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Review

Adaptive plasticity of NMDA receptors and dendritic spines: Implications for enhanced vulnerability of the adolescent brain to alcohol addiction

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Abstract

It is now known that brain development continues into adolescence and early adulthood and is highly influenced by experience-dependent adaptive plasticity during this time. Behaviorally, this period is also characterized by increased novelty seeking and risk-taking. This heightened plasticity appears to be important in shaping behaviors and cognitive processes that contribute to proper development of an adult phenotype. However, increasing evidence has linked these same experience-dependent learning mechanisms with processes that underlie drug addiction. As such, the adolescent brain appears to be particularly susceptible to experience-dependent learning processes associated with consumption of alcohol and addictive drugs. At the level of the synapse, homeostatic changes during ethanol consumption are invoked to counter the destabilizing effects of ethanol on neural networks. This homeostatic response may be especially pronounced in the adolescent and young adult brain due to its heightened capacity to undergo experience-dependent changes, and appears to involve increased synaptic targeting of NMDA receptors. Interestingly, recent work from our lab also indicates that the enhanced synaptic localization of NMDA receptors promotes increases in the size of dendritic spines. This increase may represent a structural-based mechanism that supports the formation and stabilization of maladapted synaptic connections that, in a sense, "fix" the addictive behavior in the adolescent and young adult brain.

Keywords: NMDA receptors; Dendritic spines; Experience-dependent plasticity; Adolescence; Ethanol

Contents

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1. Introduction

Alcohol and drug addiction is increasingly conceptualized as a disease of aberrant learning and memory (Hyman, 2005). While deficits in learning and memory are commonly associated with loss of function due to injury or age, prolonged use of addictive drugs is associated with the opposite problem; learned associations so strongly established they continue to influence behavior even after extended periods of abstinence. This "pathological learning" ontology is consistent with the recalcitrant and relapsing nature of addiction, and constitutes a significant conceptual advance in understanding the biology of drug addiction. Recent reviews of the neurophysiological changes that take place following exposure to addictive drugs have generally supported this concept (Kelley, 2004; Nestler, 2001). While much of the evidence for this association comes from studies of the role of dopaminergic neurotransmission in reward and motivation, the glutamatergic system has been increasingly identified as a major factor in the persistence of drug-seeking behaviors (Kalivas et al., 2005).

At the same time that learning-related processes were being identified in establishment and retention of addictive behaviors, significant progress was also made in understanding the mechanisms by which synaptic strength can be modified. Perhaps most remarkable is the capacity of synapses to undergo molecular and structural changes in response to activity. These changes include the rapid movement of glutamate receptors into and out of the synapse (Song and Huganir, 2002). Such activity-dependent events have major implications for controlling the efficacy of synapses, and may even alter their size, shape and/or number of connections (Segal, 2005). The trafficking of glutamate receptors has therefore been proposed as a mechanism for encoding information about past synaptic activity. As such, it has been suggested that alteration in synaptic structure may represent a physical manifestation of memory (Lamprecht and LeDoux, 2004).

While significant progress has been made in elucidating the effects of psychostimulants such as cocaine on the trafficking of glutamate receptors (Jones and Bonci, 2005; Malenka, 2003; Wolf et al., 2004), relatively little is known with respect to ethanol. Our results in reduced systems which examine how prolonged ethanol exposure alters glutamate receptor trafficking and related modifications to the function and morphology of glutamatergic synapses, integrate recent advances in the biology of learning with the biology of drug addiction. These observations support the suggestion that the enhanced plasticity of the adolescent brain temporally correlates with enhanced vulnerability to addiction (Chambers et al., 2003).

2. Glutamatergic neurotransmission and synaptic plasticity

Neuronal activity in the cortex, limbic system and basal ganglia has been identified as a major factor in learning and memory, and glutamatergic neurotransmission is believed to be critical in establishing and modifying synaptic connectivity and encoding mechanisms in these brain regions. The circuitry associated with the aforementioned brain regions are also believed to play a significant role in the development and maintenance of addictive behaviors. Consistent with this, accumulating evidence implicates glutamatergic neurotransmission as playing an important role in addictive processes (Tzschentke and Schmidt, 2003), and a growing body of evidence also suggests that drug addiction involves physiological processes that are common to processes of normal experience-dependent learning (Kelley, 2004). While many brain regions and neurotransmitter systems are believed to be involved, the common role of glutamate-mediated synaptic plasticity in learning and addiction has been demonstrated in numerous models and at multiple levels. The ability of addictive drugs, including ethanol, to induce changes at glutamatergic synapses within the addiction circuitry suggests a link between glutamatergic activity and the lasting changes in brain function associated with chronic drug consumption (Jones and Bonci, 2005).

2.1. *N*-methyl-D-aspartate receptors

Ethanol is well known to inhibit glutamatergic neurotransmission, and prolonged exposure produces up-regulation of the glutamate system (Fadda and Rossetti, 1998). Though the exact mechanisms of this process are not well understood, such adaptation is believed to be an important factor in the generation of ethanol tolerance, dependence and withdrawal pathology (Chandler et al., 1998). At physiologically relevant concentrations, the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors are significantly inhibited by ethanol and undergo adaptive changes in response to prