage et al., 2008; Lepsch et al., 2009). Furthermore, the cocaine metabolite benzoylecgonine has been reported to be cytotoxic (Lin and Leskawa, 1994). Oxidative stress has been implicated in cocaine toxicity. In vitro, cocaine toxicity can be attenuated by application of the superoxide dismutase-mimetic tempol (Numa et al., 2011), and in vivo high doses of cocaine lead to increased levels of oxidative stress, hydrogen peroxide, and lipid peroxidation in the striatum and HC of rats for up to 2 days after treatment. However, these changes coincide with increases in levels of glutathione peroxidase and superoxide dismutase, suggesting that compensatory mechanisms may prevent extensive long-term damage (Dietrich et al., 2005; Numa et al., 2008). Oxidative stress may be further increased due to cocaine’s reported effects on mitochondria, which include decreases in levels of complex I subunit gene expression and membrane depolarization (Dietrich et al., 2004; Cunha-Oliveira et al., 2006). These and other factors that have been implicated in cocaine toxicity, such as effects on the glutamatergic system, neurogenesis, neuroinflammation, and blood-brain barrier permeability are discussed above and reviewed in more detail elsewhere (Goncalves et al., 2014).

Cocaine purchased by consumers commonly contains adulterants such as levamisole that, together with its major metabolite aminorex, are also pharmacologically active. Although levamisole is only a rather weak reuptake inhibitor, the metabolite aminorex was found to exert strong reuptake inhibiting and releasing properties similar to amphetamine (Hofmaier et al., 2014). Aminorex was previously reported to not produce any long-lasting depletion of brain monoamines (Zheng et al., 1997), but the potential toxicity of these and other cocaine adulterants, particularly in combination with cocaine, is a topic of interest for harm reduction requiring further investigation.

Aside from possible toxic effects, it is interesting to note that cocaine actually appears to protect against toxicity of amphetamines. This effect has been linked to the reduction of cellular uptake of the amphetamines after cocaine, for instance due to translocation of DAT from the plasma membrane to endosomes (Daza-Losada et al., 2008; Peraile et al., 2010).

13. Methylphenidate and Novel Psychoactive Substances. Aside from cocaine, there are several drugs in clinical and recreational use that have a very similar mechanism of action. Most important in this regard is methylphenidate. As with cocaine, there are reports of increased oxidative stress and lipid peroxidation in certain brain regions after methylphenidate, whereas long-term depletion of monoamine levels is not observed (Yuan et al., 1997; Schmitz et al., 2012; Comim et al., 2014). Analogous with cocaine, methylphenidate also protects against toxicity of amphetamines, and it has been suggested it may also be protective against the neurodegenerative processes occurring in Parkinson’s disease (Volz, 2008).

During the increase in novel psychoactive substance use in recent years, one of the compounds that became very widely used was 3,4-methylenedioxypyrovalerone, a compound structurally related to amphetamines, but with more extensive substitutions, including an extended carbon chain instead of a methyl group at the α-carbon. Hence, the drug does not function as a transporter substrate but, like cocaine, strongly inhibits monoamine reuptake. It is most potent at the DAT and NET and to a lesser extent at the SERT (Baumann et al., 2013; Cameron et al., 2013; Iversen et al., 2014). A further investigation of the potential neurotoxic and neuroprotective effects of 3,4-methylenedioxypyrovalerone and similar novel compounds, e.g., mephedrone, could be of interest.

14. Conclusions. Basic research on cocaine has produced a large amount of detailed neurobiological, molecular biologic, and gene regulation data (for a summary, see Fig. 7), which help to understand the biologic background of various phases of cocaine addiction in different reward/emotion/cognition-related brain regions. Unfortunately, this vast database has not yet produced enough translational results to treat cocaine-addicted patients.

B. Amphetamine-type Psychostimulants

DA plays an important role in reward and motivation, two aspects of behavior, which are central to goal-directed behavior for both human as well as nonhuman
animals (Salamone, 1994; Koob, 1996). Reward can be viewed as the subjective pleasurable sensation associated with fulfilling a physiologic need, such as quenching hunger or thirst with food or water, whereas motivation is the subsequent willingness to exert effort in performing goal-directed behavior to achieve these rewards. The mesolimbic DA system, consisting of projections from the VTA to the NAc plays an important role in mediating reward and motivation (Wise, 2006) (Fig. 1). Recreational drug use is also associated with pleasurable sensations (Fischman and Foltin, 1991). So it should come as no surprise that all drugs that produce these sensations also produce increases in the DA neurotransmission in the NAc in a similar, but more robust, way as do natural or physiologically important rewarding stimuli (Wise and Rompre, 1989). Amphetamines such as AMPH and METH are particularly interesting in this respect as they, unlike other more indirectly acting drugs, except for cocaine, preferentially increase DA levels in the NAc (Wise and Rompre, 1989) via several mechanisms described below (Fig. 5; Table 1).

As will be described in more detail in the rest of this section, the primary effect of amphetamines is to increase monoamine neurotransmission by inhibiting the reuptake and enhancing the release of the monoamines...
DA, NE, and 5-HT through the DAT. However, AMPH also affects secretory vesicles, enzymes involved in DA biogenesis and metabolism, and many other targets. AMPH-type stimulants have been employed for a long time, both for medical and recreational purposes. This raises the question of what, other than its initial effects on brain neurochemistry, the long-term effects AMPH exposure may produce.

1. Many Stimulant Amphetamines for Abuse and Therapy. The biggest and most diverse group of psychostimulants is the amphetamines. In its most basic form, amphetamine (AMPH), a contraction of α-methyl-phenethyamine, consists of a phenyl ring connected to an amino group by a two-carbon side chain with a methyl group on carbon 1. The addition of a methyl group on the nitrogen atom produces methamphetamine (METH). Various other ring and side chain substitutions produce other well-known amphetamine analogs such as methylphenidate (MPH) and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). We will focus here on the amphetamines in general and make notes on specific qualities of individual compounds as necessary. The amphetamines all exist as isomers with subtle differences in their neurochemical and behavioral properties. For AMPH, the d-isomer appears to be a more potent DA releaser, whereas the l-isomer is an equally or more potent releaser of NE (Heikkila et al., 1975; Holmes and Rutledge, 1976; Mendelson et al., 2006). Furthermore, the d-isomers of AMPH and METH appear more potent than the l-isomers in producing behavioral effects such as locomotor activity, SA, and taste aversion (Balster and Schuster, 1973; Yokel and Pickens, 1973; Carey and Goodall, 1974; Segal, 1975). Illicit METH is usually distributed as either a racemic mixture of d- and l-isomers or as pure d-isomer, depending on the method of synthesis most prevalent at the time (Logan, 2002; Mendelson et al., 2006).

Psychostimulants have been widely used medically (Iversen, 2006), but presently their therapeutic use is more limited. AMPH and MPH are the first line treatment of attention deficit hyperactivity disorder (ADHD), a common disorder primarily characterized by decreased impulse control and attention, affecting approximately 4% of the population (Surman et al., 2013). They are also used for management of narcolepsy (De la Herran-Arita and Garcia-Garcia, 2013). Furthermore, although not currently approved, recent clinical trials have provided evidence that MDMA may be effective in the treatment of otherwise treatment-resistant anxiety disorders (Mitroofer et al., 2011, 2013). Recreational use of amphetamines, particularly AMPH, METH, MDMA, and more recently, various β-ketoamphetamines is also widespread, with amphetamine-type stimulants being the world's second most consumed illegal drugs after cannabis (UNODC, 2011). Like other psychostimulants such as cocaine, the subjective effects of amphetamines are associated with increases in DA and include euphoria, increased energy, insomnia, and decreased appetite. However, excessive DA stimulation can also lead to amphetamine psychosis, a condition that shows strong similarity to the psychotic episodes experienced by patients suffering from schizophrenia and which constitutes one of the primary reasons for amphetamine-related hospitalization (Snyder, 1973; McKetin et al., 2013; Medhus et al., 2013).

2. Molecular Targets and Mechanisms of Action of Amphetamines. Historically, the mechanisms that have received the most attention are the ability of amphetamines to inhibit the reuptake and enhance the release of DA via the DAT, as well as their ability to promote the release of DA from secretory vesicles (Sulzer et al., 2005; Fleckenstein et al., 2007)(Fig. 5). Recently, it has become clear that, aside from these traditional mechanisms, amphetamines are also capable of modulating action potential-dependent neurotransmitter release (Branch and Beckstead, 2012; Daberkow et al., 2013). Furthermore, amphetamines regulate DA neurotransmission via inhibition of monoamine oxidase (MAO) and enhancement of tyrosine hydroxylase (TH) and have numerous other receptor targets, including the α2-adrenergic receptor, the sigma receptor, and the trace amine-associated receptor 1 (TA1) receptor (Fung and Uretsky, 1982; Robinson, 1985; Ritz and Kuhar, 1989; Matsumoto et al., 2014; Reese et al., 2014). Many of these targets have been identified only recently, and their importance in mediating the effects of amphetamines on behavior as well as its therapeutic, reinforcing, and toxic effects are just beginning to be understood.

3. Comparison of the Mechanisms of Action of Amphetamine versus Methamphetamine. It is sometimes stated that METH is more addictive and more powerful than AMPH, but this statement has been hard to back up empirically. The changes in extracellular DA levels measured by microdialysis in rats are correlated to the striatal drug levels and are equal for the d-isomers of both drugs (Melega et al., 1995). One study confirmed that d-AMPH and d-METH produce similar increases in NAc DA levels in rats and actually found that AMPH increases PFC DA levels more than METH and also produces a higher peak locomotor activity (Shoblock et al., 2003b). In line with the effect on PFC DA levels, it was discovered that AMPH has a stronger effect than METH on working memory function. The effects of AMPH and METH on working memory are bimodal, with a small dose producing an increase in working memory performance and higher doses producing a decrease, an effect closely reminiscent of the bimodal effects of DA turnover and D1 receptor activation on working memory (Murphy et al., 1996; Vijayraghavan et al., 2007). For d-AMPH, a dose of 0.5 mg/kg produces an increase in working memory performance, whereas a 2 mg/kg dose produces a decrease. d-METH on the other hand has little effect at 0.5 mg/kg, producing the highest increase at 2 mg/kg and...
only decreasing at 4 mg/kg (Shoblock et al., 2003a). Furthermore, d-AMPH fully substitutes for d-METH in animals trained to discriminate METH from placebo (Desai and Bergman, 2010) and also causes dose-dependent increases in METH-appropriate responding in humans who have learned to discriminate 10 mg METH from placebo (Sevak et al., 2009), suggesting that the subjective effects are also very similar, if not indistinguishable. Recently, it was also shown that varying doses (between 12 and 50 mg intranasally) of d-AMPH or METH produce similar subjective effects and did not differ from each other with regards to the rate at which participants would opt for a cash reward instead of a dose of the drug (Kirkpatrick et al., 2012), thereby not corroborating the notion that METH is more addictive or reinforcing than d-AMPH.

By tradition, scientific work on the mechanism of action of amphetamines has been primarily done with AMPH, whereas studies on neurodegeneration preferentially have employed METH (Sulzer et al., 2005).

4. Effects of Amphetamine at Plasmalemmal Dopamine Transporter. The DAT as well as the other monoamine transporters, NET and SERT, belong to the SLC6 gene family of secondary active transporters consisting of 12 transmembrane domains as well as intracellular domains with phosphorylation and binding sites vital for its regulation, including by AMPH. Normally, the transporters depend on pre-existing concentration gradients of Na\(^+\) and Cl\(^-\) to cotransport one molecule of Na\(^+\) together with one neurotransmitter molecule from the extracellular space into the cytoplasm (Robertson et al., 2009).

The ability of AMPH to increase extracellular DA levels in vivo is well established, and one area particularly sensitive to AMPH-induced increases in extracellular DA is the NAc (Carboni et al., 1989). AMPH blocks the reuptake of DA via the DAT (Parker and Cubeddu, 1988). However, intracytoplasmic injections of AMPH also increase extracellular DA, and this effect is blocked by the DAT blocking drug nomifensine, indicating that AMPH, aside from blocking the reuptake, also enhances the release of intracellular DA (Sulzer et al., 1995). The uptake of radiolabeled AMPH into striatal synaptosomes is a saturable and high-affinity process that is sensitive to ouabain and cocaine and dependent on temperature, suggesting that AMPH is itself a substrate of the DAT (Sitte et al., 1998; Zaczek et al., 1991). In addition, it is important to note that AMPH is a lipophilic weak base (Mack and Bonisch, 1979; Gulaboski et al., 2007), which will, particularly at higher concentrations, diffuse through the plasma membrane and enter the cytoplasm independently of the DAT.

An early model (Fischer and Cho, 1979) known as the exchange-diffusion model, postulated that AMPH, like DA, would bind to an extracellular binding site, causing a conformational change in the DAT protein resulting in the binding site traversing the membrane and releasing AMPH into the cytoplasm and simultaneously binding a DA molecule to be transported outward. Later it would become clear that this exchange-diffusion is not limited to a 1:1 ratio of AMPH exchange for DA. AMPH also increases intracellular levels of Na\(^+\), and increases in Na\(^+\) are in itself sufficient to induce release of DA via the DAT; it is likely that it is the influx of Na\(^+\) ions via the DAT rather than AMPH itself that triggers reverse transport of DA. Furthermore, the DAT can be regulated, as discussed below, via intracellular second messengers between “reluctant” and “willing” states for AMPH-mediated DA efflux without affecting inward transport (Khoshbouei et al., 2004). AMPH is also capable of producing rapid bursts of DA release via the DAT, which is indicative of AMPH causing a conformational change to the DAT resulting in a channel-like state in which DA is rapidly transported outward via a pore in the transporter protein. The process is transient, consisting of millisecond bursts and is inhibited by the presence of DA on both sides of the plasma membrane and therefore not capable of transporting DA against its concentration gradient (Kahlig et al., 2005). Finally, recent evidence suggests that AMPH also affects the DA release associated with action potentials. One study that examined this demonstrated that AMPH at 10 mg/kg enhanced electrically evoked DA release measured 10 and 30 minutes after the initial injection. Additionally, it was shown that a low dose (1 mg/kg) of AMPH facilitated the DA release associated with a rewarding stimulus cue, suggesting that AMPH acts, at least in part, by altering the characteristics of action potential-dependent exocytotic DA release (Daberkow et al., 2013). Unlike AMPH, MPH was recently shown to decrease exocytotic DA release (Federici et al., 2014), which may help explain the differing subjective effects of this drug. The exact mechanism by which AMPH increases the exocytotic release of DA is unknown, but may be related to the ability of AMPH to induce persistent transporter-mediated ion-leakage and excitatory conductance (Ingram et al., 2002; Branch and Beckstead, 2012; Rodriguez-Menchaca et al., 2012). It has been suggested that reuptake inhibition is primarily associated with lower drug concentrations, possibly corresponding to those in the therapeutic range, whereas doses in the recreational range shift the importance to AMPH-mediated DA release (Calipari and Ferris, 2013). Furthermore, there appear to be region-specific changes in the relative importance of DAT-dependent and action potential-dependent DA release, with enhancement of vesicular release being more important in the dorsal striatum compared with the ventral striatum (Avelar et al., 2013). As discussed further below, the final increases in cortical and NAc DA levels mediate both the therapeutic and reinforcing effects of amphetamines.

5. Regulation of Dopamine Efflux Via the Dopamine Transporter by Protein Kinase C and Ca\(^{2+}\) / Calmodulin
Kinase II Phosphorylation, and by Reactive Oxygen Species. The release of DA via the DAT is highly dependent on DAT expression on the surface of the plasma membrane and is regulated by several intracellular second-messenger systems. Phosphorylation by PKC plays an important role in regulation of DAT function and surface expression. PKC-activating and phosphatase-inhibiting drugs both decrease the uptake of DA into striatal synaptosomes in vitro. The effect is inhibited by the PKC inhibitor staurosporine (Copeland et al., 1996; Zhang et al., 1997). Studies in striatal synaptosomes employing [32P]orthophosphate to assess the amount of DAT phosphorylation confirmed that phosphorylation at DAT is regulated by PKC and that the amount of DAT phosphorylation is directly related to the efficacy of DA uptake, with increased phosphorylation decreasing the uptake efficacy (Vaughan et al., 1997). PKC activation removes DAT from the membrane to endosomes, whereas PKC inhibition promotes the insertion of DAT into the membrane, something that has been confirmed using immunofluorescence confocal microscopy (Pristupa et al., 1998; Sorkina et al., 2003). In addition to affecting DA uptake, PKC-mediated DAT phosphorylation also affects AMPH-induced DA release. One study found that PKC-inhibiting drugs almost completely inhibited AMPH-induced DA efflux, while increasing baseline DA uptake in the absence of AMPH (Kantor and Gnegy, 1998). The effect of PKC-mediated DAT phosphorylation on AMPH-induced DA release appears to be mediated specifically by the PKCβII isoform (Johnson et al., 2005). Thus, whereas increases in phosphorylation appear to decrease the efficacy of baseline DA uptake and decrease cell surface DAT expression, the opposite appears to be the case for AMPH-mediated DA efflux, which is reduced by inhibition of phosphorylation.

METH is capable of inducing DAT phosphorylation both in vivo and in vitro (Cervinski et al., 2005). A single in vivo injection of METH rapidly (within 1 hour) and reversibly decreased plasmalemmal DA uptake, whereas the total binding of the membrane-permeable DAT ligand WIN35428 was not affected, indicative of METH-induced transient transporter internalization or loss of function (Fleckenstein et al., 1997b; Kokoska et al., 1998; Saunders et al., 2000). These data suggest that AMPH produces transporter internalization in a similar way as does PKC activation. However, there appear to be subtle differences between PKC-dependent and AMPH-dependent DAT internalization. Both AMPH and PKC activation results in loss of cell surface DAT in vitro, but contrary to the PKC-induced internalization that is dependent on DAT ubiquitination and subsequent sorting to LAMP1-positive endosomes for degradation, the AMPH-induced internalization sorted the DAT to Rab11-positive recycling endosomes (Hong and Amara, 2013), likely destined to be quickly reinserted into the membrane.

AMPH-mediated DA efflux appears to be influenced also by CaMKIIα. CaMKIIα binds the DAT at the distant C terminus while inducing phosphorylation at similar residues as PKC, namely the N-terminal serines. Phosphorylation by this kinase increases AMPH-induced DA efflux via DAT, and mutations at both the C-terminal binding site as well as the N-terminal phosphorylation sites inhibit the increase (Fog et al., 2006). CaMKII appears to influence AMPH-mediated DA efflux via the DAT through Syntaxin A1, a protein that, aside from being involved in synaptic vesicle release, interacts with and regulates transmembrane proteins, including DAT (Binda et al., 2008; Robertson et al., 2009). Interestingly, association of Syntaxin A1 with the DAT is also subject to regulation by AMPH itself (Binda et al., 2008), pointing to another target by which AMPH itself regulates AMPH-dependent, DAT-mediated DA efflux.

There is also evidence suggesting that ROS signaling may be involved in regulating cell surface DAT expression (Fleckenstein et al., 2007). The oxygen radical-generating enzyme xanthine oxidase reduces DA uptake into striatal synaptosomes, an effect inhibited by coapplication of the free radical scavenger superoxide dismutase (Berman et al., 1996; Fleckenstein et al., 1997a). As discussed in more detail below, AMPH is also capable of generating ROS, so this regulatory pathway is also open to modulation by AMPH itself.

Regulation of DAT function via phosphorylation and ROS are some mechanisms by which DAT surface expression and function is regulated. Because these mechanisms are affected by the presence of AMPH itself, it appears that the final amount of DA released into the extracellular space in response to AMPH depends on a complex interplay between AMPH and intracellular second-messenger systems, some of which may not have been discovered yet. To add to this complexity, AMPH also affects the amount of DA available for release via several mechanisms by affecting DA sequestration in secretory vesicles.

6. Effects on Secretory Vesicles. In addition to releasing DA from the cytoplasm to the extracellular space, AMPH also promotes the release of DA from secretory vesicles. Several mechanisms by which AMPH may release DA from secretory vesicles into the cytoplasm have been suggested, including AMPH weak base effects, competition at the vesicular monoamine transporter 2 (VMAT-2), and redistribution of VMAT-2.

AMPH is a lipophilic weak base (Gulaboski et al., 2007; Mack and Bonisch, 1979) and, as such, is capable of traversing the membranes of synaptic vesicles. The lumen of secretory vesicles is acidic, with a pH of around 5.5 (Mellman et al., 1986; Njus et al., 1986), and once inside the vesicle lumen AMPH is protonated, decreasing its membrane permeability and leading to accumulation of AMPH inside the vesicles. The protonation of
AMPH also leads to a loss of the proton gradient established by H\(^{+}\)-ATPase across the vesicular membrane, which is required by the secondary active transporter VMAT-2 to sequester DA into the vesicles, eventually leading to DA release into the cytoplasm (Sulzer and Rayport, 1990; Sulzer et al., 2005). The loss of DA from secretory vesicles leads to a decrease in quantal size due to the lower amount of DA in individual vesicles. However, after prolonged (6–48 hours) exposure to METH a rebound hyperacidification and subsequent increase in quantal size has been reported (Markov et al., 2008), indicative of a homeostatic mechanism capable of preventing long-term loss of DA from secretory vesicles.

In addition to promoting release by interfering with the membrane proton gradient, AMPH also has affinity for VMAT-2 itself, suggesting it may also interfere with the uptake of DA into vesicles and cause a net decrease of vesicle neurotransmitter content (Sulzer et al., 2005). Finally, repeated high-dose METH treatment decreases vesicular DA uptake without affecting total binding of the VMAT-2 ligand \([^{3}H]\)dihydrotetrabenazine, consistent with removal of VMAT-2 from the vesicular membrane (Brown et al., 2000; Hogan et al., 2000). However, due to the relatively low affinity of AMPH for VMAT-2 (see Table 1), the importance of VMAT-2-dependent mechanisms in affecting AMPH-induced vesicular DA efflux at physiologically relevant concentrations has been questioned (Gonzalez et al., 1994; Fleckenstein et al., 2007).

7. Other Amphetamine Targets: Monoamine Oxidase, Tyrosine Hydroxylase, other Monoamine Transporters, Trace Amine-Associated Receptor 1, and Sigma Receptors. The ability of AMPH to block MAO has been repeatedly demonstrated both in vitro and in vivo (Mantle et al., 1976; Green and el Hait, 1978; Clarke et al., 1979; Miller et al., 1980; Robinson, 1985). AMPH also increases DA levels because of enhancement of TH function (Costa et al., 1972; Kuczenski, 1975), although high concentrations appear to decrease TH function (Kogan et al., 1976; Hotchkiss and Gibb, 1980). The exact mechanism by which AMPH increases TH function is not known (Sulzer et al., 2005). This highlights that AMPH, at least to a certain extent, compensates for its enhancement of DA efflux by increasing cellular DA levels by inhibiting DA breakdown and increasing DA biosynthesis.

AMPH also increases extracellular levels of 5-HT and NE (Kuczenski and Segal, 1997) in a similar fashion by inhibiting the reuptake and enhancing the release of these neurotransmitters via their respective plasma-membrane transporters (Parada et al., 1988; Rothman et al., 2001; Hilber et al., 2005). The effects of AMPH in these other monoamine systems play an important role in mediating part of its behavioral effects (Xu et al., 2000; Gingrich and Hen, 2001; Gainetdinov et al., 2002; Perona et al., 2008).

Several other targets for AMPH and METH are known. Recently the TA\(_{2}\) (Reese et al., 2014) and sigma receptors (Matsumoto et al., 2014) have received attention. The TA\(_{1}\) is a trace amine-associated GPCR and colocalizes with DAT in certain brain regions. It exerts a modulatory effect on monoamine neurotransmission (Xie and Miller, 2009). Taar1-KO mice show clear behavioral differences and respond more strongly to the locomotor activating and rewarding effects of AMPH (Achat-Mendes et al., 2012a). The sigma receptor, a one-transmembrane domain receptor at the endoplasmic reticulum (ER) encoded by the SIGMAR1 gene that is involved in cellular Ca\(^{2+}\) and K\(^{+}\) regulation (Aydar et al., 2002; Monassier and Bousquet, 2002), is another interesting target in mediating METH neurotoxicity. METH preferentially binds to the \(\sigma_{1}\) over the \(\sigma_{2}\) subtype, and blocking the receptor acutely decreases the drug’s locomotor stimulating effects (Hayashi and Su, 2007). Sigma receptor blockade also attenuates striatal DA depletion, cytokine activation, and cognitive impairment induced by high-dose METH treatment in rodent models, something that may be related to its effects on cellular Ca\(^{2+}\) levels or the expression of \(\sigma\) receptors on microglia (Robson et al., 2013a,b; Seminero et al., 2013).

For AMPH and METH, the DA system is clearly important in mediating its neurochemical and behavioral effects, although it is clear that some of its effects are also mediated by different neurotransmitter systems, some of which only recently have been identified.

8. Action of Substituted Amphetamines and Cathinones. Once the amphetamine molecule is subjected to substitutions, its properties change in important ways. The substituted amphetamines MDMA and the cathinones mephedrone (4-MMC) and methylone (MDMC) have a mechanism of action that is reminiscent of AMPH and METH but they differ in the ratio of their effects at various monoamine systems (Table 1). AMPH and METH bind to DAT, NET, and SERT, but they show a strong preference for the catecholamine transporters DAT and NET and have much lower affinity for the SERT (Nichols, 1994; Simmler et al., 2013). Subsequently, their main effect in vivo is to increase extracellular DA levels in the NAc and FC without having strong effects on 5-HT release (Carboni et al., 1989; Kehr et al., 2011; Melega et al., 1995; Shoblock et al., 2003b).

Substitutions of the phenyl ring on the amphetamine molecule produce compounds with much higher affinity for SERT, although they often maintain DA-releasing properties as well (Nichols, 1994). Thus, MDMA, which contains a 3,4-methylenedioxy substitution, is a potent 5-HT-releasing agent with relatively weak effects on DAT (Cozzi et al., 1999; Verrico et al., 2007; Simmler et al., 2013). Furthermore, like AMPH and METH, MDMA also induces neurotransmitter efflux from synaptic vesicles (Rudnick and Wall, 1992; Mlinar and...
Corradetti, 2003). In vivo, it produces increases in NAc levels of 5-HT and DA, with 5-HT levels generally increasing more than DA (Kehr et al., 2011; Baumann et al., 2012).

The cathinones 4-MMC and MDMC, also called β-ketoamphetamines because of the ketone moiety on the β-carbon, have similar affinity for plasmalemmal monoamine transporters as their nonketoamphetamine parent compounds but display a more than 10-fold lower affinity for the VMAT-2 (Cozzi et al., 1999; Baumann et al., 2012; Martinez-Clemente et al., 2012). The exact importance of the lower VMAT-2 affinity is currently unknown, but because 4-MMC is also a weak base (Santali et al., 2011), it is likely that it is also capable of inducing neurotransmitter efflux from secretory vesicles in a similar way as other amphetamines. In vivo, 4-MMC produces an effect somewhere in between MDMA and AMPH, producing large increases in both DA and 5-HT levels in the NAc, whereas MDMC produces a neurotransmitter release pattern similar to but slightly weaker than MDMA, increasing primarily 5-HT levels (Kehr et al., 2011; Baumann et al., 2012; Wright et al., 2012). So, although the mechanism of action of these ring-substituted compounds is similar to that of AMPH and METH, they all share the property of being capable of producing large increases in extracellular 5-HT levels in addition to affecting extracellular catecholamine levels to varying extents.

9. Action of Stimulants Common in Clinical Use. In addition to AMPH and METH, the drug MPH as well as the AMPH prodrug lisdexamfetamine (LDX) is used for the treatment of ADHD.

MPH is, because of the loss of its α-methyl moiety, by chemical definition not an amphetamine. Nonetheless it is a stimulant drug and has been the mainstay of ADHD-treatment for several decades (Patrick and Markowitz, 1997). The action of MPH shows some similarity to AMPH and also produces an increase of striatal DA levels in vivo, measured using microdialysis (Aoyama et al., 1996). Studies in membrane fractions and synaptosomes show that MPH, like AMPH, inhibits the reuptake of the catecholamines by DAT and NET but has little affinity for SERT (Schweri et al., 1985; Gatley et al., 1996). In contrast to AMPH, however, MPH appears to function primarily as a reuptake blocker and not as a releasing agent (Braestrup, 1977; Patrick et al., 1987).

LDX is a prodrug consisting of AMPH covalently bound to the amino acid lysine. As a prodrug it is inactive until it is enzymatically cleaved into lysine and free AMPH. LDX does not appear to be very lipophilic, and enzymatic cleavage by hydrolysis occurs only after peptide carrier-mediated absorption in the small intestine (Pennick, 2010). The prodrug formulation has been suggested to be a clinical improvement over regular AMPH because it is longer lasting and thus allows for once daily dosing as well as possibly limiting its potential for nonmedical use (Goodman, 2007). A study investigating the effect of LDX and AMPH (both 1.5 mg/kg AMPH base) indeed shows that the onset of increases in striatal DA levels is slower with LDX, corresponding to the plasma concentration of the AMPH metabolite rather than the prodrug. Moreover, the plasma area under the curve for AMPH is similar for both drugs but with LDX showing a 50% lower maximum concentration and significantly longer delay from administration until the maximum concentration is reached. Finally, LDX induces significantly less locomotor activity (Rowley et al., 2012).

10. Efficacy and Addiction Liability of Amphetamines in Clinical Use. The ability of amphetamines to increase synaptic DA levels via various mechanisms is thought to play a pivotal role in both producing their therapeutic as well as their rewarding effects.

In ADHD patients, there is a plethora of evidence pointing toward deficits in DA neurotransmission as playing a key role in the pathophysiology of the disease. As reviewed earlier (Swanson et al., 2007), several imaging studies suggest structural abnormalities in ADHD patients, such as decreased volumes of the DA-rich caudate nucleus and its target globus pallidus. This is consistent with fMRI studies, which have reported hypoactivation of the FC as well as the frontostriatal DA circuitry while performing tasks requiring sustained attention. These abnormalities are resolved after treatment with psychostimulants. Functional abnormalities as well as the symptoms observed in ADHD patients appear to normalize after stimulant drug treatment, which increases synaptic DA levels (Lee et al., 2005). However, the long-term effects of stimulant treatments are still poorly understood. Although studies generally find sustained symptom improvement, it has been harder to demonstrate consistent improvement in standardized test scores and ultimate educational attainment (Hechtman et al., 2004; Loefeldman, 2007; Hazell, 2011).

Aside from efficacy, another interesting question is how stimulant treatment of ADHD patients affects addiction liability later in life. A number of studies, including one meta-analysis, have examined this question and concluded that stimulant treatment generally appears to lower the risk of substance abuse disorder later in life (Wilens et al., 2003; Mannuzza et al., 2008). This effect may be understood by considering that impulsive behavior is one of the primary risk factors for initiation of drug use (Kreek et al., 2005) and that stimulant treatment effectively reduces impulsivity in ADHD patients. On the other hand, misuse and diversion of stimulant medication has become increasingly common, and it is estimated that in the past year, 5 to 35% of college-aged individuals have used a stimulant drug not prescribed to them (Wilens et al., 2008).

MPH is another drug that elicits subjective effects of drug-liking similar to AMPH, and the subjective
rewards. The rewarding effect appears to be dependent upon increases in synaptic DA levels produced by the drug (Rush et al., 2001; Volkow et al., 2002; Arria and Wish, 2006; Botly et al., 2008). LDX also produces similar feelings of subjective drug-liking on questionnaires in human volunteers as the 50% lower doses of AMPH base (Jasinski and Krishnan, 2009). The higher dose need likely represents the amount needed to achieve similar peak plasma AMPH concentrations and increases in the NAc DA levels, which at an equimolar ratio emerge slower and remain lower due to its extended absorption time of LDX.

Ring substitution of amphetamines produces substances with a high capability of increasing both 5-HT and catecholamine levels (Nichols, 1994). Some of these compounds, most notably MDMA, appear to produce effects that are somewhere between those of pure stimulants and typical hallucinogens such as LSD or psilocybin. The most important effect appears to be that they, next to creating a feeling of euphoria and well-being, produce emotional opening and empathy. This observation has led to the term empathogen or entactogen being coined to describe these substances, as well as the suggestion that they may be valuable in the clinics as an aid to psychotherapy (Nichols, 1986). In fact, recent clinical trials support the idea that MDMA may be effective when used as an aid in psychotherapy for treating treatment-resistant posttraumatic stress disorder patients (Mithoefer et al., 2011, 2013). The use for treating treatment-resistant posttraumatic stress may be effective when used as an aid in psychotherapy (Nichols, 1986). In fact, recent clinical trials support the idea that MDMA may be effective when used as an aid in psychotherapy for treating treatment-resistant posttraumatic stress disorder patients (Mithoefer et al., 2011, 2013). The use for treating treatment-resistant posttraumatic stress disorder patients (Mithoefer et al., 2011, 2013). The use for treating treatment-resistant posttraumatic stress disorder patients (Mithoefer et al., 2011, 2013).

11. Neuroplasticity Related to Sensitization and Addiction to Amphetamines. The incentive sensitization theory of drug addiction states that repeated drug administration can lead to long-lasting adaptations resulting in hypersensitivity or sensitization in parts of the mesolimbic DA system involved in processing a sub-component of reward termed incentive salience or “drug wanting,” which is thought to mediate the increased drug seeking and taking characteristic of drug addiction. Repeated treatment with reinforcing drugs produces a gradual increase in locomotor activity after administration, an effect known as psychomotor sensitization. Because it is assumed that the neural adaptations responsible for developing psychomotor sensitization are similar to those involved in producing incentive sensitization, the former is often used as a model for the development of addiction (Robinson and Berridge, 2000).

Treatments with psychostimulants such as AMPH and METH produce psychomotor sensitization associated with neuroadaptations in the VTA and NAc, but also in the PFC, amygdala, and HC (Anagnostaras and Robinson, 1996; Nestler, 2001; McDaid et al., 2006; Perez et al., 2010; van der Veen et al., 2013). A number of different types of structural and synaptic plasticity have been described that may be responsible for this effect. For example, treatment with psychostimulants, including AMPH, result in upregulation of TH levels in the VTA and NAc. Exposure to psychostimulants also causes synaptic changes, such as downregulation of inhibitory D₂Rs but upregulation of excitatory D₁Rs in the VTA. It has also been suggested that NMDARs may become more permeable to Ca²⁺, due to second-messenger-mediated signaling in response to repeated psychostimulant exposure. The ultimate result of all these adaptations is enhanced DA neurotransmission in the mesolimbic system, which is critical for the development of sensitization to psychostimulants (Licata and Pierce, 2003; Kasanetz et al., 2010; Fernandez-Espejo and Rodriguez-Espinosa, 2011). VTA NMDARs appear to play a vital role in psychostimulant-induced neuroplasticity through activation of CaMKII. Increased Ca²⁺ influx after psychostimulants such as AMPH and cocaine elevate CaMKII levels, and inhibition of CaMKII blocks both behavioral sensitization and drug-seeking behavior (Licata et al., 2004; Loweth et al., 2008). The importance of CaMKII signaling is also evident because of its vital role in memory formation and because it has been shown to be directly involved in sensitization via activation of CREB and regulation of TH transcription (Lim et al., 2000; Lisman et al., 2002).

Also MDMA affects sensitization and memory. A dose of 12.5 mg MDMA administered once a week for 3 weeks increased LTP in CA1 pyramidal neurons (hippocampal slices) 1 week after in vivo treatment (Morini et al., 2011), whereas an in vitro study showed that acute MDMA increases LTP in the CA3-CA1 synapses involving the activation of presynaptic 5-HT₂ and postsynaptic D₁/5 receptors and was suppressed by a PKA inhibitor (Rozas et al., 2012). Acute administration of MDMA increased expression of c-Fos, erg-1 and erg-3 in the striatum that was suppressed by SL327, a selective inhibitor of ERK activation, and this effect was selective for the striatum (Salzmann et al., 2003). Repeated administration of MDMA, gamma-hydroxybutyrate (GHB), and their combination results in long-lasting changes in hippocampal protein expression even after a washout period (van Nieuwenhuijzen et al., 2010), whereas MDMA treatment (0.2 and 2 mg/kg) twice a day for 6 days in young rats caused dose-dependent impairment in spatial learning and reduced LTP (Arias-Cavieres et al., 2010). Furthermore, just like AMPH, MDMA also produces sensitization after repeated exposure (Bradbury et al., 2012).

Repetitive stimulant exposure that produces sensitization also results in morphologic changes to neurons in the NAc and PFC. Both SA and noncontingent injections of cocaine (15 mg/kg) or AMPH (3 mg/kg/day) produce an increase in the density of and the amount of
branching of the dendritic spines on NAc MSNs and PFC pyramidal neurons for more than 3 weeks after the final treatment (Robinson and Kolb, 1999a; Robinson et al., 2001). Meanwhile, METH-induced tissue plasminogen activator mRNA in a subgroup of cortico-striatal neurons was suggested to contribute to the development of lasting changes in the specific neuronal circuits that underlie long-term behavioral modifications (Hashimoto et al., 1998). Repeated, intermittent exposure to MDMA in rats causes behavioral sensitization and large increases in spine density and number in the NAc (multiple-headed spines in core and shell) and prelimbic regions (distal dendrites of layer V pyramidal neurons) (Ball et al., 2009). Additionally, a reduction in the basilar dendrites was observed in the anterior cingulate. These changes accounted for the long-lasting locomotor sensitization to MDMA that is accompanied by reorganization of synaptic connectivity in the limbic-corticostriatal circuit (Ball et al., 2009). The long-lasting behavioral (increased locomotion and rearing) and EEG changes (peak at 3–4.5 Hz) due to MDMA treatment were associated with DNA single- and double-strand breaks, persistent together with long-lasting metabolic changes in the hippocampal formation (Frenzilli et al., 2007).

Protein synthesis plays an important role in the induction of these morphologic changes, because proteins such as c-Fos and ΔFosB, but also Arc, a marker of neuritic outgrowth, and BDNF, which is involved in spine formation, are all elevated after AMPH or cocaine exposure (Nestler et al., 2001; Klebaur et al., 2002; Meredith et al., 2002; Grimm et al., 2003; Fumagalli et al., 2006). Particularly the rmTg appears to be an important target for AMPH-induced modulation of Fos genes that can also have strong functional and behavioral significance in terms of modulating the mesolimbic systems through its GABAergic projections (Barrot et al., 2012; Bourdy and Barrot, 2012) (Fig. 1). MDMA treatment of young rats increased 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced energy metabolism in the NAc and HC that reflects long-term brain region-specific changes in plasticity, and such an effect may result from changes 5-HT2A receptor function (Bull et al., 2006). Likewise, a dose (12.5 mg/kg) of MDMA increases basal Arc expression in the cortex and CA1 (Beveridge et al., 2004). DOI treatment (or immobilization stress) regulates neocortical Arc mRNA expression through BDNF signaling (Bencakreddy et al., 2013). Chronic MDMA treatment increased NT-4 gene expression in the brain stem, cerebellum, and cerebral hemispheres of rats (Hatami et al., 2010).

Aside from changes in a number of well-characterized proteins, AMPH treatment produces wide-ranging changes in expression of other genes as well. A recent study showed increased striatal mRNA expression of 55 genes, whereas 17 genes were downregulated after a 5-day, twice daily treatment with 5 mg/kg AMPH in spontaneously hypertensive rats, a model for ADHD. The genes showing altered expression belonged to several functional categories, including those involved in transcription, angiogenesis, cell adhesion, apoptosis, and neuronal development (Dela Pena et al., 2015). Furthermore, changes in epigenetic markers such as methylation status have been reported in several brain regions, including the NAc, after 2 weeks of amphetamine exposure (Mychasiuk et al., 2013). It is likely that these changes also have an effect on synaptic and/or structural plasticity in ways that have yet to be fully characterized.

12. Glutamate and Amphetamine/Methamphetamine Reinforcement/Extinction/Reinstatement. Like cocaine, AMPH and METH produce CPP (Thorn et al., 2012; Martin et al., 2013; Han et al., 2014) and are reliably self-administered (Pickens and Harris, 1968; Balster and Schuster, 1973; Piazza et al., 1989; Kitamura et al., 2006). Involvement of glutamate-mediated processes in AMPH reinforcement and SA has been well-documented. For instance, mGlu5 receptor antagonists produce a dose-dependent decrease in METH SA without affecting food reinforcement, suggesting that mGlu5 receptors are involved in the reinforcing effects of METH, but not natural rewards (Osborne and Olive, 2008). Similar effects have been reported with the mGlu2/3 antagonist LY379268 (1R,4R,5S,6R)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid) (Crawford et al., 2013; Kufahl et al., 2013). The mGlu5 receptor is also involved in extinction learning, because administration of an mGlu5-positive allosteric modulator resulted in more rapid extinction of responding on the active SA lever. However, this effect was not present during reinstatement tests or a second extinction session (Kufahl et al., 2012). Modulation of addictive behavior was also observed in mGlu5-KO mice, which showed decreased extinction of drug-seeking behavior and increased cue-induced reinstatement (Chesworth et al., 2013). Negative allosteric modulation of mGlu5 receptors was similarly found to attenuate both drug- and cue-induced reinstatement of METH-seeking behavior, although it produced a similar effect on sucrose and food seeking (Watterson et al., 2013).

Ionotropic glutamate receptors are also involved in AMPH-induced addictive behavior (discussed in detail in section II.A on cocaine; Fig. 6). NMDAR subunit GluN2A-KO mice fail to display behavioral sensitization and activation of Ca2+-dependent intracellular signaling pathways in the NAc in response to repeated METH treatments (Miyazaki et al., 2013). In animals trained to self-administer METH, striatal AMPAR GluA2 subunit mRNA was increased 2 hours after cessation of drug intake but decreased after 24 hours compared with control animal (Currington et al., 2011). METH-induced CPP was associated with increased surface expression of GluA1 and GluA2 subunits in the BLA (Yu et al., 2013). In the striatum, however,
a decrease in GluA1 and GluA2 mRNA and protein levels as well as in those for GluN1 subunits and increased AMPAR/NMDAR ratio has been reported in response to repeated METH exposure (Jayanthi et al., 2014). Furthermore, this treatment decreased enrichment of acetylated histone H4 on GluA1, GluA2, and GluN1 promoters as well as a number of other epigenetic markers, whereas the expression of these AMPAR and NMDAR subunits was blocked by the histone deacetylase inhibitor, valproic acid, suggesting an epigenetic mechanism, despite valproate’s limited specificity (Jayanthi et al., 2014). Changes in AMPAR surface expression may also be mediated by striatal-enriched tyrosine phosphatase isoform 61, which internalizes AMPARs, as one study demonstrated that the decreases in striatal-enriched tyrosine phosphatase isoform 61 increases in GluA2 surface expression and behavioral sensitization after repeated METH treatments were blocked by an mGluR negative allosteric modulator (Herrold et al., 2013). Treatment with cetriaxone, which increased glutamate transporter mRNA in the mPFC, blocked METH-induced reinstatement of CPP (Abulseoud et al., 2012).

An interesting question is whether observed plasticity phenomena are drug dependent or if they are primarily associated with development of SA or sensitization. METH-induced glutamatergic neuroplasticity appears to be at least partially dependent upon concurrent development of addictive behaviors, rather than the drug itself. One study investigated the effect of noncontingent METH administration versus METH SA and found important differences in the glutamatergic response to a subsequent METH challenge after drug withdrawal. In noncontingently dosed rats the challenge decreased extracellular glutamate levels in the NAc, whereas in previously self-administering rats, an increase in glutamate levels was observed. This increase was also associated with a larger increase in DA levels (Lomina et al., 2012). Another study found reduced basal glutamate levels in both the NAc and PFC, but increased glutamate levels after reinstatement in self-administering rats compared with yoked saline controls (Parsegian and See, 2014).

How does plasticity differ between METH and cocaine exposures? One study demonstrating the similarities between glutamate involvement in METH and cocaine SA directly compared the effect of a selective mGlu1 receptor antagonist on both METH- and cocaine-induced behavior in squirrel monkeys. It reported that the antagonist produced a reduction in SA of both drugs and attenuated the increase in responding for food rewards produced by both drugs (Achat-Mendes et al., 2012b). DA receptors play an important role in mediating synaptic plasticity (Wolff et al., 2003; Luscher and Malenka, 2011), and because both METH and cocaine produce increases in extracellular DA levels in vivo, it is not surprising that they produce similar neuropsychological effects that may be related to the development of drug dependence. On the other hand, differences also exist, and it has been reported that METH, unlike cocaine, did not alter NAc or PFC cell surface expression of AMPARs or NMDARs during withdrawal (Boudreau and Wolf, 2005; Nelson et al., 2009), indicative of important differences between the two drugs. Cotreatment with a spin-trapping agent prevented AMPH-induced increases in glutamate efflux, suggestive of a ROS-dependent signaling pathway (Wolf et al., 2000). ROS signaling may be more important during amphetamine exposure compared with cocaine because of the increased intracellular ROS after release of DA from vesicles and cytoplasmic oxidation. Clearly, further work is needed to outline the similarities and differences between AMPH/METH and cocaine neuroadaptation, as well as its behavioral implications.

13. Long-term Neuroadaptation/Neurotoxicity after Exposure to High Doses of Psychostimulants. In research on psychostimulant neuroplasticity there has been a focus on the long-term after-effects of psychostimulants such as METH and MDMA. These amphetamine-type psychostimulants are sometimes referred to as club drugs, because they are commonly consumed recreationally at music festivals and dance clubs. This pattern of consumption is not generally associated with the development of addiction, but users will sometimes redose the drug repeatedly during one sitting (binge), which has led to concern about the possible long-term effects of this pattern of consumption on the brain in terms of long-term neuroadaptation and cognitive function, such as deficits in memory and attention.

a. Effects on brain structure. Ricaurte et al. (1982) demonstrated that high doses of METH (3 injections of 50 mg/kg spaced over 8 hours) to rats caused destruction of nerve terminals in the striatum as observed 4 days after the treatments with a silver staining method, considered the gold standard of neurotoxicity testing (Switzer, 2000). Furthermore, they reported that similar regimens also reduced levels of DA and 5-HT both in the striatum and other brain regions such as the FC and amygdala (Ricaurte et al., 1980, 1982). The observation that high doses of METH produced neurotoxicity, visible as swollen fibers and nerve terminals indicative of chromatolysis and axonal damage, has also been found by other investigators (Escalante and Ellinwood, 1970; Lorez, 1981). Recently, evidence of toxicity, measured by silver staining, was observed for METH at even lower doses (3 × 5 mg/kg) (Ares-Santos et al., 2014). Similar results were reported by Molliver et al. (1990) for MDMA (20 mg/kg twice daily for 4 days), who found evidence of structurally damaged axons that appeared as swollen stumps in cortical regions after visualization with silver staining. The damage coincided with loss of fine 5-HT axons in cortical regions 2 weeks after treatment visualized by 5-HT immunocytochemistry.
b. Effects on neurochemistry. Quantitative ligand binding and neurochemical methods suggest alterations in monoamine systems after METH. Numerous studies have reported decreases in striatal DA and 5-HT levels after treatment with METH, with at least partial recovery over time (Wagner et al., 1980; Seiden et al., 1988; O'Dell et al., 1991; Cass, 1996; Callahan et al., 1998; Cass and Manning, 1999). DAT and SERT levels, as measured by ligand binding or uptake measurements, are decreased after METH (Hirata and Cadet, 1997; Brown et al., 2000; Ladenheim et al., 2000; Schroder et al., 2003; Armstrong and Noguchi, 2004). Finally, decreases in the levels of and function of the synthesizing enzymes TH and tryptophan hydroxylase (TPH) have been reported repeatedly as well, also with recovery occurring as the survival interval increases (Hotchkiss and Gibb, 1980; Bakhit et al., 1981; Haughey et al., 1999; Schroder et al., 2003).

In rodents, numerous studies demonstrate that METH can affect 5-HT and DA axons. For instance, METH produces axons that appear swollen and varicose and display a decrease in 5-HT immunoreactive boutons (Fukui et al., 1989). Similarly, both METH and AMPH were reported to produce reduction in TH-positive neurons in the striatum and FC, with AMPH also producing swollen and enlarged axons (Kadota and Kadota, 2004; Bowyer and Schmued, 2006). MDMA appears to have an effect on 5-HT, but not on DA axons. For instance, decreased 5-HT-positive neurons were observed in the parietal cortex and forebrain regions after treatment with high doses of MDMA, and similar to METH, the remaining axons were ablated or thick and varicose. However, no damage was observed to the cell bodies in the raphe nucleus or to catecholaminergic neurons and, furthermore, the loss of 5-HT axons recovered to a large extent after 1 year (O’Hearn et al., 1988; Molliver et al., 1990; Scanzello et al., 1993).

The available evidence for MDMA suggests that high doses affect only the 5-HT system in rats, having no long-lasting effects on the DA system. In rats, decreased levels of 5-HT and SERT binding in several brain regions including the striatum, HC, and hypothalamus have been reported, as well as decreased TPH activity. The alterations of these markers commonly recover with time, and no studies report any alterations in the catecholaminergic systems (Armstrong and Noguchi, 2004; Stone et al., 1986; Battaglia et al., 1987, 1988; De Souza et al., 1990; Sprague et al., 1994). In mice, conversely, even very high doses of MDMA (60 mg/kg) do not affect serotonergic markers such as brain 5-HT levels or TPH activity (Stone et al., 1987) but instead decrease the DA levels, particularly in the striatum (Logan et al., 1988; O’Callaghan and Miller, 1994). Thus, there appears to be an important species difference, with MDMA affecting the 5-HT system in rats but the DA system in mice.

Studies in nonhuman primates generally support the neurotoxicity or plasticity profile also observed in rats, with METH decreasing 5-HT, DA, DAT, TH, and VMAT-2 binding in various brain regions such as the striatum and cortex (Woolverton et al., 1989; McCann et al., 1998b) and MDMA affecting only the 5-HT system as observed by decreased levels of 5-HT and SERT binding as well as loss of 5-HT-positive axons in several brain regions including cortical regions and the HC, again with at least partial recovery occurring as the survival interval increases (Insel et al., 1989; De Souza et al., 1990; Hatzidimitriou et al., 1999).

c. Effects on behavior. In addition to the neurochemical after-effects of high doses of METH and AMPH, research has also focused on the potential consequences on behavior, primarily by means of animal models of cognition, anxiety, and depression and including memory tests such as the novel object recognition test and the Morris water maze, tests of anxiety-like behavior in emergence tests, elevated plus maze tests, and social interaction tests as well as the forced swim test, said to measure aspects of depression (McGregor et al., 2003b; Clemens et al., 2004, 2007 Clemens et al., 2004). Overall, however, the results supporting long-term effects of amphetamines on these behavioral tests are much less consistent than the data from neurochemical studies, often showing contradictory effects.

METH was shown to decrease recognition memory in the novel object recognition test for up to several weeks after drug treatments (Schroder et al., 2003; Marshall et al., 2007; Herring et al., 2008). Also decreases in spatial memory have been observed using tests such as the radial arm maze and Morris water maze (Nagai et al., 2007; Camarasa et al., 2010). However, other studies report no effect on memory using the novel object recognition test (Clemens et al., 2007), whereas inconsistent results are obtained for anxiety-related behavior depending on the type of test used, and no effect was found on the forced swim test (Clemens et al., 2004, 2007).

With MDMA, the evidence of persistent long-lasting effects on rodent behavioral tests is also difficult to interpret. Decreased recognition memory performance (Mechan et al., 2002; Piper and Meyer, 2004) and decreased performance in a delayed nonmatching to place test (Marston et al., 1999) have been reported. Evidence of depressive behavior measured as increased immobility in the forced swim test and evidence of anxiety-related behavior measured using several tests, such as the social interaction and emergence test and the elevated plus maze, have also been described (McGregor et al., 2003a,b). On the other hand, decreases in anxiety-like behavior after MDMA have been observed using these same tests (Mechan et al., 2002; Piper and Meyer, 2004). Also memory deficits after MDMA are not always observed. For instance, MDMA has been reported not to affect object recognition memory (McGregor et al., 2003a). Other studies also found no evidence of impairment in animals tested on
a wide range of behavioral tests, including the radial arm maze and the Morris water maze, despite the fact that large decreases in cortical 5-HT levels were present (Seiden et al., 1993; Marston et al., 1999). These results indicate that the relationship between observed monoamine system deficits and functional outcome in terms of behavior and cognitive function is not straightforward.

A number of limitations must be kept in mind when interpreting the results from preclinical rodent studies. Foremost, the use of allometric scaling to adjust for differences in basal metabolic rate between humans and rodents has led to rodents commonly being administered relatively high drug doses, often in the 10–20 mg/kg range or higher. However, the use of allometric scaling has been criticized for drugs such as MDMA, which display nonlinear kinetics and are extensively metabolized (Baumann et al., 2007). When employing effects scaling—based on selecting doses required to achieve similar amounts of 5-HT release, prolactin secretion, reinforcement, or drug discrimination—the required doses are in the 1–3 mg/kg range, closely corresponding to the doses used recreationally by humans. More importantly, when administered at these doses (<5 mg/kg) there is no evidence of long-lasting monoamine depletions (O’Shea et al., 1998; Baumann et al., 2007). Furthermore, high doses of the drugs are often given to animals without any preconditioning to lower doses, something that would more correctly model human use and has shown to be protective in animal models (see below). Finally, a majority of studies report on the effect on measures such as levels of monoamines and their transporters and synthesizing enzymes. One can contend whether this reflects actual toxicity or rather long-term adaptation, particularly as these changes are not always associated with deficits in behavioral tests and because higher doses are commonly needed to produce toxicity as measured by methods such as Fluoro Jade B staining and gliosis marker levels (Baumann et al., 2007).

14. Limited Evidence for Long-term Effects of Substituted Cathinones in Rodent Models. Recent studies on the effects of the substituted cathinones 4-MMC and MDMC show much more limited long-term effects of these drugs on the monoamine systems compared with METH and MDMA. When 4-MMC is administered at three injections of up to 10 mg/kg there was no long-lasting decrease in striatal or cortical monoamine levels in rats (Baumann et al., 2012). Interestingly, also intense, high-dose binge treatments of four injections of up to 40 mg/kg spaced 2 hours apart failed to produce any decreases in striatal DA levels (Angoa-Perez et al., 2012). The lack of effect of 4-MMC on the monoamine system is corroborated by two other studies employing various dosing regimens such as 30 mg/kg once daily for 10 days (Motbey et al., 2013) or twice daily for 4 days (den Hollander et al., 2013), which both failed to observe any decreases in monoamine levels in the striatum, cortex, and HC at a survival interval between 14 and 43 days after the final 4-MMC treatment. The only studies so far that did observe an effect of 4-MMC on markers of the monoamine system, such as DAT, SERT, TPH, and TH levels and functions, were the studies in which the drugs were administered at elevated (>26°C) ambient temperatures (Hadlock et al., 2011; Martinez-Clemente et al., 2014). High ambient temperatures produce a general increase in amphetamine neurotoxicity (see below), and these studies indicate that this also holds true for cathinones. Furthermore, there is evidence that 4-MMC can increase the loss of striatal DA induced by METH when the two drugs are coadministered (Angoa-Perez et al., 2013b), but does not exacerbate the loss of 5-HT after METH or MDMA (Angoa-Perez et al., 2013a).

MDMC has so far been less studied, but the available evidence suggests that it does not have an effect on monoamine levels at doses of up to three times 10 mg/kg (Baumann et al., 2012), whereas high doses (30 mg/kg twice daily for 4 days) produce decreases in 5-HT levels in the cortex, striatum, and HC at a 2-week survival interval (den Hollander et al., 2013).

Regarding the effects of these drugs on behavioral tests of cognition, the data are less clear. Although a decrease in recognition performance in the novel object recognition test was observed 1 month after treatment with 4-MMC (Motbey et al., 2012), as well as a decrease in working memory measured using the T-maze spontaneous alternation test, there were no effects on spatial memory in the Morris water maze test. Furthermore, both 4-MMC and MDMC have no effect on anxiety-like behavior measured using the emergence test or elevated plus maze or on depressive behavior in the forced swim test and actually appear to improve aspects of spatial reversal learning (den Hollander et al., 2013).

15. Mechanisms Involved in Long-term Adaptation and Neurotoxicity. The four primary mechanisms responsible for producing neurotoxicity after exposure to amphetamines are oxidative stress, mitochondrial dysfunction, excitotoxicity, and hyperthermia (Fig. 8). Additionally, a number of other factors including inflammation, microglial activation, blood-brain barrier disruption, and altered neurogenesis play a role, whereas neuroplasticity associated with low-dose AMPH preconditioning may provide protective effects. A summary of the primary mechanisms is provided here, whereas more in-depth information can be found in relevant reviews (Yamamoto et al., 2010; Carvalho et al., 2012; Goncalves et al., 2014; Halpin et al., 2014).

a. Oxidative stress: DA oxidation and drug metabolites as sources of reactive species. Support for the involvement of oxidative stress as a causal mechanism in amphetamine toxicity comes from a number of studies demonstrating attenuation of METH-
MDMA-induced monoamine loss after coadministration of spin-trapping agents or antioxidants such as N-acetylcysteine, ascorbic acid, vitamin E, and selenium (Wagner et al., 1985; De Vito and Wagner, 1989; Colado and Green, 1995; Fukami et al., 2004; Barayuga et al., 2013). Accordingly, depletion of glutathione, inhibition of superoxide dismutase, and selenium-deficient diets increase toxicity (De Vito and Wagner, 1989; Sanchez et al., 2003; Chandramani Shivalingappa et al., 2012).

One of the primary sources of oxidative stress after amphetamines is the large increase in cytoplasmic and extracellular DA levels and the subsequent metabolism of DA into toxic metabolites (Yamamoto and Raudensky, 2008). DA can be both enzymatically and nonenzymatically metabolized, with both routes resulting in production of reactive products. When MAO metabolizes large amounts of DA, the resulting byproduct hydrogen peroxide may rise to levels exceeding the detoxification capacity of the glutathione system. Subsequently, the hydrogen peroxide can react with Fe^{2+}, resulting in the production of the highly reactive hydroxyl radical. Moreover, both DA and its metabolite 3,4-dihydroxyphenylacetaldehyde can undergo auto-oxidation to form superoxide and reactive semiquinones (LaVoie and Hastings, 1999; Sulzer and Zecca, 2000; Anderson et al., 2011; Munoz et al., 2012). Accordingly, reducing intracellular DA through blockade of DAT or inhibition of DA synthesis reduces the METH toxicity (Schmidt et al., 1985; Marek et al., 1990; Yamamoto and Zhu, 1998).

Other potential sources of reactive species are amphetamine molecules themselves. Particularly MDMA has received attention in this respect, because certain catechol-like hepatic metabolites of MDMA are thought to be capable of auto-oxidizing and producing reactive quinones capable of forming thioether conjugates with sulfur-containing compounds such as glutathione and N-acetylcysteine. These thioether compounds retain the ability to redox cycle and are toxic to 5-HT neurons. In fact, they are more toxic than MDMA itself (Monks et al., 2004; Ferreira et al., 2013) and have also been detected in vivo after MDMA exposure (de la Torre and Farre, 2004; Perfetti et al., 2009). Nonetheless, DA remains an important factor also in MDMA-induced 5-HT toxicity (Stone et al., 1988; Schmidt et al., 1990; Shankaran et al., 1999; Falk et al., 2002; Breier et al., 2006).

b. Mitochondrial dysfunction: ATP deficit, superoxide leakage, and apoptosis. METH and MDMA are known to decrease the levels and activity of mitochondrial complexes I, II, and IV in the striatum (Burrows et al., 2000; Brown et al., 2005; Klongpanichapak et al., 2006; Puerta et al., 2010). MDMA was also shown to cause deletions in mitochondrial DNA coding for subunits of complexes I and IV (Alves et al., 2007). More recently it was shown that even relatively low doses of both

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**Fig. 8.** Summary of various mechanisms and factors involved in cellular toxicity by amphetamine-type stimulants.
AMPH and METH (2 mg/kg or less) decrease complex I, II, or IV activity in various brain regions, including the striatum, FC, and amygdala, obtained from animals killed while still under the influence of the drugs (Feier et al., 2012). Inhibition of the mitochondrial electron transport chain (ETC) by amphetamines can result in reduced ATP levels but also causes excessive leakage of superoxide, increasing oxidative stress and possibly triggering apoptosis.

METH decreases ATP levels in the striatum and cerebral cortex while increasing AMP levels (Shiba et al., 2011). The importance of decreased ATP levels in amphetamine toxicity is demonstrated by an experiment where neural glucose uptake was prevented by administration of 2-deoxyglucose, a manipulation that enhanced both the decrease in ATP levels observed directly after METH treatment as well as the loss of striatal DA 1 week later (Chan et al., 1994). Similarly, inhibition of metabolism by directly interfering with the ETC also increases toxicity because the potent complex II inhibitor malonate exacerbated both METH and MDMA toxicity (Burrows et al., 2000; Darvesh et al., 2005), whereas application of the energy substrates decylubiquinone or nicotinamide 6 hours after METH treatment reduced toxicity (Stephans et al., 1998).

ETC inhibition can also exacerbate amphetamine toxicity by increasing oxidative stress. During normal oxidative phosphorylation, approximately 1–4% of mitochondrial oxygen will be reduced to superoxide. However, during inhibition of mitochondrial complexes, the electron leak and superoxide production will increase dramatically. The resulting increase in oxidative stress adds to the stress already occurring from DA oxidation and represents a key factor in mediating amphetamine toxicity (Brown and Yamamoto, 2003; Adam-Vizi, 2005).

The mitochondrial-dependent apoptotic pathway has also been suggested to play a role in amphetamine toxicity (Cadet et al., 2005). METH increases the expression of proapoptotic genes BAD, BAX, and BID, while decreasing the expression of antiapoptotic genes Bcl-2 and Bcl-XL (Jayanthi et al., 2001; Krasnova et al., 2005; Beauvais et al., 2011). Indeed, METH has been shown to trigger the release of apoptosis inducing factor, cytochrome c, and smac/DIABLO from mitochondria, eventually leading to the recruitment of caspases and the initiation of apoptotic cell death (Jayanthi et al., 2004). As expected, both overexpression of Bcl-2 and inhibition of caspases protect against METH toxicity (Cadet et al., 1997, 2003; Uemura et al., 2003). However, the apoptotic effect has primarily been observed after extremely high doses of METH or in vitro, and its relevance for human users remains unclear.

c. Excitotoxicity: glutamate receptor involvement and intracellular excitotoxic processes. Studies employing in vivo microdialysis have shown that METH increases glutamate release in the striatum (Mark et al., 2004; Nash and Yamamoto, 1992; Stephens and Yamamoto, 1994) and that blockade of glutamate receptors attenuates METH-induced depletion of striatal DA and other markers of toxicity (Farfel et al., 1992; Bowyer, 1995; Battaglia et al., 2002; Shah et al., 2012), indicating that glutamate receptor-mediated processes are involved in METH toxicity. Conversely, MDMA appears to reduce glutamate release in certain brain regions, and the importance of excitotoxicity in mediating MDMA-induced 5-HT toxicity is still unclear (Capela et al., 2009).

One event associated with increased Ca²⁺ levels that is observed after amphetamine exposure is proteolysis of the cytoskeletal proteins, such as calpain-mediated cleavage of spectrin (Lee et al., 1991). Spectrin proteolysis has been observed in the rat striatum after METH treatments, whereas METH- and MDMA-induced increases in calpain activation and spectrin proteolysis were blocked by the calpain inhibitor calpastatin in vitro (Staszewski and Yamamoto, 2006; Warren et al., 2007; Suwanjarg et al., 2012).

Excitotoxic processes can also lead to increased production of reactive species. Nitric oxide synthase is elevated during METH exposure, and NO can readily react with superoxide to produce peroxynitrite, a highly reactive anion that produces toxicity through nitrosylation. Direct evidence for its involvement in METH toxicity comes from data showing that METH-induced depletion of DA is accompanied by increased levels of striatal nitrate and nitrotyrosine (Anderson and Itzhak, 2006; Pacher et al., 2007; Wang et al., 2008b). Furthermore, blockade of nitric oxide synthase provides full protection against METH-induced loss of DA (Itzhak and Ali, 1996), whereas coadministration of a peroxynitrite decomposition catalyst partially prevents toxicity (Imam et al., 1999). Specific targets have also been identified as METH-induced nitrosylation, coupled with loss of function, and have been observed on the VMAT-2 protein (Éyerman and Yamamoto, 2007). In addition to nitric oxide synthase, other enzymes may also be involved. Activation of xanthine oxidase can result in increased superoxide production, whereas cleavage of membrane phospholipids and release of arachidonic acid by phospholipase A₂ may also result in production of superoxide after lipoxygenase- and cyclooxygenase-mediated metabolism of arachidonic acid (Battelli et al., 1995; Yamamoto and Raudensky, 2008).

d. Hyperthermia: a catalyst of other toxic processes. Hyperthermia plays a well-established and important role in mediating amphetamine toxicity. Both METH and MDMA produce hyperthermia, and preventing the hyperthermia provides protection against subsequent neurotoxicity. Furthermore, lower ambient temperatures attenuate, whereas higher ambient temperatures exacerbate, both METH and MDMA toxicity (Ali et al., 1994; Bowyer et al., 1994; Broening et al., 1995; Malberg and Seiden, 1998; Goni-Allo et al., 2008). Hypphyseotomy,
The effect of body temperature on amphetamine toxicity appears to depend on a specific interaction, because hyperthermia on its own does not produce METH-characteristic toxicity (Bowyer, 1995). The effect of temperature is most likely due to a general catalytic effect on the enzymatic and nonenzymatic reactions, such as those resulting in production of reactive species. For instance, it has been shown that hypothermia prevents METH-induced oxidative stress (Fleckenstein et al., 1997c; Yamamoto et al., 2010), whereas hyperthermia significantly increases oxidative stress and other measures of toxicity (Silva et al., 2014).

Interestingly, the cathinone 4-MMC produces transient hypothermia rather than hyperthermia in rats (Shortall et al., 2013), and its neurotoxicity is enhanced in mice at elevated ambient temperature (26°C) when it causes hyperthermia (Miller et al., 2013). By using telemetric body core temperature monitoring in mice, it was found that high doses of 4-MMC produced complex changes, first inducing hypothermia that was followed by hyperthermia (Angoa-Perez et al., 2012).

e. Other factors: inflammation, blood-brain barrier disruption, protective preconditioning. Recently, there has been more focus on additional factors that may mediate amphetamine neurotoxicity. METH- and MDMA-induced activation of microglia and subsequent increases in proinflammatory cytokines contribute to toxicity. In contrast, 4-MMC has failed to induce striatal microglial activation and astrogliosis even at high doses in mice, although METH was active in the same conditions (Angoa-Perez et al., 2012). Similarly, amphetamines are reported to produce defects in the ubiquitin proteasomal system and blood-brain barrier integrity, whereas stress and human immunodeficiency virus infection also represent modulatory factors (Yamamoto et al., 2010). Alterations in neurogenesis from the subventricular zone stem/progenitor cells and the dentate gyrus have also been reported (Goncalves et al., 2014), and toxic products such as ammonia, resulting from METH-induced damage to peripheral organs may play a further role in mediating neurotoxicity (Xie and Miller, 2009; Seminerio et al., 2013; Halpin et al., 2014).

There are also factors that mediate neuroprotection, such as preconditioning with low doses of amphetamines before a toxic challenge. Preconditioning with escalating doses of METH (0.5–2.5 mg/kg) for up to 2 weeks before a subsequent toxic METH challenge provides full protection against METH-induced DA toxicity (Graham et al., 2008; Cadet et al., 2009; Hodges et al., 2011). Possible mechanisms have been investigated in an in vitro study that found that low concentrations of METH produced changes in the pERK1/2 level, by decreasing it in basal conditions and increasing it in response to the toxic 6-hydroxydopamine challenge (El Ayadi and Zigmond, 2011). METH also upregulated levels of the antiapoptotic protein Bcl-2. Also voluntary running wheel endurance-type exercise in mice has been shown to be protective against METH toxicity, an effect that appears to be mediated by antioxidant adaptations in brain microvasculature and tight-junction proteins at the blood-brain barrier (Toborek et al., 2013). Meanwhile, for MDMA it was shown that pretreatment with a low doses (3 mg/kg) protected against toxicity of a subsequent toxic dose of MDMA (12.5 mg/kg) administered 1 week later. The pretreatment was associated with increased levels of the inflammatory cytokine IL-1β after 6 hours, while slowly producing a significant increase in the interleukin receptor antagonist IL-1ra reaching a peak 4 days after the preconditioning (Cohen et al., 2013). The neuroplasticity that occurs in response to changes in cellular inflammatory and oxidative stress processes may play an important role in modulating potential neurotoxicity in humans, because such preconditioning may happen naturally during dose escalation that occurs in human users.


a. Cognitive behaviors. Deficits in abstinent METH users compared with control subjects have been observed on tests such as immediate and delayed recall and digit span tests and measuring performance on memory-related domains, such as verbal memory, long-term declarative memory, and working memory. Deficits are also observed on tests such as the Wisconsin card sorting test, Stroop test, and trail-making test, indicating deficits in the cognitive domains of executive function and attention (Simon et al., 2000; Kalechstein et al., 2003; Han et al., 2008; Rendell et al., 2009). Furthermore, METH users appear to experience higher levels of anxiety and depression than nonusers (Zweben et al., 2004; Vik, 2007).

Similar to METH, abstinent MDMA users also show deficits in certain cognitive domains. The most common observations include deficits in short-term and long-term memory, verbal reasoning, attention, and executive functions as well as decreased performance in tests.
reflecting aspects of general intelligence (Parrott et al., 1998; McCann et al., 1999; Gouzoulis-Mayfrank et al., 2000; Heffernan et al., 2001; von Geusau et al., 2004). Other studies of abstinent MDMA users have also reported higher ratings of depression and anxiety in addition to decreases in memory performance (McCardle et al., 2004; Thomasius et al., 2006). However, many studies do not properly match MDMA users and controls in terms of education, intelligence quotient, and other variables. When subjects are properly matched, no consistent detrimental effects of MDMA are observed (Halpern et al., 2011).

b. Brain imaging of receptors, transporters and activity/metabolism. PET scans have been used to assess monoamine-related ligand binding in human amphetamine users. Similar to what is seen in animals, decreased DAT and VMAT2 binding has been observed in the striatum and FC of abstinent METH users, who also performed lower than controls on measures of cognitive function, including memory. However, correlations between DAT binding and the time of abstinence or between DAT binding and measures of cognitive function were not always observed (McCann et al., 1998a, 2008; Sekine et al., 2001; Johanson et al., 2006). PET studies measuring cerebral metabolism have also reported differences between abstinent METH users and nonusers. For instance, it was reported that METH users showed decreased metabolism in the parietal cortex and thalamus (Volkow et al., 2001), whereas hypometabolism is observed in the FC of METH users, who also made more errors of preservation on the Wisconsin card sorting test than control subjects (Kim et al., 2005, 2009). Decreased performance of METH users on a vigilance task and increases in anxiety and depression-like symptoms were associated with lower metabolism in the cingulate and insula but higher metabolism in the FC. Moreover, in METH users, task performance was negatively correlated with metabolism in the cingulate and insula, whereas a positive correlation was observed in controls (London et al., 2004, 2005).

Abstinent MDMA users show decreases in SERT binding in several brain regions, including cortical regions, thalamus, hypothalamus, and striatum, and the extent to which the binding is decreased is correlated with the amount of previous MDMA use. Additionally, abstinent MDMA users perform worse on tests of memory function, and their performance on these tests is correlated with insular and hippocampal SERT binding (McCann et al., 1998a, 2008; Kish et al., 2010). However, a previous SPECT study found decreased SERT binding only in abstinent female, but not male, subjects. Furthermore, the decreased binding appeared to recover with protracted abstinence, reaching a level higher than control subjects (Reneman et al., 2001a). Decreased metabolism has also been reported in MDMA users in the cingulate, HC, amygdala, and striatum; however, no correlation was found between metabolism and the amount of MDMA consumed (Obrocki et al., 1999; Buchert et al., 2001). Brain 5-HT_{2A} receptor binding in MDMA users appears to be decreased in all cortical regions, whereas abstinent users show increased 5-HT_{2A} receptor binding, but only in the occipital cortex (Reneman et al., 2002). Furthermore, the abstinent MDMA users scored lower on a test of verbal memory, and memory performance was positively correlated to the amount of 5-HT_{2A} receptor binding (Reneman et al., 2000). However, the relationship between memory function and 5-HT_{2A} receptor binding is hard to interpret, because the abstinent users had higher levels of 5-HT_{2A} binding than controls but an overall lower memory performance.

c. Magnetic resonance imaging studies. Both structural and functional MRI studies have reported differences between METH users and nonusers. It has been reported that abstinent METH users have lower gray matter density in the FC as well as decreased Wisconsin card sorting test task performance compared with drug-free control subjects (Kim et al., 2006). Decreased cingulate and hippocampal volumes that correlated with performance on a verbal memory test (Thompson et al., 2004) and correlations between increased METH exposure and reductions in orbital and medial PFC volumes have also been noted (Daumann et al., 2011). Interestingly, striatal volume is reportedly increased in abstinent METH users in a study that, however, did not find any evidence of decreased cognitive performance (Chang et al., 2005). Another study also found increased putamen volume in METH users that correlated negatively with performance on the go/no-go test for impulsivity (Jan et al., 2012). Decreased volumes of certain cortical regions and the HC have also been reported in MDMA users (Cowan et al., 2003; den Hollander et al., 2012).

Functional MRI studies also report differences between amphetamine users and nonusers. Two studies reported that METH users showed increased impulsivity as measured by a delay-discounting test and showed decreased activity in the cingulate and dorsolateral PFC (Monterosso et al., 2007; Hoffman et al., 2008). Decreased activation was also observed in the frontal and insular cortices of METH users in response to a Stroop task, although task performance was not consistently different from control subjects (Salo et al., 2009; Nestor et al., 2011).

In fMRI studies of MDMA users, a weaker BOLD signal during working memory task performance was noted in the frontal and temporal cortex in heavy compared with light users, but when comparing users to nonusers there was no clear effect on BOLD signal and, furthermore, task performance was also similar between MDMA users and controls (Daumann et al., 2003). Increases in BOLD signal in the frontal gyrus in response to a memory task on which MDMA users also were not impaired have been reported as well (Moeller
et al., 2004). Differences in hippocampal BOLD signal in response to episodic or associative memory tasks revealed decreases in MDMA users versus controls and in continuing versus abstinent MDMA users; however, here also there were no differences observed on the actual task performance (Daumann et al., 2005; Becker et al., 2013). It is possible that differing cognitive strategies between users and nonusers may account for the observed variability in BOLD signal intensity (Honey et al., 2000).

d. Magnetic resonance spectroscopy and analysis of postmortem tissue. In addition to alterations in structural and functional MRI studies, alterations have also been reported in cerebral blood flow and white matter integrity using perfusion and fractional anisotropy MRI techniques (Iyo et al., 1997; Chang et al., 2002; Alicata et al., 2009). Furthermore, magnetic resonance spectroscopy has been employed to detect changes in chemical markers, such as N-acetylaspartate, choline, creatine, and phosphocreatine, putatively reflecting abnormalities in neuronal cell membrane integrity or metabolism (Panenka et al., 2013). Decreases in phosphocreatine and correlations between N-acetylaspartate/creatine ratio and verbal memory performance have been reported in METH and MDMA users, respectively (Reneman et al., 2001b; Sung et al., 2013).

Analyses have also been made of postmortem brain material, and a decrease in striatal DA, DAT, and TH levels has been reported (Wilson et al., 1996). Decreases in D1,R-mediated stimulation of adenylyl cyclase (AC) in response to DA, suggestive of alterations in the level and function of DA-related proteins, have also been reported (Tong et al., 2003). However, because brains in these studies are primarily sourced from individuals who overdosed on METH, their relevance for normal recreational users must be questioned.

e. Prospective and experimental studies. Neurocognitive studies in humans have produced vastly divergent results, with certain studies reporting decreased performance on various measures of cognitive performance, whereas others report no difference. Furthermore, specific deficits are not often replicated between studies. Neuroimaging studies also suffer from large result variability, with various differences in task-related signal intensities, which often occur without any overt effect on task performance. Another major issue confounding the interpretation is that causality cannot be determined from cross-sectional studies, and it is likely that pre-existing differences between MDMA users and control subjects account for a large part of the observed differences.

Prospective studies have been performed to address some of these issues. In a Dutch study, subjects who were considered to be at high risk of commencing MDMA use were recruited for an in-depth set of neuroimaging experiments, both at baseline before any MDMA and at a follow up within 3 years after the baseline scans. The individuals who started using MDMA displayed a slight decrease in regional relative cerebral blood flow in the globus pallidus and putamen as well as decreased fractional anisotropy in the thalamus and frontoparietal white matter. However, no effect was found on N-acetylaspartate/creatine ratio, and, importantly, no difference was found on the most important measure, namely SERT binding between new MDMA users and those who remained MDMA naive. A dose-response relationship between cumulative MDMA use and SERT binding was also not present, suggesting that low-dose MDMA use (mean of six tablets) has no effect on SERT-binding in novel MDMA users (de Win et al., 2008).

The same population was also subjected to a wide array of neurocognitive tests. Subjects who commenced MDMA use scored significantly lower on immediate and delayed verbal recall compared with MDMA-naive subjects; however, no effects were observed on any other neurocognitive tests, and all scores were within the normal ranges. Importantly, the verbal memory scores of MDMA users did not decrease between the baseline and follow up, and the difference between users and nonusers was actually due to an increase in performance of the nonusers during the follow up, suggesting that low-dose MDMA use has no effect on measures of neurocognitive function, including memory (Schilt et al., 2007; Krebs and Johansen, 2008).

A second prospective study focused specifically on users who had a higher MDMA consumption, excluding subjects who had used less than 10 tablets and thus comparing heavier users (mean of 34 tablets) to individuals having consumed cannabis only. Like in the Dutch study (de Win et al., 2008), no effects were found on verbal memory, and significant differences between groups being reported only for immediate and delayed visual memory. Here, the difference was due to a decreased performance in the MDMA users (Wagner et al., 2013).

Although prospective studies are more informative than cross-sectional studies, there are still a number of confounding factors that only experimental studies can address. Although a number of studies have administered METH or MDMA experimentally to humans (Mas et al., 1999; Harris et al., 2002; Hart et al., 2012; Carhart-Harris et al., 2014), long-term cognitive outcomes have usually not been reported. One study that did reported no effects on cognitive function measured at 1 year or longer after the drug treatments (Mithoefer et al., 2013). In summary, although a number of cross-sectional studies report cognitive and neurologic deficits or alterations as a result of the use of amphetamines, these studies often suffer from methodological limitations that could lead to overestimation of the actual drug effect. Most results are not replicated in prospective or experimental studies and, furthermore, although amphetamine users sometimes score lower
than control subjects, they still score well within the normal range (Hart et al., 2012), suggesting that any existing drug effect is very limited and does not produce any clinically significant impairment.

17. Conclusions. Amphetamines are a class of drugs that exert powerful stimulatory effects on brain function by mechanisms that include the inhibition of monoamine reuptake and enhancement of monoamine release. They are used medically to treat disorders such as ADHD, but nonmedical recreational use of amphetamines is also widespread, because amphetamines are the second most commonly used illicit drug after cannabis. A large number of animal studies indicate that amphetamines, in addition to being addictive, are neurotoxic and even lethal at high doses. It is still unclear whether similar neurotoxicity occurs in human recreational users. Some studies suggest that amphetamine users may display certain abnormalities in the monoamine systems reminiscent of neurototoxicity observed in animals after high-dose treatments. However, because most studies are cross-sectional, no clear conclusions can be drawn with regards to causality. Furthermore, amphetamine users do not show any clear evidence of persistent functional deficits and consistently score within normal ranges on neuropsychological tests of memory function.

C. Nicotine

Smoking has been estimated to cause smokers to lose one decade of their lives compared with never-smokers (Jha et al., 2013). Early smoking cessation (<40 years of age) seems to give back most of those years, indicating some reversibility of smoking-related damage in the body.

Nicotine is the principal neuroactive alkaloid responsible for tobacco addiction (Jaffe and Kanzer, 1979; Stolerman and Jarvis, 1995; Pontieri et al., 1996; Pich et al., 1997). Despite widespread prevalence of tobacco smoking, it was not until the mid-1990s that there was a scientific consensus that nicotine is addictive in a similar manner to other drugs of abuse such as cocaine (Stolerman and Jarvis, 1995; Pontieri et al., 1996; Pich et al., 1997). Recent studies have shown that non-nicotine components in tobacco are also reinforcing or influence nicotine SA. Anabasine dose dependently increased nicotine SA at 0.02 mg/kg s.c. in rats, whereas both anabasine and anatabine reduced nicotine SA at 2 mg/kg s.c. (Hall et al., 2014). Another non-nicotine tobacco component, norharmane, was behaviorally reinforcing on its own, and these reinforcing effects were additive with those of nicotine (Arnold et al., 2014). Norharmane is a MAO inhibitor. It has been suggested that the inhibition of MAOs contributes to the rewarding effects of nicotine (reviewed in Kapelewski et al., 2011).

Smoking is well known to be associated with many adverse effects, including increased risk of cancers and cardiovascular disease. Although the abuse potential and harmful effects of nicotine were recognized, there continued to be interest in the potential beneficial acute effects of nicotine for cognitive enhancement (Everitt, 1997; Robbins et al., 1997; Changeux et al., 1998; Levin, 2013) and neuroprotection (Buckingham et al., 2009; Hernandez and Dineley, 2012; Kawamata et al., 2012). Others pointed to cognitive impairment in heavy smokers during abstinence as evidence of adverse effects on neural plasticity associated with learning and memory (Abrous et al., 2002). The diversity of nicotinic acetylcholine receptor subtypes contributes to the diverse functional effects of nicotine.

1. Nicotinic Acetylcholine Receptors. Nicotinic acetylcholine receptors (nAChRs) belong to the "Cys-loop" superfamily of multisubunit transmembrane ionotropic neurotransmitter receptors (Albuquerque et al., 2009). High-resolution structural studies have shown that nAChRs are assembled from five subunits, which form to create a central pore (Unwin, 2005; Baenziger and Corringer, 2011). Sixteen different subunits have been identified in mammals (Millar and Gotti, 2009). Of the mammalian nAChR subunits, only α7 and α9 can form homopentamers and only α7 forms endogenous homopentameric receptors (Vetter et al., 2007; Millar and Gotti, 2009). The other α subunits usually form heteropentamers containing two α subunits and three β subunits. Receptor subtypes are named according to their subunit composition, with an asterisk used to indicate cases where one or more additional subunit types may also be present (Lukas et al., 1999; Millar et al., 2014). nAChRs composed of different subunits are differentially distributed throughout the brain. α4β2, α4β5β2, α3β4*, α6β2β3*, and α7 are expressed in the VTA and SN; α4β2 and α7 in the hypothalamus; α4β2, α4α5β2, α6β2β3, and α6α4β2β3 in the striatum; α4β2, α4α5β2, α3β4, and α7 in the HC; and α4β2, α4α5β2, and α7 are expressed in the cerebral cortex (Gotti et al., 2006, 2007). Nicotine is an agonist at all nAChRs except α9/10 nAChRs, expressed by vestibular and cochlear mechanosensory hair cells (Elgoyhen et al., 2001; Scholl et al., 2014) and a subset of dorsal root ganglion sensory cells (Papadopoulo et al., 2004), at which nicotine is an antagonist (Elgoyhen et al., 2001; Millar et al., 2014).

nAChRs are widely expressed in the midbrain DAergic neurons projecting to the dorsal striatum, ventral striatum and NAc, and PFC. A remarkable diversity of β2* nAChR subtypes modulates DA release in these projections (Grady et al., 2007; Gotti et al., 2009; Livingstone and Wonnacott, 2009). These projections, in particular those to the NAc and ventral striatum, are likely involved in reward pathways and the reinforcing properties of nicotine contributing to addiction. Nicotine increases firing of midbrain DA neurons (Grenhoff et al., 1986; Mereu et al., 1987) and increases DA release in the NAc (Imperato et al., 1986; Rahman et al., 2003). Selective blockade of nAChRs in the VTA is sufficient to prevent nicotine-induced DA release in the NAc,
nicotine-induced locomotion and nicotine SA (Nisell et al., 1994). In rodents, when nicotine injections are repeated, there is a sensitization of the nicotine-induced locomotor activation (Clarke and Kumar, 1983; Ksir et al., 1985) and the nicotine-induced NAc DA release (Benwell and Balfour, 1992; Benwell et al., 1995; Balfour et al., 1998; Schoffelmeer et al., 2002). Human studies confirm a role for the DAergic system in the effects of nicotine but paint a more complex picture. In human PET studies, reduction of $[^{11}C]$raclopride binding potential is used as an indirect measure of increase in DA release. After nicotine administration by nasal spray, there was no overall change in $[^{11}C]$raclopride binding in any striatal regions, but baseline $[^{11}C]$raclopride binding potential in the associative striatum correlated negatively with the Fagerström score, an index of nicotine dependence, and nicotine concentration correlated negatively with binding potential in the limbic striatum (Montgomery et al., 2007a). However, in tobacco-dependent individuals, smoking a regular cigarette resulted in greater reduction in ventral striatal $[^{11}C]$raclopride binding potential than smoking a denicotinized cigarette (Brody et al., 2009).

At higher doses, nicotine is aversive rather than rewarding. Nicotine also often has aversive effects on first exposure. Stronger aversive reactions in first-time smokers are associated with reduced likelihood of development of habitual tobacco use (Sartor et al., 2010). There is therefore increasing interest in understanding the mechanisms of aversive responses to nicotine, because aversive reactions on initial exposure may reduce propensity to addiction. The MHB-IPN pathway, because aversive reactions on initial exposure may involve increases in mRNA expression (Marks et al., 1992) but rather reflects posttranscriptional mechanisms. The $\alpha 4\beta 2$ nAChR subtype has fairly consistently been implicated in nicotine-induced upregulation. In vitro studies in fibroblast and HEK cell culture systems expressing $\alpha 4\beta 2$ nAChRs showed that nicotine exposure resulted in receptor upregulation as determined by ligand binding (Peng et al., 1994; Bencherif et al., 1995; Rothhut et al., 1996; Eilers et al., 1997; Gopalakrishnan et al., 1997; Warman et al., 1998; Whiteaker et al., 1998; Harkness and Millar, 2002). Upregulation of these subunits was further confirmed by transfection of $\alpha 4$ and $\beta 2$ subunits tagged with fluorescent protein into both HEK cells and neurons from the ventral midbrain (Nashmi et al., 2003). In vivo, upregulation of $\alpha 4$ in response to nicotine treatment was shown in a transgenic mouse expressing $\alpha 4$ tagged with yellow fluorescent protein (Nashmi et al., 2007).

Replacement of $\beta 2$ with $\beta 4$ in receptors containing $\alpha 4$ or $\alpha 3$ reduces nicotine-induced upregulation assayed by radioligand binding (Wang et al., 1998), whereas addition of $\alpha 5$ or $\beta 3$ may block upregulation (Perry et al., 2007; Mao et al., 2008). Association of $\alpha 4\beta 2$ with $\alpha 5$ may explain why nicotine-induced nicotinic receptor upregulation is not observed in some brain regions (Mao et al., 2008). Chronic nicotine failed to induce nAChR upregulation in the adrenal gland, which has little $\alpha 4\beta 2$ nAChRs (Flores et al., 1997; Davila-Garcia et al., 2003). Comparison of nicotine-induced upregulation of $\alpha 3\beta 2$, $\alpha 4\beta 2$, and $\alpha 6\beta 2$ nAChRs in transfected cells revealed subtype differences in sensitivity to nicotine concentration and the time course of upregulation (Walsh et al., 2008). $\alpha 4\beta 2$ nAChRs were most sensitive, upregulating at lower nicotine concentration, whereas $\alpha 6\beta 2$ nAChRs required higher concentrations of nicotine, and $\alpha 3\beta 2$ nAChRs required the highest concentrations of nicotine. $\alpha 6\beta 2$ nAChRs showed the fastest upregulation, followed by $\alpha 3\beta 2$ nAChRs, whereas $\alpha 4\beta 2$ nAChRs showed the slowest response. On chronic nicotine exposure, $\alpha 6\beta 2^*$ nAChRs are downregulated rather than upregulated (Lai et al., 2005; Perry et al., 2007; Doura et al., 2008; Marks et al., 2014). $\alpha 6\beta 2^*$ nAChR downregulation was more sensitive to the dose of chronic nicotine treatment than $\alpha 4\beta 2^*$ nAChR upregulation (Marks et al., 2014).

Thus, different receptor nAChR receptor subtypes may respond differently to different doses, durations, and patterns of nicotine exposure. For example, within the VTA, it appears that chronic nicotine upregulates $\alpha 4^*$ nAChRs on GABAergic neurons but not on DA neurons (Nashmi et al., 2007). Similarly, in the SN, chronic nicotine increased functional $\alpha 4\beta 2^*$ nAChRs on GABAergic neurons of the SN pars reticulata but not on DA neurons of the SN pars compacta (Xiao et al., 2009). It has been proposed that exposure to nicotine leads to nAChR upregulation in the VTA, increasing excitability and favoring induction of LTP resulting in sensitization
to the effects of DA leading to behavioral sensitization and drug addiction (Govind et al., 2009). Further studies are required to establish how the doses and patterns of nicotine exposure associated with smoking differentially regulate the various nAChR subtypes in different brain regions.

Various mechanisms have been proposed to underlie nicotine-induced upregulation of nAChRs. Importantly, it was earlier shown that the mechanisms underlying upregulation must be posttranslational rather than transcriptional, because nicotine exposure did not alter nAChR subunit mRNA levels (Marks et al., 1992). Proposed mechanisms have included decreased turnover of receptors (Peng et al., 1994), desensitization of surface receptors (Fenster et al., 1999), enhancement of receptor maturation (Sallette et al., 2005), and slow stabilization of receptors in a high-affinity state that is more easily activated (Vallejo et al., 2005). However, recent consensus of opinion, at least for upregulation of \( \alpha 4\beta 2^* \) nAChRs, has converged on the notion that nicotine acts as a pharmacological chaperone (Kuryatov et al., 2005; Henderson et al., 2014; Srinivasan et al., 2011, 2012). Pharmacological chaperoning of intracellular nAChRs is proposed to facilitate subunit assembly in the ER and enhance export of assembled receptors from the ER and their transport and insertion into the membrane.

3. Nicotine-priming Effects and Upregulation of Catecholamine Synthesis. Nicotine does not only sensitize or prime catecholaminergic systems via upregulation of nicotinic receptors, there is also evidence that nicotine exposure sensitizes or primes certain catecholaminergic systems more directly by upregulating expression of TH, the rate-limiting enzyme for catecholamine synthesis. Acute nicotine administration increased catecholamine synthesis in the NAc, hypothalamus, and HC, whereas chronic administration of nicotine by daily subcutaneous injections in rats for 28 days potentiated this response in the HC (Mitchell et al., 1989). This chronic treatment increased TH activity in the HC (Joseph et al., 1990). Studies of chronic treatment of 7 to 28 days showed that the first increase in TH activity from 3 days in the NEergic locus ceruleus (LC) and DAergic nuclei. Subsequently, the TH protein expression spread in the NEergic projections to the forebrain, reaching the terminals up to 3 weeks later, consistent with axonal transport of the enzyme from the cell bodies (Smith et al., 1991). Subsequently, it was shown that 7-day treatment or even a single injection of nicotine was sufficient to produce an increase in TH activity in the terminal fields 3–4 weeks later (Smith et al., 1991; Mitchell et al., 1993). A single acute nicotine injection (0.8 mg/kg) was sufficient to increase mRNA for TH in the LC cell bodies 2 to 6 days later and, 4 weeks later, to increase TH activity in the terminals and NE release in the hippocampus on challenge injection of nicotine (0.4 mg/kg) (Mitchell et al., 1993).

NE is known to induce synaptic plasticity in the hippocampal dentate gyrus (Neuman and Harley, 1983; Stanton and Sarvey, 1985; Burgard et al., 1989; Dahl and Sarvey, 1989; Harley, 1991, 1998, 2007), suggesting the possibility that nicotine-induced priming of nicotine-induced NE release in the HC could induce neuroplasticity. After priming with seven daily injections of nicotine, a challenge dose of nicotine produced a long-lasting potentiation of medial perforant path-dentate gyrus evoked responses (Hamid et al., 1997). This effect was blocked by both mecamylamine and...
propranolol, indicating involvement of nAChRs and β-adrenoceptors. Subsequently, it was shown that this nicotine priming and challenge effect cross-primes with other mechanisms, increasing TH expression in the LC. Theta driving at 7.7 Hz, which is suggested to mimic aspects of anxiety or stress responses, increased TH activity in rat HC 15 to 33 days later (Graham-Jones et al., 1985) and 0.4 mg/kg challenge injection of nicotine induced medial perforant path-dentate gyrus long-lasting potentiation in rats primed by theta driving but not in rats that did not receive theta driving (Markevich et al., 2007). Chronic clozapine treatment activated the LC (Souto et al., 1979; Ramirez and Wang, 1986; Nilsson et al., 2005) and increased TH expression in the LC (Verma et al., 2007). Rats primed with clozapine (30 mg/kg) daily for 7 days showed nicotine (0.4 mg/kg) challenge-induced medial perforant path-dentate gyrus long-lasting potentiation 21 to 28 days later (Rajkumar et al., 2013). These data suggest that nicotine alone or in conjunction with other stimuli can induce TH expression in the LC, resulting in delayed transport of TH to the afferent terminal fields facilitating nicotine-induced NE-mediated synaptic plasticity. Nicotine may also induce synaptic plasticity within catecholaminergic nuclei themselves.

4. Nicotine and Neuroplasticity within the Ventral Tegmental Area. DAergic neurons of the VTA and SN are tonically active, firing irregularly at low frequency, but can switch to burst firing (Grace and Bunney, 1984a,b) (Fig. 9). The switch between tonic and burst firing has been associated with signaling of novel events and reward (Chergui et al., 1993; Cooper, 2002; Hyland et al., 2002). A similar switch between tonic and burst firing occurs in response to nicotine (Grenhoff et al., 1986; Zhang et al., 2009). This burst firing is thought to be mediated by activation of NMDARs on DAergic cell bodies by glutamatergic afferents from the PFC (Chergui et al., 1993; Tong et al., 1996). It is proposed that stimulation of presynaptic α7 nAChRs on glutamatergic afferents (Jones and Wonnacott, 2004) releases glutamate activating NMDARs on DAergic cell bodies to produce the burst firing (Livingstone and Wonnacott, 2009). The activation of NMDARs not only induces acute changes in firing but can induce long-lasting presynaptic facilitation and postsynaptic NMDAR-dependent LTP, resulting in sustained increases in the sensitivity of the DAergic neurons to burst firing (Mansvelder and McGehee, 2000; Mansvelder et al., 2002). Likely, these mechanisms translate to behavioral nicotine-induced increases in responsiveness to reward.

However, the essential role of presynaptic α7 nAChRs in induction of burst firing is questioned by the observation that nicotine still triggers burst firing in α7-KO mice (for discussion, see Livingstone and Wonnacott, 2009). Meanwhile, the effect of nicotine on DAergic cell firing mode is abolished in β2-KO mice (Mameli-Engvall et al., 2006). The situation is further complicated by the effects of nicotine on inhibition and excitability of the VTA. α7 nAChRs are also expressed by GABAergic neurons in the VTA (Klink et al., 2001; Jones and Wonnacott, 2004). A model integrating these data (McKay et al., 2007; Livingstone and Wonnacott, 2009) proposes that nicotine desensitizes α4β2 nAChRs on GABAergic interneurons rapidly and preferentially (Woolorton et al., 2003), thus disinhibiting DAergic neurons while simultaneously activating α7 nAChRs on DAergic cell bodies and glutamatergic afferent terminals (Mansvelder and McGehee, 2000). It is suggested that β2* nAChRs on DAergic cell bodies contribute by providing depolarization enhancing excitability and thus bringing the cell closer to the threshold to change firing patterns or to induce LTP (Livingstone and Wonnacott, 2009).

The nicotinic control of DAergic firing is further complicated by essential and complex roles of the balance between activation and desensitization of nAChR mechanisms (Picciotto et al., 2008). Although activation and desensitization likely occur over too fast a time scale to contribute directly to maintaining nicotine-induced neuroplasticity, it is likely that this balance plays a role in determining the conditions permissive to induction of neuroplasticity. As mentioned earlier, chronic nicotine treatment upregulates α4* nAChRs on GABAergic interneurons, but not on DAergic neurons, of the VTA and on glutamatergic terminals in forebrain. This is suggested to be sufficient to explain tolerance of DAergic neuron firing in midbrain and sensitization of synaptic transmission in forebrain (Tapper et al., 2004; Nashmi et al., 2007). The role of α7 nAChRs remains more controversial. Although most reports suggest that upregulation of α4* nAChRs alone is sufficient to explain sensitization and tolerance, others studying in vitro expression of human nicotine receptors have suggested that functional inactivation of α4β2 nAChRs and α7 nAChRs but sustained inhibition of α3* nAChRs by chronic exposure to nicotine contributes to tolerance (Olale et al., 1997).

In addition to the roles of nicotine receptors on GABAergic interneurons versus DAergic neurons in the VTA, effects of nicotine on AMPAR/NMDAR ratios in VTA DA neurons have been implicated in nicotinic regulation of VTA plasticity. Drugs of abuse, including nicotine, were found to increase AMPAR/NMDAR ratios in midbrain DA neurons (Saal et al., 2003). In the case of nicotine, a single dose of 0.5 mg/kg i.p. was administered to mice, and 24–30 hours later AMPAR/NMDAR ratios were increased in VTA DA neurons in midbrain slices (Saal et al., 2003). Subsequent investigations reported that increases in AMPAR/NMDAR ratios likely occur via α7 or β2* nAChR-mediated mechanism (Gao et al., 2010a) and can occur as fast as within 1 hour of a single nicotine injection.

A recent study by Doyon et al. (2013) on rats adds further complexity, as a single dose of nicotine dramatically reduced DA release compared with baseline in the
NAC for at least 15 hours. Furthermore, nicotine-treated animals made more responses for obtaining alcohol solution during the first operant sessions. These effects were blocked by dihydro-β-erythroidine (β2*), but not by methyllycaconitine (α7*). Nicotine pretreatment enhanced bath-applied ethanol-mediated increase in the frequency of sIPSCs from GABA neurons in the VTA slices, which was also seen during bath application of GABAA receptor BZ agonist diazepam. Importantly, nicotine sensitivity of DA release was not affected at 15 hours after the nicotine pretreatment, and also the bath applied nicotine similarly enhanced the frequency of sIPSCs in slices from saline- and nicotine-treated animals. Therefore, further mechanisms were examined. A glucocorticoid/progesterone receptor antagonist, mifepristone (RU486), given systemically or intra-VTA before nicotine, attenuated the increases in sIPSC frequency and responses for alcohol solution and rescued the impaired ethanol-induced DA release. Whether the nicotine effect is peripherally or centrally initiated is not known. Interestingly, both acute and chronic nicotine as well as acute morphine reduce the release of GABA in the VTA of mice (Vihavainen et al., 2008), whereas morphine after nicotine enhanced the release. In summary, these findings suggest that nicotine can enhance the activity of VTA DA neurons via multiple mechanisms, including by influencing the function of GABA neurons.

5. Nicotine and Neuroplasticity in the Lateral Hypothalamic Orexin System. The lateral hypothalamic orexin (hypocretin) system has also been implicated in mechanisms of addiction and drug-induced neuroplasticity in the VTA (for discussion, see Baimel and Borgland, 2012; Baimel et al., 2012). Orexin signaling to the VTA promotes synaptic plasticity by potentiating glutamatergic inputs to DA neurons.

Acute nicotine administration excited orexin-expressing neurons in the lateral hypothalamus/perifornical area (Huang et al., 2011) and dose dependent increased Fos expression in these brain regions (Pasumarthi et al., 2006). Chronic administration of nicotine by repetitive injection over 10–14 days altered expression of mRNAs for preproorexin and orexin receptors (Kane et al., 2001, 2001).

The OX1 (hcrt-R1) receptor antagonist, SB-334867 [N-(2-methyl-6-benzoxazolyl)-N',1,5-naphthyridin-4-yl urea], reduced nicotine SA in animals on both fixed ratio and progressive ratio schedules (Hollander et al., 2008). Fixed ratio schedules are thought to measure drug reinforcement, whereas progressive ratio schedules are thought to measure motivation to obtain the drug. Furthermore, infusion of SB-334867 into the insular cortex also reduced SA on the fixed ratio schedule consistent with the observation that tobacco smokers with damage to the insula are more likely to quit smoking without relapse and the recurrence of urges to smoke (Naqvi et al., 2007). Systemic administration of SB-334867 also prevented reduction of the threshold for intracranial self-stimulation by nicotine (Hollander et al., 2008). Together these data suggest that nicotine may act via the orexin system to induce synaptic plasticity in the VTA, thus modulating responses to reward. Involvement of the orexin system raises the question of effects of nicotine on feeding. Overall, nicotine decreases food intake, but likely this is mediated by actions in the brain stem rather than the VTA, because it is blocked by mecamylamine infusion into the fourth ventricle (Guan et al., 2004).

6. Nicotine and Glutamatergic Neuroplasticity. There are also numerous reports on nicotine-induced neuroplasticity in the HC and cerebral cortex. For example, investigations of the effects of nicotine agonists and antagonists on LTD induction in wild-type, α2-, α7-, and β2-KO mice revealed that LTD-inducing stimulation releases ACh activating α7 nAChRs to suppress LTD at hippocampal CA3-CA1 synapses, but this effect is reversed by nicotine via a non-α7, non-α2, and non-β2 receptor mechanism (Nakauchi and Sumikawa, 2014). In hippocampal slices from animals chronically treated with nicotine, expression of α4β4 nAChRs on glutamatergic terminals is increased, and acute exposure to nicotine during tetanic stimulation increases induction of LTP in medial perforant path (Nashmi et al., 2007) (Figs. 9 and 10). Meanwhile, in cultured hippocampal neurons, activation of presynaptic α4β2* nAChRs produced a glutamatergic neurotransmission-dependent enlargement of postsynaptic dendritic spines (Oda et al., 2014). Chronic treatment with nicotine (2 mg/kg/day s.c.) for 6 weeks reversed beta-amyloid (Aβ)-induced memory and hippocampal LTP deficits (Srivareerat et al., 2011) and chronic stress-induced intensification of Aβ-induced long-term memory and synaptic plasticity deficits (Alkadhi, 2011; Alkadhi et al., 2010, 2011). Although the interaction between acetylcholine receptors (AChRs) and Aβ is complex, overall activation of AChRs can have protective effects against Aβ toxicity, leading to an interest in developing α7 agonists for treatment of Alzheimer’s disease (reviewed in Lombardo and Maskos, 2015).

In freely moving mice, acute nicotine administration (1 mg/kg i.p.) induced medial perforant path-dentate gyrus synaptic potentiation by a mechanism dependent on DA receptors in the HC and activity of VTA neurons (Tang and Dani, 2009). Adult rats trained to intravenously self-administer nicotine 0.04 or 0.08 mg/kg per infusion, achieving daily nicotine intakes of approximately 200 and 300 μg/kg, respectively, showed decreased cell proliferation and increased cell death in the dentate gyrus of the HC (Abrous et al., 2002). Nicotine SA under extended access (0.03 mg/kg/infusion, 21 hours/day for 4 days) with intermittent periods of deprivation (3 days) for 14 weeks, but not nicotine intake under limited access (1 hour/day for 4 days), enhanced proliferation and differentiation of hippocampal neural progenitors (Cohen et al., 2015). Together these data represent evidence for effects of nicotine on plasticity in cortical and hippocampal...
pathways, but it is less clear that this plasticity is of any functional relevance to the persistent neuroplasticity underlying behaviors associated with addiction. The evidence for involvement in addiction is stronger for midbrain pathways.

7. Nicotine and the Habenula. Among midbrain pathways involved in nicotine addiction, the habenulo-interpeduncular pathway has recently received considerable attention (Velasquez et al., 2014; Antolin-Fontes et al., 2015). The mammalian habenula is located at the posterior medial end of the dorsal thalamus next to the third ventricle. The habenula is phylogenetically highly conserved across vertebrates (Concha and Wilson, 2001; Aizawa et al., 2005; Dadda et al., 2010) and connects limbic forebrain nuclei with midbrain and hindbrain nuclei (Sutherland, 1982). The MHB receives major inputs from the septum through the stria medullaris and has a major output to the IPN through the fasciculus retroflexus, whereas the LHb makes a minor contribution to this projection (Herkenham and Nauta, 1979; Swanson and Cowan, 1979; Qin and Luo, 2009).

The MHB-IPN tract highly expresses α-bungarotoxin-insensitive nAChRs (Mulle et al., 1991), likely containing α4, α5, α3, and β4 nAChR subunits encoded by the CHRNA5-A3-B4 gene cluster (Aizawa et al., 2012; Shih et al., 2014). This gene cluster has been linked with increased nicotine consumption and dependence in human genetics studies (Berrettini et al., 2008; Bierut et al., 2008; Lips et al., 2010; Liu et al., 2010; Ware et al., 2011). Consistent with these human genetic studies, animal models indicate a role for the MHB-IPN pathway in modulation of nicotine aversion and nicotine withdrawal (Salas et al., 2009; Fowler et al., 2011; Frahm et al., 2011) (Fig. 10). Mice with null mutation of Chrna5, the gene encoding for the α5 nAChR subunit, showed a marked increase in nicotine intake that could be rescued by expression of α5 subunits in the MHB (Fowler et al., 2011). The β4 nAChR subunit is important for receptor activation. Asp398Asn mutation in the α5 nAChR subunit is associated with increased tobacco usage in humans (Bierut et al., 2008; Saccone et al., 2009; Bierut, 2010), and α5 Asp398Asn was found to interact closely with a β4 Ser435 residue important in increasing nAChR currents (Frahm et al., 2011). In mice overexpressing Chrm4, the gene encoding for the β4 nAChR subunit, viral-mediated expression of α5 Asp398Asn subunits reduced nicotine aversion (Frahm et al., 2011). In mice chronically treated with nicotine, null mutations of the α2 and α5 subunits highly expressed in the IPN reduced mecamylamine-induced withdrawal, and in wild-type mice microinfusion of mecamylamine into the Hb or IPN precipitated withdrawal (Salas et al., 2009). Moreover, lentiviral expression in the MHB of a gain-of-function Thr374Ile variant of the β4 nAChR subunit associated with reduced risk of smoking resulted in strong nicotine aversion in mice, whereas expression of a loss-of-function Arg348Cys variant failed to induce nicotine aversion (Slimek et al., 2014). Nicotine-induced excitation of the MHB neurons in mice was mediated by tachykinin NK1 and NK3 receptors (Dao et al., 2014). In chronic nicotinetreated mice, intra-MHB infusions of NK1/3 antagonists provoked withdrawal symptoms, suggesting that the tachykinin receptors were in a sensitized state.

Although, as discussed above, the α5, α3, and β4 nAChR subunits that are strongly expressed in the Hb are likely resistant to nicotine-induced upregulation; nicotine produces persistent changes in the MHB and the fasciculus retroflexus projection to the IPN through selective neurotoxicity. Administration of high doses of

Fig. 10. Summary of the effects of nicotine on various brain regions, with a special reference to adolescent exposure and to neurotoxicity in the medial habenula. DG, dentate gyrus; fr, fasciculus retroflexus; HC, hippocampus; IPN, interpeduncular nucleus; LH, lateral hypothalamus; MHB, medial habenula; mPFC, medial prefrontal cortex; MPP, medial perforant path; NAc, nucleus accumbens; TH, tyrosine hydroxylase; VTA, ventral tegmental area.
nicotine, continuous infusion of 5.72, 6.44, 7.13, 20.41, and 43.1 mg/kg/day nicotine tartrate via osmotic minipump or intermittently at 11.32 mg/kg/day via once daily subcutaneous injection over 5 days resulted in neurodegeneration of the axons of the MHb neurons that form the fasciculus retroflexus (Carlson et al., 2001). More recent neurochemical investigations indicate that cholinergic neurons of the MHb are also a major target of this nicotine-induced neurodegeneration (Ciani et al., 2005). Previously, rather than affecting the MHb, continuous administration of stimulants amphetamine or cocaine had been found to cause neurodegeneration in the LHb, which has a weaker contribution to the fasciculus retroflexus (Ellison, 1992; Carlson et al., 2000). It has been suggested that as the fasciculus retroflexus is a phylogenetically primitive tract carrying much of the negative feedback from forebrain back onto midbrain reward circuitry, the destruction of these descending control pathways by drug binges could result in loss of forebrain control of addictive behavior (Ellison, 2002). This is consistent with the role for the MHb-IPN pathway in modulation of nicotine aversion and nicotine withdrawal (see above).

It is not known whether human smokers show similar neurotoxicity in the MHb-IPN pathway. It is also not known whether there is any subsequent adaptive neuroplasticity and functional recovery. An important question that remains to be further addressed is the extent to which this neurotoxicity in the MHb is reversible. Further investigations are needed to understand the implications for behavior and whether the effects of the neurotoxicity are permanent. More recently, it has been found that repeated nicotine exposure in adolescent rats reduces MHb activity and increases nicotine preference, suggesting that repeated phases of nicotine exposure induce a functional switch in the activity of MHb neurons in adolescent rats leading to increased nicotine consumption (Lee et al., 2015).

8. Long-term Sequelae of Adolescent Exposure to Nicotine. It has been suggested that nicotine has especially profound effects on neuronal networks (Fig. 10), in particular in the PFC, when exposure occurs during adolescence (Goriounova and Mansvelder, 2012a,b). The onset of puberty is a period of significant development of the emotional brain, including the midbrain D\text{\textalpha}ergic nuclei, the hypothalamus, the N\text{\textalpha}c, the striatum, and amygdala. In contrast, development of the frontal cortical areas responsible for self-control and decision-making lags behind, maturing throughout adolescence and into adulthood (Sowell et al., 2003; Giedd, 2004; Casey et al., 2005) (Fig. 4). Asynchronous brain development may thus be responsible for the characteristic traits of adolescence (Goriounova and Mansvelder, 2012a), such as mood swings, impulsivity, risk-taking, and susceptibility to peer influence in social behavior (Orr and Ingersoll, 1995; Spear, 2000; Galvan et al., 2006). Together, impulsivity, risk-taking, and susceptibility to peer pressure can contribute to increased risk of onset of smoking in adolescents. Once they smoke, adolescents appear more susceptible to dependence at lower levels of cigarette consumption (Colby et al., 2000; Kandel and Chen, 2000). Similarly, in mice and rats there is increased vulnerability to nicotine SA during early adolescence (Adriani et al., 2002; Ahsan et al., 2014). It has been proposed that adolescents may show this greater vulnerability to nicotine addiction, because nicotine has greater positive effects and smaller negative effects in adolescents than adults (O’Dell, 2009). Moreover, the adolescent brain appears to be exceptionally sensitive to nicotine-induced neuroplasticity.

Acutely, smoking during adolescence is associated with disruption of working memory and attention consistent with reduced PFC activation (Jacobsen et al., 2005, 2007; Musso et al., 2007). Moreover, smoking is a prospective risk factor for impaired cognitive function in later life (Cervilla et al., 2000; Richards et al., 2003), and adolescent tobacco use is associated with increased risk of developing mental and behavioral problems, including abuse of other drugs, later in life (Brown et al., 1996; Johnson et al., 2000; McGee et al., 2000; Ellickson et al., 2001; Brook et al., 2004). In rodents, adolescent animals are more susceptible to nicotine-induced CPP than adults (Vastola et al., 2002; Belluzzi et al., 2004; Shram et al., 2006; Kota et al., 2009; Shram and Le, 2010; Brielmaier et al., 2012; Ahsan et al., 2014). Moreover, nicotine administration during, but not after adolescence, has long-lasting effects on cognitive, emotional, and addiction-related behaviors (Adriani et al., 2003, 2004; Cournette et al., 2009, 2011; Iniguez et al., 2009). Together these data point to the conclusion that adolescent nicotine exposure has long-term effects on attention, cognition, and emotional function in adulthood (Adriani et al., 2003; Cournette et al., 2009, 2011; Goriounova and Mansvelder, 2012a,b).

A model synthesizing the data on effects of adolescent exposure to nicotine on PFC in rodents (Goriounova and Mansvelder, 2012a,b) proposes that, in the short-term, adolescent nicotine exposure increases local inhibition via nAChRs on GABAergic interneurons, while simultaneously decreasing glutamatergic excitatory inputs to PFC neurons by increasing presynaptic mGlu_2 expression. However, in the long-term, local inhibitory activity normalizes, but there is a persistent and long-lasting downregulation of presynaptic mGlu_2 on glutamatergic afferent terminals, resulting in increased excitability of PFC pyramidal neurons associated with long-lasting downregulation of synaptic short-term depression and impaired attention. In rodents, the adolescent PFC is especially sensitive to nicotine-induced changes in gene expression (Schochet et al., 2005, 2008; Polesskaya et al., 2007). In adolescence, exposure to nicotine increases expression of immediate early genes used as functional
markers of neuronal activation (Leslie et al., 2004; Schochet et al., 2005) and dendritic expression of mRNA for dendrin, a protein associated with synaptic plasticity (Schochet et al., 2008). Meanwhile, chronic nicotine exposure increases expression of a wide range of genes involved in neurotransmission, signal transduction, and synaptic architecture (Polesskaya et al., 2007), and repeated nicotine exposure leads to increased phosphorylation of ERK and CREB involved in neuroplasticity (Brunzell et al., 2003). After adolescent nicotine exposure, nAChR levels in the PFC, likely representing primarily nAChRs on GABAergic interneurons, are elevated in the short-term but return to normal by 5 weeks (Counotte et al., 2012), whereas in contrast, mGlu2 receptors are strongly downregulated at this time point (Counotte et al., 2011). In rats, acute nicotine exposure during adolescence reduced PFC LTP in response to timed presynaptic and postsynaptic activity (tLTP) but subsequently increased this LTP in adulthood (Goriounova and Mansvelder, 2012b). These changes in LTP could be explained by the changes in mGlu receptors, because activation of mGlu2 receptors mimicked the acute decrease in tLTP and blocking of mGlu2 receptors mimicked the long-term increase in tLTP. Increases in glutamate release in the PFC also occur in NMDAR antagonist models of aspects of schizophrenia. NMDAR antagonist-induced increases in PFC glutamate release are associated with deficits in attention and loss of impulse control, and these behavioral effects can be reversed by administration of an mGlu2/3 agonist (Poatti et al., 2011). Administration of mGlu2 agonist also improved attention performance in animals that had been exposed to nicotine during adolescence (Counotte et al., 2011).

The effects of adolescent nicotine exposure on mGlu2 are not unique to the PFC. Long-lasting changes in mGlu2 function after adolescent nicotine exposure have also been reported in the VTA and NAc, where they modulate the rewarding properties of nicotine (Helton et al., 1997; Kenny et al., 2003; Kenny and Markou, 2004; Liechti et al., 2007; Counotte et al., 2011). In these brain regions, activation of mGlu2/3 receptors reduces SA of nicotine (Liechti et al., 2007), and these receptors are thought to play a role in development of drug dependence and manifestation of affective withdrawal symptoms (Kenny and Markou, 2004). However, the precise role of mGlu2 downregulation in the VTA-NAc system in the mechanisms of increased vulnerability to nicotine addiction after adolescent nicotine exposure requires further elucidation.

Recent findings suggest that long-term cigarette smoking in adulthood is associated with accelerated cerebral cortical thinning (Karama et al., 2015). Various degrees of cortical thinning may be associated with a number of neuropsychiatric diseases, such as schizophrenia, depression, dementias, and fetal alcohol spectrum disorders, and with an increased risk for them (Narr et al., 2009; Peterson et al., 2009; Zhou et al., 2011; Byun et al., 2012), and accelerated cortical thinning may be a normal physiologic phenomenon during adolescence and even associated with better neuropsychological performance (Squeglia et al., 2013). However, smoking increases the prevalence of Alzheimer’s disease (Barnes and Yaffe, 2011), and, therefore, it is important that smoking-induced cortical thinning seems to be at least partially reversible, albeit very slowly. Further studies of the mechanism and extent of nicotine-induced structural changes in the adult brain likely will be informative.

There is a strong association between habitual smoking and alcohol dependence, and there may be common genetic risk factors (Bierut et al., 2000; Grucza and Bierut, 2006; Mingione et al., 2012; Vrieze et al., 2013; Anantharaman et al., 2014) (reviewed in Sturgess et al., 2011). In particular, patients with chronic pain may be susceptible to tobacco, alcohol, and opioid dependence (Fishbain et al., 2012), and there is a strong association between smoking and chronic pain (reviewed in Ditre et al., 2011; Parkerson et al., 2013). Meanwhile, antidepressants have been used in treatment of alcohol addiction, and antidepressants and MAO inhibitors have been used in chronic pain management. It has also been noted that MAO inhibitors increase tolerance to alcohol in mice (Popova et al., 2000), and that tobacco smoke exposure increases alcohol consumption in adolescent C56BL/6 mice (Burns and Proctor, 2013). Acetaldehyde is produced in high concentrations when cigarettes are burned. There appear to be synergistic interactions between acetaldehyde and both nicotine and alcohol. Rats preferred a combination of acetaldehyde and nicotine to either substance alone (Rabinoff et al., 2007). Moreover, rats could be trained to self-administer acetaldehyde and subsequently consumed more alcohol (Amit and Smith, 1985). Although most alcohol metabolism occurs in the liver, some alcohol is metabolized to acetaldehyde by microbial action already in the oral cavity. Deficient oral hygiene, heavy alcohol drinking, and smoking modify oral microbiome to increase acetaldehyde from local alcohol metabolism (reviewed in Salaspuro, 2007). The acetaldehyde produced in this way may contribute not only to association between smoking and alcohol dependence but also to increased risk of gastrointestinal tract cancers.

9. Conclusions. Nicotine addiction leads to increased risk of severe adverse health effects caused by tobacco. Distribution of nAChRs with different subunit combinations in various brain regions contributes to diverse mechanisms of plasticity. Mechanisms of nicotine-induced plasticities include upregulation and downregulation of nAChRs; interactions with catecholaminergic, GABAergic, and glutamatergic neurotransmission; and neurodegenerative structural changes in fasciculus retroflexus and cortex. The extent to which the changes induced by nicotine exposure are reversible remains incompletely understood. There is need for further investigation of the long-term sequelae of
nicotine exposure, particularly those of adolescent exposure. These mechanisms and relevant brain pathways are summarized in Fig. 10. Nicotine addiction is of particular concern because of the ready availability of legally available tobacco products in many societies. There is increasing evidence that early exposure to nicotine leads to increased risk of later life dependence not only on nicotine but also to other drugs of abuse including alcohol. There is need for further investigation of the mechanisms by which nicotine-induced plasticity can increase risk of dependence to other drugs of abuse.

D. Neural Adaptations Induced by Ethanol

Ethanol is one of the most widely used psychoactive substances worldwide, with both reinforcing and sedative-hypnotic properties. Acutely, these effects arise from many molecular targets, and they most prominently involve alterations in synaptic function. Repeated ethanol exposure leads to homeostatic changes in synaptic and intracellular functions. Behaviorally, these neuroadaptations are expressed as tolerance, dependence, somatic withdrawal symptoms upon cessation of chronic ethanol exposure, and so-called protracted withdrawal characterized by susceptibility to negative reinforcement that is postulated to contribute to relapse and compulsive features of ethanol addiction. Figure 11 depicts the cycle of alcohol intoxication/animal adaptation/withdrawal effects in the brain, with proposed roles of the neurotransmitter/modulator systems. In the following, we will focus primarily on the synaptic effects of ethanol and their persistent alterations in key brain regions during chronic ethanol administration.

Adjunct pharmacological means to treat alcoholism have been expanded from one specific drug, disulfiram, to opioid receptor antagonists naltrexone and nalmefone and to glutamate receptor antagonists acamprosate (Heilig and Egli, 2006). Benzodiazepine therapy during alcohol withdrawal is still important, especially to protect the brain from potential neurotoxicity of withdrawal-induced seizures (Sellers et al., 1983; Walker et al., 2013). Recent neurobiological research on ethanol addiction, relapse, and withdrawal has produced many other potentially efficient drug targets, which are mentioned below within a discussion on mechanism of alcohol action and addiction.

1. Cell Membrane Ion Channels as Primary Targets of Ethanol. Compared with many other drugs of abuse that exert their actions through specific neurotransmitter receptors or transporters, ethanol is a relatively nonspecific and nonpotent pharmacological agent. Ethanol was believed to fluidize membrane lipids and cause intoxication by perturbing neuronal function (for reviews, see Deitrich et al., 1989; Franks and Lieb, 1994; Peoples et al., 1996; Spanagel, 2009). Then, protein targets emerged for anesthetics and for alcohols of various carbon chain lengths (Franks and Lieb, 1984). A general binding pocket for ethanol has not been settled, although in several ligand-gated ion channels the action of alcohol is dependent on specific single amino acid residues, as modeled in a prokaryotic member of ligand-gated ion channels, GLIC (Howard et al., 2011). Although many drug targets have been revealed by using high-affinity (nanomolar) radiolabel binding to brain membranes or recombinant receptors, with ethanol this approach has not been possible because of its 1000- or even 100,000-fold lower affinity. However, there is accumulating evidence that ethanol has a few well-defined molecular targets (Trudell et al., 2014). At intoxicating ethanol concentrations, from low to approximately 100 mM, the most important targets of ethanol include ion channels, neurotransmitter receptors, and intracellular signaling molecules. The best characterized of these targets are ligand-gated ion channels (Lovinger and Roberto, 2013) that bind extracellular neurotransmitters, which leads to opening of an intrinsic ion pore.

In short, there are three major classes of ion channels activated by neurotransmitters. The so-called cys-loop channels are protein pentamers that have a Cys-Cys bond in the N-terminal binding domain, four transmembrane domains forming the ion channel, and an intracellular loop between TM3 and TM4 for modification by phosphorylation/dephosphorylation. This major class of receptor channels passes either anions, such as the γ-aminobutyric acidA (GABA A) and strychnine-sensitive glycine receptors, or cations, such as the nicotinic acetylcholine nACh and serotonin 5-HT3 receptors. The general action of acute ethanol is to enhance the function of cys-loop ligand-gated ion channels (Lovinger, 1997; Harris, 1999; Perkins et al., 2010), although ethanol can also produce inhibition in some subtypes/receptor domains (Johnson et al., 2012). Ethanol exerts its action by potentiating channel opening in the presence of low agonist concentrations. This is achieved either by increasing the probability of channel opening or increasing the agonist affinity (Tonner and Miller, 1995; Zhou et al., 1998; Welsh et al., 2009).

The potentiation of the GABA A receptor by ethanol has been investigated widely. The GABA A receptor is assembled as variable combinations of 19 subunit proteins (Collingridge et al., 2009), and many subunit combinations have been characterized in heterologous expression systems and in native receptor preparations, including cultured and isolated neurons. In summary, ethanol potentiates the function of various GABA A receptor subtypes containing α, β, and γ subunits, as well as those containing α4 and α6 together with β and δ subunits (McCool et al., 2003; Olsen et al., 2007; Lobo and Harris, 2008). In brain slices from the cerebellum, HC, and thalamus, ethanol potentiation has also been observed in extrasynaptic, δ subunit-containing high-affinity GABA A receptors mediating a tonic inhibitory current (Wei et al., 2004; Hanchar et al., 2005; Glykys et al., 2007; Jia et al., 2008). However, the potentiation
has not been universally replicated in all neuron types or experimental settings (Borghese et al., 2006; Korpi et al., 2007; Lovinger and Homanics, 2007). Ethanol-induced potentiation of GABA<sub>A</sub> receptor function may also depend on protein phosphorylation. For example, the epsilon subunit of protein kinase C (PKC<sub>ε</sub>) appeared to be necessary for ethanol potentiation of GABA<sub>A</sub> receptors containing the g<sub>2</sub> subunit (Qi et al., 2007), whereas PKC<sub>d</sub> was required for ethanol’s action on the d subunit-containing GABA<sub>A</sub> receptors (Choi et al., 2008).

It is noteworthy that ethanol potentiation of GABAergic inhibition often results from presynaptic actions of ethanol. Ethanol enhances GABA release from presynaptic terminals, which contributes to synaptic inhibition (for reviews, see Siggins et al., 2005; Lovinger and Roberto, 2013). These data are obtained mostly from brain slices and isolated neurons from the cerebellum, HC, VTA, and amygdala (Artwodola et al., 2003; Roberto et al., 2003; Kelm et al., 2007; Theile et al., 2008). The mechanism of ethanol enhancement of GABA release is not well established, but ethanol may interact with mechanisms involved in intracellular calcium release, increasing presynaptic calcium concentrations (Kelm et al., 2007). The possible role of intracellular signaling pathways in this ethanol effect is suggested by findings that GABA release is diminished in cerebellar Purkinje neurons by PKA inhibitors and in the CeA in mice lacking PKC<sub>ε</sub> (Bajo et al., 2008; Kelm et al., 2008).

Ethanol potentiation of the strychnine-sensitive glycine receptor channels also depends on the receptor subunit composition. Greatest potentiation is seen in α1 subunit-containing receptors in the *Xenopus leavis* oocytes (Valenzuela et al., 1998b), whereas receptors with the α2 subunit are less sensitive (Mascia et al., 1996). Expression of the β subunit together with α2 abolishes ethanol potentiation (McCool et al., 2003). As both the GABA<sub>A</sub> and glycine receptors are inhibitory, ethanol enhancement of their function increases neuronal inhibition. The net effect on neural networks is determined by the primary target, either interneurons or principal neurons. Intracellular mechanisms for the glycine receptor potentiation by ethanol have been revealed, e.g., in isolated VTA neurons (Ye et al., 2001; Zhu and Ye, 2005). The activated G protein βγ-subunits enhance the ethanol effect in recombinant glycine receptors by binding to specific intracellular domains (including two lysines at 385/386) of the α1 subunits (Ye et al., 2004). In addition to GABA release, ethanol also augments glycine release via Ca<sup>2+</sup>-dependent mechanisms (Mariqee et al., 2014). Strychnine blocks the ethanol-induced DA release from the nucleus accumbens and attenuates nicotine- and THC-induced release, but not the release induced by cocaine and morphine (Jonsson et al., 2014), indicating the importance of glycine...
receptors in the modulation of mesolimbic DA pathway activity.

Acute ethanol also potentiates the function of serotonin 5-HT₃ receptors that gate an intrinsic cation channel (Lovinger and White, 1991; Machu and Harris, 1994). As these receptors are expressed both pre- and postsynaptically, the site of ethanol action remains to be determined. The roles of the five different 5-HT₃ receptor subunits (Thompson, 2013) are not well elucidated. Interestingly, in rat brain, the 5-HT₃ receptors have been localized primarily to cholecystokinin-containing (CCK) GABAergic interneurons (Morales and Bloom, 1997). Furthermore, they are also expressed in vagal afferents from the stomach, where they can be activated by serotonin released from enterochromaffin cells, which together with the area postrema 5-HT₃ receptors participate in nausea and/or emesis reflexes (Hornby, 2001).

Like the GABAₐ receptor, the nACh receptor is often composed of multiple subunits (see section II.C on nicotine), but homomeric receptors also exist. High ethanol concentrations (>100 mM) potentiate the majority of nACh receptor subtypes without selectivity (Narahashi et al., 1999; Zuo et al., 2002). However, the effects of ethanol concentrations lower than 100 mM are dependent on the α subunit, with potentiation of α2β4, α4β4, α2β2, and α4β2 nACh receptors, with no effect on α3β4 and α3β2 receptors, and inhibition of the α7 receptors. In chimeric recombinant homomeric receptors with the N-terminal ligand-binding domain from the nACh α7 subunit and the rest from 5-HT₃a subunits, nicotine responses were inhibited by ethanol similarly to α7 receptors, suggesting that the ethanol inhibition was dependent on the N-terminal ligand binding domain (Yu et al., 1996).

The second major class of ligand-gated ion channels consists of the ionotropic glutamate receptors (iGluRs) that have three major subclasses, the AMPARs, the NMDARs, and the kainate receptors (Collingridge et al., 2009). All these subclasses are cation permeable, but differ in selectivity between Na⁺, K⁺, and Ca²⁺. These ligand-gated receptor channels are homo- or heterotramers, with subunits having large N-terminal ligand-binding domains, three TM regions, and a fourth re-entrant loop as in potassium channels. Although ethanol partially inhibits all iGluRs, inhibition of NMDARs with intoxicating ethanol concentrations has been most extensively investigated (Dildy and Leslie, 1989; Hoffman et al., 1989; Lovinger et al., 1989; Kuner et al., 1993; Frye and Fincher, 2000). Ethanol produces the inhibition by promoting desensitization of the AMPARs (Moykkynen et al., 2003). The kainate receptors (composed of GluK1-5) are inhibited by ethanol at low concentrations, but it is unclear whether this action involves direct effects on the receptor protein (Valenzuela et al., 1998a; Lack et al., 2008). The GluK1 receptors are especially interesting, because the presence of a polymorphism in GRIK1 gene correlated with a robust response to topiramate in reducing heavy drinking of alcohol-dependent subjects (Kranzler et al., 2014). Taken together, inhibition of the iGluRs by ethanol decreases neuronal excitability and also inhibits synaptic plasticity that requires activation of iGluRs.

The purinergic trimeric P2X receptors constitute the third major class of ligand-gated ion channels. At intoxicating concentrations, ethanol has inhibitory effects on many P2X subtypes, with the P2X4 receptors being the most sensitive subtype to inhibition (Li et al., 1993; Davies et al., 2002, 2005). Interestingly, inhibition of the ATP action on native P2X receptors or recombinant rat P2X4 receptors by series of alcohols provides evidence for a cut-off point at butanol, allowing smaller alcohols to inhibit presumably via binding to a binding pocket of a restricted size (Li et al., 1994; Asatryan et al., 2010). The anthelmintic agent ivermectin has a positive allosteric action at this subtype, among several other ligand-gated ion channels, such as GABAₐ receptors (Zemkova et al., 2014). Ivermectin reduces alcohol drinking in mouse models (Yardley et al., 2012, 2014). Further functional significance of ethanol actions on P2X1/3 receptors comes from findings that ethanol reduces GABA release in isolated VTA DA neurons via inhibition of ATP effects on P2X receptors (Xiao et al., 2008).

Also the nonligand-gated ion channels are targets of ethanol. Ethanol inhibits high-voltage-activated dihydropyridine-sensitive Ca²⁺ channels (L-type, Ca₉.1.x) (Wang et al., 1994) and potentiates the function of G protein-activated inwardly rectifying K⁺ channels (GIRKs, Kᵢ₃.₃.x) (Kobayashi et al., 1999; Lewohl et al., 1999), which both inhibit neural activity. The large conductance Ca²⁺- and voltage-activated K⁺ (BK)
channel has emerged as a most interesting molecular target for ethanol, based on genetic studies on Caenorhabditis elegans and Drosophila, and on recent results from mouse knockout and human genetic association studies (Treistman and Martin, 2009; Bettinger and Davies, 2014; Dopico et al., 2014). BK channels are also involved in neuroadaptation to alcohol. They are formed as tetrameric complexes from two α subunits and two β subunits (usually either β1 or β4), and they are expressed throughout the body; in the brain they are present in all areas with presynaptic and extrasynaptic compartments. Ethanol usually activates these channels in neurons, which should produce neuronal inhibition and reduction in neurotransmitter release depending on the β subunits. Tolerance to ethanol appears in minutes as reduced channel sensitivity, followed by declustering and internalization of the channels. The β1 subunits make the BK channels insensitive to ethanol, while β4 subunits provide ethanol sensitivity (Feinberg-Zadek et al., 2008). This seems to correlate in mouse models with quicker escalation of limited access alcohol drinking after induction of dependence by chronic intermittent ethanol inhalation in the absence of the β1 subunit (Kreifeldt et al., 2013) and quicker tolerance development to motor impairment in the absence of the β4 subunit (Martin et al., 2008). The search for the site of action of ethanol and other n-alcohols on BK channel-forming α subunits is very actively pursued (Bukiya et al., 2014; Davis et al., 2014). The discovery of this site of action might allow the rational development of an alcohol antagonist for BK channels. In addition, establishing more detailed roles of the accessory β subunits in the regulation of channel function by phosphorylation (Liu et al., 2006) might open novel ideas for translational experiments.

Ethanol may also affect the function of the GPCRs, but generally these effects by acute ethanol are weak and the mechanisms are unclear, unlike those of opioids and cannabinoids acting on their respective GPCRs. There is early evidence that ethanol can stimulate cAMP production, perhaps by activating ACs, which can underlie ethanol-induced neurotransmitter release (Luthin and Tabakoff, 1984; see above for discussion on GABA release).

2. Ethanol-Induced Changes in Glutamatergic Transmission. Repetitive patterned activation of afferent inputs to postsynaptic neurons induces long-lasting changes in the efficacy of synaptic transmission. The most common forms of these changes, LTP and LTD, have also been examined after acute ethanol (McCull, 2011). Consistent with the inhibition of NMDARs at relevant ethanol concentrations, acute ethanol inhibits induction of LTP at various synapses and experimental preparations, such as Schaffer collateral inputs to the CA1 pyramidal neurons in the HC (Zorumski et al., 2014). Acute ethanol also modulates types of LTD in many brain regions, including LTD in the HC involving activation of mGluRs (Overstreet et al., 1997) and the eCB-mediated signaling in the dorsal striatum (Clarke and Adenmark, 2010). Ethanol inhibition of these types of plasticity is consistent with acute ethanol-induced impairment of working and short-term memory (Schweizer and Vogel-Spratt, 2008).

Considering the development of alcoholism in humans, however, the most relevant neural changes recapitulated by animal models probably include those produced by chronic ethanol exposure. Chronic ethanol produces tolerance, manifested by decreased behavioral response to ethanol, and dependence, characterized by a specific symptomology after ethanol withdrawal. In addition, chronic alcohol exposure can also produce a “postdependent state,” which is understood as a combination of behavioral and neuroadaptive consequences that are produced as the individual becomes dependent and which remain for extended periods even in the absence of the drug-specific withdrawal symptoms. Typical behavioral correlates of the postdependent state include anxiety, dysphoria, and exaggerated stress responses (Heilig and Koob, 2007).

Various experimental paradigms have been used for inducing alcohol dependence, including repeated intraperitoneal or intragastric administration, ethanol-containing liquid diets, and either continuous or intermittent ethanol vapor chamber exposure. These paradigms typically produce peak blood ethanol concentrations of 35–69 mM (150–300 mg/dl), which cannot be attained by most voluntary alcohol drinking models. However, alcohol-preferring mice consume enough ethanol to achieve blood ethanol levels exceeding 23 mM (100 mg/dl) in the recently described binge-drinking model (“drinking in the dark”) (Rhodes et al., 2005), and the reintroduced intermittent ethanol access model results in escalation of ethanol drinking in rats, with intoxicating blood ethanol levels (Wise, 1973; Simms et al., 2008). Even if induction of alcohol dependence in these binge models is debatable, long-term binge drinking has been shown to produce a variety of neuroadaptations (Stuber et al., 2008; Wilcox et al., 2014).

Chronic ethanol increases NMDAR function at both synaptic and extrasynaptic compartments in many brain regions and experimental preparations. Enhanced NMDAR function is seen as an increased intracellular level of NMDAR agonist-induced Ca2+ or directly as increased ion current through the NMDAR pore (Gulya et al., 1991; Iorio et al., 1992; Smothers et al., 1997; Grover et al., 1998; Floyd et al., 2003). However, the ability of acute ethanol to inhibit NMDARs is not diminished by chronic ethanol treatment (Roberto et al., 2004b). The mechanism underlying the enhanced NMDAR function is not well known, but it may involve increases in the levels of the GluN2B subunit (Floyd et al., 2003; Carpenter-Hyland et al., 2004; Kash et al., 2009). In the VTA, chronic ethanol has also been associated with a higher order plasticity,
described as metaplasticity, that affects synapses of postsynaptic neurons in a global manner, increasing their susceptibility to undergo activity-dependent LTP/LTD (Abraham, 2008). Thus, chronic ethanol enhances the susceptibility of VTA DA neurons to the induction of LTP of NMDAR-mediated transmission. This plasticity results from an increase in the potency of inositol 1,4,5-trisphosphate (IP$_3$) in producing amplification of action potential-evoked Ca$^{2+}$ signals, lasting for 1 week after ethanol exposure (Bernier et al., 2011). This kind of metaplasticity could be an important neuroadaptation that drives the formation of drug-associated cues both during drug intoxication and withdrawal.

Acute doses of many drugs of abuse, including ethanol, increase the AMPAR/NMDAR current ratio in VTA DA neurons (Saal et al., 2003), reflecting an enhancement of AMPAR-mediated excitatory synaptic transmission. The findings of the chronic ethanol effects on AMPARs are sparse and variable, suggesting increases in AMPAR expression in the HC (Bruckner et al., 1997) and increased AMPAR function, as shown by enhanced AMPAR-mediated synaptic responses in the BLA (Lack et al., 2007). In the VTA neurons, voluntary intermittent ethanol consumption enhances postsynaptic AMPAR function without altering presynaptic glutamate release (Stuber et al., 2008). The enhanced AMPAR function in the BLA could contribute to drug seeking, whereas in the VTA, AMPAR activity could regulate firing of the mesolimbic DA neurons and thereby contribute to the reinforcing and activating effects of ethanol.

In the NAc, another key component of the reward circuitry, glutamatergic innervation, interacts with the mesolimbic DA system and participates in drug reinforcement, drug seeking, and relapse. The Homer proteins are integral components of the PSD that appear to be necessary for alcohol-induced neuroplasticity within the NAc. For example, chronic ethanol consumption under continuous access produces a robust increase in the NAc Homer2 protein levels, which was observable even 2 months after withdrawal from alcohol drinking (Szumlinski et al., 2008). The increased Homer2 expression was accompanied by elevation in total mGlu$_4$ receptor and GluN2B subunit levels during the first 2 weeks after withdrawal. Furthermore, both virus-mediated knockdown of Homer2 expression and intra-NAc antagonism of mGlu$_4$ and PI3K attenuated ethanol intake, suggesting that upregulation of NAc mGlu$_4$-Homer2-Pi3K signaling constitutes an important neuroadaptation to chronic ethanol (Cozzoli et al., 2009).

Chronic ethanol produces elevated extracellular glutamate levels in the brain, especially during withdrawal and repeated cycles of withdrawal, thus contributing to the hyperglutamatergic state implicated in the development of ethanol dependence. These findings have been derived from both in vivo microdialysis and magnetic resonance spectroscopy studies (Rossetti and Carboni, 1995; Hermann et al., 2012), and they do not directly indicate whether the source of glutamate is synaptic, extrasynaptic, glial, or metabolic. However, there is evidence of increased synaptic glutamate release from the CeA after chronic ethanol treatment, pointing to adaptations in the presynaptic terminal function (Roberto et al., 2004b).

3. Ethanol-Induced Changes in $\gamma$-Aminobutyric Acidergic Transmission. GABAergic neurotransmission is one of the primary targets of ethanol and is hypothesized to be involved in multiple long-lasting neuroadaptations underlying ethanol tolerance, dependence, and reinforcement. Many early studies focused on elucidating changes in the expression profiles of specific GABA$_A$ receptor subunits by chronic ethanol, with the hypothesis that these changes could account for alterations in the functional properties of GABAergic signaling after chronic ethanol exposure (Kumar et al., 2009). Generally, these studies failed to show dramatic changes in the number of GABA$_A$ receptors across the preclinical models, but pointed to a number of region-specific and temporally dependent alterations in GABA$_A$ receptor subunit mRNA and peptide levels (reviewed in Uusi-Oukari and Korpi, 2010). For example, in the cerebral cortex, chronic ethanol consumption elicited an increase in $\alpha$4 subunit mRNA levels and a decrease in $\alpha$1 subunit mRNA levels. There was also an increase in $\gamma$2$\alpha$, but not $\gamma$2L, subunit mRNA levels after chronic ethanol consumption. In addition, $\gamma$1 subunit expression was increased, whereas $\alpha5$, $\beta1$, $\beta2$, $\beta3$, $\gamma3$, and $\delta$ subunit mRNA levels did not change (Devad et al., 1995). These changes correlated well with the receptor subunit peptide levels in ethanol-dependent rats, but not necessarily with subunit expression during withdrawal (Devad et al., 1997). However, other brain regions did not exhibit similar patterns of subunit changes, and subregions of the cerebral cortex differed from the whole cortex (Grobin et al., 2000). Direct comparison of cerebral cortex and HC revealed that also in the HC the $\alpha$4 subunit peptide level was increased, but $\alpha1$, $\alpha2$, $\alpha3$, $\beta2/3$, or $\gamma2$ subunits were not altered, suggesting that chronic ethanol changes GABA$_A$ receptor gene expression in the HC but differently from that in the cerebral cortex (Matthews et al., 1998). Moreover, the changes were dependent on the length of ethanol exposure. Recent data from human postmortem brain areas from controls and alcoholics have also revealed brain region-specific changes in GABA$_A$ and glutamate receptor subunit expression in alcoholics (Jin et al., 2011; Bhandage et al., 2014; Jin et al., 2014a,b).

The hypothesis derived from the adaptive changes in the GABA$_A$ receptor expression by chronic ethanol is that those adaptations lead to a hypofunction of GABAergic synaptic functions, expressed as tolerance and increased neuronal excitability during ethanol withdrawal, which forms the basis for the treatment of severe alcohol
withdrawal with benzodiazepines (Sellers et al., 1983). There is evidence that ethanol-induced alterations in the expression of GABA_A receptor subunits leads to diminished GABA_A-mediated transmission. For example, chronic intermittent ethanol exposure induces a decrease in α1 and δ subunits and an increase in α4 subunit in rat hippocampal slices. These changes were reflected in recordings made in hippocampal slices from ethanol-exposed animals. Thus, the decay time of GABA_A-mediated miniature IPSCs was decreased and potentiation of these current by positive modulators was reduced by chronic ethanol, except for the α4-preferring benzodiazepine ligands, with which potentiation was either maintained or even increased (Cagetti et al., 2003). Synaptic and extrasynaptic components of GABA_A receptor activation were investigated in the same animal model and hippocampal preparation. After chronic ethanol, potentiation of tonic current by the α1 subunit-prefering agonist zolpidem was lost, whereas potentiation by the α4 subunit-prefering THIP was only partially reduced. Also the potentiation of synaptic GABA_A receptor currents by zolpidem was eliminated by chronic ethanol, whereas THIP enhanced them after ethanol exposure. These data are consistent with α1 subunit decreases in both synaptic and extrasynaptic compartments, and with increased α4 subunits in synaptic and decreased α4 subunits in extrasynaptic GABA_A receptors (Liang et al., 2004). The cause for these changes is not clear, but one possibility is that the putative primary reduction in α1 expression leaves the γ2 subunit (that is needed for synaptic targeting) available to assemble with α4 subunits, which increases the synaptic α4 subunit-containing receptors. In these receptors, the inverse agonist of the benzodiazepine-site Ro 15-4513 acts as an agonist [like in α6 subunit-containing receptors of the cerebellar granule neurons (Malminiemi and Korpi, 1989)] and enhances the synaptic responses only in the ethanol-dependent rats (Liang et al., 2006).

The CeA, a major component of the extended amygdala, has emerged as an important brain area for both the acute positive reinforcement of drugs of abuse and negative reinforcement associated with protracted abstinence. Ethanol drinking in nondependent rats is inhibited by antagonism of GABA_A receptors in the CeA (Hytytä and Koob, 1995), whereas GABA_A activation modulates ethanol reinforcement only in animals after chronic ethanol exposure (Roberts et al., 1996), suggesting altered CeA GABAergic transmission by chronic ethanol. Acute ethanol augments GABA_A-mediated inhibition in the CeA, which can be at least partially mediated by ethanol-induced presynaptic GABA release (Roberto et al., 2003). In CeA slices from rats treated chronically with ethanol, the baseline evoked IPSPs and IPSC amplitudes were increased and the paired-pulse facilitation ratios were lower than in naive rats, consistent with increased GABAergic transmission involving postsynaptic effects after chronic ethanol treatment. Ethanol administered in vitro enhanced IPSPs and IPSCs similarly in slices from ethanol-naive and ethanol-dependent rats, suggesting that tolerance did not develop to this acute effect of ethanol. The frequency of miniature IPSCs was higher in chronically ethanol-treated rats, in line with the idea that enhanced GABAergic transmission both by acute and chronic ethanol could also be ascribed to a presynaptic mechanism involving vesicular GABA release. Verification with microdialysis showed a fourfold increase in baseline GABA release in ethanol-treated rats compared with controls (Roberto et al., 2004a), although this method cannot necessarily distinguish between neuronal and glial GABA release. Significant GABA release seems to take place from glial cells (astrocytes) that may have a “GABAergic phenotype” (Lee et al., 2011) and release GABA via bestrophin 1 anion channels (Lee et al., 2010). These data show that both acute and chronic ethanol effects on GABAergic transmission could involve pre- and postsynaptic alterations. More recently, ethanol-induced increase in firing rates of subpopulations of CeA neurons has been shown to be mediated by CRF_1 receptors and tonic GABA currents, with the network effect being increased output neuron activity to the BNST (Herman et al., 2013), a brain region involved in sustained anxiety and relapse. Furthermore, the ethanol-induced increase in GABA release and IPSC amplitudes in the CeA slices can be blocked by activation of presynaptic CB_1 receptors (Roberto et al., 2010a), demonstrating multiple presynaptic mechanisms being involved in ethanol modulation of amygdala activity.

Further evidence for involvement of presynaptic mechanisms in ethanol effects on GABAergic transmission has been obtained from the VTA. A single ethanol dose induced a long-lasting facilitation of GABA transmission, as suggested by paired-pulse depression in ethanol-treated animals compared with paired-pulse facilitation in controls (Melis et al., 2002). Also, as the frequency of spontaneous miniature IPSCs was increased, a presynaptic mechanism can be inferred. Indeed, a GABA_A antagonist shifted paired-pulse depression to paired-pulse facilitation, indicating presynaptic GABA_A receptor activation by hypothesized GABA spillover by ethanol treatment. Similarly in the VTA, a single ethanol exposure increased GABA release onto DA neurons (Wanat et al., 2009). The importance of these cellular, possibly long-lasting signaling events for ethanol consumption and development of addiction remains to be investigated. In contrast to GABA neurotransmission in the amygdala, chronic alcohol treatment makes the VTA GABA system hyposensitive, which may be based on persistent changes in gene expression (Arora et al., 2013).

Dorsal striatum and associated sensorimotor structures have been implicated in the development of addiction, particularly in the mediation of habit formation, and this region has also emerged as a site of alcohol-induced neuroadaptations. In cynomolgus monkeys, prolonged...
intermittent alcohol drinking decreased the frequency of GABA_A receptor-mediated miniature IPSCs recorded from the MSNs in the caudoventral area of the putamen (Cuzon Carlson et al., 2011). This area corresponds to the dorsolateral striatum in rodents, and a similar reduction of IPSC frequency was produced by repeated binge drinking in mice in this striatal area, but also in the dorsomedial striatum (Wilcox et al., 2014). These data suggest that heavy, long-term alcohol exposure causes a depression of GABAergic transmission in the dorsal striatum, contributing to an increased output from this structure.

4. Ethanol-Induced Changes in Neuropeptide Mechanisms. Various neuropeptide systems have been hypothesized to be recruited or downregulated during chronic ethanol exposure. Particularly in the extended amygdala, neuropeptides may underlie the negative emotional states that contribute to compulsive ethanol seeking and drinking via negative reinforcement (Koob, 2008).

CRF plays a role in coordinating hormonal, autonomic, and behavioral stress responses in the body. In ethanol-dependent animals, ethanol withdrawal increases CFR levels in the CeA and BNST (Merlo Pich et al., 1995; Olive et al., 2002). Administered into the CeA, a CRF antagonist attenuated ethanol withdrawal-related anxiety (Rassnick et al., 1993). Increased ethanol consumption during protracted abstinence after induction of ethanol dependence was reduced by selective CRF_1 receptor antagonism, but in nondependent animals CRF antagonist failed to modulate ethanol intake, suggesting that CRF contributes to increased ethanol consumption induced by dependence (Funk et al., 2007). CRF_1 and CRF_2 receptors mediate their most physiologic functions in the brain via coupling to heterotrimeric G proteins and activating AC (Grammatopoulos, 2012). Increased cAMP levels then turn on a plethora of cytoplasmic (e.g., activation of PKA) and nuclear (e.g., nuclear factor κ-light-chain-enhancer of activated B cells (NFkB), ERK1/2, and GSK-3B/Wnt signaling) functions. Also, activation of the PLC-PKC pathway has been implicated in the dose dependently increasing effects of CRF on VTA DA neuron firing (Wanat et al., 2008). In midbrain slices from binge-drinking mice, CRF-potentiation of NMDAR responses was found in VTA DA neurons, and the binge drinking was significantly reduced by intra-VTA infusion of CRF_1 receptor antagonist CP-154526 [N-butyl-N-ethyl-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrrolo[2,3-d]pyrimidin-4-amine hydrochloride] (Sparta et al., 2013).

In the CeA, ethanol-induced enhancement of GABAergic transmission is mediated by CRF_1 receptors, perhaps even by CRF release, as shown by lack of ethanol-stimulated GABA release in CRF_1 receptor-KO mice and after CRF_1 but not CRF_2 receptor antagonists (Nie et al., 2004). Moreover, CRF_1-mediated ethanol effects on GABA in the CeA involve activation of the PKCe pathway (Bajo et al., 2008). In CeA slices of ethanol-dependent rats, the sensitivity of GABA release on CRF and CRF_3 antagonists was increased, and in vivo intra-CeA administration of a CRF_1 antagonist reversed dependence-related elevations in baseline extracellular GABA and blocked ethanol-induced increases in GABA in both dependent and nondependent rats. Chronic treatment with a CRF_1 antagonist blocked withdrawal-induced increase in alcohol drinking in ethanol-dependent rats (Roberto et al., 2010b). Collectively, these findings suggest a specific chronic ethanol-induced synaptic neuroadaptation in the interaction of CRF and GABAergic transmission that could contribute to the development and maintenance of ethanol dependence, together with other described functional alterations in the CeA (Hansson et al., 2006; Sommer et al., 2008). Also the BNST has a rich expression of CRF, and the region is well connected with many brain regions, including the midbrain DA neurons and the CeA. In the juxtacapsular nucleus of the anterior BNST, a form of LTP in response to HFS of the stria terminalis was impaired during withdrawal from chronic ethanol, and this impairment was reversed by CRF_1 receptor antagonism (Francesconi et al., 2009). Therefore, also the BNST CRF system may undergo persistent changes during development of ethanol dependence. Novel modulators have been found to play a role in reinstatement of alcohol drinking, because blockade of the relaxin-3 receptor RXFP3 by intra-BNST infusions of an antagonist attenuated cue- and stress-induced reinstatement of alcohol drinking in rats (Ryan et al., 2013). Importantly, the neurons in the brain stem-located nucleus incertus, which is the major source of relaxin-3 in the mammalian brain, are highly sensitive to stress and CRF (Banerjee et al., 2010; Smith et al., 2011; Farooq et al., 2013).

Another relevant peptide neurotransmitter system uses orexin A/hypocretin-1 and orexin B/hypocretin-2 peptides that are synthesized selectively in the lateral hypothalamus, perifornical area, and dorsomedial hypothalamus (de Lecea et al., 1998; Sakurai et al., 1998), with the lateral hypothalamus orexin system being most strongly associated with rewarding behaviors (Harris et al., 2005, 2007). In the VTA, both orexins and CRF have similar excitatory effects on DA neurons (Borgland et al., 2010). Orexin effects are mediated by OX_1 and OX_2 receptors, which mainly couple to Go_11 and PLC-PKC pathways. Both receptor subtypes appear to be expressed in various neuronal populations of the VTA, and both DA and GABA neurons increased their firing rates by orexins (Korotkova et al., 2003). The OX_1 receptor is expressed on VTA DA neurons. Its stimulation induces increased membrane targeting of the NMDARs, thereby potentiating their currents (Borgland et al., 2006). Importantly, initial findings on the effects of OX_1 receptor antagonists suggest that they do reduce alcohol drinking in some, but not all,
paradigms in rodent models (Lawrence et al., 2006; Moorman and Aston-Jones, 2009; Daher et al., 2010; Jupp et al., 2011; Voorhees and Cunningham, 2011). Further work is needed to establish the ways by which orexin antagonists could be useful in the treatment of alcohol abuse and other drug addictions. The OX₁ and OX₂ receptor antagonists have very strong effects on arousal and they thus might be emerging as the future generation of hypnotic drugs for insomnia (Michelson et al., 2014), provided they pass safety screening, such as possible induction of narcolepsy, which is related to deficiency of orexin and its receptors (Thannickal et al., 2000).

Other neuropeptides that have been shown to modulate the interaction of ethanol with GABAergic neurotransmission in the CeA include neuropeptide Y (NPY) and nociceptin/orphanin FQ (N/OFQ). NPY mediates many behavioral responses to ethanol (Thiele et al., 1998), and NPY injections into the CeA suppress ethanol drinking, particularly in ethanol-dependent animals (Gilpin, 2012). Superfusion of NPY decreased baseline CeA GABAergic transmission and reversed ethanol-induced enhancement of inhibitory transmission by suppression of GABA release through presynaptic GABA transporters (Gilpin et al., 2011). NPY is anxiolytic and may mediate acute ethanol-induced anxiolysis. By using histone deacetylase inhibitors, Sakharkar et al. (2012) showed in alcohol-prefering P rats that a rapid tolerance is produced to this anxiolytic effect during chronic ethanol treatment via possible epigenetic changes causing a reduction in NPY expression. It is quite possible that these epigenetic changes are far more frequent and affect the transcription of many genes important for plasticity (Nestler, 2014) and that epigenetic mechanisms can also contribute to genetic vulnerability to increased alcohol consumption, at least in animal models (Moonat et al., 2013).

Also the effects of the peptide nociception/orphanin FQ (N/OFQ) on GABAergic transmission are altered by chronic ethanol. N/OFQ decreases CeA GABAergic transmission by reducing GABA release, and this N/OFQ-induced decrease was more pronounced in ethanol-dependent rats (Roberto and Siggins, 2006). N/OFQ is the endogenous ligand of the GABAergic N/OFQ (NOP) receptor, also referred to as the opioid receptor-like 1 receptor. Because N/OFQ has been shown to attenuate many ethanol-related behaviors, including cue- and footshock stress-induced ethanol seeking and anxiety during ethanol withdrawal (Witkin et al., 2014), there has been a growing interest to test brain penetrating NOP receptor agonists for their effects on ethanol drinking. Recently, a novel NOP agonist, MT-7716 ((R)-2-[3-[1-acenaphthen-1-yl]piperidin-4-yl]-2-oxo-2,3-dihydro-1H-benzimidazol-1-yl]-N-methylacetamide hydrochloride hydrate), was shown to reduce ethanol drinking persistently even for 1 week after discontinuation of the drug and to attenuate ethanol somatic withdrawal symptoms (Cicoccioppo et al., 2014), presumably by inhibiting presynaptically GABA and glutamate release, as shown in the amygdala slices (Kallupi et al., 2014a,b). Furthermore, presumably via presynaptic mechanisms, NOP activation counteracts the CRF-induced increase in GABA_A receptor-mediated IPSPs in the CeA slices from naive rats and even more robustly in ethanol-dependent rats (Cruz et al., 2012), suggesting that dependent animals have developed sensitized responses to NOP activation in the amygdala.

A large body of evidence suggests that the endogenous opioid system is involved in the behavioral actions of ethanol. Acute ethanol stimulates the release of the endogenous opioid peptides β-endorphin, enkephalin, and dynorphin in the brain (Gianoulakis, 2001), and μ- and δ-opioid receptor antagonists decrease ethanol SA and seeking in many experimental models (Koob et al., 2003), suggesting that the GABAergic, mainly presynaptic, μ- and δ- receptors are involved in the mediation of the positive reinforcing effects of ethanol. These results have been translated into the treatment of alcoholism by using nonselective opioid receptor antagonists, naltrexone or nalmefene, to block alcohol reward either chronically or on demand (Volpicelli et al., 1992; Hyttia and Sinclair, 1993; Mann et al., 2013). Dynorphin, an endogenous ligand of the δ-receptor/dynorphin system is linked to aversive states, and accumulating data implicate the dynorphin/κ-receptor system in the processes of negative reinforcement in ethanol-dependent subjects (Sirohi et al., 2012). In animals chronically exposed to ethanol, both the predynorphin mRNA and dynorphin B expression was increased in the NAc (Przewlocka et al., 1997; Lindholm et al., 2000), suggesting an upregulation of the κ-receptor/dynorphin system during ethanol dependence, although the κ-receptor mRNA level was decreased (Rosin et al., 1999). The fact that either intracerebroventricular, intra-accumbal, or intra-amygdalar administration of κ-receptor antagonists selectively suppressed ethanol SA in dependent animals with no effect in nondependent subjects (Walker and Koob, 2008; Nealey et al., 2011; Kissler et al., 2014) suggests that the upregulated κ-receptor/dynorphin system contributed to escalation of ethanol intake after dependence induction. The functional consequences of the alteration in the κ-receptor/dynorphin system are not known, but increased signaling through the κ-receptors was shown to reduce DA release locally in the NAc (Spanagel et al., 1992) and in the PFC terminal area via inhibition of VTA DA neurons (Margolis et al., 2006), possibly leading to dysphoric and depression-like behavioral states. The transcriptional processes underlying these events may include the CREB that mediates the increases in the dynorphin levels in the mesolimbic DA system (reviewed in Sirohi et al., 2012). One of the important targets of CREB is the BDNF that modulates dynorphin
expression (Lonze and Ginty, 2002). Ethanol exposure increases BDNF expression in the dorsal striatum, which could lead to initiation of MAP kinase signaling and a subsequent increase in the downstream gene products, including preprodynorphin (McGough et al., 2004; Logrip et al., 2008). This could be one of the mechanisms contributing to the hypothesized dysregulation of the dynorphin signaling linked with ethanol dependence (Walker and Koob, 2008).

Ghrelin is a circulating gut-brain hormone that promotes food consumption through hypothalamic growth hormone secretagogue receptors (GHS-R1A) (Horvath et al., 2001). In addition to the role of ghrelin in body weight regulation, it has also been suggested to be involved in mediation of the reinforcing effects of drugs, including alcohol (Jerlhag et al., 2009). For example, GHS-R1A antagonists suppress alcohol intake in many animal models of alcohol SA, and a recent study demonstrated that no tolerance developed to repeated antagonist administration (Suchankova et al., 2013). The GHS-R1A receptors are also widely expressed in the mesocorticolimbic DA system, and the VTA expression of Ghsr was downregulated in the rats drinking high amounts of alcohol compared with the low-drinking individuals (Suchankova et al., 2013). Similar to ghrelin, other gut-brain hormones have also been implicated in the regulation alcohol drinking, including glucagon-like peptide 1, leptin, and galanin (Blednov et al., 2004; Lewis et al., 2004; Egecioglu et al., 2013). Also histamine has been as a gut-based mediator of smooth muscle function and a central neurotransmitter that participates in regulation of food intake (among a wide variety of important CNS roles), and recently it has been shown to modulate alcohol consumption and alcohol-induced place preference via H3R histamine receptors (Panula and Nuutinen, 2013).

5. Role of Acetaldehyde in Ethanol’s Reinforcing Actions. Although it is widely believed that the actions of the ethanol molecule itself are responsible for ethanol reinforcement, there is evidence to suggest that ethanol metabolism and its products, particularly acetaldehyde, could have a role in ethanol consumption and abuse (reviewed in Quertemont, 2004; Deng and Deitrich, 2008; Deehan et al., 2013). The bulk of ethanol is metabolized in the liver by alcohol dehydrogenase and aldehyde dehydrogenase (ALDH) enzymes. Alcohol dehydrogenase is not physiologically active in the brain. However, the first metabolic product, acetaldehyde, can also be formed in the brain through the peroxidative activity of catalase and by oxidation via other enzymes, such as the cytochrome P450 2E1. Significant acetaldehyde concentrations are formed in vitro in brain tissue at concentrations of ethanol achieved from voluntary consumption by rodents.

Although there are many demonstrations of the reinforcing actions of acetaldehyde in the brain, either by experimenter- or self-administered acetaldehyde (Deehan et al., 2013), the mechanisms of action remain to be clarified. Converging evidence suggests that acetaldehyde could contribute to ethanol’s stimulatory action on the VTA DA neurons. Acetaldehyde stimulates VTA DAergic activity at remarkably lower concentrations than ethanol in vitro (Brodie and Appel, 1998; Diana et al., 2008), and in vivo a catalase inhibitor applied in the VTA prevents ethanol-stimulated increase in DAergic activity (Melis et al., 2007; Diana et al., 2008). Further support for the involvement of catalase and acetaldehyde in ethanol’s central actions comes from studies using viral vectors altering catalase activity. For example, introduction of an anticalatalase viral vector into the VTA decreased ethanol drinking and ethanol-induced DA release in the NAc (Karahanian et al., 2011; Quintanilla et al., 2012). However, the DA-releasing effect of ethanol in the NAc is not mediated by acetaldehyde (Clarke et al., 2014). It is also to be noted that acetaldehyde is a highly reactive molecule that interacts with many endogenous substances in the brain to form additional biologically active products (Deehan et al., 2013).

Acetaldehyde’s putative central reinforcing actions notwithstanding, peripheral acetaldehyde is aversive. This is amply demonstrated by the ALDH blocking drug disulfiram (Antabuse, Odyssey Pharmaceuticals, East Hanover, NJ) that causes increased accumulation of acetaldehyde and thereby highly aversive effects shortly after ethanol consumption, thus curbing further drinking. In some Asian populations, the ALDH2*2 allele that encodes an inactive form of the mitochondrial ALDH2 enzyme, has a high prevalence. Individuals homozygotic for ALDH2*2 exhibit high blood acetaldehyde concentrations even after light to moderate alcohol drinking, and it has been shown that this allele strongly protects against alcoholism (Quertemont, 2004).

6. Structural Plasticity in Alcohol Dependence. Because dendritic spines are both structural and functional units of excitatory synapses and include many critical postsynaptic components of the synapse, any changes in spine number or morphology could have long-reaching consequences for the connectivity of neural networks. Similar to other drugs of abuse (reviewed in other sections), chronic ethanol regulates the spine dynamics in many brain areas important for learning, memory, reward, executive function, and stress (reviewed in Cui et al., 2013). The general picture emerging from these studies is that chronically administered ethanol decreases many measures related to dendrites and their spines, accompanied by functional or behavioral alterations. For example, withdrawal from long-term ethanol exposure decreases spine density in the amygdala, concomitant with increased anxiety and decreased BDNF-Arc signaling (Pandey et al., 2008). In the NAc, both chronic alcohol drinking and alcohol withdrawal decreased spine density, accompanied by reduction in NMDAR-mediated synaptic.
currents and hampered LTD (Zhou et al., 2007; Spiga et al., 2014). On the other hand, prolonged intermittent alcohol drinking in cynomolgus monkeys increased the density of dendritic spines and glutamatergic transmission in the putamen, but not in the caudate nucleus (Cuzon Carlson et al., 2011). Similarly, chronic intermittent alcohol exposure increased spine density in the mPFC in mice (Kroener et al., 2012). These findings suggest that the direction of alcohol-induced alterations in dendritic spine plasticity is dependent on brain region. The functional consequences of these structural alterations in the brain networks remain to be elucidated.

It is noteworthy that some structural changes seen in ethanol exposure in adult animals can also be seen if ethanol treatment has been given during the prenatal period. Prenatal ethanol treatment retarded the growth of the SN pars compacta TH-positive neurons and produced abnormalities in dendritic branching (Shetty et al., 1993). In the forebrain, ethanol exposure produced a sexually dimorphic effect on dendritic branching in the NAc and reduced spine density and distribution in the PFC (Lawrence et al., 2012). In humans, heavy ethanol consumption during pregnancy produces fetal alcohol spectrum disorders, of which the most severe form is the fetal alcohol syndrome. Although it is accepted that high ethanol doses can produce detrimental effects on brain development, the mechanisms remain elusive. Data from animal models suggest that mechanisms of ethanol action on the developmental trajectory of neural cells include alterations in many neurotransmitter and signaling systems, neural migration, neurogenesis, and synaptic plasticity (Valenzuela et al., 2012).

7. Conclusions. Ethanol exerts its acute and chronic effects via a number of molecular targets, altering synaptic transmission, brain function, and behavior (Fig. 11). Acutely, ethanol increases synaptic and extrasynaptic inhibition by enhancing GABAergic mechanisms and decreases excitation by inhibiting NMDARs. The net effect of these neurochemical events is dampening of synaptic excitation, which also either hampers or modulates various forms of synaptic plasticity. Chronic ethanol exposure leads to compensatory homeostatic effects in these neurotransmitter systems. Thus, the function of the NMDARs is enhanced and extracellular glutamate levels are elevated, contributing to neuronal hyperexcitability implicated in ethanol dependence and withdrawal. Chronic ethanol effects on GABAergic transmission lead to a hypofunction of GABAergic systems. Particularly in the extended amygdala, chronic synaptic neuroadaptations involve interactions of the GABAergic neurotransmission with various neuropeptides that either mediate or counteract ethanol-induced alterations. The between-systems adaptations could contribute to the development of ethanol dependence and mediate the interactions between stress, anxiety, and chronic ethanol effects. In addition, these neuropeptide systems could be important future targets for pharmacological treatment of alcoholism.

E. Benzodiazepines and Other GABAergic Drugs

Benzodiazepines (BZs) were introduced to clinical use as anxiolytics in the 1960s, and soon they displaced barbiturates and similar compounds as safer and more efficient anxiolytics and hypnotics. BZs were acutely well-tolerated and patients are usually compliant with the therapy. Unfortunately, anxiety did not always vanish. Soon, problems with their use emerged as some patients developed tolerance, and in many users attempts to withdraw from the medication lead to a return of the initial anxiety symptoms. This apparently led to a strong urge for continued use, with, in some patients, considerable difficulty in cutting down chronic BZ medication and possibly a negative effect on concurrent cognitive therapy (e.g., Westra and Stewart, 1998; Vorma et al., 2002). Whether BZ therapy persistently affects cognitive functions has been difficult to assess. For example, Puustinen et al. (2007) found no association of cognitive impairment (assessed with Mini-Mental State Examination) with long-term use of BZs in a nonrandomized study comparing patients admitted to acute hospital wards during 1 month with and without BZ treatment, whereas a small meta-analysis found significant impairments (Barker et al., 2004a). The long-term BZ effect was clearer when the reversal of cognitive impairment in different domains of neuropsychological tests was monitored during withdrawal from prolonged BZ use (Barker et al., 2004b). The results showed significant reversal during abstinence, which was not quite complete in comparison with the scores in controls or normative data. Recently, several epidemiologic studies reported increased risk of dementia symptoms in older BZ users (Billioti de Gage et al., 2015), with the risk increasing with cumulative dose and treatment time. Causality of the association is not yet known, because BZ drugs may also have some protective effect against Alzheimer’s disease (Fastbom et al., 1998) or be associated with insomnia and increased risk for this disease (Chen et al., 2012).

BZs are still used in clinical medicine as anxiolytics, sedatives, and hypnotics, although guidelines have suggested that their use in anxiety disorders should be indicated only in severe cases that do not respond to certain antidepressants (particularly serotonin selective reuptake inhibitors), gabapentin/pregabalin, or psychotherapy (Baldwin et al., 2014). BZ-site acting hypnotics, preferably short-acting ones, should be prescribed for shorter treatment periods with careful tapering off the dosages. Antihistamines and melatonin should be tested for insomnia and α2-adrenoceptor agonists or anesthetics be used for sedating agitated and aggressive patients before using BZs. Furthermore, nonpharmacological therapies, such as exposure and
cognitive psychotherapies, may provide good results for many patients with less severe insomnia (Hofmann and Smits, 2008).

Like all other abused drugs, only a portion of patients will show increase in tolerance and develop troublesome dependence during BZ therapy. Therefore, these efficacious drugs should not be fully forgotten (e.g., see Starcevic, 2014). Therefore, it is important to understand their mechanisms of action and their long-term effects on brain structure and function. In this section, we will also shortly review the effects of related drugs, such as GABA<sub>B</sub> agonists, γ-hydroxybutyrate (GHB), and anesthetetic agents.

1. Molecular Targets for Benzodiazepines. The GABA<sub>A</sub> receptor is the main fast-acting inhibitory neurotransmitter receptor in the mammalian brain (for reviews for molecular biology and receptor subtypes, genetics, structure, and function, see Sieghart, 1995; Whiting et al., 1995; Olsen and Sieghart, 2008, 2009; Uusi-Oukari and Korpi, 2010). It is a pentameric integral plasma membrane ligand-gated ion channel, assembled from 19 different subunits (integral plasma membrane ligand-gated ion channel, 2009; Uusi-Oukari and Korpi, 2010). It is a pentameric GABA<sub>A</sub> receptor is the main fast-acting inhibitory neurotransmitter receptor in the mammalian brain (for reviews for molecular biology and receptor subtypes, genetics, structure, and function, see Sieghart, 1995; Whiting et al., 1995; Olsen and Sieghart, 2008, 2009; Uusi-Oukari and Korpi, 2010). It is a pentameric integral plasma membrane ligand-gated ion channel, assembled from 19 different subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, and ρ1-3), which are products of different genes residing in several clusters in the human and rodent genomes (Olsen and Sieghart, 2008). Most GABA<sub>A</sub> receptors have the subunit structure of 2α-2β-γ/δ/ε.

Subunits are heterogeneously expressed in various brain cell types and regions, thus producing pharmacologically and functionally different GABA<sub>A</sub> receptor subtypes (Wisden and Seeburg, 1992; Luddens et al., 1995; Hevers and Luddens, 1998; Korpi et al., 2002; Sigel and Steinmann, 2012), and some of them are produced in alternative spliced or RNA edited isoforms.

Positive BZ-site modulators, such as diazepam, midazolam, and zolpidem, typically need the α and γ subunits for their allosteric actions increasing affinity of GABA binding (Sigel, 2002). The most important site of the receptor molecule for BZ binding is the “100th” residue of the α subunits, which is histidine (His) in all diazepam-sensitive receptors with α1, α2, α3, and α5 subunit-containing receptors and arginine in diazepam-insensitive α4 and α6 subunit-containing receptors (Luddens et al., 1995). This residue was originally detected in an “abnormal” subunit α6 (Luddens et al., 1990; Wieland et al., 1992), with restricted expression to cerebellar granule neurons. Receptors with this subunit do not bind diazepam but bind an inverse agonist imidazobenzodiazepine Ro 15-4513, correlating with the pharmacological profile of the cerebellar granule cell layer (Sieghart et al., 1987; Malminieni and Korpi, 1989; Uusi-Oukari and Korpi, 1990; Turner et al., 1991). Furthermore, a spontaneous mutation changing arginine (Arg) to glutamine (Gln) was detected in the cerebellar α6 subunit of alcohol-sensitive, and particularly BZ-sensitive, ANT rat line (Korpi et al., 1993; reviewed in Korpi et al., 2007). Glutamine behaved in the receptor similarly as histidine, drastically increasing the receptor sensitivity to benzodiazepines for binding and positive allosteric modulation (Korpi et al., 1993). These observations were extended by Uwe Rudolph, Hanns Möhler, and colleagues by using His to Arg knock-in mutations for understanding the roles of various BZ-sensitive GABA<sub>A</sub> receptors in the behavioral and physiologic effects of diazepam in mouse models (Table 2 with the references). Importantly, in mice, these findings suggested that anxiolytic actions could be separated from sedative actions, because the former effects were absent in α2(His101Arg) knock-in mice, whereas the α1(His101Arg) mice exhibited limited sedative effects of diazepam (Low et al., 2000) or even hyperactive phenotype after a sedative dose of diazepam (McKernan et al., 2000). These mouse models have also indicated that tolerance and dependence-inducing effects of diazepam are dependent on α1 and α5 subunit-containing receptors (van Rijnsoever et al., 2004; Tan et al., 2010). All these effects are also associated with γ2 subunit-containing receptors, because this subunit is obligatory for BZ binding (Pritchett et al., 1989). Because the γ2 subunit gives the subcellular address for GABA<sub>A</sub> receptors to be trafficked into inhibitory synapses (Essrich et al., 1998), all these knock-in mouse models are related to BZ actions on phasic synaptic inhibition.

More recently, the focus has moved to extrasynaptic GABA<sub>A</sub> receptors, which usually contain the δ subunit instead of γ2, with preferential expression together with either α6 (in cerebellum) or α4 (in forebrain) subunits (Wisden et al., 1992; Jones et al., 1997). These receptors do not form high-affinity BZ binding sites (but see Wallner et al., 2006), but they form a receptor population with high GABA sensitivity, low conductance, and slow desensitization, and their main function is to provide tonic extrasynaptic inhibition regulated by ambient GABA (Mody, 2001; Stell and Mody, 2002; Farrant and Nusser, 2005). When this kind of tonic inhibition is abolished from the cerebellar granule cells by genetically knocking out the α6 subunit, it is compensated by increased expression of two-pore leak K<sup>+</sup> channels of TASK-1 type (Brickley et al., 2001). For these neurons, it has been estimated that the charge transfer via tonic inhibition is at least 10 times greater than that via phasic synaptic inhibition (Brickley et al., 1996), further indicating the physiologic importance of tonic inhibition. The δ subunit-containing receptors have high affinity to GABA-site agonists muscimol and gaboxadol, which are less potent in inducing behavioral effects in GABA<sub>A</sub> δ-KO mice (Chandra et al., 2010). Also, the effects of neurosteroid agonists, such as ganaxolone, are reduced in δ-KO mice (Mihalek et al., 1999).

The peripheral BZ receptor (PBR) has been detected in many tissues using the same BZ ligands as for GABA<sub>A</sub> receptors. The PBR is now known to be the mitochondrial translocator protein (TSPO), which is believed to be particularly involved in cholesterol
transport into inner mitochondrial matrix for the initial step of steroidogenesis by P450 side chain-cleavage enzyme producing pregnenolone (reviewed in Rupprecht et al., 2010). TSPO-KO from testicular Leydig cells, however, failed to affect steroidogenesis (Morohaku et al., 2014). In the brain, the PBR is enriched in glial cells, astrocytes, and microglia, but it is also found in some principal neurons. Although most actions of BZs can be explained by facilitation of GABA_A receptors (see Table 2), it is possible that they also partly act via stimulation of neurosteroid synthesis (progesterone = > allopregnanolone; deoxycorticosterone = > 3α,5α-tetrahydrodeoxycorticosterone) as recently shown for some brain mechanisms with a neurosteroid synthesis blocker finasteride abolishing the effects of BZs (Rupprecht et al., 2009; Tokuda et al., 2010). Importantly, these neurosteroids have rapid effects via allosteric modulation of GABA_A receptors. Novel TSPO ligands have shown efficacy in reducing anxiety in animal models and initial human experiments (Rupprecht et al., 2010), in the absence of affinity to GABA_A receptors. TSPO ligands are being also developed as PET ligands to monitor glial cell dynamics in vivo in the brain, including microglial activation as a measure of neuroinflammation (e.g., Van Camp et al., 2010).

2. Neuroplasticity Induced by Benzodiazepines and Related Compounds. Sedative compounds like BZs are generally known to depress brain activity, both in animal models and humans (Kelly and McCulloch, 1982; Kelly et al., 1986; Wang et al., 1996; Freo et al., 2008). Ito et al. (1994) compared clonazepam (that acts selectively on BZ sites of the GABAA receptor, not on TABLE 2

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Subtypes</th>
<th>Location</th>
<th>Behavior/Physiology</th>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1</td>
<td>α1βγ2</td>
<td>60%, syn and extrasyn</td>
<td>BZ: sedation, anterograde amnesia, anticonvulsant effect, addiction, reward, anxiety?</td>
<td>s.d.: zolpidem</td>
<td>(Low et al., 2000)</td>
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<tr>
<td></td>
<td>α1βδ</td>
<td>all brain regions, incl. interneurons</td>
<td>α1(His101Arg)</td>
<td>(Smith et al., 2012)</td>
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<td></td>
<td></td>
<td></td>
<td>- developmental plasticity</td>
<td>α2(β101Arg)</td>
<td>(Crestani et al., 2001)</td>
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<td></td>
<td></td>
<td></td>
<td>- depression of brain activity?</td>
<td>α2-KO</td>
<td>(Blednov et al., 2013)</td>
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<td></td>
<td></td>
<td></td>
<td>- conditioned fear</td>
<td>(Smith et al., 2012)</td>
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<td></td>
<td></td>
<td></td>
<td>- neuroplasticity of VTA DA neurons</td>
<td>α2-KO</td>
<td>(Reynolds et al., 2012)</td>
</tr>
<tr>
<td>α2</td>
<td>α2βγ2</td>
<td>syn, limbic areas, cortex, principal neurons</td>
<td>BZ: anxiety, analgesia, anesthesis, myorelaxation, addiction?</td>
<td>s.d.: (Low et al., 2000)</td>
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<td></td>
<td></td>
<td></td>
<td>- adult neurogenesis</td>
<td>α3(β126Arg)</td>
<td>(Crestani et al., 2001)</td>
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<td></td>
<td></td>
<td></td>
<td>- CTA to ethanol</td>
<td>α3-KO</td>
<td>(Christian et al., 2013)</td>
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<td></td>
<td></td>
<td></td>
<td>- conditioned fear</td>
<td>(Reynolds et al., 2012)</td>
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<td></td>
<td></td>
<td></td>
<td>- reduction of ICSS threshold</td>
<td>α3-KO</td>
<td>(Witschi et al., 2011)</td>
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<td></td>
<td></td>
<td></td>
<td>(reward)</td>
<td>(Knabl et al., 2008)</td>
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<td></td>
<td></td>
<td></td>
<td>- thalamocortical oscillations, regulated by endozepines?</td>
<td>(Duveau et al., 2011)</td>
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<td></td>
<td></td>
<td></td>
<td>- sensory-motor gating</td>
<td>(Kristna et al., 2012)</td>
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<tr>
<td>α3</td>
<td>α3βγ2</td>
<td>syn and extrasyn, limbic areas, monoamine neurons, reticular nucleus of thalamus, principal neurons</td>
<td>BZ: myorelaxation, analgesia, anesthetic, addiction?</td>
<td>s.d.: TP003</td>
<td>(Low et al., 2000)</td>
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<td></td>
<td></td>
<td></td>
<td>- myorelaxation</td>
<td>α4(β126Arg)</td>
<td>(Crestani et al., 2001)</td>
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<td></td>
<td></td>
<td></td>
<td>- anxiety, anesthesis</td>
<td>α4-KO</td>
<td>(Christian et al., 2013)</td>
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<td></td>
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<td>- conditioned fear</td>
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<td>- reduction of ICSS threshold</td>
<td>α4-KO</td>
<td>(Knabl et al., 2008)</td>
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<td>(reward)</td>
<td>(Yee et al., 2005)</td>
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<td></td>
<td></td>
<td></td>
<td>- sensorimotor gating</td>
<td>(Sohal et al., 2003)</td>
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<tr>
<td>α5</td>
<td>α5βγ2</td>
<td>extrasyn, hippocampus, cortex, principal neurons</td>
<td>BZ: learning and memory</td>
<td>zolpidem-4S</td>
<td>(Hauser et al., 2005)</td>
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<td></td>
<td></td>
<td></td>
<td>- sensorimotor gating</td>
<td>s.d.: L-655708</td>
<td>(Yee et al., 2004)</td>
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<td></td>
<td></td>
<td></td>
<td>- BZ tolerance</td>
<td>α5(β105Arg)</td>
<td>(van Rijnsoever et al., 2004)</td>
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<td></td>
<td></td>
<td></td>
<td>α5-KO</td>
<td>(Crestani et al., 2002)</td>
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<tr>
<td>α4, δ, θ</td>
<td>α4/6βγ2</td>
<td>syn (γ2), extrasyn (δ), cerebellum (α6), forebrain (α4)</td>
<td>BZ-IS</td>
<td>s.d.: gaboxadol, neurosteroids</td>
<td>(Duveau et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>α4/6βδ</td>
<td></td>
<td>sedation, sleep, anesthesia</td>
<td>α4-KO</td>
<td>(Vashchinkina et al., 2012, 2014a)</td>
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<td></td>
<td></td>
<td></td>
<td>- tonic inhibition</td>
<td>α6-KO</td>
<td>(Stell et al., 2003)</td>
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<td>- depression of brain activity</td>
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<td>- adult neurogenesis</td>
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<td>(Jones et al., 1997)</td>
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<td></td>
<td></td>
<td></td>
<td>(α4)</td>
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<td>(Brickley et al., 2001)</td>
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</table>

BZ, benzodiazepine; CTA, conditioned taste aversion; ICSS, intracranial self-stimulation; KO, knockout; IS, insensitive; s.d., selective drug; syn, synaptic; extrasyn, extrasynaptic; VTA, ventral tegmental area.

*aReceptor subtypes are from the review of Olsen and Sieghart (2008).
TSPO) and the GABA-site agonist muscimol in rats using \[^{14}\text{C}]\text{deoxy-d-glucose autoradiography, and found reductions of brain glucose uptake by both of them. However, although clonazepam reduction plateaued at 30\% reduction, the effect of muscimol was linearly related to estimated receptor occupancy at least until 60\% reduction of glucose uptake. These results suggest a major difference in how ligands affecting preferentially synaptic and extrasynaptic \(\text{GABA}_A\) receptors influence brain metabolism. In addition, these results generally agree with depressed neuronal activation after treatments with \(\text{GABA}_A\) agonist drugs using immediate early gene assays, such as immunohistochemistry of c-Fos expression (see below).

Acute BZs generally depress also gene expression in the brain (Huopaniemi et al., 2004), except for the CeA (Beck and Fibiger, 1995; Hitzemann and Hitzemann, 1999; Shaw et al., 2011; Panhelainen and Korpi, 2012). Induction of immediate early genes in the CeA is common for various anxiolytics, but not specific for them (Thompson and Rosen, 2006). Huopaniemi et al. (2004) used a high dose of diazepam in mice (30 mg/kg) and found a short reduction in expression of neuroplasticity-related genes such as BDNF and c-Fos, but, interestingly, the expression of \(\text{CaMKII}\alpha\) remained diminished for at least 40 hours after a single diazepam administration. These effects on gene expression were not observed in \(\alpha_1(\text{His101Arg})\) knock-in mice. Thus, ligands promoting the activity of the main type of \(\text{GABA}_A\) receptor (\(\alpha_1\) subunit-containing) depress brain activity and gene expression, and by that they might not be expected to induce neuroplasticity changes in the brain. Acute BZ treatments might thus strongly prevent neuroplasticity. In addition, Licata et al. (2013) found that single doses of triazolam and zolpidem reduced BDNF in the mouse HC, but unexpectedly a 7-day twice daily treatment with diazepam or zolpidem failed to change BDNF expression. However, concurrent 2- to 3-week diazepam treatment prevents the stimulation of neurogenesis by fluoxetine in normal rats (Wu and Castren, 2009) and in socially isolated anxious rats (Sun et al., 2013). It remains to be tested whether even a single administration of BZs would prevent antidepressant-induced neuroplasticity in the rat models.

Acute effects of BZs on DA release and synthesis have been contradictory, not fully supporting any role for midbrain DA neurons in reinforcing and dependence-producing effects of BZs. However, Heikkinen et al. (2009) observed similar increase in AMPAR/NMDAR current ratios of VTA DA neurons ex vivo 1–3 days after a single administration of diazepam and the \(\text{GABA}_A\) receptor \(\alpha_1\) subunit-preferring zolpidem. This effect could be blocked by the benzodiazepine-site antagonist flumazenil and, interestingly, by the NMDAR antagonist dizocilpine. This finding was confirmed and extended by Tan et al. (2010) by showing that midazolam, either systemically or intra-VTA, induced a persistent change in DA neuron glutamate synapses. AMPAR-mediated responses showed inward rectification at positive holding potential in the presence of intracellularly added polyamines, indicating increased targeting of Ca\(^{2+}\)-permeable non-GluA2 AMPA receptor subunits (Jonas and Burnashev, 1995; Dingleedine et al., 1999). Furthermore, they showed that the primary target of midazolam was the local \(\text{GABA}_A\)ergic interneuron expressing \(\alpha_1\) subunit-containing \(\text{GABA}_A\) receptors. This result was confirmed by using \(\alpha_1(\text{His101Arg})\) mutant mice, indicating that the effect by BZs was mediated by disinhibition of DA neurons. In proof, by recording spontaneous IPSCs, Tan et al. (2010) observed that midazolam increased the phasic synaptic inhibition of the VTA \(\text{GABA}_A\) neurons, whereas at the same time the synaptic inhibition of the DA neurons was diminished. Furthermore, in \(\alpha_1(\text{His101Arg})\) mouse midbrain slices, midazolam was ineffective in inhibiting \(\text{GABA}_A\) neurons, which was now accompanied with greater inhibitory current in DA neurons.

These recent findings clearly indicate that BZ addiction may also have a similar positive reinforcing component as most other drugs of abuse (e.g., see Ator et al., 2005; Straub et al., 2010). This component adds to the better-known negative reinforcement, such as BZ-induced relief from punishment or from anxious dysphoria, guilt, and shame. It is also plausible that BZs have more widespread neuroplasticity effects in other brain regions such as the HC (for chronic BZ withdrawal effects, see Shen et al., 2009), although the persistency and efficacy of single versus chronic dosing are still poorly known. However, it should be kept in mind that BZ agonists are especially efficient in reducing conflict arising from internal or external cues in simple and more complicated experimental animal tests, such as Vogel and Geller-Seifter conflict tests (Nazar et al., 1997; Millan and Brocco, 2003), and conditioned taste aversion and neophobia tests (Yasoshima and Yamamoto, 2005; Wislowska-Stanek et al., 2008; Callaerts-Vegh et al., 2009). Furthermore, the BZ lorazepam can reverse punishment-suppressed operant responding for the opioid remifentanil in rats (Panlilio et al., 2005), indicating its efficacy in reversing the aversion-induced change in behavior (a conflict situation, punishment by mild footshocks). However, it was inefficient in reinstating extinguished responding for remifentanil, whereas heroin was effective in both reversing punishment-suppressed and extinguished responding. Thus, BZs are efficient in counteracting learned aversion-induced behaviors, by negative reinforcement, and in addition to their direct reinforcing effect. Most likely, other related anxiolytic \(\text{GABA}_A\) agonists and other drugs showing efficacy in conflict situations (Millan and Brocco, 2003) would reinstate punishment-suppressed responding. Whether this effect is a long-lasting one is not known. Anyway, the possibility that an abstinent alcoholic or a drug addict might lose the abstinence-promoting effect...
of social control/punishment should be taken into account when prescribing anxiolytic drugs. One interesting example of the short-term BZ effect is the midazolam-induced attenuation of conditioned (LiCl, i.p.) taste aversion to sucrose, which unfortunately returned back to full aversion in the next session (Yasoshima and Yamamoto, 2005).

Does the acute stimulation of tonic extrasynaptic GABAergic inhibition by selective drugs induce neuroplasticity in VTA DA neurons? This question was studied by Vashchinkina et al. (2012, 2014a). First, the GABA-site agonist gaboxadol (4,5,6,7-tetrahydroisoxazolo(5,4-c) pyridin-3-ol), which is a superagonist at δ subunit-containing receptors, whereas GABA is a partial agonist (Storustovu and Ebert, 2006; Saarelainen et al., 2008), induced prolonged (>6 days) increase in AMPAR/NMDAR current ratios and AMPAR current rectification in VTA DA neurons 24–144 hours after a single sedative dose in a dose-dependent manner (Vashchinkina et al., 2012). Second, this finding was extended to a neurosteroid agonist ganaxolone, which does not have genomic effects like endogenous neurosteroids (Carter et al., 1997). A single mildly sedative dose of ganaxolone induced a similar long-lasting neuroplasticity in VTA DA neurons ex vivo as a dose of gaboxadol (Vashchinkina et al., 2014a). By using a GAD67-GFP knock-in mouse line, in which GABA neurons are fluorescently labeled, ganaxolone in vitro provoked a fourfold increase in tonic GABAA-GABA neurons are fluorescently labeled, ganaxolone in vivo as a dose of gaboxadol (Vashchinkina et al., 2014a). However, the level of δ subunit protein expression in VTA GABA neurons is not very high, at least compared with the cerebellar granule neurons, and, therefore, direct immunohistochemical demonstration of its presence has failed because of background labeling even in the δ-KO mice by available δ subunit antibodies (unpublished). These results indicate that drugs potentiating tonic inhibitory conductance also target primarily GABAergic interneurons in the VTA and induce neuroplasticity effects in DA neurons by disinhibition.

Surprisingly, both gaboxadol and ganaxolone failed to induce CPP, and if anything they induced aversion (Vashchinkina et al., 2012, 2014a). Gaboxadol failed to sustain the acute nose-poking response in mice using the yoked control paradigm of intravenous administration over a wide dose range nor was it self-administered in cocaine-primed baboons, although the BZ agonist triazolam could replace cocaine (Vashchinkina et al., 2012). In line with the electrophysiological results, conditioned aversion to ganaxolone was abolished in δ-KO mice (Vashchinkina et al., 2014a). Because a short aversive swimming stress induces neuroplasticity in VTA DA neurons (Saal et al., 2003), it appears possible that there are several populations of midbrain (and VTA) DA neurons that may be similarly/differentially adapted to various rewarding and aversive stimuli (Matsumoto and Hikosaka, 2009; Bromberg-Martin et al., 2010; Lammel et al., 2011, 2012; Fiorillo, 2013; Fiorillo et al., 2013; Jennings et al., 2013; Ilango et al., 2014). It is also possible that triggering conditioned aversion takes place primarily in other brain regions, such as the BNST (Kim et al., 2013b) and/or the LHB (Matsumoto and Hikosaka, 2007; Hikosaka, 2010; Stamatakis and Stuber, 2012), which have reciprocal connections with the VTA.

Ca2+-permeable NMDARs are often involved in LTP, but in NMDAR-independent forms of LTP, VGCC are important (Morgan and Teyler, 1999; Bauer et al., 2002; Schroeder and Shinnick-Gallagher, 2004; Bloodgood and Sabatini, 2007). Tietz et al. (see below for references) made a series of detailed experiments on the mechanisms of tolerance to and withdrawal from subchronic flurazepam treatment in the hippocampal CA1 area. They treated rats with a high oral dose of flurazepam for at least 1 week and studied the brains under tolerant and 1–2 day withdrawal conditions. Several interesting transient changes were observed in their studies. Downregulation of GABA_A receptor-associated ligand binding and GABA_A receptor subunit levels took place in several brain regions of tolerant animals (Rosenberg et al., 1985; Tietz et al., 1999) (for a review on GABA_A subunit expression after various GABA_A drugs, see Uusi-Oukari and Korpi, 2010). Surprisingly, the changes in the GABA_A system and tolerant behavior were abolished in the tolerant, withdrawal BZ-free state by a single administration of the BZ antagonist flumazenil (Tietz et al., 1999), with the testing being carried out 1 day after the injection of the short-acting flumazenil. Increased AMPAR currents and binding sites were detected in the hippocampal CA1 and CA3, but not dentate gyrus, region of withdrawing animals (Van Sickle and Tietz, 2002), the increased currents correlating with increased withdrawal anxiety (Xiang and Tietz, 2007; Xiang et al., 2008). GluA1, but not GluA2, subunit protein was increased synaptically (Das et al., 2008) because of GluA1 subunit phosphorylation at Ser831 by CaMKII (Earl et al., 2012). Whereas the increase in AMPAR function could not be abolished by the NMDAR antagonist dizocilpine (MK-801), it was abolished by the L-type VGCC blocker nimodipine, which also reversed withdrawal anxiety. At day 2 of withdrawal, the levels of GluN1 and GluN2B subunits and NMDAR function were also downregulated, which could be reversed by blocking AMPARs and their upregulation (Van Sickle et al., 2004; Xiang and Tietz, 2007), suggesting that the change in AMPAR was the primary alteration in the glutamate system in BZ tolerance. These results
demonstrate an important cascade of transient (1–4 days) functionally interrelated changes in the hippocampal neurons that are caused by chronic, but not acute (Van Sickle et al., 2002), BZ administration. These results suggest that even chronic heavy BZ treatment induces rather short-lasting effects at least on behavior and HC, but that they also show some potential for inducing neuroplasticity.

3. Behavioral After-effects of \(\gamma\) -Aminobutyric Acid A Drugs. Once the single-dose neuroplasticity effect of the BZ agonists was found (Heikkinen et al., 2009; Tan et al., 2010), it was of interest to study whether behavioral responses to other drugs of abuse would be affected by pretreatment (and neuroplasticity effects) of BZs. Panhelainen et al. (2011) gave a single injection of diazepam (5 mg/kg i.p.) and tested adolescent and adult mice for psychomotor stimulation by three daily doses of morphine and d-AMPH. In contrast to a hypothesis of enhanced psychomotor activation, based on the sensitizing effects of viral-mediated upregulation of GluA1 subunit in the midbrain (Carlezon and Nestler, 2002), we found that diazepam pretreatment reduced locomotor responses to morphine (in both sexes) and attenuated behavioral locomotor sensitization to d-AMPH, with the effects being detectable, although slightly diminished, also in adult mice (Panhelainen et al., 2011). The effects on d-AMPH-induced sensitization could be blocked by a single injection of dizocilpine prior to diazepam pretreatment. These preliminary results suggest that occlusion of neuroplasticity attenuates behavioral effects induced by acute and repeated morphine and amphetamine.

Because gaboxadol also induces neuroplasticity in VTA DA neurons, which lasted even longer than that induced by diazepam (Vashchinkina et al., 2012), we repeated Panhelainen’s study (Panhelainen et al., 2011) to test whether the pretreatment with gaboxadol would also affect the psychomotor stimulation by morphine and d-AMPH. However, we observed no alterations in the responses in adolescent or adult mice (unpublished results). This suggested that rewarding BZs and aversive gaboxadol might induce neuroplasticity in different populations of VTA DA neurons, but preliminary examination did not indicate any regional variation in the VTA of DA neurons with altered glutamate transmission after gaboxadol (Vashchinkina et al., 2012). It is also possible that GABA_A receptor drugs persistently affect many brain regions other than the VTA. To start examining this possibility, we counted c-Fos positive neurons in averse-associated brain regions 2 hours after a single injection (6 mg/kg) of gaboxadol (when sedation was gone), and, interestingly, found a significant activation exclusively in the oval part of the BNST (E. Vashchinkina et al., submitted manuscript). In parallel behavioral tests, gaboxadol provoked a mild anxiety-like withdrawal state, which was preceded by transiently increased plasma corticosterone level.

In addition, we examined whether selective blockade of CRF_1 receptors would attenuate gaboxadol plasticity in VTA DA neurons, because CRF signaling is often involved in oval BNST-mediated behaviors (Erb et al., 2001b; Beckerman et al., 2013). Indeed, gaboxadol-induced VTA DA neuron plasticity was totally blocked by pretreatment with CP-154526, a selective CRF_1 receptor antagonist (Shaham et al., 1998) (E. Vashchinkina et al., submitted manuscript). Thus, VTA DA plasticity and anxiogenic/aversive consequences of activation of extrasynaptic GABA_A receptors seem to be linked to activation of oval BNST and CRF_1 signaling.

In contrast to gaboxadol experiments, 2 hours after diazepam administration, a strong increase in the number of c-Fos-positive cells was found in the CeA (Panhelainen and Korpi, 2012). In addition, by using the Golgi method for counting dendritic spines, we found that a single dose of gaboxadol increased spine counts in the HC, whereas diazepam was inactive (Vashchinkina et al., 2014b). These results suggest that the effects on brain-wide cellular activity are different in diazepam- and gaboxadol-treated mice, making it possible that synaptic and extrasynaptic GABA_A neurotransmissions modulate different pathways to the VTA DA neurons.

4. Effects of Flumazenil on Benzodiazepine and Alcohol Tolerance. Flumazenil (Hunkeler et al., 1981) is acutely very efficient and specific in clinically antagonizing the actions of all BZ-site agonists, whether they have the BZ structure or other chemical structures, such as zolpidem and zopiclone. It is available as intravenous injection and its half-life is short (about 1 hour) in humans (Klotz et al., 1984; Roncari et al., 1993). It is primarily used in BZ intoxications or in the treatment and diagnosis of drug intoxications (Hoffman and Warren, 1993; Kreshak et al., 2012). As a competitive BZ antagonist, its acute actions can be well understood as counteracting the effects of agonists.

Interestingly, flumazenil has shown variable intrinsic activity in preclinical and clinical settings, varying from weak partial agonism (positive allosteric modulation) to partial inverse agonism (negative modulation) (e.g., File et al., 1982a,b; Nutt et al., 1990; Da Cunha et al., 1992; Smolnik et al., 1998; Izumi et al., 1999; Goulenok et al., 2002). Its intrinsic efficacy has been explained by antagonism of endogenous positive or negative modulators. Indeed, there is evidence for endogenous production of compounds with binding and functional activity at BZ sites (“endozepines”), such as diazepam and its metabolite desmethyl-diazepam (Sangameswaran et al., 1986; Klotz, 1991), and molecules with other structures, including heme derivatives (Ruscito and Harrison, 2003) and the diazepam binding inhibitor (DBI) and its proteolytic peptide fragments, such as octadecaneuropeptide (Alho et al., 1985; Costa and Guidotti, 1991). Recently, DBI and octadecaneuropeptide have been shown to increase neurogenesis in postnatal mouse subventricular zone by negative
modulation of GABA_A receptors (Alfonso et al., 2012), and, surprisingly, DBI was described to positively modulate reticular thalamic α3 subunit-containing GABA_A receptors, an effect that appears to be needed for antiabsence seizure effect in a mouse model (Christian et al., 2013). Production of endozepines is especially high during acute hepatic failure with and without encephalopathy (Olasmaa et al., 1990; Avallone et al., 1998). This state is also associated with increased levels, expression, and function of the DBI peptide and TSPO protein, which also leads to increased levels of neurosteroids THDOC and allopregnanolone (Rothstein et al., 1989; Goulenok et al., 2002), although it does not counteract the effects of neurosteroids. Flumazenil has a therapeutic effect in patients with hepatic encephalopathy and coma (Bansky et al., 1989; Goulenok et al., 2002), although it does not counteract the effects of neurosteroids. Although the setpoint of the GABA_A receptors determining positive or negative BZ modulation might be different between different subjects or it might change because of illness or drug treatment, it is possible that at least a part of the intrinsic activity of flumazenil can be accounted for by antagonism of various endogenous ligands.

In regard to the present review, a most interesting finding has been the efficacy of flumazenil to reverse BZ and alcohol tolerance and dependence in preclinical models by a single dose or by a pretreatment (Buck et al., 1991; Flaishon et al., 2003; but see Nutt and Costello, 1988; Loscher and Rundfeldt, 1990; Tietz et al., 1999). Although this phenomenon has been tested in BZ-dependent patients with inconsistent efficacy for countering tolerance in acute settings (Cittadini and Lader, 1991; Savic et al., 1991; Lader and Morton, 1992), a small clinical study has reported preliminary results on prolonged subcutaneous flumazenil administration in withdrawing BZ-dependent patients (Hulse et al., 2013), suggesting that perhaps longer treatments are needed in human populations. Another study using twice daily intravenous injections of flumazenil showed that flumazenil significantly blocked withdrawal symptoms and kept the patients better in abstinence (Gerra et al., 2002). Because flumazenil increases membrane targeting of GABA_A receptors (Flaishon et al., 2003; Pericic et al., 2004), its efficacy in reversing BZ tolerance and in alleviating withdrawal in dependent subjects may at least partly be based on increased number of functional GABA_A receptors.

5. Treatment of Addictions by γ-Aminobutyric Acid B Receptor Agonists. Although the ligand-gated anion channel GABA_A receptors mediate fast phasic inhibition and prolonged tonic inhibition in the mature nervous system, the GPCR metabotropic GABA_B receptors mediate neuronal inhibition by slower actions on neuronal metabolism and K' and Ca^{2+} channels both presynaptically at inhibitory and excitatory terminals and postsynaptically on dendrites and spines (Bowery et al., 2002; Bettler et al., 2004). GABA_B receptors are heterodimers of two subunits, GABA_B1 and GABA_B2, that are large (110 kDa) proteins with seven transmembrane domains and long extracellular N terminus. GABA_B1 seems to be responsible for GABA binding and GABA_B2 in turn for G protein coupling (Bettler et al., 2004). Knockout of either subunit eliminates the typical GABA_B receptor responses (Schuler et al., 2001; Gassmann et al., 2004). In the simplest case, activation of presynaptic GABA_B autoand heteroreceptors inhibits, via released G_i/o protein βγ subunits, the P/Q-type (Ca_{2.1}) and N-type (Ca_{2.2}) currents of high VGCC. Stimulation of postsynaptic GABA_B receptors activates in the same way multiple types of K' channels, including inwardly rectifying Kir3 channels. After both presynaptic and postsynaptic activation, there is the release of Gbg subunits that inhibits AC, leading to reduced cAMP production and PKA activity (Bettler et al., 2004; Koyrakh et al., 2005). The signaling of GABA_B receptors is most likely more complicated and neuronal cell-population specific because of differences in the receptor's cell surface expression and localization (Becher et al., 2004), isoform diversity (Bettler et al., 2004), crosstalk with various ionotropic (e.g., GABA_A, NMDA), and metabotropic (e.g., mGlu, TrkB) receptors (reviewed in Xu et al., 2014) and GABA_B receptor interacting proteins. Notably, GABA_B receptors interact with several other proteins, including activating transcription factor 4, inducing a novel signaling pathway (Nehring et al., 2000; Vernon et al., 2001; Chen et al., 2003), regulators of G protein signaling (RGS) (Labouebe et al., 2007), and, most recently, potassium channel tetramerization domain-containing proteins that bind to the intracellular carboxy terminus and alter agonist sensitivity and desensitization (Schwenk et al., 2010). In relation to neuroplasticity, it is important to note that GABA_B receptor activation reduces the Ca^{2+} flux via NMDARs both by producing hyperpolarization due to opening of K' channels and/or by reducing the activities of AC and PKA, which reduces the phosphorylation of NMDAR subunits (Morrisett et al., 1991; Chalifoux and Carter, 2010).

Activation of GABA_B receptors inhibits the spontaneous pacemaker-like firing of VTA DA neurons ex vivo (Seutin et al., 1994; Wu et al., 1999) and attenuates the firing rate and burst firing of these neurons in vivo (Erhardt et al., 2002). Therefore, facilitation of the GABA system is being tested for the treatment of various drug addictions, with two main lines of approach. Stimulation of GABA_B receptors reduces rewarding effects and consumption of several addictive drugs in various preclinical models (Roberts, 2005; Vlachou and Markou, 2010), but so far the results from the treatment trials on human addictions, especially on alcoholism, with the selective direct sedative agonist baclofen ([3R]-4-amino-3-(4-chlorophenyl)butanoic acid) have been inconsistent (Tyacke et al., 2010; Agabio et al., 2013; Brennan et al., 2013; Agabio and Colombo, 2014). To date, baclofen is the only GABA_B receptor agonist approved in clinical use, in addition to γ-hydroxybutyrate (see below).
The research focus is now on positive allosteric modulators that act on a GABA\textsubscript{B} receptor site distinct from GABA and baclofen (Pin and Prezeau, 2007). Because positive allosteric modulators facilitate the effects of GABA-site ligands (Jensen and Spalding, 2004), they alter activated receptors only. Hence they rather enhance the physiologic pattern of GABA\textsubscript{B} receptor activation, and so may have less adverse effects than ligands that alter all GABA\textsubscript{B} receptors. In agreement, allosteric modulators interfere less with locomotor activity (Ong and Kerr, 2005) and do not induce hypothermia (Jacobson and Cryan, 2008).

It is not known whether the activation of GABA\textsubscript{B} receptors would persistently reverse any addiction-related neuroplasticity. Importantly, these drugs have not shown any rewarding potential in intracranial self-stimulation or CPP paradigms, although they attenuate addiction-related behavior such as sensitization, drug-induced CPP, and SA of various addictive substances (for review and references, see Filip et al., 2015). Furthermore, there are features in GABA\textsubscript{B} receptor activation that indicate reduction of neuroplasticity in adult animals. These are inhibition of neurogenesis (Giacino et al., 2014), increased expression of activating transcription factor 4 that reduces brain gene expression and neuroplasticity (Nehring et al., 2000; Vernon et al., 2001; Chen et al., 2003), reduced expression of BDNF (GABA\textsubscript{B} antagonists and receptor subunit knockouts increase BDNF (Heese et al., 2000; Enna et al., 2006) and, importantly, PKA-mediated blunting of NMDAR-dependent Ca\textsuperscript{2+}-transients of dendritic spines in the PFC layer 2/3 (Chalifoux and Carter, 2010). In addition, GABA\textsubscript{B} receptors retard the synaptic vesicle priming in glutamate synapses by reducing cAMP (Sakaba and Neher, 2003). It is still unclear whether activation or inhibition of GABA\textsubscript{B} receptors is antidepressant (Cryan and Slattery, 2010), which may turn out to be a complicating factor in the therapeutic use of GABA\textsubscript{B} ligands.

Another interesting alternative for antiaddiction drug treatment might be the antiepileptic, irreversible GABA transaminase (GABA-T) blocker $\gamma$-vinyl-GABA, vigabatrin, which increases brain and cerebrospinal fluid GABA concentration (Halonen et al., 1988; Petroff et al., 1996). Vigabatrin reduces neurochemical changes produced by cocaine in the brain reward areas, including cocaine-induced DA release and cocaine consumption in preclinical models (Dewey et al., 1997; Kushner et al., 1997, 1999; Peng et al., 2008). At least some of its actions can be abolished by GABA\textsubscript{B} receptor antagonists (Ashby et al., 1999). Vigabatrin also affects synaptic and extrasynaptic GABA\textsubscript{A} transmission (Overstreet and Westbrook, 2001). Importantly, vigabatrin and a reversible GABA-T blocker ACC (1R,4S-4-amino-cyclopent-2-ene-carboxylic acid) do not induce CPP (Paul et al., 2001; Ashby et al., 2002). Vigabatrin has shown neuroprotective effects in epilepsy models (Halonen et al., 1995; Pitkanen et al., 1996). Initial experiments showed some efficacy in human cocaine addicts (Brodie et al., 2009), but a larger study failed to show significant effects, perhaps due to poor adherence of the addicts to vigabatrin treatment (Somoza et al., 2013). This drug was originally developed for epilepsy, in which indication it was efficient, but permanent, slowly progressing vigabatrin-associated visual field loss, possibly linked to its cumulative dosing, has limited its use in epilepsy (Malmgren et al., 2001; Maguire et al., 2010; Clayton et al., 2013). The mechanism of retinal damage is not known (Ravindran et al., 2001). All these efforts have led to the development of a more potent GABA-T blocker, (1S,3S)-3-amino-4-difluoromethylenyl-1-cyclopentanoic acid (CPP-115) (Pan et al., 2012), which is being further studied. As with GABA\textsubscript{B} agonists, there is no clear information on possible long-term reversal of addiction-related neuroplasticity by GABA-T blockers.

6. $\gamma$-Hydroxybutyrate as a Drug of Abuse and a Therapeutic Compound. GHB was originally synthesized for sedation and anesthesia, and as a GABA analog it was supposed to enter the brain and act on GABA receptors easier than exogenous GABA (Laborit, 1964). This short-chain fatty acid compound [half-life in plasma about 1 hour (Abanades et al., 2006)] was used for anesthesia (Kam and Yoong, 1998), but now it is mainly used, with the non-proprietary name sodium oxybate, in narcolepsy with cataplexy. It improves consolidation of nonrapid eye movement sleep and normalizes sleep stage shifts, and through these mechanisms particularly reduces appearance of daytime cataplexic attacks in narcolepsy patients (Mamelak, 2009; Boscolo-Berto et al., 2012). It is also used in some European countries in the treatment of alcohol withdrawal symptoms and in the maintenance of alcohol abstinence (Leone et al., 2010). Interestingly, when used at low doses in the treatment of narcolepsy, very little tolerance develops to its sleep-improving effects, but when abused for its euphoric and intoxicating effects at high doses, tolerance and dependence develops and withdrawal symptoms appear spontaneously (or in nonhuman primates after administration of GABA\textsubscript{B} receptor antagonists) (Goodwin et al., 2013). GHB is an endogenous compound, synthesized from GABA and found in the brain at about 1 $\mu$M concentration (Maitre, 1997), but when exogenously given in preclinical settings for sedative and hypothermic effects, the doses needed are so high that brain concentrations are elevated up to 1 mM (Klein et al., 2009), which are in agreement with EC\textsubscript{50} of about 1 mM to activate GABA\textsubscript{B} receptors in midbrain slices (Madden and Johnson, 1998). The preclinical effects of high GHB doses are dependent on GABA\textsubscript{B} receptor activation (Kaumann et al., 2003). The identity (and significance) of the high-affinity GHB binding site(s) in the brain is not known. These sites might represent specific GHB receptor, unaffected in GABA\textsubscript{B} knockout mice (Kaumann et al., 2003), and/or partly reflect the most sensitive GABA\textsubscript{A} receptors, the
extrasynaptic α4β1δ receptors (Absalom et al., 2012). However, these highly GHB-sensitive GABA_A receptors could not be found in specific thalamic, NAc, or HC dentate gyrus neurons with known tonic inhibitory currents examined with electrophysiological patch-clamp methods in the brain slices from juvenile rats (Connelly et al., 2013).

GHB has variable effects on firing of midbrain DA neurons; both inhibition and activation of VTA DA have been produced in animal models (Pistis et al., 2005). Interestingly, RGS2 protein has been suggested to be responsible for the high and low efficacy coupling of the GABA_B receptors to somatodendritic Kir3 K+ channels in the VTA GABA and DA neurons, respectively (Labouebe et al., 2007), which seems to explain why GHB can acutely inhibit the GABA neurons similarly to baclofen (Cruz et al., 2004). But, when GHB was given subchronically at a high dose, animals showed aversion to consuming the initially preferred GHB-containing sucrose solution. This change coincided with the reduction of RGS2 expression in the VTA DA neurons and the enhanced inhibition of these neurons by GABA_B activation (Labouebe et al., 2007). Recent experiments on the effects of acute GHB on brain gene expression have further indicated that GHB induces neuroplasticity changes in the brain (van Amsterdam et al., 2012). This is consistent with the development of tolerance and rewarding effects to repeated GHB administration (Itzhak and Ali, 2002). Similarly to sedative GABA_A agonists, GHB globally reduces rat brain glucose metabolism (Kuschinsky et al., 1985). However, GHB provoked surprisingly robust activation of c-Fos protein in many subcortical brain areas, shared by similar effects by an equipotent sedative dose of baclofen (van Nieuwenhuijzen et al., 2009). The activation of thalamic nuclei, including the LHb in the epithalamus, after high GHB doses that induce cortical spiking (modeling absence seizures) can be explained by the oscillating thalamocortical circuits (Zhang et al., 1991; van Nieuwenhuijzen et al., 2009). Importantly, the some of the reward-related areas, such as the VTA and NAc, were clearly not activated by GHB or baclofen (van Nieuwenhuijzen et al., 2009). Thus, GHB remains an interesting, although a difficult compound in relation to addictions, with still much to be learned about basic neurobiology and mechanisms of action that contribute to its endogenous physiologic functions, to mechanisms of abuse of exogenous GHB and to its use in the treatment of alcohol addiction. Finally, it should be remembered that GHB has similar abuse liability to sedative drugs and alcohol (Griffiths and Johnson, 2005; Carter et al., 2006; Johnson and Griffiths, 2013), with severe delirium presenting in many cases where use has been extensive and tolerance has developed. This withdrawal state is hard to treat with BZs, and GHB may need to be reinstigated and then tapered slowly (de Jong et al., 2012). Baclofen might be useful here, too.

7. Anesthetics and Neuroplasticity. Several inhalational (isoflurane, halothane, and sevoflurane) and intravenous (propofol, etomidate, barbiturates, and BZs) anesthetics facilitate the function of GABA_A receptors, most likely as a part of their clinical mechanisms of action (Sonner et al., 2003; Franks and Lieb, 2004; Rudolph and Antkowiak, 2004). In addition to BZs and barbiturates, these drugs might have addiction potential, but this question has not been much studied. Propofol abuse has been reported (Wilson et al., 2010; Bonnet and Scherbaum, 2012). In a recent study, propofol showed abuse liability and euphoria on the first exposure in patients undergoing sedation for endoscopic examination (Kim et al., 2013a).

Because GABA_A receptors are, at least in early brain development, excitatory and essential for the normal development of neuronal circuits (Akerman and Cline, 2007; Ben-Ari et al., 2007), prolonged facilitation or activation of these receptor channels may have strong effects on brain structure and function. Similarly, the blockade of the NMDARs with ketamine, phencyclidine, and alcohol affects the developing nervous system (Olney et al., 1991; Ikonomidou et al., 1999, 2000). Early studies with chronic halothane administration (10 ppm halothane/air, 8 hours/day, 5 days/week, throughout development or in adulthood for about 1.5 months) showed in rats that structural changes evaluated with the Golgi method (retarded synaptogenesis, impaired dendritic branching, and disruption of organelle structure) were more abundant in the young than adult rodent nervous system (Quimby et al., 1974; Levin et al., 1991), the changes generally correlating with impaired results in tests for cognitive function. On the other hand, the persistent effects of anesthetics on the nervous system have recently been studied using prolonged (4–6 hours) anesthesia at the age when brain development is still occurring. At that stage, many clinically used anesthetics and also other neuropsychiatric drugs such as antiepileptics seem to induce neurotoxicity (apoptosis) in rodents and nonhuman primates (Vutsikits et al., 2006; Briner et al., 2010, 2011; Creelley and Olney, 2010; 2013; Brambrink et al., 2012a,b; Creelley et al., 2013; Jevtovic-Todorovic et al., 2013), with often a single administration being neurotoxic. Prolonged pediatric anesthesias for more than 4 hours, which are presently clinically justified and often unavoidable to provide the best treatment of the pediatric patient, have putatively been associated with cognitive impairment later in life (Jevtovic-Todorovic et al., 2013). Therefore, because proper anesthesia is important in surgical treatment, future efforts should be aimed at shortening durations of anesthesia, finding novel mechanisms of action for anesthetics that would not so globally involve the ligand-gated receptors of the main neurotransmitter systems, and/or finding pretreatment options that would limit the neurotoxicity. For example, lithium and clonidine might be two compounds with some efficacy to prevent
anesthetic-induced toxicity (Young et al., 2008; Straiko et al., 2009; Ponten et al., 2012), whereas hypothermia may be an option to protect the brain from ischemia/excitotoxicity and effects of prolonged anesthetics (Ikonomidou et al., 1989). Ketamine-induced apoptosis is enhanced by cotreatment with diazepam or midazolam in a rodent model (Fredriksson et al., 2004; Young et al., 2005), but diazepam has still been long used to alleviate psychotomimetic adverse effects of ketamine in humans. In addition, magnesium loading that has been used in (pre) eclampsia, may be neurotoxic for the developing nervous system (Dribben et al., 2009), and at least one clinical trial with MgSO4 supplementation to pregnant women with preterm labor had to be preliminarily stopped due to increased pediatric mortality (Mittendorf et al., 1997), especially to neonatal intraventricular hemorrhage (Mittendorf et al., 2002). These last cases are just two examples of problems recently encountered in the treatment affecting negatively the developing fetal or neonatal brain.

In the VTA DA neurons of juvenile mice, a single sedating dose of diazepam or midazolam induces persistent increases in AMPAR/NMDAR current ratios (Heikkinen et al., 2009; Tan et al., 2010). Intravenous anesthetic propofol in vitro at low micromolar concentrations activates AMPARs on VTA DA neurons (Li et al., 2008). Interestingly, isoflurane anesthesia alone has been tested in treatment-refractory depressed patients and found to produce long-lasting antidepressant effect, which was similar to electroconvulsive therapy (under isoflurane anesthesia) (Langer et al., 1985, 1995). These results were recently confirmed in a study on refractory depression, which also showed that electroconvulsive therapy (on an average ten 15-minute sessions within 3 weeks) had modestly more efficient antidepressant effect than isoflurane anesthesia alone at 4-week follow up, but produced more severe neurocognitive adverse effects (Weeks et al., 2013). In rats, 7 days after a single 2-hour isoflurane anesthesia, avoidance learning and HC LTP at CA1 synapses were impaired (Uchimoto et al., 2014). However, a similar single anesthesia in mice induced only some deficits in attention tests 1 week afterward, and most results of an extensive battery of behavioral and physiologic tests were not altered (Yonezaki et al., 2015). In any case, further research on possible prolonged neuroplasticity effects of GABAergic compounds appears warranted.

8. Conclusions. Although the GABAergic system is important for neuronal development and circuit functions in the mature nervous system, drugs like benzodiazepines that facilitate the actions of the GABA receptors have many effects on the nervous system that limit neuroplasticity. This may be due to their strong sedative effect. However, these drugs act by negative reinforcement, and more recently, benzodiazepines have been shown to induce similar glutamate synapse neuroplasticity in the DA neurons of the reward system as stimulants, opioids, and alcohol, suggesting also potential for positive reinforcement. Prolonged use of these drugs even in normal therapy may cause cognitive impairment that may not be fully reversible. Because GABAA receptors exist in multiple heterogeneous subtypes, subtype-selective BZ ligands have been studied, but this work has not yet translated into less addictive drugs. Initial studies on activation of extrasynaptic GABAA receptors suggest that they might show aversive features, perhaps by inducing glutamate neuroplasticity in a stress/aversion-sensitive subpopulation of DA neurons and/or by activating other stress/aversion-related brain areas. GABA receptors hold promise as a target for novel addiction therapies with specific positive allosteric modulators or via increased GABA levels.

F. N-methyl-D-aspartate Receptor Antagonists

NMDA antagonists are also known as dissociatives or dissociative hallucinogens because of their subjective effects. These are described as feelings of sensory detachment or dissociation from the outside world coupled with perceptual disturbances as well as catatonia-like motor phenomena. They are clearly distinct from the overt visual hallucinations produced by serotonin-type hallucinogens (Gouzoulis-Mayfrank et al., 2005; Muetzelfeldt et al., 2008). The most commonly used dissociatives are ketamine and phencyclidine (PCP), whereas a third NMDAR antagonist known as MK-801 or dizocilpine has been widely used in pharmacological research but never gained popularity among recreational users. In recent years there has also been an increase in the use of structural analogs with similar pharmacological activity in the so-called legal highs scene (Morris and Wallach, 2014; NIDA, 2015).

As NMDAR antagonists, primarily ketamine, are also widely used medically, their effects on the nervous system have been studied extensively, yet they remain enigmatic because of their widely differing and dose-dependent effects. PCP is known to produce psychotomimetic effects in humans and it was first proposed to be a candidate for a model of drug-induced schizophrenia in the late 1950s (Domino and Luby, 2012; Luby et al., 1959). This finding later opened the idea of glutamatergic model of schizophrenia in the past two decades (Javitt and Zukin, 1991; Javitt et al., 2012). NMDAR antagonists have neuroprotective effects, but they also have proven neurotoxic effects in rodents, although their effects on nonhuman primates and humans are still unclear (Low and Roland, 2004; Shirakawa et al., 2013; Wang et al., 2013a). Much effort has been put in to identify the putative mechanisms of NMDAR antagonist-induced neuroplasticity (Olney et al., 1989). What follows is a review of ketamine, PCP, and MK-801 and their effects on neurogenesis, neurotoxicity, and behavior.
1. Ketamine: a Dissociative Anesthetic with Rapid Antidepressant Effects in Patients. Ketamine is a non-competitive NMDAR antagonist and a dissociative anesthetic developed in 1962 (Jansen, 2000). Recently, it has received a lot of attention for its rapid-onset antidepressant effects (Krystal et al., 2013). After the first report on the surprisingly rapid antidepressant effect after intravenous infusion of ketamine in depressed patients (Berman et al., 2000), many reports reproduced the results and demonstrated that the antidepressant effects after a single dose (intravenous or intramuscular) is sustained for 3 to 10 days (Diazgranados et al., 2010; Zarate et al., 2012b; Chilukuri et al., 2014). Also repeated infusions of ketamine produced long-term effects (aan het Rot et al., 2010; Murrough et al., 2013; Diamond et al., 2014). Because of these data, there is a strong interest to understand the mechanisms of action of ketamine.

With regards to the neurochemical effects underlying the antidepressive effects of ketamine, chronic ketamine intake is associated with increases in serum BDNF in humans (Ricci et al., 2011). Although one report in adult rats and mice claimed that ketamine showed a transient effect (at 50 mg/kg) in forced swim and tail suspension tests without any persistent antidepressant effect (Popik et al., 2008), many preclinical studies have recently focused on understanding the molecular mechanisms underlying the rapid-onset prolonged antidepressant effects. These studies show that plasticity-related pathways, especially the BDNF signaling pathway, are influenced by ketamine treatment (Duman and Voleti, 2012; Kavalali and Monteggia, 2012; Reus et al., 2014; Yang et al., 2014; Zhou et al., 2014). These effects are dependent on the mammalian target of rapamycin (mTOR) signaling pathway (Li et al., 2010a), because they can be blocked by rapamycin. This effect could be mimicked by GluN2B subunit-containing NMDAR antagonist Ro 25-6981 [(αR,βS)-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanoic maleate], which induced antidepressant-like behavioral effects. mTOR is a serine-threonine protein kinase, the subtypes of which, mTORC1 and mTORC2, form active protein complexes with Raptor or Rictor, respectively. mTORC1 via p70S6K promotes cell growth, mRNA biogenesis, and the translation of ribosomal proteins. mTORC2 via Rictor activates and phosphorylates Akt to enhance cell survival, relying upon PKCα for cytoskeleton remodeling, and controlling cell migration through the Rac guanine nucleotide exchange factors and through Rho signaling (Laplante and Sabatini, 2009; Buffington et al., 2014; Maiese, 2014). A single dose of ketamine was reported to upregulate BDNF via activation (phosphorylation) of 5′-adenosine monophosphate-activated protein kinase in the rat HC (Xu et al., 2013). Rats receiving ketamine (75 mg/kg) on postnatal day 7 (P7) developed cognitive impairments in adulthood (Huang et al., 2012). These effects were associated with suppressed expression of pPKC, pERK1/2, and Bcl-2 with unchanged tPKC or tERK levels during postnatal phase. Long-term ketamine treatment in adult mice may cause delayed and persistent upregulation of subcortical DAergic system via a mechanism involving the BDNF pathway (Tan et al., 2012b). Whereas acute and chronic treatment of ketamine caused an antidepressant-like effect in the forced swim test, only chronic treatment (0.5 mg/kg/day for 10 days) increased AMPAR/NMDAR density ratio in the HC of the “depressed” female Wistar-Kyoto rats (but not in control Wistar rats) at 20 hours after the treatment (Tizabi et al., 2012). Synaptic potentiation appears to underlie the ketamine efficacy also in depressive patients, as suggested by increased cortical excitability measured by means of magnetoencephalography (Cornwell et al., 2012). Importantly, AMPAR antagonists have been shown to prevent the antidepressant effects of ketamine in rodents, suggesting that AMPAR activation is required for more persistent antidepressant effects that are at least partly mediated by upregulation of mTOR and BDNF pathways (Koike et al., 2011; Koike and Chaki, 2014; Zhou et al., 2014).

Stereoomers (R,S) of ketamine and its metabolites norketamine and (2S,6S)-hydroxynorketamine increase phosphorylation of mTOR and its downstream targets Akt, ERK1/2, 70S6K, and 4E-BP1 in a cell culture model (Paul et al., 2014b). As mentioned earlier, the activation of mTOR pathways is crucial for synaptic plasticity. Pharmacokinetic studies in treatment-resistant bipolar patients show that norketamine is the initial but not the main metabolite and that other metabolites, namely dehydronorketamine, (2S,6S;2R,6R)-hydroxynorketamine, and hydroxyketamine are detected during the first 48 hours (Zhao et al., 2012). These metabolites may play a role in the antidepressant effects. Intriguingly, higher plasma levels of (2S,5S;2R,5R)-hydroxyketamine were related to nonresponse to ketamine in bipolar disorder patients (Zarate et al., 2012a), some metabolites being perhaps responsible for psychotomimetic effects of ketamine.

Ketamine and some metabolites have affinity to α7-nAChRs at relevant concentrations and behave as negative allosteric modulators (Moaddel et al., 2013). Furthermore, several neuropharmacological studies have shown direct and indirect effects of ketamine on other neurotransmitters. A very recent study showed that ketamine treatment-associated restoration of plasticity in the ventral subiculum-NAc shell is caused by activation of D1 receptors (Belujon and Grace, 2014). In vitro receptor binding studies show that ketamine has very similar affinity to NMDARs and the high-affinity state of the D2 receptors (where it works as an antagonist) and a slightly lower affinity to 5-HT2 receptors (Kapur and Seeman, 2001, 2002; Seeman and Kapur, 2003; Seeman et al., 2005). Ketamine (40 mg/kg) pretreatment prevented bicuculline-induced seizures and reversed changes in [3H]QNB binding to muscarinic receptors induced by bicuculline, showing the roles of muscarinic.
and GABA<sub>A</sub> receptors (Schneider and Rodriguez de Lores Arnaiz, 2013). Indeed, ketamine, but not PCP or MK-801, acts as a positive modulator at specific cerebellar extrasynaptic GABA<sub>A</sub> receptor populations (Hevers et al., 2008). An earlier study showed that ketamine inhibits muscimol signaling in CHO cells as observed from intracellular Ca<sup>2+</sup> release in response to the agonist acetyl-β-methylcholine (Durieux, 1995). Finally, ketamine has been shown to inhibit the inward pacemaker current <i>I_h</i>, which is mediated by ketamine binding to hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 (HCN1) subunits in forebrain neurons (Chen et al., 2009; Zhou et al., 2013). HCN1-KO mice are less sensitive to ketamine-induced hypnosis (Chen et al., 2009), indicating that this mechanism may explain the high-dose anesthetic effects, but no data are available on whether this mechanism is involved in other behavioral or neuroplasticity-inducing effects of ketamine. It is noteworthy that all these studies draw parallels to the anesthetic dose levels and not the subanesthetic antidepressant dose levels.

Ketamine has strong and prolonged effects on neuronal synaptic physiology. In conscious rats, ketamine induces synaptic depression in the Schaffer collateral-CA1 pathway sustaining beyond 4 hours but normalizing by 24 hours (Duan et al., 2013). Ketamine effects on memory impairment were anyway predicted to last for days or even weeks, because the long-lasting synaptic change is expected to influence protein synthesis and gene expression. Ketamine and PCP prevent the LTP in the CA1 region in response to cornu ammonis stimulation in the HC of anesthetized rats (Stringer and Guyenet, 1983), and, in agreement, a recent in vitro study showed that ketamine, at its antidepressant concentration level, negatively modulated both LTP and LTD in CA1 of juvenile rats (Izumi and Zorumski, 2014). LTD inhibition and enhancement of somatic EPSPs occurred shortly after ketamine administration, whereas modulation of LTP occurred after 2 hours or later. Clinically relevant micromolar concentrations of ketamine dose dependently decreased LTP without affecting paired-pulsed facilitation in Schaffer collateral-CA1 pyramidal neuron synapses in a mouse HC slice preparation, but higher concentrations affected basal excitatory synaptic transmission and presynaptic volley amplitude (Ribeiro et al., 2014). In a model for psychosis, administrations of ketamine (20 mg/kg s.c. given six times, once every 2 hours.) on P4-7 impaired LTP at 3–4 weeks of age when recorded in slices from the anterior cingulate cortex, which was accompanied by increased AMPAR-mediated excitatory transmission and decreased GABAergic inhibitory transmission (Wang et al., 2014).

Ketamine in high anesthetic doses is thought to be neurotoxic in developing brain of rodents and nonhuman primates and in selected regions of the mature nervous system (Olney et al., 1989; Slikker et al., 2007; Wang et al., 2013a), and chronic ketamine treatment in mice (6 months of daily 30 mg/kg i.p.) caused persistent damage in brain function as observed from deterioration in neuromuscular strength and nociception (Sun et al., 2011). A case report suggested that chronic ketamine use could impair memory and synaptic plasticity (Jansen, 1990). Furthermore, one MRI study provided anecdotal evidence of brain atrophy (affecting both gray and white matter) in the prefrontal, parietal, occipital, limbic cortical regions, brain stem, and striatum after 2–4 years of use in some ketamine-dependent subjects, although no clear conclusions can be drawn from this study because of the lack of statistics and a control group (Wang et al., 2013b).

The psychedelic effects of ketamine may cause psychologic dependence that is cyclical in nature like cocaine and amphetamine but has been described to be devoid of any clear withdrawal syndrome (Jansen and Darracott-Cankovic, 2001). However, recent studies have shown withdrawal effects from ketamine use. Female ketamine users may show more severe withdrawal-associated cognitive impairment than male users (Pattore et al., 2009; Chen et al., 2014). Furthermore, in humans the abuse potential of oral ketamine (65 and 100 mg) is robust enough to have it being suggested as a positive control for evaluating abuse potential of other psychedelic compounds (Shram et al., 2011). Ketamine intake is higher in rats that were transferred to SA chambers during the test sessions than in rats that also were housed in those chambers, indicating the significant influence of the setting on ketamine taking (De Luca and Badiani, 2011). Withdrawal of ketamine after 5 days of high dosing (twice daily 150 mg/kg oral doses) in male cynomolgus macaques increased activity and stereotypic behaviors, including head bobbing, repetitive grooming, repetitive body/limb or hand movements, and repetitive nail biting during the withdrawal period (Walgren et al., 2014). Withdrawal syndrome has also been frequently reported in rodents. Subchronic ketamine (10 mg/kg twice daily for 10 days) in rats causes persistent working memory impairment in the delayed spatial win-shift task after a withdrawal for 10 days (Enomoto and Floresco, 2009). Chronic administration (100 mg/kg/day i.p. for 10 days) of ketamine in mice produces hyperactivity, enhanced immobility period in forced swim test, and reduced latency period in passive avoidance test that persisted at least for 10 days after the withdrawal (Chatterjee et al., 2011). Notably, all these studies used high ketamine doses and/or models of prolonged abuse, indicating stronger neuroadaptation in that situation than in low-dose antidepressant treatment studies. In contrast, in clinical settings ketamine has been shown to reduce cravings in heroin addicted patients when dispensed in combination with psychotherapy sessions (Krupitsky et al., 2002).

In summary, ketamine produces strong dose-dependent behavioral and neurochemical effects. At low doses, it
produces significant changes in gene expression, resulting in enhanced synaptic plasticity that is likely to be related to its rapid-onset antidepressive action in both animal models and humans. On the other hand, high doses of ketamine impair neuroplasticity and may cause neurotoxic effects. Finally, in terms of behavior, both addictive and antiaddictive properties of ketamine have been described. It is therefore of interest to compare and contrast the effects of ketamine with that of the second commonly used NMDAR antagonist, PCP.

2. Phencyclidine. PCP, an NMDAR antagonist and dissociative anesthetic, long known for its delirium- and thought disorder-inducing (cognitive deficits) properties (Javitt and Zukin, 1991; Domino and Luby, 2012) can acutely dissociate the CNS from peripheral sensory input and induce metabolic hypofunction in several brain regions, including the FC, HC, and thalamus. PCP acts on many mechanisms that have been reported to underlie its neuropharmacological and neurotoxicological effects. At the receptor level, it has a high affinity to NMDA, 5-HT 2A, and D 2 receptors (Yamada et al., 1999; Kapur and Seeman, 2002; Seeman et al., 2005, 2009). Sigma-1 receptors are involved in cognitive deficits associated with PCP treatment in rodents (Hashimoto et al., 2007; Kunitachi et al., 2009). At the functional level, PCP impairs LTP in both in vivo and in vitro systems (Stringer et al., 1983), e.g., PCP (and ketamine) prevents both LTP and LTD in rat perforant path-dentate gyrus synapses (Tizabi et al., 2012). NMDAR antagonists, such as 2-amino-5-phosphovaleric acid, N-acetyl-aspartyl-glutamate, and PCP, prevent both LTP and primed burst potentiation, produced by variants of electrical stimulation modeling plasticity, in the CA1 area of rat HC slices (Wiescholleck and Manahan-Vaughan, 2013; Xu et al., 2013). Subchronic PCP treatment to rats caused impaired LTP in the lateral amygdala and Schaffer collateral to HC-CA1 pathway and marked increases in the NMDAR and AMPAR-mediated currents in the lateral amygdala (Pollard et al., 2012).

Effects of PCP in rodents are especially strong and persistent when given during brain development. Administration of PCP to pregnant rats during gestational days 12–20 days caused a decrease in PCP binding sites in the fetal brains (Ali et al., 1988, 1989). The prenatal administration (embryonic days 12–18) of PCP (5–20 mg/kg) caused an increased response to PCP-induced ataxia and an increased number of [3H]PCP binding sites in the striatum and cortex of the offspring (Fico and Vanderwende, 1989). Early oligodendrocyte progenitor cell markers were decreased in rat brains prenatally exposed to PCP (10 mg/kg), suggesting that surviving cells are arrested at an immature stage, which might have implications for the role of glutamate and glutamate receptors in white matter abnormalities in neurodevelopmental disorders (Lindahl et al., 2008). These findings were strengthened by another recent report on prenatally PCP-treated mice, demonstrating aberrant gene expression (Notch2 and Ntn1) and consequential decrease in the density of glutamatergic neurons in the PFC, deficits in cognitive memory, and sensorimotor gating at adulthood (Toriumi et al., 2012).

Prenatal PCP treatment (20 mg/kg) in mice induced hypersensitivity to a low dose of PCP (3 mg/kg) in locomotor activity and impairment of recognition memory in the novel object recognition test at the age of 7 weeks and reduced the phosphorylation of GluN1 subunit, although it increased the expression of this subunit (Lu et al., 2010b). Prenatal PCP (5 mg/kg) treatment caused impairment in recognition memory in a novel object recognition test, enhanced locomotion in a forced swim test, reduced the extracellular glutamate level, and increased the expression of a glial glutamate transporter in the PFC at 8 weeks of age (Lu et al., 2011). Likewise, prenatal administration of PCP (10 mg/kg) showed disruption of acquisition of passive avoidance response and pole-climbing avoidance response (Nabeshima et al., 1988). 5-Bromo-2′-deoxyuridine-labeled newborn cells in the dentate gyrus granule cell layer were significantly increased in rat offspring that were subjected to prenatal exposure of PCP (Tanimura et al., 2009). Prenatal and neonatal exposure of PCP caused increased $B_{\text{max}}$ for muscarinic cholinergic receptors in the HC and reduced performance in HC-related radial arm and water maze tasks (Yanai et al., 1992).

A single administration of PCP caused an increase in cataleptic freezing and sporadic recurrences of backing up and weaving behavior for up to 21 days in adult rats (Haggerty et al., 1984). In rats, PCP initially also reduces the brain weight, and, interestingly, withdrawal from PCP causes a transitory rebound associated with increased molecular layer depth and number of synapses in the occipital cortex, suggesting a transitory burst of synaptogenesis (Brooks et al., 1997). After a 72-hour withdrawal from PCP, sensitized locomotor activation, a marked increase in GluN1 subunit mRNA in several brain regions, including the FC and anterior striatum, were detected (Wang et al., 1999). Furthermore, NMDAR-stimulated DA release was not affected by PCP treatment, but the inhibition of this release by NMDAR blockers (PCP, 7-chlorokynurenic acid, and DL-2-amino-5-phosphovaleric acid) was blunted by chronic PCP, indicative of tolerance. Most likely, PCP treatment alters the subunit stoichiometry of NMDARs that may contribute to behavioral plasticity (Wang et al., 1999). Although there seems to be a discrepancy between the persistent behavioral deficits (rotarod and open field tests) and the amount of cell loss in relation to the time of maximal prenatal PCP effect, exposure to PCP increases agoraphilic cells in the entorhinal cortex and subiculum, but reduces them in the ventromedial hypothalamus, indicating that neurodegenerative and antiapoptotic effects are dependent on the brain region (Jebelli et al., 2002). Postnatal (P5–15) phencyclidine
Disrupts water maze performance in adult rats, which was associated with maximal $[^{3}H]$MK-801 binding ($B_{\text{max}}$) in the HC and FC (Sircar, 2003), although similar treatment reduced NMDA receptor expression in juvenile rats. Male rats treated with PCP (8.7 mg/kg s.c.) on P7, 9, and 11 showed slight impairment in performing a spatial reference memory task and strong impairment in reversal and spatial working memory tasks (Andersen and Pouzet, 2004). A similar perinatal treatment schedule with 10 mg/kg of PCP induced disruption of cognition as tested in progressive ratio operant schedule task beyond postnatal 120 days (Wiley and Compton, 2004). PCP administration (10 mg/kg) on P2, 6, 9, and 12 caused a constellation of anatomic changes in rats at early adulthood (P70), because it reduces densities of principal neurons in the CA3 and DG of the HC, those of interneurons in all cortical and HC regions, density of reelin- and somatostatin-positive cells, and increases the number of perisomatic inhibitory terminals around the principal cells in the motor cortex and HC dentate gyrus (Radonjic et al., 2013). These changes were correlated with increased expression of neuregulin-1, a protein involved in synaptic development and schizophrenia (Mei and Xiong, 2008; Shamir et al., 2012), in the cortex and HC.

Subchronic PCP treatment (twice a day for 7 days) followed by 7 days of abstinence causes significant impairment in the reversal phase of a operant reversal learning paradigm, demonstrating a persistent cognitive deficit (Abdul-Monim et al., 2006). The deficit was reversed by acute treatments with atypical antipsychotic compounds ziprasidone, olanzapine, and clozapine. PCP withdrawal impaired performance in object-place-context task in rats, which was reversed by donepezil but not by clozapine (Le Cozannet et al., 2010). Subchronic PCP treatment induced a persistent deficit in novel object recognition that was attenuated by lurasidone (a potent 5-HT$_{1A}$ partial agonist, 5-HT$_{2A}$ antagonist), tandospirone (weak D$_{2}$ antagonist), and F15599 (selective postsynaptic 5-HT$_{1A}$ agonist), and interaction studies with these various ligands showed that 5-HT$_{1A}$ agonism was adequate to attenuate the PCP-induced effects (Horiguchi and Meltzer, 2012). These deficits were also reversed by D$_{3}$R agonism, the effect of which was facilitated by 5-HT$_{1A}$ and mGlur$_{5}$/G receptor signaling (Horiguchi et al., 2013). Recently, Lu AE58054 (2-(6-fluoro-1H-indol-3-yl)-N-[[3-(2,2,3,3-tetrafluoropropoxy)phenyl]methyl]ethanamine), a 5-HT$_{6}$ receptor antagonist, was found to reverse subchronic PCP-induced deficits in novel object recognition test (Arnt et al., 2010). It is not known whether any of the drug treatments can produce persistent reversal of subchronic PCP effects, but, interestingly, clozapine and haloperidol prevented the metabolic hypofunction and the reduced Pv expression in several brain regions in rats treated intermittently with PCP (Cochran et al., 2003).

In the PFC of rats, acute PCP treatment caused significant changes in about 500 genes, including many that are associated with neural plasticity (Kaiser et al., 2004). Single and repeated administrations in adult rats causes transient BDNF upregulation in the cortico-limbic system, and subchronic postnatal administration of PCP increased BDNF (protein and mRNA) in the HC and entorhinal cortex that sustained till 8 weeks of age, being responsible for the persistent decrease in the prepulse inhibition in adults (Takahashi et al., 2006). PCP treatment (20 mg/kg/day) for 14 days caused reduction in the levels of pERK1/2, pCaMKII$_{a}$, and pCREB in the PFC (Molteni et al., 2008). Two injections of PCP reduced Pv and GAD67 levels in the PFC, and this effect persisted after 10 days (Amitai et al., 2012). A single injection of PCP (10 mg/kg) to rats on P7 caused selective reduction in Pv-containing, not calcitomin- or calbindin-containing, interneurons in cortical areas in early adulthood (P56) (Wang et al., 2008a). A persistent decrease in the number of PFC spine synapses, in parallel to the cognitive deficits, was evident with twice daily injection of 5 mg/kg of PCP for 7 days (Elsworth et al., 2011b). These PCP effects on gene regulation are important examples of the widespread actions of PCP on neural tissue, which appear to produce long-term changes in the nervous system. An in vitro study employing cortical neuronal culture showed that PCP causes a transient increase within 3 hours followed by a decrease in the intracellular BDNF, whereas activation of TrkB receptors and downstream cascades (MAPK/ERK1/2 and PI3K/Akt) were decreased. The number of synaptic sites and expression of synaptic proteins were reduced 48 hours after treatment, without affecting the cell viability (Adachi et al., 2013). Interestingly, exogenous BDNF completely reversed the downregulating effect of PCP on the TrkB signaling and synaptic protein expression (Adachi et al., 2013). Postnatal treatment (P7, 9, and 11) with PCP (10 mg/kg) reduced Nrg1 and erbB4 expression and phosphorylated Akt during development in the cerebral cortex (du Bois et al., 2012) and displayed hyperactivity in hole-board and forced swim tests in adulthood (du Bois et al., 2008).

Cognitive impairment and anatomic and neurochemical effects after PCP administrations have also been reported in mice. Repeated administration of low doses (0.5–2.0 mg/kg) of PCP causes cognitive impairment associated with alterations in Arc and spinophilin mRNA expression in the HC, striatum, and retrosplenial cortex (Beraki et al., 2009). Repeated PCP treatment (10 mg/kg for 10 days) decreased the expression of Pv and synaptophysin mRNA in the mouse PFC and increased basal Arc mRNA expression (Thomsen et al., 2009). Lastly, in primates, chronic PCP (0.3 mg/kg twice a day for 14 days) caused a 40% reduction in Pv-containing axo-axonic structures in the PFC (Morrow et al., 2007). PCP treatment in primates also leads to
persistent deficits of cortical DA (for 9 days) (Jentsch et al., 1999). Subchronic PCP treatment (0.3 mg/kg twice a day for 14 days) caused a reduction in the number of asymmetric spine synapses in layer II/III of the dorsolateral PFC that may perhaps account for the cognitive dysfunction (Elsworth et al., 2011a). Thus, persistent effects of PCP appear to rely at least in part on its cortical neurotoxicity and neuregulin downregulation.

In terms of addiction potential, PCP is also abused by humans (Crider, 1986). Rats self-administer PCP as well as MK-801 and the competitive NMDAR antagonist 3-((+/-)2-carboxypiperazin-4-yl)propyl-1-phosphate into the NAc and PFC in a DA-independent manner, unaffected by D2 receptor blockade by sulpiride (Carlezon and Wise, 1996). Many NMDAR antagonists have often, but not in all studies, dose dependently induced CPP in rodents (Tzschentke, 2007) and PCP, at least somewhat, lowered the threshold for lateral hypothalamic self-stimulation (Kornetsky and Esposito, 1979).

PCP, like ketamine, has strong effects on synaptic plasticity and is capable of altering LTD and LTP as well as the expression of genes known to be involved in neuronal plasticity, effects that are often associated with behavioral abnormalities and changes in cognition and prepulse inhibition as well as the development of SA of the drug. This leads to the question of how PCP and ketamine compare with the prototypical and highly selective NMDA antagonist MK-801.

3. Dizocilpine, a Prototypic Noncompetitive N-methyl-D-aspartate Receptor Antagonist. Unlike ketamine and PCP, the recreational use of dizocilpine (MK-801) has been very limited, although it has been reported to have clear psychoactive effects comparable with other NMDAR antagonists, suggesting that MK-801 has somehow remained undesirable or unobtainable for prospective users (Morris and Wallach, 2014). However, it is the most selective of the noncompetitive inhibitors of NMDARs and is known to cause behavioral sensitization (Vanderschuren et al., 1998).

The long-lasting and persistent effects of MK-801 on synaptic plasticity and memory in rodents have been recently reviewed (Wiescholeck and Manahan-Vaughan, 2013). Administration of MK-801 to rats causes facilitation of LTP at the HC CA1-subiculum synapses 24 hour after treatment, suggesting metaplasticity (Buck et al., 2006). However, a 4-week treatment reversed the magnitude of LTP to control level. Intrahalamic administration of MK-801 changes local field potentials and paired-pulse facilitation in rats, indicative of changes in short-term plasticity (Kiss et al., 2011). After application of MK-801, homosynaptic LTP of the direct cortical input to CA1 was abolished for at least 1 week, with partial recovery after 4 weeks (Wöhrl et al., 2007). MK-801 treatment causes long-lasting potentiation in HC-mPFC pathway and impairments in mPFC-dependent cognitive flexibility and HC-mPFC-dependent working memory in rats, which were reversed by mGluR2/3 receptor agonist LY379268 [(1R,AR,5S,6R)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid] (Blot et al., 2013). Thus, it is important to take into consideration the strong effects of MK-801 treatments themselves on neuroplasticity and metaplasticity, when one wants to assess the roles of the NMDARs in plasticity processes by using this antagonist. Furthermore, in rodents, high neurodegenerative doses of MK-801 induce prolonged hypothermia, which can acutely affect cognitive behavior but may not as such have lasting impairing effects on cognition (Zajaczkowski et al., 2000).

A small study showed that primates with the history of PCP use, but not those with a history of cocaine use, self-administered MK-801 (Beardsley et al., 1990; Steinpreis et al., 1995). MK-801 also reversed the withdrawal effects of PCP (Carroll et al., 1994). In rodents, MK-801 elicits CPP by itself (Layer et al., 1993). Importantly, it inhibits CPP induced by METH (Kim and Jang, 1997) and morphine (Tzschentke and Schmidt, 1995; Kim et al., 1996) and blocks the persistent neuroplasticity in VTA DA neurons and the blunting of d-AMPH sensitization after a single dose of BZs (Heikkinen et al., 2009; Panhelainen et al., 2011). Interestingly, combination treatment of the rewarding doses of NMDA and MK-801 did not result in CPP (Panos et al., 1999). MK-801 significantly reduces fixed ratio maintained operant responding for cocaine (Pierce et al., 1997), but it can reinstate extinguished cocaine-seeking behavior (De Vries et al., 1998) and disrupt the reconsolidation of a cocaine-associated memory for CPP (Brown et al., 2008). MK-801 produces variable effects on DA release, because systemic injection of MK-801 significantly suppressed striatal DA release in conscious rats, which was not related to its behavioral effects (Kashihara et al., 1990). Infusion of MK-801 into the NAc prevented intrapallidal morphine-induced NAc DA release (Anagnostakis et al., 1998). Its systemic administration, however, increased DA release in the NAc, which could be prevented by prazosin, an α1 adrenoceptor antagonist (Mathe et al., 1996). Systemic administration or infusion into the PFC, ventral pallidum, NAc, or striatum increased local DA release (Wedzony et al., 1993; Hondo et al., 1994; Kiss et al., 1994; Kashihara et al., 1995; Schmidt and Fadayel, 1996; Yan et al., 1997; Mathe et al., 1999; Kretschmer, 2000). In rodent striatal slices, MK-801 attenuated ischemia-, hypoxia-, hypoglycemia-, and L-glutamate-induced DA release (Milusheva, 1992; Kim et al., 1995; Antonelli et al., 1997), suggesting neuroprotective effects. MK-801-induced behavioral sensitization was associated with changes in the kinetics of DA release characterized by a delayed (after 2 hour) release in the PFC (Cui et al., 2014). But MK-801 failed to alter the striatal DA release caused by METH (Baldwin et al., 1993), and a few other reports showed that MK-801 had a weak or no effect on
MK-801 has profound effects on gene expression in the rodent brain. For example, it decreases BDNF mRNA in the HC and superficial layers of cerebral cortex and increases BDNF mRNA in the middle layer (single cells) of cerebral cortex, midline thalamic nuclei, and retrosplenial and medial entorhinal cortices (Castren et al., 1993). The treatment increases DNA binding activity of the activator protein-1 complex, mRNAs of BDNF (Linden et al., 2000), neuronal Src in superficial layers of the parietal cortex, tyrosine phosphorylation of GluN2A subunit (Linden et al., 2001), c-Fos, ΔFosB, Fra-2, andJunB expression in the entorhinal cortex (Kontkanen et al., 2000; Vaisanen et al., 2004), possibly related to psychotic effects. Expression profiling analysis showed that MK-801 regulates ER proteins Erp29, a stress-inducible liver protein, and (at higher doses) RTN1, a reticulon, and ABC transporters involved in small molecule transport, reflecting possible toxicity in the cingulate and entorhinal cortices (Toronen et al., 2002).

The mechanisms involved in MK-801-induced neurotoxicity have been described and include 1) in the adult brain, occurrence of paradoxical neurodegeneration, disinhibition of glutamatergic and cholinergic projections of the cerebral cortex due to blockade of NMDARs in inhibitory neurons in many subcortical regions (Farber et al., 2002; Li et al., 2002), and 2) in the developing brain, apoptotic neurodegeneration (Olney et al., 1989; Dzietko et al., 2004) that is also reported for ketamine (Zou et al., 2009; Liu et al., 2011) and PCP (Rahbar et al., 1993; Deutsch et al., 1998). The MK-801-induced toxicity in adult rats can be prevented by injections of scopolamine and NBQX (6-nitro-2,3-dioxo-1,4-dihydrobenzo[f]quinoxaline-7-sulfonamide) (into the cerebral cortex) and clonidine (into basal forebrain) (Farber et al., 2002) and by systemic scopolamine (Shirakawa et al., 2013), erythropoietin (partial effect, counteracting the reduction in BDNF and GNDF expression by NMDAR antagonists) (Dzietko et al., 2004), and ethanol (Farber et al., 2004), but not by increased BDNF (Vaisanen et al., 2003). Neurotoxicity by PCP and ketamine can also be attenuated by diazepam and haloperidol (Nakao et al., 1996; Nakki et al., 1996). It is noteworthy that ethanol-antagonism of NMDARs induces more neurotoxicity in developing than adult rat brain, presumably due to the inhibitory GABA mimetic effects of ethanol in adult brain (Olney et al., 2000, 2002). It may also reflect the role of the NMDARs in neuronal development and excitotoxicity, both of which can be blocked in cultured neurons by ethanol (Wegelius and Korpi, 1995).

In summary, MK-801 as a prototypic NMDAR antagonist produces widespread changes in brain gene expression, which might have prolonged effects on neuronal function and structure. The high-dose toxicity of MK-801 and other NMDAR antagonists is associated with brain region-dependent and time-dependent astrogliosis and microglial activation (Fix et al., 1996; Nakki et al., 1996). On the other hand, recent work has also revealed that MK-801 and other NMDAR antagonists attenuate microglial activation induced by various drugs and insults (Thomas and Kuhn, 2005; Nair and Bonneau, 2006), which may at least partly explain the neuroprotective effects of these drugs. Interestingly, MK-801 and cocaine have distinct effects on gene expression, with the interactions between their effects being complex (Storvik et al., 2006).

4. Conclusions. Potent NMDAR antagonists are drugs that affect neuroplasticity processes in the brain, lead to addiction, and suffer from induction of psychotomimetic effects and thought-imparing cognitive deficits in many subjects, with a high potential to induce paradoxical neurodegeneration. Ketamine and its analogs are now studied for the rapid, long-lasting antidepressant effect, with early results indicating strong activation of neuronal plasticity mechanisms. Phencyclidine and dizocilpine are used in preclinical settings to induce a model of adult psychosis by adolescent exposure or to assess the role of NMDARs in behavior and physiologic functions, respectively.

G. Opioid-Induced Neural Adaptations

Opioids are widely used as powerful analgesic agents that are also highly addictive. A sizable proportion of individuals that either misuse opioids or use them clinically become addicted. With chronic use, adaptive neural mechanisms are initiated at various levels of neural organization, leading to both short-term and protracted changes in the functioning of opioid-sensitive neurons and neural networks (Williams et al., 2001; Christie, 2008; Dacher and Nugent, 2011b; Lutz and Kieffer, 2013). Some of these adaptations, such as opioid tolerance, have been intensively investigated. However, the neural mechanisms underlying protracted features of withdrawal, such as opioid seeking and relapse contributing to the compulsive features of opioid dependence, still remain largely unknown. In the following sections, we will focus on describing the most important findings related to chronic opioid use, including the mechanisms of...
opioid tolerance, the withdrawal syndrome, and opioid-induced synaptic plasticity.

Opioids exert their actions through three major receptor subtypes, μ, δ, and κ, opioid receptors that were first identified by pharmacological tools and later verified by molecular cloning (Goldstein and Naidu, 1989; Evans et al., 1992; Kieffer et al., 1992; Reisine and Bell, 1993). Further diversification of opioid receptor functions may be achieved by alternate splicing, post-translational regulation, or receptor dimerization. All opioid receptors are GPCRs, coupled with pertussis toxin-sensitive G proteins G\(_i\)/G\(_o\) (Connor and Christie, 1999). The coupling profile to G proteins of all three opioid receptors is similar, including inhibition of AC, activation of potassium conductance, inhibition of calcium conductance, and inhibition of transmitter release (Williams et al., 2001). Opioid receptors and their mRNAs display distinct anatomic distribution in neuronal systems, as well as paracrine and exocrine tissues important for opioid drug actions (Mansour et al., 1987, 1995; Zhu et al., 1998).

The primary target of most opioid drugs is the μ receptor, through which the addictive properties of opioids are also mediated. The levels of neural adaptations to chronic opioids can be conceptualized as 1) loss of the μ receptor coupling to its cellular effectors, 2) adaptations in the intracellular signaling cascades, 3) systems feedback adaptations in neuronal and glial networks, and 4) changes in synaptic plasticity (Christie, 2008).

1. Desensitization and Internalization in Opioid Tolerance and Dependence. Development of opioid tolerance is partly dependent on a partial loss of capacity of the μ receptor to signal to its intracellular effectors (Kieffer and Evans, 2002; Christie, 2008; Allouche et al., 2014). This "receptor tolerance" can be achieved by different mechanisms, primarily by decreased cell surface expression of μ receptors and/or attenuation of coupling efficacy of the surface receptors. Because chronic morphine administration generally produces little changes in either μ receptor mRNA or protein expression (Patel et al., 2002), most research has focused on the reduced coupling of μ receptor to its effectors. However, considering the limited reduction in potency of opioid agonists in functioning neurons after prolonged morphine exposure (Williams et al., 2001), it does not seem likely that behavioral opioid tolerance can fully be ascribed to decreased receptor coupling. Tolerance at systems levels also involves interaction of mechanisms of tolerance at molecular, cellular, and neural network levels. The magnitude of μ receptor uncoupling from G protein activation has been found to differ in different neuron types, where μ receptors are coupled with diverse signaling systems (Sim-Selley et al., 2000).

For receptor uncoupling, the mechanism of opioid receptor desensitization and internalization are assumed to resemble the model established for the β2-adrenergic receptor. In this model, agonist activation induces μ receptor phosphorylation by G protein-coupled receptor kinase 2, which increases the affinity of the receptor to arrestin 3 (Arr3). The binding of Arr3 uncouples the receptor from G protein signaling (desensitization) and initiates the receptor sequestration and internalization by an Arr3- and dynamin-dependent mechanism (Gainetdinov et al., 2004). The importance of this sequence of events was tested using Arr3-KO mice (Bohn et al., 2000). In these mice, chronic morphine treatment did not induce desensitization of the μ receptor and failed to produce either acute or chronic antinociceptive tolerance. However, these mice still became physically dependent on morphine and exhibited upregulation of AC activity that has been used as a cellular marker of opioid dependence (see below), suggesting that antinociceptive tolerance and physical dependence could have different underlying mechanisms. Although homologous desensitization thus seems to have a role in morphine tolerance at the receptor level, the importance of internalization is far from clear. Compared with other efficacious μ receptor agonists, such as [D-Ala\(_2\), NMe-Phe\(^4\), Gly-ol\(^5\)]-enkephalin (DAMGO), methadone, or sufentanyl, morphine has a low efficacy for producing receptor internalization (Whistler et al., 1999; Borgland et al., 2003), consistent with its profile as a partial agonist. Therefore, the effects of chronic morphine on desensitization are probably more important than internalization for the functional uncoupling of the receptor.

2. Cellular Signaling in Opioid Tolerance and Dependence. Behavioral tolerance can also result from mechanisms subsequent to receptor activation that adapt to restore function in the presence of drug. Continued presence of an inhibitory agonist leads to homeostatic compensation of the downstream signaling systems. Subsequent removal of the agonist can then lead to a rebound activation of the signaling systems and cause neural excitation. This overshoot may underlie the initial phases of withdrawal, but could also lead to more persistent effects of signaling systems that are reflected in synaptic events or structural changes. Particularly interesting for opioid-induced alterations are the signaling systems directly downstream from μ receptor activation, such as the AC-cAMP-PKA cascade and the MAPK cascades (Christie, 2008). Most likely, however, the mechanisms underlying chronic opioid effects are not limited to signaling systems directly downstream of receptor activation, but could reflect general homeostatic compensations in response to enhanced neural inhibition by opioid agonists.

The AC-cAMP-PKA cascade is a well-studied example of the tolerance resulting from compensation. Acute opioids inhibit AC, but depressed concentrations of cAMP return to normal during chronic opioid exposure as the AC activity is upregulated. Upon removal of the...
agonist, this compensatory increase persists and has therefore been regarded as a cellular marker of withdrawal (Sharma et al., 1975; Williams et al., 2001). This general picture is complicated by the existence of several isoforms of AC that could be regulated by distinct mechanisms (Watts and Neve, 2005). Three of these isoforms, ACI, ACII, and ACV, are neuronal and they are highly localized to synapses (Mons and Cooper, 1995). In cell lines and expression systems, ACI and ACV isoforms were supersensitized by chronic opioid treatment, but at neuronal tissue levels, the magnitude of upregulation is rather small, which could be due to the heterogeneity of cell types, presence of many opioid receptor subtypes, and AC isoforms (Williams et al., 2001). However, there is abundant evidence that the opioid-induced upregulation of the AC-cAMP-PKA cascade in specific synapses in different brain areas could contribute to neural and behavioral adaptations associated with morphine withdrawal.

Adaptations of the NEnergic LC have been implicated in opioid withdrawal behavior. Acute opioids decrease LC neuronal activity and inhibit the cAMP signaling pathway (Duman et al., 1988). Chronic opioids produce tolerance, demonstrated by upregulation of cAMP-dependent PKA during chronic morphine treatment (Ivanov and Aston-Jones, 2001), suggesting that this signaling pathway is involved in the withdrawal-induced hyperactivity of LC neurons. The LC hyperactivity may influence the activity of several other brain areas through widespread efferent projections. The phosphorylation state of CREB exerts transcriptional control over a large number of neurotransmitter receptors and signaling molecules. In the LC, overexpression of the constitutively active CREB mutant aggravated morphine withdrawal symptoms, whereas the dominant-negative CREB mutant suppressed them (Han et al., 2006). CREB overexpression did not affect neuronal firing at baseline, but it enhanced the effects of the AC activator forskolin, suggesting sensitization of the cAMP pathway. Therefore, morphine-induced increase in CREB activity could contribute to adaptations underlying morphine dependence and withdrawal. Similar to the LC, physiologic and biochemical studies have indicated a role for the periaqueductal gray area (PAG) in the expression of several withdrawal signs. Because the inhibition of GABAergic transmission by opioids causes disinhibition of PAG projection neurons, leading to activation of the descending pathways, adaptations in the PAG could be associated with the somatic and aversive withdrawal symptoms (Bandler and Shipley, 1994). In line with this idea, opioid withdrawal increased inhibitory transmission mediated by GABA_A receptors, which was blocked by PKA inhibitors and occluded by cAMP analogs (Ingram et al., 1998). It therefore seems that in the PAG, opioid dependence reflects μ receptor coupling to presynaptic inhibition in GABAergic nerve terminals via AC- and PKA-dependent

processes, with the withdrawal being associated with the abrupt increase in GABA release.

Opioid-induced adaptations in the nuclei of the mesolimbic DA pathway, such as the VTA and NAc that lead to alterations in the DAergic tone, could potentially modulate the motivational aspects of opioid withdrawal. In the VTA of morphine-treated guinea pigs, both inhibitory postsynaptic currents and the potency of forskolin and the cAMP-dependent protein kinase to increase these currents were augmented (Bonci and Williams, 1997), suggesting upregulation of the cAMP-dependent cascade. Similarly, in the NAc during withdrawal from chronic morphine, forskolin activation increased inhibitory transmission, suggesting that opioid dependence induced an opioid-sensitive cAMP-dependent mechanism that regulates transmitter release and could contribute to opioid withdrawal (Chieng and Williams, 1998).


Because chronic opioid administration produces alterations in the excitability in opioid-sensitive neurons, these changes can indirectly induce adaptations in other neurons and synapses in the circuitry. For example, this is illustrated by opioid-induced adaptations in the VTA DA neurons that are not primary targets of opioids. It has also been postulated that specific systems that functionally oppose the μ opioid receptor signaling could modulate opioid tolerance and dependence. One of these systems is the cholecystokinin (CCK) system that has been studied especially in relation to analgesic effects of μ-opioid receptor stimulation (reviewed in Wiesenfeld-Hallin et al., 1999). For example, CCK peptides, G protein-coupled CCK_1 and CCK_2 receptors show complex expression patterns and interact with a number of neurotransmitter/modulator mechanisms to both oppose and reinforce opioid-induced analgesia and anxiolysis within the PAG (Mitchell et al., 2011). CCK is also strongly expressed in the VTA (Ballaz et al., 2013) and can influence drug-induced reward processes. Indeed, CCK infusion into the NAc attenuated intracranial electrical self-stimulation in the VTA (Vaccarino and Koob, 1984). Systemic CCK_2, but not CCK_1, receptor antagonism attenuated expression of morphine-induced CPP (Lu et al., 2000; Mitchell et al., 2006) and naloxone-induced withdrawal symptoms (Lu et al., 2000). Furthermore, the CCK_2 antagonism in the anterior NAc attenuated morphine-induced CPP, whereas that in the posterior NAc enhanced the CPP in a manner dependent on D_2 receptors (Mitchell et al., 2006).

Another important one of these modulating systems is the dynorphin/κ-opioid receptor system that has been suggested to participate in mediating opposing alterations in behavior and brain neurochemistry that occur in response to repeated drug use, particularly stimulants and alcohol (Shippenberg et al., 2007). Therefore,
aberrations in this opioid system could also contribute to the neural dysregulations underlying the development of addiction. In addition, increasing evidence is accumulating that the opioid receptor-like NOP receptor and its endogenous ligand N/OFQ contribute to changes in plasticity observed with morphine tolerance and dependence. In particular, increased antiopioid activity of the nociceptin system was suggested to participate in the development of opioid tolerance and dependence, because either genetic or pharmacological blockade of NOP signaling prevented them (Ueda et al., 2000; Chung et al., 2006).

Glia activation is also emerging as a novel modulator of opioid effects, although glia have mainly been studied in the context of pain at the spinal level. The glial cells, particularly astrocytes and microglia in this context, release several substances that can modulate glia-neuron communication and shape neuronal plasticity (Watkins et al., 2005). Chronic morphine treatment induces glial activation in many brain areas, including the spinal cord, cingulate cortex, hippocampus, and PAG (Song and Zhao, 2001; Hao et al., 2011). Morphine and other μ receptor agonists bind to an accessory protein of glial toll-like receptor 4 (TLR4), myeloid differentiation protein 2, and thereby induce TLR4 oligomerization and trigger subsequent glia-mediated proinflammatory responses. TLR4 activation causes the release of proinflammatory and neuroexcitatory cytokines, such as tumor necrosis factor-α and interleukin-1β (Wang et al., 2012b). Tumor necrosis factor-α has been shown to modulate neuronal functions in various ways, including an increase of AMPAR activity, spontaneous and evoked transmitter release, and cell surface expression of AMPARs and NMDARs (reviewed in Ouyang et al., 2012). Both the opioid-active (+)-naloxone and (-)-naloxone and the opioid-inactive isomers (+)-naloxone and (+)-naltrexone block activation of TLR4 signaling, providing a means of specifically inhibiting glial activity by the nonopioid isomers (Watkins et al., 2009). They have been shown to attenuate morphine analgesic tolerance and withdrawal symptoms (Hutchinson et al., 2010), as well as opioid addiction-like behavior, such as morphine-induced CPP, remifentanil SA (Hutchinson et al., 2012), and abstinence enhancement of cue-induced heroin seeking (Theberge et al., 2013). However, the potential of (+)-naloxone and (+)-naltrexone as TLR4 antagonists has been contested on the basis of in vitro assays (Skolnick et al., 2014). Further indications of the role of glial cells in morphine behaviors come from interesting experiments by Schwarz and coworkers (Schwarz et al., 2011; Schwarz and Bilbo, 2013), who first showed that neonatal handling of rats (from P2 to P20) strongly decreased the methylation of the gene encoding the cytokine IL-10 in microglia-enriched brain samples and increased IL-10 expression in adulthood. This was negatively correlated with morphine-induced reinstatement of CPP (Schwarz et al., 2012b). Tumor necrosis factor-

4. Opioid-Induced Changes in Excitatory and Inhibitory Synaptic Plasticity. The compulsiveness of drug use has been conceptualized to be mediated by pathologic forms of memory that are based on synaptic adaptations resembling other forms of activity-dependent plasticity, such as LTP and LTD (Kauer and Malenka, 2007). For example, synaptic plasticity in the VTA has been suggested to be a common cellular substrate for abused drugs in the initial phases of drug addiction. Thus, a single administration of morphine also induces plasticity in the VTA DA neurons that lasts long after the administration, as evidenced by increased ratio of AMPAR/NMDAR currents, which is consistent with morphine-induced LTP of glutamatergic synapses onto DA neurons (Saal et al., 2003). In addition, there is now increasing evidence for GABAergic inhibitory plasticity after morphine administration. It has long been known that DA-dependent opiate actions involve VTA GABA neurons. By inhibiting local GABAergic interneurons, opiates disinhibit DA neurons (Johnson and North, 1992), which results in increased DA cell firing and DA release in the VTA projection areas, including the NAc.

GABA	extsubscript{A} receptor-mediated synaptic transmission onto VTA DA neurons expresses long-term potentiation, known as LTP	extsubscript{GABA} (Nugent et al., 2007; Niehaus et al., 2010). This form of LTP has been described as heterosynaptic, because it requires postsynaptic NMDAR activation but results from increased GABA release at neighboring inhibitory nerve terminals. Activation of NMDARs on postsynaptic DA neurons produces the retrograde signaling molecule NO, which in turn activates guanylate cyclase in presynaptic GABAergic nerve terminals. Increased levels of guanylate cyclase promote potentiation of GABA release, thereby initiating LTP	extsubscript{GABA}. Further work showed that NO-cGMP signaling activates cGMP-dependent kinase PKG and that NO-cGMP signaling potentiates GABA	extsubscript{A} receptor-mediated, but not GABA	extsubscript{B} receptor-mediated, synaptic responses (Nugent et al., 2009). Morphine in vivo blocks LTP	extsubscript{GABA} by interrupting signaling from NO to guanylate cyclase and therefore reduces normal inhibitory control (Nugent et al., 2007).

In addition to LTP	extsubscript{GABA}, the same GABAergic synapses can also exhibit long-term depression (LTD	extsubscript{GABA}) in response to 1-Hz LFS. This form of synaptic downregulation is not dependent on NMDA or CB	extsubscript{1} receptors but on D	extsubscript{2}R-like receptor activation. This D	extsubscript{2}R activation...
releases a neurochemical signaling via inhibitory
G protein-PKA-inositol phosphate receptor IP$_3$-R-Ca$^{2+}$-
calcineurin-A-kinase anchoring protein 150 AKAP150
at GABAergic synapses (Dacher et al., 2013). Importantly, LTD$_{GABA}$ is abolished/occluded by a single dose
of morphine 24 hours before the measurements (Dacher
and Nugent, 2011a). Collectively, morphine-induced
changes in LTP$_{GABA}$ and LTD$_{GABA}$ represent forms of
inhibitory plasticity that together with changes in
excitatory synapses provide a mechanism to enhance
mesolimbic DA transmission and thereby increase the
incentive properties of morphine (Dacher and Nugent,
2011b).

More recently, Atwood et al. (2014) found in vitro LTD
of glutamate inputs to the dorsolateral striatum in-
duced by agonists of $\mu$, $\delta$, and $\kappa$ receptors, with $\mu$ and $\delta$, but not $\kappa$, agonists also inducing the LTD in the
dorsomedial striatum. The depression of synaptic re-
sponses could be blocked by receptor subtype-specific
antagonists, but once induced and the agonists washed
away, the LTD responses were insensitive to opioid
receptor antagonists. Importantly, a single in vivo dose of
oxycodone occluded the $\mu$ receptor-LTD and endocannabinoid-LTD for 2 days. These novel mecha-
nisms may contribute to striatum-dependent behaviors,
such as habit learning.

5. Chronic Opioids and Synaptic Plasticity. Plasticity associated with chronic opioid exposure could underlie various aspects of opioid addiction, such as sensitization, dependence, withdrawal, and opioid seek-
ing. Opioid-induced plasticity in VTA excitatory and
inhibitory synapses could also provide mechanisms for
these chronic opioid effects. Work assessing the alter-
ations in glutamate receptor subunit expression found
that continuously delivered morphine had no influence
on glutamate receptor levels. However, intermittent
morphine administration schedule that has been associ-
ated with behavioral sensitization elevated the VTA
GluA1 levels (Fitzgerald et al., 1996). Upregulated
GluA1 subunit levels could contribute to increased
excitability of VTA DA neurons, which was confirmed
by selective viral-mediated increase of GluA1 subunit
in the VTA bringing up a sensitized response to morphine in
locomotion and place preference conditioning (Carlezon
et al., 1997).

Because the pattern of VTA DA neuron firing depends
on excitatory glutamate drive, any adaptive changes in
excitatory synaptic transmission produced by chronic
morphine are reflected in the activity of DA neurons and
could thus also contribute to decrease of mesolimbic
DAergic neuronal activity observed in vivo in morphine-
withdrawn rats. Manzoni and Williams (1999) found
that during acute withdrawal from chronic morphine
treatment there was a fundamental change in the
presynaptic regulation of the glutamate release at this
synapse. First, the transduction pathway responsible
for the opioid receptor-mediated inhibition was less
sensitive to agents that disrupt the normal inhibition,
including the potassium channels blocker 4-aminopyr-
dine and the lipoxygenase inhibitor baicalein. Second,
they found that in withdrawn animals, the sensitivity of
presynaptic inhibitory control mediated by GABA$_{B}$ and
mGlu$_{2/3}$ receptors was enhanced. Together, these find-
ings suggested that one of the consequences of withdraw-
alone from chronic morphine could be augmented
presynaptic inhibition of the excitatory inputs to the
VTA DA cells, which would add to the inhibition of these
neurons. Increased GABA release from interneurons
further potentiates the inhibition of VTA DA neurons
during withdrawal (Bonci and Williams, 1997). The
enhanced GABA release appears to be correlated with
physical dependence on morphine, as measured by
somatic withdrawal signs during withdrawal (Madhavan
et al., 2010). As previously discussed, morphine-activated
$\mu$ receptors do not exhibit substantial endocytosis and
recycling. In a knock-in mouse, in which a genetic change
in the $\mu$ receptors promotes morphine-induced receptor
desensitization and endocytosis in the VTA GABA inter-
eurons, the increased GABA release onto VTA DA
neurons was abolished during withdrawal. These data
suggest that receptor trafficking is a compensatory cellular
mechanism that could modulate synaptic plasticity
produced by chronic opioid administration (Madhavan
et al., 2010).

GABA$_{A}$ergic neurotransmission in the VTA has been
suggested to mediate the switch from the drug-naive to
the dependent and addicted state. A discrete population
of VTA GABA$_{A}$ receptors was shown to undergo a switch
from inhibitory to excitatory in heroin-dependent and
withdrawn rats (Laviolette et al., 2004). The precise
mechanism of this transformation is not known, but it
seems to be mediated by the BDNF, as demonstrated by
promotion of the switch by BDNF injection into the VTA
and attenuation of the aversive motivational states of
heroin withdrawal by local knockdown of the BDNF
receptor TrkB expression (Vargas-Perez et al., 2009,
2014). The cellular phenotypes of the circuit compo-
ents for this model require still further work in the
VTA and associated areas. More generally, these results
may relate to stress vulnerability and social defeat
stress, which affect drug sensitivities and motivation to
seek reward and drugs (Piazza et al., 1993; Shaham
et al., 1997; Kauer, 2003; Yap et al., 2005; Niehaus et al.,
2010; Ungless et al., 2010; Cabib and Puglisi-Allegra,
2012; McEwen, 2012; Wang et al., 2013c; Walsh et al.,
2014). Recently, BDNF signaling in the NAc has been
shown to be involved in susceptibility to social defeat
stress, actually mediating stress effects, such as social
aversive (Berton et al., 2006). Phasic optogenetic acti-
vation of VTA-NAc pathway had no effects on social
interaction and BDNF levels in the NAc in stress-naive
mice, but strongly reduced social interaction and in-
creased BDNF in mice subjected to a subthreshold
social stress (Walsh et al., 2014), confirming that
stressful events induce prolonged changes in the mesolimbic DA pathway.

In addition to augmentation of VTA presynaptic mGlu2/3 receptor functions in morphine-withdrawn subjects (Manzoni and Williams, 1999), chronic morphine also alters mGlu2/3-mediated events in the NAc. Activation of these receptors induces LTD at NAc glutamatergic synapses. However, this mGlu2/3-dependent LTD was abolished during withdrawal from chronic morphine (Robbe et al., 2002). Together with enhancement of VTA presynaptic mGlu2/3 function and the ability of mGlu2/3 agonists to reduce morphine withdrawal symptoms, these findings suggest that regulation of mGlu2/3 synaptic plasticity in the mesolimbic system could be important for the development of opioid addiction. Other cellular events underlying chronic opioid actions in the NAc include trafficking of AMPARs in the D2R-MSN. Chronic morphine administration was associated with a decrease in surface trafficking of the GluA1 subunit, which could be a substrate of morphine-induced alterations in neuronal activity within the NAc (Glass et al., 2008).

The involvement of opioid receptors in the modulation of hippocampal plasticity has been amply documented. Chronic opioids produce cognitive deficits in human opioid users and reduce neurogenesis in the rat HC (Guerra et al., 1987; Eisch et al., 2000), suggesting that maladaptive alterations in hippocampal plasticity could partly contribute to opioid addiction, craving, and relapse. In accordance with this idea, chronic morphine or heroin treatment attenuated the hippocampal CA1 LTD during withdrawal and produced deficits in spatial learning (Pu et al., 2002; Salmanzadeh et al., 2003). Hippocampal LTD could be restored by re-exposure to opioids, including the μ receptor agonist [D-Ala2, NMe-Pheβ, Gly-ol]-enkephalin (DAMGO) and inhibitors of PKA, suggesting that upregulation of the cAMP pathway was one of the underlying mechanisms (Pu et al., 2002; Bao et al., 2007). In addition, chronic morphine exposure increased hippocampal extracellular adenine concentrations, which contributed both to the inhibition of hippocampal LTD and impairment of spatial memory retrieval, and both deficits could be reversed by an adenosine antagonist or adenosine deaminase (Lu et al., 2010a). There is also evidence to suggest that opioid-induced changes in hippocampal synaptic plasticity could have a role in drug-context associations. Morphine-induced CPP was associated with increased basal synaptic transmission and impaired hippocampal LTD, accompanied with increased synaptic expression of the NMDAR subunits GluN1 and GluN2B. After extinction of CPP, impaired LTP and elevated GluN2B expression persisted, but morphine-primed reinstatement of CPP was associated with LTP similar to controls (Portugal et al., 2014). These findings implicate hippocampal LTD and glutamatergic transmission in context-dependent morphine effects, consistent with blockade of morphine CPP by GluN2B-selective antagonists (Ma et al., 2006).

In agreement with the hypothesized role of the AMPARs in drug-induced plasticity, a 12-hour withdrawal from four escalating morphine doses (5, 8, 10, 15 mg/kg at 12-hour intervals) increased the expression of GluA2-lacking (Ca2+-permeable) glutamate receptors (containing GluA1 and/or GluA3 subunits) in mouse hippocampal synaptic and extrasynaptic fractions, correlating with reduced magnitude of LTD in HC neurons without changes in sensitivity to AMPA (Billa et al., 2010). When this same morphine dosing was used in a specific setting (locomotor activity chambers), the treated mice challenged with 5 mg/kg morphine 1 week later showed robust context-dependent locomotor sensitization in the same chambers compared with saline treatment or unpaired morphine challenge (Xia et al., 2011). Interestingly, this persistent effect correlated with increased phosphorylation of GluA1 Ser845 and impaired LTP in the HC. However, earlier work revealed that GluA1-KO and GluA1-Arg (Ca2+ impermeable) mutant mice show enhanced context-dependent sensitization to 3 mg/kg morphine in comparison with wild-type littermates 5 days after 6-day treatment with 10 mg/kg morphine (Vekovischeva et al., 2001), indicating that GluA1 subunits are not obligatory for context-dependent morphine sensitization and that their impaired function can be compensated. Indeed, although a single dose of morphine induces persistent increase in AMPAR/NMDAR current ratios in VTA DA neurons 24 hours after administration in wild-type mice (Saal et al., 2003; Heikkinen et al., 2009), it fails to alter this ratio in GluA1-KO mice that had already increased ratio at the baseline (Aitta-aho et al., 2012). The lack of the VTA DA neuron plasticity might correlate with impaired state-dependent place preference, because the GluA1-KO failed to show place preference under morphine testing (Aitta-aho et al., 2012). Taken together, these data support the role of the AMPARs in adaptation to opioid effects, although the details of subunit roles are still poorly known and most likely differ in different brain regions.

In the mPFC of rats with a history of heroin SA, GluA2 subunit-containing AMPARs are downregulated in the principal pyramidal cells, leading to reduced AMPAR/NMDAR current ratios and increased rectification (Van den Oever et al., 2008), which permitted heroin seeking in abstinent and extinguished rats by a compound heroin-related cue. Blocking the GluA2 subunit downregulation (and subsequent LTD?) also prevented relapse to heroin seeking. Furthermore, exposure to heroin cues after abstinence almost doubled the frequency of GABAergic spontaneous IPSCs in mPFC pyramidal neurons in brain slices prepared 30 minutes after cue exposure (Van den Oever et al., 2010). The heroin seeking was also associated with a reduction in ECM proteins around the Pv-containing interneurons, suggesting that the PPN was downregulated to
enhance the inhibition of principal neurons. These data indicate that mPFC output is reduced after relapse-inducing cue exposure and that relapse to heroin seeking is associated with altered structural and functional aspects of both glutamatergic and GABAergic systems in the mPFC.

Drug-induced changes in the structural plasticity of dendrites are potentially an important component of synaptic plasticity. Basic research into the dynamics of the dendritic spines suggests that the size and shape of individual spines correlates with forms of synaptic plasticity, such as LTP and LTD at glutamate synapses. First, stabilization of the transient immature spines into functional spines is activity dependent. Second, experimental protocols that induce LTD are linked with shrinkage or retraction of spines, whereas induction of LTP enhances formation of new spines and enlargement of existing spines (Nagerl et al., 2004). In sharp contrast to psychomotor stimulants that increase dendritic branching and density, chronic morphine exposure reduces the size of VTA DA neurons (Sklar-Tavron et al., 1996; Russo et al., 2007; Mazei-Robison et al., 2011) and the density of dendritic spines in brain areas considered relevant for drug addiction, such as the NAc, OFC, and HC (Robinson and Kolb, 1999b; Robinson et al., 2002). Because stimulants and opioids similarly induce transcription factors (e.g., CREB, ΔFosB) that regulate expression of cytoskeletal proteins, the disparate findings on spine density are perplexing. It is possible that stimulants and opioids recruit different signaling pathways regulating synaptogenesis acting under CREB or ΔFosB control or that additional pathways are involved (Russo et al., 2010). For example, the reduced VTA DA neuron soma size induced by chronic opioids is hypothesized to be due to downregulation of BDNF signaling, because it was reversed by local infusion of BDNF (Sklar-Tavron et al., 1996). Further downstream neurotrophic signaling pathways mediating this effect include the downregulated Akt-mTORC2 signaling that increases the excitability of VTA DA neurons and could thereby trigger a compensatory decrease in neuron soma size and DA output (Mazei-Robison et al., 2011). The results are consistent with the view that drug state-associated activity in the brain is also based on rapid changes in the neuronal structures.

6. Conclusions. Opioids are effective in the acute treatment of pain, but they are also abused due to their addictive properties. After chronic exposure, opioids produce a wide range of neuroadaptations that are thought to underlie opioid tolerance, withdrawal, and compulsive use. These adaptations can be identified at different organizational levels, including those at the μ opioid receptors that undergo desensitization and internationalization, depending on the μ opioid receptor agonist. The alterations in intracellular signaling systems are complex, but opioid-induced upregulation of the AC-cAMP-PKA cascade in specific synapses in different brain areas is suggested to contribute to neural and behavioral adaptations associated with morphine withdrawal. Several neuropeptide systems have been described to functionally oppose the μ opioid receptor signaling, thus inducing development of opioid tolerance and dependence. In accordance with the idea of aberrant forms of synaptic plasticity driving addictive behavior, opioids influence plasticity both at glutamatergic and GABAergic synapses in many brain regions, including the mesolimbic DA system. However, further work is needed to understand how these adaptations together produce the addicted state of compulsive opioid use and relapse.

H. Cannabinoids: Multiple Mechanisms and Possible Indications

Cannabis is one of the oldest and most used “illegal” drugs in the world. There are also many potential therapeutic indications for cannabinoi-based drugs (Izzo et al., 2009; Pertwee, 2012), with the treatment of spasticity and pain in multiple sclerosis and of nausea, lack of appetite, and subsequent wasting in AIDS having already been translated into clinical medicine. The importance of normal endocannabinoid function for brain health was dramatically revealed during chronic treatment of extreme obesity with rimonabant, the cannabinoid type 1 (CB1) receptor inverse agonist. Although rimonabant (SR141716) was as efficient as any other pharmacological treatment in producing sustained reduction in weight and normalizing lipid and glucose metabolism in clinical trials (Despres et al., 2005; Van Gaal et al., 2005), it was withdrawn from the market rapidly after its launch because of treatment-associated psychiatric adverse effects, such as anxiety, depression, and suicidality in a minority of the population studied (depressed mood was an exclusion criterium for the clinical studies) (Christensen et al., 2007; Van Gaal et al., 2008).

Cannabis intoxication is rarely considered lethal for otherwise healthy subjects (Weissenborn and Nutt, 2012; Hall, 2015). Pediatric cases of cannabis-induced coma and young- to middle-age adult cases of cannabis-induced cardiovascular fatalities and arrhythmias were recently reported (Bachs and Morland, 2001; Tormey, 2012; Le Garrec et al., 2014). The low toxicity may be related to low abundance of CB1 receptors in vital brain stem nuclei regulating cardiovascular and respiratory functions (Herkenham et al., 1990), although the receptors are present in the PAG and rostroventral medulla, in which activation of CB1 receptors may produce analgesia by disinhibition of descending pain-relieving neurons (Vaughan et al., 1999, 2000). Another mechanism related to the inherent limits of acute cannabinoid toxicity may be that treatment with Δ9-tetrahydrocannabinol (Δ9-THC) activates the ERK1/2-MAPK pathway via CB1 receptors producing an increase in the cytochrome P450 side-chain-cleavage enzyme (P450scct),
rapidly increasing the brain synthesis of pregnenolone from cholesterol (Vallee et al., 2014). Among other actions, pregnenolone is an allosteric inhibitor of CB1 receptors, thereby significantly reducing a wide range of pharmacological effects of Δ9-THC, the main active substance in cannabis. Moreover, Δ9-THC is only a partial agonist at cannabinoid receptors (Howlett et al., 2002), which may limit its toxicity. It should be noted that synthetic cannabinoids of various chemical structures and receptor binding profiles are much more potent and efficient than Δ9-THC, and an alert on their potential toxicity and lethality recently appeared (Trecki et al., 2015).

1. Endocannabinoid System as an Endogenous Lipid Messenger System. The cannabinoid system mediates its main effects via the CB1 and CB2 receptors (Pertwee and Ross, 2002; Pertwee et al., 2010). They are modulated by the endocannabinoids (eCB) N-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG), plant cannabinoids (phytocannabinoids) such as Δ9-THC, as well as synthetic cannabinoid agonists [e.g., WIN 55,212-2 [(R)-5-[(2R,3R)-4,5-dihydro-2-methyl-3-(1,1-dimethylpropyl)-1H-indol-3-yl]-N-methyl(pyridin-2-yl)methanamide] mesylate, CP55,940 [(−)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol], HU-210 [(6αR)-3-(1-naphthalenylmethyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol], and antagonists (often pyran-9-methanol, and JWH-081 [(1-pentyl-1H-indol-3-yl)-1-naphthalenylmethanone mesylate], GW405833 [1-(2,3-dichlorobenzoyl)-5-methoxy-2-methyl-3-[4-(morpholinyl)-ethyl]-1H-indole] (agonized by AM630) were found to reduce DA neuron firing and cocaine SA in the wild-type mice but not in CB2 receptor partial knockout mice (Xi et al., 2011; Zhang et al., 2015a).

Most of the brain CB1 receptors are localized in axons and presynaptic terminals of GABAergic and glutamatergic neurons (Howlett et al., 2002). This presynaptic localization is illustrated by the mismatch of CB1 receptor mRNA (enriched in the soma) and the autoradiographic signal for CB1 receptor-specific ligand (e.g., [3H]CP55940) in the rodent and human brain. A clear mismatch is also seen in glutamatergic cerebellar granule neurons displaying CB1 mRNA in the cerebellar granule cell layer and CB1 ligand binding in the molecular layer, where the axons impinge on dendritic trees of Purkinje neurons. Another example is the mismatch in the GABAAergic neurons of the basal ganglia, showing CB1 mRNA in the caudate-putamen and presynaptic CB1 ligand binding in the external segment of the globus pallidus and the substantia nigra reticulata. Yet the definitive proof for presynaptic localization of CB1 receptor comes from direct visualization using antibodies at the electron microscopy level especially in hippocampal GABAergic CCK-expressing basket cells (Hajos et al., 2000; Katona et al., 2000). In conclusion, the CB1 receptors are predominantly presynaptic in both glutamate and GABA neurons, and thus have the possibility to modulate synaptic efficacy and neuronal circuit functions by controlling the release of the main neurotransmitters (Freund et al., 2003). As an example, activation of CB1 receptors on striatopallidal axon terminals leads to presynaptic inhibition of GABAergic transmission to globus pallidus neurons (Engler et al., 2006), whereas activation of CB1 receptors on subthalamic neuron axon terminals leads to presynaptic inhibition of glutamatergic excitation of globus pallidus neurons (Freiman and Szabo, 2005).

The eCB system is tightly coupled to regulation of synaptic efficacy. The main eCBs, 2-AG and anandamide, are formed from membrane phospholipid precursor molecules in the postsynaptic neuron in response to increased intracellular Ca2+ after intense activation, after which they are retrogradely released onto presynaptic CB1 receptors (Figs. 2, 6, and 12). The most abundant eCB 2-AG (Mechoulam et al., 1995) is formed from diacylglycerol by the action of specific postsynaptic membrane-associated diacylglycerol lipase (DG لا) (Bisogno et al., 2003; Matyas et al., 2008), and degraded by presynaptic monoacylglycerol lipase (MGL) that colocalizes with the CB1 receptor (Dinh et al., 2002; Gulyas et al., 2004). Although MGL is responsible for about 85% of 2-AG hydrolysis, two other serine hydrolase family enzymes

Van Sickle et al., 2005). Specifically, CB2 receptors are present in the VTA DA neurons, and the selective agonists JWH133 [(6αR,10αR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran] and GW405833 [1-(2,3-dichlorobenzoyl)-5-methoxy-2-methyl-3-[4-(morpholinyl)ethyl]-1H-indole] (agonized by AM630) were found to reduce DA neuron firing and cocaine SA in the wild-type mice but not in CB2 receptor partial knockout mice (Xi et al., 2011; Zhang et al., 2015a).
have been discovered with potential roles in hydrolyzing excess 2-AG, namely postsynaptic ABHD6 and microglia-enriched ABHD12 (Savinainen et al., 2012). Anandamide (Devane et al., 1992) is synthesized by several enzymes and degraded by the fatty acid amide hydrolase localized mainly in the postsynaptic neuron (Gulyas et al., 2004). Genetic inactivation of DGLα, but not that of DGLβ, in mice abolished retrograde eCB-mediated short-term synaptic plasticity processes in the cerebellar, hippocampal, and striatal slices using various electrical and pharmacological ways to induce eCB release (Gao et al., 2010b; Tanimura et al., 2010).

2. Short-term Plasticity Involving Retrograde Endocannabinoid Signaling. In hippocampal slices, a train of action potentials of CA1 neurons induces a suppression of GABAA receptor-mediated inhibition (Pitler and Alger, 1994) with reduced frequency of spontaneous IPSPs and reduced amplitudes of IPSCs but with unchanged sensitivity to iontophoretically applied GABA. This reproducible phenomenon was called DSI (Fig. 2), and it was further shown that there was a lag of about 1 second between the train of action potentials and maximal DSI and that it was prevented by pertussis toxin (Pitler and Alger, 1994). Therefore, presynaptic mechanisms were hypothesized, and a few years later, it was found that this transient DSI in pyramidal neurons was produced by the short-distance “retrograde” release of eCBs that inhibited GABA release from interneurons via an action on presynaptic CB1 receptors (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Retrograde eCB signaling is very widespread and affects both inhibitory and excitatory synapses. DSE was also observed in the cerebellar cortex, where a short depolarization of Purkinje neurons induces the inhibition of glutamatergic inputs from parallel and climbing fibers (Kreitzer and Regehr, 2001). Similar Purkinje neuron depolarization also suppresses the GABAergic synapses from cerebellar cortical interneurons (Tanimura et al., 2010), such as the GABAergic basket cells (Szabo et al., 2004). In addition to the HC and cerebellum, DSI and/or DSE is also observed, e.g., in the VTA DA neurons (Melis et al., 2004), NAc MSNs (Tanimura et al., 2010; Shonesy et al., 2013), and in the BLA neurons (Zhu and Lovinger, 2005; Patel et al., 2009; Yoshida et al., 2011; Shonesy et al., 2014). Together these findings suggest that the retrograde eCB signaling is a very general “local” transient process that tunes neurotransmission (reviewed in Wilson and Nicoll, 2002; Kano et al., 2009).

Upon firing of a neuron containing the eCB synthesis machinery, its Ca2+ level increases to micromolar levels via VGCCs and NMDAR channels, maybe also from release from intracellular Ca2+ stores (Kano et al., 2009). Through as yet poorly known mechanisms, 2-AG synthesis is then promoted, part of that being dependent on the phospholipase PLCβ. For a single activated neuron, diffusion of endocannabinoids extends to a sphere of about 40 μm (Wilson and Nicoll, 2001), the effect thus remaining very local. Other important activators of PLCβ are all GPCRs that signal through Gq/11 proteins (Kano et al., 2009), such as group I mGlu receptors and M1/M3 muscarinic ACh receptors (Katona and Freund, 2012). The eCB-CB1 receptor system is thus an important part of a versatile mechanism in presynaptic regulation of brain circuits.
Signal transduction of CB1 (and CB2) receptors is mediated via G_{i/o} proteins (reviewed in Heifets and Castillo, 2009)). Thus, the activation of CB2 receptors generally leads to inhibition of AC (leading to reduced phosphorylation of PKA targets) and P/Q-type calcium channels and stimulation of inwardly rectifying potassium channels. Many other signaling alternatives have been proposed for these receptors, which may also be present as homodimers or heterodimers.

3. Long-term Plasticity and CB1 Receptors. It is thus clear that the activation of CB1 receptor by endogenous or exogenous ligands has the potential to influence synaptic plasticity. An early in vitro study showed that a low concentration (10 pM) of Δ^9-THC prolonged LTD, whereas higher concentrations (100 and 1000 pM) reduced the duration of LTP in rat CA1 hippocampal slice preparation (Nowicky et al., 1987). Cannabinoid receptor activation in vitro impairs LTP and LTD by reducing presynaptic glutamate release to a level below that which is required to depolarize the postsynaptic membrane to relieve the Mg^{2+}-blockade of NMDARs (Misner and Sullivan, 1999). Cannabinoid receptor agonists (WIN 55,212-2 and CP55,940) and an antagonist (rimonabant) favor LTD and LTP, respectively, in the glutamatergic synapses of rat PFC slice preparation (Auclair et al., 2000). WIN 55,212-2 blocks the induction of LTP and suppresses paired-pulse depression in rat CA1 slice preparation, an effect that is blocked by rimonabant (Paton et al., 1998). Striatal LTD at glutamate synapses is produced by postsynaptic release of eCBs, because it is not detected in CB1-KO mice, it is blocked by rimonabant and activated by postsynaptic anandamide (Gerdenman et al., 2002). Also, the synthetic CB1 ligand, JWH-081, impairs LTD in mouse CA1 slice preparation (Basavarajappa and Subbanna, 2014).

A single administration of Δ^9-THC (3 mg/kg) to mice blocked the eCB-dependent LTD in the NAc and HC for at least 1 day and reduced the hippocampal DSI after 20 hours by almost 60% (Mato et al., 2004). A single dose of Δ^9-THC (≥5 mg/kg) impairs the LFS-induced LTD in the HC (Chen et al., 2013b), which is absent in cyclooxygenase-2 (COX-2) knockout mice. Seven-day treatment of mice with Δ^9-THC (10 mg/kg) impaired fear memory (reduced footshock-induced freezing 24 hours after the last dose) and spatial learning and memory in a water maze test (Chen et al., 2013b). These CB1 receptor-mediated effects were dependent on increased COX-2 activity and production of PGE_2. Furthermore, in the HC, this treatment downregulated the expression, phosphorylation, and surface trafficking of GluA1, GluN2A, and GluN2B subunits and consequently reduced LTD at CA3-to-CA1 synapses, which was apparently correlated with reduced dendritic spine density of HC-CA1 neurons. Interestingly, Δ^9-THC-induced CB1 receptor activation upregulates COX-2 expression, whereas that by 2-AG suppresses the COX-2 expression in cell culture models (Chen et al., 2013b). This suggests that exogenous agonists and eCBs may have different signaling routes, with Δ^9-THC acting via G_{i/o} subunits and 2-AG via the G_{i/o} subunit. In cultured hippocampal neurons, synaptic release sites are greatly reduced by incubation with Δ^9-THC, which is mediated by decreased cAMP (Kim and Thayer, 2001). It should be further noted that the COX-2 enzyme is known to oxidize eCBs, anandamide and 2-AG, and to produce derivatives with prostaglandins, the selective inhibition of which has been proposed to be used in drug development to enhance eCB actions (Hermanson et al., 2014).

In rats, acute treatment with Δ^9-THC increased ERK, pCREB, and c-Fos in the caudate-putamen and cerebellum, whereas chronic Δ^9-THC exposure induced increases in ERK, pCREB, and FosB in the PFC and HC (Rubino et al., 2004). Blocking ERK activation in Ras-GRF1-KO mice prevented tolerance to Δ^9-THC in sedation and analgesia (Rubino et al., 2004). In mice, it has been reported that a very low dose (0.002 mg/kg) of Δ9-THC induced long-lasting (7 weeks) modifications of ERK activity in the HC, FC, and cerebellum and elevated pCREB in the HC and BDNF in the FC (Fishbein et al., 2012). The effects of cannabinoids on the ERK1/2 signaling in vivo were regulated by both D1 and NMDARs (Daigle et al., 2011). Chronic exposure of rats to Δ^9-THC (1.5 mg/kg for 7 days) upregulated BDNF in the NAc, posterior VTA, hypothalamic paraventricular nucleus, and mPFC (Butovsky et al., 2005). Chronic exposure to Δ^9-THC prevents synaptic plasticity in the NAc and reduces sensitivity of GABAergic and glutamatergic synapses to cannabinoids and opioids (Hoffman et al., 2003), indicating a possible tolerance and cross-tolerance development. It is noteworthy that subchronic treatment of mice with Δ^9-THC induced CB1 receptor desensitization in [35S]GTPγS autoradiography in several brain regions, but not in the striatal regions, in which the treatment induced the greatest expression of ΔFosB (Lazenga et al., 2014). This suggests that gene expression regulated by ΔFosB counteracted the CB1 receptor desensitization.

4. Cannabinoid Effects on Brain Development. Recent rodent studies have indicated that cannabinoid exposure to the developing brain during pregnancy or early development may cause alterations in offspring behavior and physiology via CB1 receptor-mediated mechanisms (reviewed in Calvigioni et al., 2014). One study revealed that maternal exposure to Δ^9-THC affected the stability of cortical neurites and connections and disrupted CB1 receptor-positive perisomatic baskets in the cortex (Tortoriello et al., 2014). This was mediated by a reduction in a microtubule-binding protein Superior Cervical Ganglion 10 (stathmin-2) via phosphorylation by CB1 receptor-mediated activation of JNK. Importantly, reduced expression of Superior Cervical Ganglion 10 mRNA and protein was also found in human hippocampal tissue from cannabis-exposed fetuses (Tortoriello...
et al., 2014). Behavioral effects are also evident, because CB1 receptor blockade in the prenatal period decreases immobility behavior, demonstrating that the cannabinoid system plays a crucial role in ontogeny of psychomotor behaviors (Moreno et al., 2005).

Cannabinoid receptors in the white matter tracts are abundant in rats during early brain development but dramatically reduce by P30 (Romero et al., 1997). In prenatal brains, WIN55,212 activated [35S]GTPγS binding in white matter areas, which could be antagonized by the CB1 receptor antagonist rimonabant. Indeed, various components of the brain eCB system are present at early development (Fernandez-Ruiz et al., 2000), suggesting a possible involvement of cannabinoid signaling in neurogenesis and gliogenesis (Berghuis et al., 2005; Arevalo-Martin et al., 2007; Garcia-Ovejero et al., 2013; Zhou et al., 2015), development and myelination of neuronal pathways, and thus the macro- and microstructure of the brain during adolescence and adulthood (Keimpema et al., 2011). Adult neurogenesis in the HC is compromised in DGL−/− KO and DGLβ−/− KO mice (Gao et al., 2010b), indicating a role for eCBs. Importantly, CB1 receptors are present in myelin-forming oligodendrocytes and CB2 receptors in microglia, with both being increased in white matter plaques of multiple sclerosis (Benito et al., 2007). As in rodents, CB1 receptor binding is remarkable in the white matter tracts of human fetal brains but hardly detectable in postmortem brains from juvenile or adult ages (Mato et al., 2003). In heavy cannabis users, axonal connectivity, as assessed by diffusion-weighted magnetic resonance imaging, was impaired in comparison with matched controls (Zalesky et al., 2012), with the impairment correlating with age of starting regular cannabis use. In another recent study, marijuana use was associated with reduced gray matter volume of the OFC and with increased functional and structural connectivities in the forceps minor pathway (Filbey et al., 2014). The connectivity changes in the latter study were associated with early onset of use, being first increased and then decreased during protracted use. Thus, longitudinal studies are obviously needed, especially because the reversibility of the changes is not known. Although the effects of prenatal Δ9-THC are not as dramatic as those produced by alcohol abuse during brain development in pregnancy, it is possible that early cannabis use could affect brain development during critical periods and, therefore, be a contributing factor to later cognitive impairment, substance abuse/addictions, and symptoms related to mental illnesses.

5. Cannabinoid-Induced Cognitive Impairment. Cannabis acutely impairs various cognitive, perceptual, and executive functions (see Ameri, 1999; Iversen, 2000). In one study, memory impairment after acute cannabis in occasional cannabis users could be counteracted by the acetycholinesterase inhibitor rivastigmine (Theunissen et al., 2015), which might also activate the same presynaptic G proteins as Δ9-THC via indirect stimulation of mACh receptors. However, similarly to prolonged use of benzodiazepines that might produce persistent cognitive impairment, but which has been hard to prove (c.f., section II.E), the cognitive deficiencies after chronic, heavy cannabis abuse have not been proven to be persistent (Pope et al., 2002) nor purely transient (Solowij et al., 2002), although at least one report showed heavy users still performing worse than light users after a 4-week abstinence (Bolla et al., 2002). It should be noted that Δ9-THC levels remain at a significant level for a few weeks after stopping the use of cannabis (Bergamaschi et al., 2013), which could explain the results of the latter report on cognitive impairment during the first few abstinent weeks (Bolla et al., 2002). Although adolescents might be more sensitive to cognitive impairment by cannabis use, any persistent impairment is still difficult to assess, and there appears to be a need for larger longitudinal studies (James et al., 2013).

Longitudinal studies would also be needed to establish possible gross abnormalities in human brain structural features produced during cannabis/marijuana use. Some of the recent studies using MRI on subjects with a history of cannabis use compared with nonusers, have suggested statistically significant alterations in gray matter density and/or volumes of brain regions involved in reward and cognition, such as the HC, NAc, and amygdala (Yucel et al., 2008; Demirakca et al., 2011; Gilman et al., 2014; Lorenzetti et al., 2015). Most recently, the report by Weiland et al. (2015) controlled for the alcohol use between the daily marijuana user/nonuser groups by matching the scores on the Alcohol Use Disorders Identification Test (AUDIT). This study found no significant effects of marijuana use on gray matter density and/or volumes of the bilateral NAc and amygdala, the HC, and the cerebellum. Although the cannabis-use associated fine structural alterations cannot be excluded by this result, alcohol use may have been a significant confounding factor in the earlier studies in which the groups might have been more heterogeneous in relation to alcohol use. Even in the study of Weiland et al., alcohol drinking averaged five drinks a day in all study groups.

In rats, cannabinoid treatments impair learning and memory processes, but again the persistence of effects is not known. Acute pharmacological blockade of MGL, which increases 2-AG levels, also impairs learning and memory in rats (Griebel et al., 2015). Chronic 15-day treatment with a potent cannabinoid receptor agonist HU-210 (100 μg/kg i.p.) caused impaired learning in Morris water maze with longer intertrial delays and reduced LTP in the CA1 region of HC tested 18 hours after the last dose (Hill et al., 2004). Both Δ9-THC and WIN 55,212-2 decrease PFC DA turnover for up to 2 weeks, suggesting that persistent cognitive disturbances after cannabinoid exposure are possible and perhaps
at least partly related to the reduction in the DA transmission in the PFC (Verrico et al., 2003).

Repeated Δ⁹-THC administration in adolescent rhesus monkeys impaired age- and practice-related improvements in accuracy on a spatial working memory task and neither tolerance nor sensitization developed to the acute effects after 6 months (Verrico et al., 2014). This result is consistent with the acute impairment of cognition by cannabis in adolescent humans.

6. Rewarding and Aversive Behaviors, Specific Actions on VTA DA Neurons. Cannabis produces euphoria and also aversive effects, such as anxiety, in humans. Δ⁹-THC induced striatal DA release in humans in some experiments (Bossong et al., 2009) but not in others (Barkus et al., 2011). Similarly, in rodent microdialysis or firing rate studies, Δ⁹-THC induced DA release in some experiments (French, 1997; Tanda et al., 1997) but failed to do so in others (Castañeda et al., 1991). Importantly, high-resolution vivo voltammetric analyses suggest that CB1 receptor blockade by rimonabant can block DA transients in the NAc induced by ethanol, nicotine, and cocaine in freely moving rats (Cheer et al., 2007). Indeed, CB1 receptors are present in the VTA GABA neurons and in afferent GABAergic MSNs from the NAc (Matsuda et al., 1993; Marsicano and Lutz, 1999), which is in keeping with cannabinoid-induced decrease in GABA release in the VTA (Szabó et al., 2002). There are no CB1 receptors in the VTA DA neurons, but they contain the machinery for eCB synthesis and thus for retrograde signaling. These results indicate that the CB1 receptor activation can lead to increased mesolimbic DA release, although this effect seems to be in several cases more modest than that induced by other drugs of abuse. Direct infusion of Δ⁹-THC to the posterior VTA and the shell of the NAc in rats induces rewarding behavior, such as lever pressing for the infusion and CPP (Zangen et al., 2006). Also, Δ⁹-THC increases GluA2-lacking AMPA receptors and causes LTD in the pedunculopontine nucleus, a subcortical nucleus that influences reward and motivational functions (Good and Lupica, 2010). Early studies on mediodorsal bundle self-stimulation at the hypothalamus in rats indicated that, like other drugs of abuse (Wise, 1996), Δ⁹-THC also facilitated the ICSS, especially in Lewis rats (Lepore et al., 1996; Gardner et al., 1988). However, like the effect on DA release, this effect of Δ⁹-THC has been difficult to replicate in rats or mice (Vlachou et al., 2007; Fokos and Panagis, 2010; Wiebelhaus et al., 2015). A recent study with Sprague-Dawley rats suggested that a lower dose of 0.1 mg/kg of Δ⁹-THC reduced the threshold for self-stimulation, whereas the often-used 1.0 mg/kg failed to do so, both effects being antagonized by rimonabant (Kwikasz and Negus, 2012).

There are two important behaviors that appear to be under the control of eCB system: habit formation by modulation of dorsolateral striatal neurotransmission and extinction of aversive memories by modulation of BLA neurotransmission. Recent development of behavioral assays for goal-directed versus habitual drug-seeking behaviors have established specific roles for the ventromedial striatum in goal-directed behavior and for the dorsolateral striatum for habitual instrumental responding (Hilario and Costa, 2008; Everitt, 2014) (Fig. 1). Experiments comparing responses to immediate rewarding effects versus responding to devalued rewards, e.g., after a preceding drug or food reward, have been important to distinguish the goal-directed and habitual instrumental responding (Balleine and Dickinson, 1998). Repeated responding for drugs of abuse starts with activation of ventral pathways from the VTA to the NAc, but later overtraining preferentially activates the dorsolateral nigrostriatal pathways, previously suggested as a spiraling activation of midbrain-basal ganglia-cortical loops (Haber et al., 2000; Haber and Knutson, 2010). There exists a steeply increasing gradient of CB1 receptors from the medial to lateral striatum (Herkenham et al., 1991), which correlates with the presynaptic CB1 receptor actions and retrograde actions of eCBs on striatal excitatory transmission (Gerdenman and Lovinger, 2001; Gerdenman et al., 2002). Furthermore, pharmacologically isolated EPSCs and IPSCs were found to be attenuated by neuronal firing-induced eCB release (Adermark et al., 2009). Thus, both LTD and LTP processes can be produced in the striatal MSNs by activity-driven eCB retrograde signaling, which is more prominent in the dorsolateral than dorsomedial striatum and can promote more habitual than goal-directed responding. Habit formation is impaired under cannabinoid receptor blockade and in CB1-KO mice (Hilario et al., 2007). Stimulant drugs initially induce goal-directed drug-seeking behavior, which after overtraining and prolonged drug exposure alters to more habitual instrumental behavior (Everitt and Robbins, 2005). This correlates with drug-induced changes in synaptic functions (LTP, LTD) in the dorsal striatum (Gerdenman et al., 2003) and possibly agrees with chronic stimulant treatment-induced changes in the sensitization of behavioral stereotypy and striosomal early gene activation in the lateral striatum (Canales and Graybiel, 2000; Vanderschuren and Everitt, 2005). In THC-dependent mice that show no difference in responding to valued versus devalued rewards, bilateral infusion of apamin (a SKCa channel blocker) into the dorsolateral striatum rescued the eCB-dependent LTD (lost during tolerance development) for up to 5 days and changed the behavior from habitual responding to goal-directed responding with a significantly greater responding for valued than devalued rewards (Nazzaro et al., 2012).

D₂R activation (D₂R-like) has been linked to LTD and D₃R activation (D₃R-like) to LTD in MSN glutamate synapses (Calabresi et al., 2007). This is correlated with DA inducing increased NMDAR currents in D₁R-MSNs.
and decreased currents in D2R-MSNs (Andre et al., 2010), with both DA responses being modulated/partly mediated the eCB system. The role of DA and DA receptors in MSN plasticity has been restudied using mouse models with selectively labeled MSNs of the direct (D1R) or indirect (D2R) pathways (Surmeier et al., 2009), revealing a complex interplay between timed firing and dendritic activity and activation of a number of GPCRs and intracellular signaling cascades (Shen et al., 2008). In the striatum, CB1 receptors are present in both direct (striatonigral) and indirect (striatopallidal) MSNs populations (Marsicano and Lutz, 1999; Holtmann and Herkenham, 2000), presynaptically in both GABAergic and glutamatergic axon terminals, but not in TH-positive axon terminals (Fitzgerald et al., 2012). The differential DA receptor distribution between the direct and indirect GABAergic striatal projection neurons (MSNs) is well known (Gerfen et al., 1990). D1R activation stimulates anandamide production (Giuffrida et al., 1999), and this DA receptor subtype has been shown to functionally interact with CB1 (Kearn et al., 2005) and adenosine A2A receptors (Ferre et al., 2010). Cocaine decreases striatal GABA transmission, enhances anandamide synthesis, and inhibits its breakdown via D2R-like receptor activation (Centonze et al., 2002, 2004). However, the HFS-eCB-dependent LTD at glutamate synapses of striatal MSNs can be induced by opening of L-type VGCCs (Adermark and Lovinger, 2007), suggesting that the action of D2R activation on indirect pathway LTD is not obligatory. Thus, the effect of D2R activation here is mediated perhaps via suppression of adenosine A2A receptor signaling that enhances retrograde eCB signaling (Fuxe et al., 2007; Lerner et al., 2010). In the direct pathway, the activation of D1R induces LTD (Calabresi et al., 2007), after activation PKA-mediated phosphorylation of DA- and cAMP-regulated phosphoprotein DARPP-32 and other plasticity-related signaling cascades (Greengard, 2001; Nairn et al., 2004; Svenningensson et al., 2004). Activation of this cascade has been linked to the effects of many drugs of abuse (Nairn et al., 2004; Svenningensson et al., 2004), and some behaviors induced by Δ9-THC are altered in DARPP-32 knockout mice (Lazenna et al., 2015). In line, subchronic Δ9-THC selectively induces accumulation of ΔFosB in D1R-MSNs (Lobo et al., 2013). This induction by Δ9-THC can be blocked by D1R antagonist, but not by genetic deletion of DARPP-32 (Lazenna et al., 2015). In conclusion, the present data indicate a central role for CB1 receptors and eCB signaling in the regulation of short- and long-term plasticities in the main striatal projection neuron populations. Further studies will be needed to better understand the roles of these plasticity processes in behaving animals.

Cocaine SA in rhesus monkeys was initially associated with reduced [14C]deoxyglucose uptake in the ventral striatum and PFC, which, after prolonged SA, shifted to the dorsolateral striatum (Porrino et al., 2004). It is not known whether the cocaine-induced reduction in striatal activation involves the CB1 receptor mechanisms or some other signaling pathways that are affected by chronic cocaine. In rodents at least, antagonism of CB1 receptors can abolish the rewarding effects of cocaine (Xi et al., 2008), whereas the effects of CB2 receptor blockade may show species differences between mice and rats (Zhang et al., 2014). The cocaine SA experiments in rhesus monkeys stress the importance of serial engagement of medial versus lateral midbrain-striatal-cortical loops. However, these changes in neuronal activity may be also explained by prolonged changes in DA release, receptors, and transporters (Beveridge et al., 2009), not necessarily by eCB-induced LTD.

Aversive memories induced by drugs might be an important deterrent of drug abuse. Marsicano et al. (2002) found that eCB activation promotes extinction of aversive fear memories by inhibiting GABAergic circuits in the amygdala. By using brain slices, HFS of lateral amygdala induces LTD of GABAergic transmission in the BLA, which then increased amplitudes of EPSCs in the neurons of the CeA, the final output nuclei from the amygdala (Azad et al., 2004). This activation might then induce a novel memory trace for extinction of fear conditioning. Notably, CB1-KO animals failed to show extinction of freezing response to an auditory tone previously coupled to footshocks. Extinction of appetively motivated responding was not affected in CB1-KO animals (Holter et al., 2005). More recently, infusion of the CB1 receptor antagonist AM251 [N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] into the BLA was shown to abolish the freezing response if infused after the reactivation of fear memory (Ratano et al., 2014), suggesting that reconsolidation of fear memory is dependent on CB1 receptor activation. This effect of AM251 could in turn be reversed by infusion of bicuculline, a GABA receptor antagonist, indicating that CB1 receptor blockade enhanced GABA transmission. Altogether these results suggest that cannabinoid mechanisms affect the amygdaloid circuitry and produce long-term alterations in responding to aversively conditioned stimuli.

7. Cannabinoids during Adolescence: Increased Risk for Schizophrenia?. Older literature on cannabis abundantly recognizes that heavy use is associated with sensory modality alterations and hallucinations (Iversen, 2000). However, an association between cannabis use and the development of schizophrenia has been more difficult to demonstrate. A study following Swedish conscripts from adolescence found a relationship between cannabis use and subsequent development of schizophrenia, with an odds ratio (OR) of about 6 when cannabis had been used chronically more than 50 times (Zammit et al., 2002). This hints toward severe...
adolescence may cause long-lasting effects on behavior, especially in female rats. Importantly, the experiments by Schneider and Koch (2003) indicate that the time of cannabinoid treatment is crucial. Chronic WIN 55,212-2 treatment during puberty (from P40 to P65), but not during prepubertal or adult age, induced prolonged effects on sensorimotor gating (loss of prepulse inhibition of acoustic startle, which could be normalized by acute haloperidol 0.1 mg/kg), object recognition memory, and a progressive ratio task in adulthood (P75–P120). Thus, as in humans, the significance of the timing of cannabinoid exposure for persistent effects is important (Schneider, 2008), which may be related to the increased activity of the brain cannabinoid system during adolescence in humans and experimental animals (Mato et al., 2003; Rodriguez de Fonseca et al., 1993).

8. Conclusions. Cannabis is a relatively safe drug, with low potential for lethality in cannabis intoxications of young people. But because the endocannabinoid system is wired into mechanisms of brain development and synaptic plasticity, they have the potential for producing long-term changes in the nervous system structure and function. Particularly the heavy cannabis exposure during adolescence may induce early psychosis and other changes in cognitive and emotional behaviors. Furthermore, more longitudinal research is needed for several aspects of cannabinoid-induced long-term alterations in brain structure and function, especially with an advent of more potent synthetic cannabinoids and their entering the illegal markets.

1. Hallucinogens

The study of the persistent effects of 5-HT, lysergic acid diethylamide (LSD), and other serotonergic hallucinogens on perception, cognition, and mood started more than four decades ago but has long been hampered by legislative hurdles (Geyer and Vollenweider, 2008; Nutt et al., 2013). Therefore, it is only recently that proper guidelines for psychedelic drug research have been established (Johnson et al., 2008) and studies have begun to produce some understanding of the neurochemical events directly responsible for producing hallucinogen-induced neuroplasticity (Catlow et al., 2013; Lepack et al., 2015), although many questions still remain.

In this section, the basic pharmacology of hallucinogens is discussed, followed by a review of their acute effects on neurochemistry and behavior. Finally, the neuroplasticity of hallucinogens will be discussed in terms of their long-term effects on sensory processing, mood, and anxiety.

1. Effects of Serotonergic Hallucinogens Are Mediated by the 5-HT$_{2A}$ Receptor. The typical serotonergic hallucinogens can be divided into several chemical classes including tryptamines, phenethylamines, ergolines, and amphetamines. They all display high affinity for the 5-HT$_2$ receptors but also show varying degrees of affinity
for a range of other 5-HT receptors including the 5HT₁, 5HT₄, 5HT₅, 5HT₆, and 5HT₇ receptors (Nichols, 2004). The ergoline hallucinogen LSD, for instance, has the highest affinity to, and (partial) agonistic effects on, 5-HT₂₅A, 5-HT₂₅C, 5-HT₁₈A, and 5-HT₆ receptors in the low nanomolar range, but also shows intrinsic activity at the DA D₂ and α-adrenergic receptors (Nichols, 2004; Passie et al., 2008). The tryptamine hallucinogen, psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine), is synthesized de novo in several species of hallucinogenic mushrooms and, together with its active metabolite psilocin, it also displays a low nanomolar affinity for 5-HT₂₅A receptors, and a somewhat lower affinity for 5-HT₁₈A and 5-HT₂₅B receptors (McKenna et al., 1990). With a Kᵢ of 0.7 nM, the 5-HT₂₅A receptor is also the primary target of the synthetic amphetamine hallucinogen DOI, although it also targets 5HT₂₅B and 5HT₂₅C receptors with slightly lower affinities (Canal and Morgan, 2012). Also other hallucinogens, such as N,N-dimethyltryptamine and the phenethylamine hallucinogen mescaline have significant affinity mainly to the Gₒ/₁₁-coupled 5-HT₂₅C receptors (Aghajanian and Marek, 1999; Ray, 2010). Moreover, efavirenz [(4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H,3,1-benoxazin-2-one], a nonnucleoside reverse-transcriptase inhibitor used for treatment of HIV-1 infection, shows a risk for neuropsychiatric adverse effects such as night terrors and treatment of HIV-1 infection, shows a risk for neuro-psychiatric adverse effects such as night terrors and hallucinations and is a potent 5-HT₂₅A agonist (Gatch et al., 2013). MDMA, however, a stimulant with secondary hallucinogenic properties, produces its hallucinogenic effects mainly indirectly, by increasing synaptic 5-HT levels through SERT-mediated 5-HT release (Liechti et al., 2000).

Although hallucinogens have a number of 5-HT receptor targets, the hallucinogenic effects in fact appear to be mediated primarily by one receptor subtype (Fig. 13). The hallucinogen-induced head shaking response can be restored in transgenic mice lacking the 5-HT₂₅A receptor by selectively restoring this receptor in cortical pyramidal neurons (Gonzalez-Maeso et al., 2007), although the discriminative stimulus properties of LSD and subjective effects of hallucinogens can be fully blocked by 5-HT₂₅A antagonists in both experimental animals and humans (Vollenweider et al., 1998; Gresch et al., 2007). Furthermore, the 5-HT₂₅C antagonist ketanserin blocks the effect of psilocybin on visual EEG and event-related potentials and on prepulse inhibition of the acoustic startle in healthy volunteers (Quednow et al., 2012; Kometer et al., 2013). This indicates that the hallucinogenic effects of serotonergic hallucinogens are primarily mediated by the 5-HT₂₅A receptor. This receptor subtype is enriched in the deeper cortical layers (Andrade, 2011).

Aside from serotonergic hallucinogens, other drugs that produce hallucinogenic effects include substances such as ketamine, nitrous oxide, and phencyclidine, which belong to a subtype of hallucinogens often referred to as dissociatives, acting primarily as NMDAR antagonists. In recent years, a number of new analogs, such as methoxetamine and 3-MeO-PCP have become available on the recreational drug market. They have been shown to have a similar mechanism of action (Morris and Wallach, 2014; Roth et al., 2013). Additionally, drugs such as dextromethorphan and salvinorin A that act primarily as μ-, δ- or κ-opioid receptor agonists or as monoamine reuptake inhibitors, are also included in the class of dissociative hallucinogens (Codd et al., 1995; Burns and Boyer, 2013; Morris and Wallach, 2014). So, although the 5-HT₂₅A receptor is the most important for mediating the hallucinogenic effects of serotonergic hallucinogens, many other receptors mediate the hallucinogenic effects of other classes of psychedelics. As the receptor targets of serotonergic hallucinogens have been clearly identified, it raises the question of what downstream effects result after modulation of receptor activity by these substances.

2. Hallucinogen Action—A Perturbation of Sensory Gating or Desynchronization of Cortical Rhythms?. The involvement of 5-HT₂₅A receptors in the apical dendrites of cortical pyramidal neurons in producing hallucinogenic effects (Gonzalez-Maeso et al., 2007) suggests involvement of cortical circuit mechanisms. Evidence of thalamocortical glutamate pathway involvement comes from a study demonstrating hallucinogen-induced changes in glutamate release (Aghajanian and Marek, 1999). These results were supported by human imaging studies performed in subjects under acute exposure to hallucinogens (psilocybin, mescaline, and ketamine) that indicate activation of the frontal, cingulate, and insular cortices, with reduced activation in the striatum, thalamus, and parietal cortex (Geyer and Vollenweider, 2008). Interestingly, a more recent experiment using intravenous psilocybin (2 mg) indicated reduced blood flow and blood oxygen level signals in the anterior and posterior cingulate cortex and thalamus of psilocybin-experienced healthy volunteers reporting strong subjective acute effects of the drug (Carhart-Harris et al., 2012). This was replicated by a subsequent magnetoencephalography study showing large decreases in spontaneous oscillatory power after psilocybin, especially in cortical association areas and default-mode network (Muthukumaraswamy et al., 2013). These changes were of neuronal origin, consistent with excitation of 5-HT₂₅A receptors on deep layer 5 pyramidal neurons, which results in activation of inhibitory mechanisms in more superficial layers (Andrade, 2011; Bastos et al., 2012).

The sensory gating theory of hallucinogenic action states that hallucinations occur because of drug-induced perturbations of the limbic cortico-striato-thalamic-cortical feedback loop and subsequent reduction of thalamic sensory gating, leading to sensory overload that manifests as hallucinations (Vollenweider, 2001). The action of other drugs capable of inducing hallucinations,
such as NMDAR antagonists and even amphetamine, may be explained using the same model, suggesting that alterations of the cortico-striato-thalamic-cortical loops may be a final common pathway for all hallucinogenic drugs. Moreover, the drug-induced hallucinatory state also shows strong phenomenological similarity to acute psychosis in patients suffering from schizophrenia, and both states are reversed by antipsychotic drugs, which has led to use of the hallucinogen-induced state as a pharmacological model of psychosis (Vollenweider et al., 1997a,b). More recently, however, the cortical desynchronization theory of hallucinogen action (see the previous paragraph) has replaced the sensory gating theory, because the drug effects can be explained by direct actions on cortical 5-HT2A receptors. Moreover, pharmacological models of psychosis can be induced by many drugs activating or inhibiting the main cortical neurotransmitter systems (reviewed in Steeds et al., 2015).

3. Behavioral Effects and Addiction Potential. Serotonergic hallucinogens, and also some other hallucinogens such as salvinorin A, do not have any clear rewarding potential in animal models (Passie et al., 2002; Nichols, 2004; Cunningham et al., 2011). Similarly, although these substances are used frequently by humans, addiction appears to be very uncommon, and indeed they have been used to treat alcohol addiction (Gable, 1993; Studerus et al., 2011; van Amsterdam et al., 2011; Krebs and Johansen, 2013). This is consistent with the rather low-affinity, mixed and delayed efficacy of LSD on various types of DA receptors (Passie et al., 2008); with a very low affinity of psilocybin/psilocin to DA receptors (Passie et al., 2002) and with salvinorin A having selective high affinity to \( \kappa \)-opioid receptors (Roth et al., 2002; Chavkin et al., 2004). Although the \( \kappa \)-opioid receptor agonists may also influence DAergic mechanisms, they are generally aversive (decreasing DA release) and in fact produce conditioned place aversion (Gable, 1993; Zhang et al., 2005; Cunningham et al., 2011). Interestingly, dose-dependent rewarding/aversive effects in place conditioning with salvinorin A via \( \kappa \)-opioid and CB1 receptors have been reported in zebrafish (Braida et al., 2007).

On the other hand, a 3-month LSD treatment of rats (0.16 mg/kg i.p. every 2nd day) induced hyperlocomotion for at least 3 months after discontinuation of the treatment (Marona-Lewicka et al., 2011), which suggests an action on DA receptors. Indeed, using a drug discrimination paradigm, Marona-Lewicka and coworkers showed that LSD produces initial generalization to a 5-HT2A agonist (and suppression of locomotion), followed at a later phase of cue responding, and generalization to a D2 agonist (and locomotor activation) (Marona-Lewicka et al., 2005; Marona-Lewicka and Nichols, 2007). The prolonged LSD treatment shows development of persistent psychosis-like behavioral and neurochemical features in rats (Marona-Lewicka et al., 2011), with at least the hyperlocomotion being antagonized by antipsychotic drugs blocking D2 and/or 5-HT2A receptors.

The picture is somewhat different with regard to NMDAR antagonism-based psychedelics, because there is some evidence that dissociative psychedelics such as ketamine and PCP are self-administered, at least under some circumstances (Broadbear et al., 2004; Carroll et al., 2005; De Luca and Badiani, 2011). These effects are likely related to the ability of these substances to increase accumbal DA release and turnover (Carboni et al., 1989; Irifune et al., 1991) (see section II.F).

4. Long-term Residual Effects of Serotonergic Hallucinogens: the Role of Neuroplasticity. Hallucinogens produce clear neurochemical and acute behavioral and subjective effects. However, they also produce more long-term, persistent effects on behavior and sensory processing, as well as on mood and attitude.

a. Sensory processing. Hallucinogen-induced long-term alterations of sensory processing have been
reported, initially as LSD-like recurrences or "flashbacks" in the 1950s. Later, diagnostic criteria were proposed for flashbacks in the DSM-III-R that were further refined and termed hallucinogen persisting perception disorder (HPPD) in the DSM-IV (American Psychiatric Association, 1994). Although this disorder has a very low prevalence (Halpern and Pope, 2003), HPPD is characterized by perceptual symptoms, most often visual in nature, that manifest themselves in patients previously intoxicated with hallucinogens, even long after the cessation of drug intake (and beyond the accepted time of the drug’s acute pharmacological actions), associated with significant distress, and without being attributed to any other medical condition or psychiatric disorder (Horowitz, 1969; Abraham et al., 1996; Halpern and Pope, 2003). Several discrete case studies report flashback phenomenon days to months after the use of hallucinogens such as LSD (Duncan, 1974) and psilocybin (Espiard et al., 2005) alone or in combination with alcohol or other drugs of abuse, but there are hardly any controlled studies on the subject. It is noteworthy that pharmacological treatment, e.g., with antipsychotics, benzodiazepines, and clonidine, has proved useful in controlling the symptoms in most of the few cases published (Halpern and Pope, 2003).

Alterations in visual processing may be better described without reference to HPPD, because this diagnosis requires the presence of significant distress associated with the symptoms. Persistent effects such as perception disorders have also been observed in healthy individuals after experimentally administered doses of psilocybin (Studerus et al., 2011), and these could all be managed by interpersonal discussion and support. Furthermore, drug-free visual experiences were observed in 62% of the respondents in a web-based study that analyzed a population of 2455 participants for abnormal experiences associated with a history of hallucinogen use, especially LSD (Baggott et al., 2011). The persistent perceptual alterations in subjects with past hallucinogen use is clear evidence of neuroplasticity. Even in the absence of recent LSD use, patients with HPPD display an augmented neural coherence in the occipital regions (isolated from anterior regions) that is proposed to underlie their illusions and hallucinations (Abraham and Duffy, 2001). However, little is known about the exact mechanisms responsible for these changes or about the factors involved in producing susceptibility to long-lasting sensory alterations or HPPD.

b. Mood and anxiety. It has been known for a long time that serotonergic hallucinogens can produce long-lasting effects on mood, anxiety, and other aspects of neuropsychiatric functioning, thereby providing indirect evidence of neuroplasticity. For instance, LSD was studied early on as a drug or an adjunct to other treatment modalities for neuropsychiatric illnesses, but research collapsed in the late 1960s after LSD and other psychedelics were made illegal. Subsequently, a long period largely devoid of any psychedelic research followed, something that has been attributed largely to the fact that their illegality made it excessively difficult for researchers to work with these substances (Nutt et al., 2013). However, after a long hiatus, there now appears to be a renewed interest in the potential clinical applications of psychedelic drugs, such as psilocybin and LSD and also other psychedelic drugs including ketamine and MDMA, for treating disorders ranging from alcohol and nicotine addiction to depression, anxiety disorders, and cluster headaches (Sewell et al., 2006; Chabrol, 2013; Grob et al., 2011; Krebs and Johansen, 2012; Mithoefer et al., 2013; Gasser et al., 2014; Moaddel et al., 2015). Evidence of long-term effects on behavior comes also from a set of studies where psilocybin was administered to healthy subjects. They reported that doses of psilocybin that resulted in strong visual and perceptual effects, including those described as mystical-type experiences, subsequently also produced persistent positive changes in mood, attitude, and behavior that were sustained for more than 1 year after the drug was administered (Griffiths et al., 2006, 2011; MacLean et al., 2011). This suggests that serotonergic hallucinogens may possess anxiolytic, antiaddictive, and antidepressive properties, but the question remains what mechanisms mediate the neuroplasticity required to produce these long-lasting effects.

One recent study attempted to shed light on the relationship between the “therapeutic” effects of serotonergic hallucinogens and their effect of neuroplasticity by measuring the effects of varying doses of psilocybin on extinction of fear conditioning and neurogenesis in mice (Catlow et al., 2013). They demonstrated that psilocybin significantly accelerated the extinction of fear conditioning, and this was associated with a trend toward increased neurogenesis in the dentate gyrus. Although further work is needed, one could speculate that the effects of psilocybin on neurogenesis are due to 5HT2A receptor-mediated upregulation of BDNF (Vaidya et al., 1997), suggesting a mechanism strongly resembling that of SSRIs (Martinowich and Lu, 2008; Catlow et al., 2013; Quesseveur et al., 2013) and possibly the antidepressive mechanism of ketamine (Lepack et al., 2015).

Considering the vital role of the 5HT2A receptor, it is important to note that although the 5-HT2A receptor is the primary target for hallucinogens, there are other 5-HT2A agonists, such as lisuride, that do not produce any hallucinogenic effects. The exact reason for this difference is not known, but may be related to differing recruitment of intracellular second messengers; although both LSD and lisuride activate PLC, only LSD also recruits Gαo proteins and Src (Gonzalez-Maeso et al., 2007). Interestingly, the specific 5HT2A receptor activation caused by hallucinogens seems to be of key importance in producing neuroplasticity. Hallucinogens
such as LSD induce brain-region dependent mRNA expression of various immediate early genes, such as c-Fos, arc, and homer1a splice isofrom ania3 (Nichols and Sanders-Bush, 2002), that influence regulation of gene expression and synaptic responses, whereas DOI has been shown to produce changes in dendritic spine morphology on cortical neurons (Jones et al., 2009). Chronic LSD administration to rats causes a persistent increase in synthesis and turnover of 5-HT as well as downregulation of cortical 5HT2A receptors (Diaz and Huttunen, 1971; Lee and Geyer, 1980; Gresch et al., 2005), and a 2-week LSD treatment followed by 14 days of abstinence still showed an augmented 5-HT level in the midbrain and cerebral cortex (Peters and Tang, 1977). Continuous delivery, but not daily injections, of LSD in rats caused long-lasting increases (measured after 30 days) in [3H]LSD binding in the FC, amygdala, septum, and NAc that coincided with behavioral alterations (King and Ellison, 1989). LSD (1 mg/kg) causes a sustained increase in Nor1 gene expression in the PFC, which could play a role in synaptic dysregulation (Nichols et al., 2003). Similar effects of LSD and DOI on transcriptional responses in mouse somatosensory cortex were dependent on 5-HT2A receptors, because they were absent in 5-HT2A-KO mice (Gonzalez-Maeso et al., 2003).

Importantly, lisuride, a 5-HT2A agonist not producing the hallucinogenic activity-correlating head-twitching behavior in rodents, failed to produce similar transcriptomic changes as LSD and DOI. These results are consistent with 5-HT2A receptor antagonists abolishing the behavioral effects of hallucinogenic drugs (Fiorella et al., 1995; Vollenweider, 1998; Winter et al., 1999).

5. Scopolamine: Another Hallucinogen Revisited for Depression. Scopolamine (hyoscine or burundanga) is a nonselective muscarinic antagonist well known for its use in preclinical animal models to induce cognitive impairment and for its use to treat, e.g., motion sickness. Scopolamine disrupts working memory and other parameters in rodents, canines, and primates (Rupniak et al., 1989; Araujo et al., 2004; Winsauer et al., 2004; Klinkenberg and Blokland, 2010). Some case reports have revealed hallucinogenic effects of scopolamine, largely from patients of different age groups in whom hallucinations unexpectedly followed peripheral administrations (transdermal patch or eye drops) of scopolamine (Warburton et al., 1985; Sennhauser and Schwarz, 1986). A sparse amount of literature predicts that scopolamine could be addictive and it is likely to cause CNS effects upon withdrawal (Luetje and Wooten, 1996; Patel and Ezzo, 2009), but its recreational or predatory use has not been convincingly identified (King et al., 2014). On the other hand, scopolamine might have a beneficial effect in opioid and cocaine addiction (Gambelunghe et al., 2014; Liu et al., 2013). With the recent interest in exploration for quick-onset antidepressants, scopolamine, like ketamine, was reported to be a prospective candidate and interestingly the clinical improvement lasted for more than 2 weeks (Furey and Drevets, 2006). These reports show that although the cognitive impairments are due to mAChR antagonism, the persistent effects of scopolamine that might be mediated via NMDAR gene expression and mTOR signaling-mediated synaptic plasticity (Drevets et al., 2013); these ideas need to be investigated in the future.

6. Conclusions. The hallucinogenic effects of serotonergic hallucinogens are mediated primarily by the 5HT2A receptor, activation of which produces hallucinations by a range of functional changes thought to result in deficient thalamic sensory gating and/or cortical desynchronization. Furthermore, 5HT2A receptor activation by hallucinogens also mediates many changes in gene expression, receptor regulation, and synaptic morphology likely to be associated with the long-term, persistent effects of hallucinogens on perception, mood, and anxiety. However, the molecular mechanisms linking the neuropharmacological actions, human brain functional changes, and behavioral outcomes need to be further studied.

III. General Discussion

We have reviewed neuroplasticity induced by cocaine, amphetamine, and related psychostimulants, nicotine, ethanol, benzodiazepines and other GABA ligands, NMDAR antagonists, opioids, cannabinoids, and hallucinogens. Brain regions implicated across the various classes of drugs of abuse were diverse but again certain commonalities emerged. As might be expected, the VTA was recurrently implicated, but other brain regions that featured repeatedly across classes of drug of abuse were the striatum, amygdala, BNST, Hb, mPFC, and HC. Although there are many diverse mechanisms of neuroplasticity specific to the pharmacology of each of these classes of drug of abuse, many common themes surfaced.

Among the drugs of abuse that might be characterized as having stimulant and/or disinhibitory effects—cocaine, amphetamine and related psychostimulants, nicotine and ethanol, and benzodiazepines—a recurrent theme was the role of neuroplasticity of glutamate synapses in the VTA DA neurons. However, the specific mechanisms of early induction of the persistent changes in the AMPAR/NMDAR ratios varied between the different drugs. Additional investigations are required to further elucidate whether different drugs induce glutamate neuroplasticity in different populations of VTA DA neurons, perhaps one of them linking to reinforcing mechanisms and another one to aversive mechanisms.

Another commonality was the recurrent role of GABAergic mechanisms, by disinhibition of the DA neurons, in the VTA in the neuroplasticity induced by cocaine, nicotine, ethanol, benzodiazepines and other...
GABA ligands, opioids, and cannabinoids. The specific mechanisms of induction of changes in GABAergic function in the VTA varied between the various classes of drugs, and further studies are required to investigate whether there would be possibilities in controlling GABAergic function at specific neuronal populations in the midbrain to modulate addictive behaviors across these different classes of drugs.

An overarching theme that has repeatedly emerged is concern over the possibility that neuroplasticity induced by one drug of abuse may increase the risk of dependence on other drugs of abuse. In particular, there is concern that relatively easily available drugs of abuse such as nicotine and alcohol may trigger mechanism of neuroplasticity that predispose to increased risk of abuse of other drugs. However, further studies are required to determine whether the underlying mechanism of neuroplasticity induced by nicotine and alcohol predispose risk to addiction to all other drugs of abuse equally or preferentially to specific classes of drug of abuse. Moreover, the question of the reversibility of the neuroplastic changes induced has seldom been exhaustively addressed. For many of the drugs of abuse the question of whether there is long-lasting and irreversible alteration of function has not been fully resolved. Further studies are required to determine how long these changes continue to increase the risk of drug abuse.

Another overarching theme that has repeatedly emerged across our discussion of the various classes of drugs of abuse is the particular vulnerability of the adolescent brain to drug-induced neuroplasticity. Evidence is accumulating for concern over significantly stronger and more insidious induction of plasticity on adolescent drug exposure compared with adult drug exposure. Adolescent exposure to drugs of abuse is a major concern because the relatively easy availability of alcohol, nicotine, and cannabis in many societies could lead to risk of exposure. Even limited exposure may be sufficient to induce neuroplastic changes in the highly sensitive adolescent brain, leading to increased risk of later life addiction not only to nicotine and alcohol but also to other drugs of abuse (Table 3). The adolescent/early adulthood period in rodents is also sensitive to nondrug interventions, such as effects of environmental enrichment (reviewed in Nithianantharajah and Hannan, 2006).

A. Novel Methods for Future Studies

Further investigations of the mechanisms of action and persistent neuroplasticity induced by drugs of abuse are clearly warranted. Recent methodological advances in systems neurobiology may provide new tools for such investigations. In the past decade or so, a variety of techniques with unprecedented resolution has been developed. Research on long-term neuroplasticity in addiction can benefit tremendously from the continued use of these techniques and incorporation of others. In this regard, advances in optical techniques have enabled us to manipulate the activity of certain classes of neurons and determine their causal role in brain function. As such manipulations (e.g., optogenetics) have high temporal resolution, their effect on the firing characteristics of neurons, using optical and electrical readouts, can be simultaneously monitored along with their effect on behavior. This has allowed us to observe how changes in behavior correlate with changes in the "neural code" (the firing pattern of populations of neurons).

Closed-loop optogenetics (i.e., optical stimulation of the brain when a certain criteria is met, e.g., on or before SA or when a certain pattern of activity is observed in the brain) are helping us further understand addiction. Indeed, it is conceivable that in the future we might be able to manipulate, using optogenetics and chemogenetics, increasingly more specific classes of neurons (e.g., only the projections from and to certain regions in the brain that selectively express three or more genes). Furthermore, recent developments in optogenetic technologies including red-shifted opsins (Chuong et al., 2014) will enable simultaneous optogenetic stimulation of two genetically distinct cell types or projections and hence, elucidate the causal role of different populations of neurons with unprecedented temporal precision (e.g., the behavioral effects of simultaneous manipulation of D2R- and D1R-MSNs). Additionally, direct optical control of intracellular pathways (reviewed in Stuber and Mason, 2013) can provide an understanding of the role of specific intracellular signaling pathways in a specific population of neurons with similar temporal resolution. At the other end of the spectrum, optoFMRI, which involves the combination of optogenetics and fMRI, can elucidate changes in functional connectivity at the level of the brain (Lee, 2011). Similarly, recent advances in optical imaging techniques, including development of genetically encoded calcium indicators (for instance, Gcamp6; Chen et al., 2013c) with the necessary temporal resolution to detect action potential-associated calcium ion changes and suprathreshold subcellular activity and development of red-shifted genetically encoded calcium indicators (Rcamp; Akerboom et al., 2013), will soon enable simultaneous optical imaging from different populations of neurons in vivo (using implantable imaging devices, albeit at a lower resolution) or ex vivo (similar to the ex vivo brain slice patch-clamping techniques currently in widespread use but allowing the visualization of multiple neurons simultaneously) (see Deisseroth and Schnitzer, 2013).

Monitoring calcium ion dynamics associated with neuronal activity is advantageous, because calcium ions play a fundamental role in induction of synaptic plasticity and hence, possibly in the long-term neuroplasticity induced by drugs of abuse. On the other hand, recent
advancements in genetically encoded voltage indicators enable the simultaneous detection of voltage fluctuations in multiple neurons and combined with optogenetics enable "opto-patching" of neurons ex vivo, if not in vivo yet (Hochbaum et al., 2014). These advancements can greatly benefit the drug research and addiction fields because ex vivo techniques have been very successfully used thus far.

Similarly, the burgeoning field of chemogenetics provides the opportunity to specifically modulate (via GPCRs or ion channels) a certain genetically defined and/or spatially defined population of neurons using systemic administration of synthetic ligands, which do not affect other neurons. This provides the advantage over optogenetics of performing noninvasive chronic interventions even across spatially nonrestricted population of neurons (for instance, D2R-expressing neurons across the brain) and possibly simultaneous activation or inhibition of multiple pathways across the brain (see Sternson and Roth, 2014).

Furthermore, optrodes with high-density silicon microelectrodes coupled with multiple micro-optogenetic stimulation points (for optical tagging and stimulation of small regions) allow chronic acquisition of neuronal activity from large-scale "identified" neuronal ensembles. In addition, novel techniques have been developed to allow visualization of receptor expression and the anatomic pathways in unprecedented detail without sectioning the brain (e.g., Hama et al., 2011; Chung and Deisseroth, 2013). These techniques will allow for an improved visualization of structural and biochemical changes induced across the brain during addiction.

Finally, recent development of genome engineering technologies based on CRISPR-associated RNA-guided endonuclease Cas9 (CRISPR-Cas9) enable easy editing or removal of any DNA sequence within the endogenous genome at any age. Indeed, multiple CRISPR-Cas9 mediated genetic manipulations can be performed simultaneously (Hsu et al., 2014). This technique holds potential to elucidate the roles of different genes, at different time points, in the development of addiction. Targeted knockdown of multiple genes will allow us to determine the role of non-Mendelian genes in addiction. Furthermore, this technique will enable us to understand the complex interplay of genes in such disorders, which was not possible with knockdown of a single gene.

The techniques discussed in this section allow unprecedented access to probe brain form and function. Therefore, great insights in to the neural mechanisms underlying addiction can be expected in the years to come.

Understanding addiction will also require elucidation of the genetic and epigenetic changes, the alterations in the intracellular biochemical pathways, changes in neural excitability and information integration, changes in release of neurotransmitters and receptor expression levels, systems-level neural processing in different brain structures and the resultant change in information processing across structures, and, finally, understanding how these changes translate into altered behavior. This is by no means a small feat. The issue is further complicated by the fact that different drugs affect the brain differently and the role of each of the aforementioned factors changes as the disease progresses. One of the principal goals of addiction research is to understand and develop therapies for addiction by finding commonalities and differences between various drugs of abuse. Our review of neuroplasticity induced by cocaine, amphetamine, and related psychostimulants, nicotine, ethanol, benzodiazepines and other GABA

<table>
<thead>
<tr>
<th>Drug/Intervention</th>
<th>Period</th>
<th>Effects in Adulthood</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>P25–45</td>
<td>Increased social anxiety</td>
<td>(reviewed in Spear, 2014)</td>
</tr>
<tr>
<td></td>
<td>P30–43</td>
<td>Increased alcohol drinking</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>P36–48</td>
<td>Disruption of locomotor habituation</td>
<td>(Adriani et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>P34–43</td>
<td>Increase in nicotine intake, disturbed attentional behavior, increased impulsiveness</td>
<td>(Adriani et al., 2003; Couinotte et al., 2011)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>P30–44</td>
<td>Decreases reward sensitivity, increased stress sensitivity, vulnerability to depression</td>
<td>(Iniguez et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>P35–46</td>
<td>Increased stimulant effects of cocaine, abnormal shift in attention, decreased anxiety, deficient contextual fear response</td>
<td>(Black et al., 2006; Sillivan et al., 2011)</td>
</tr>
<tr>
<td>Morphine</td>
<td>P30–32</td>
<td>Increased locomotor response to morphine</td>
<td>(White and Holtzman, 2005)</td>
</tr>
<tr>
<td></td>
<td>P37–42</td>
<td>Increased reinstatement of morphine CPP</td>
<td>(Schwarz and Bilbo, 2013)</td>
</tr>
<tr>
<td>Δ2-THC</td>
<td>P30–51</td>
<td>Reduced social interaction and novel object recognition</td>
<td>(reviewed in Rubino and Parolaro, 2008)</td>
</tr>
<tr>
<td></td>
<td>P28–49</td>
<td>Increased heroin SA</td>
<td></td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>P5–15</td>
<td>Impaired cognitive behavior, model for psychosis</td>
<td>See the section II/F.</td>
</tr>
<tr>
<td></td>
<td>P9–42</td>
<td>Reduced exploration, normal social interaction</td>
<td>(Metaxas et al., 2014)</td>
</tr>
<tr>
<td>Environmental enrichment</td>
<td>Prolonged, at variable times after weaning</td>
<td>A great number of neuroplasticity changes in neuropsychiatric animal models</td>
<td>(reviewed in Nithianantharajah and Hannan, 2006)</td>
</tr>
<tr>
<td></td>
<td>P60–96</td>
<td>Multiple structural and gene expression changes in the brain</td>
<td>(Mychasiuk et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>P30–60</td>
<td>Transient decrease in cocaine seeking</td>
<td>(Chauvet et al., 2012)</td>
</tr>
</tbody>
</table>
ligands, NMDAR antagonists, opioids, cannabinoids, and hallucinogens offers a step toward consolidating the current status of knowledge of such commonalities and the identification of directions for future research.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Korpi, den Hollander, Farooq, Vashchinkina, Rajkumar, Nuth, Hyttaße, Dawe.

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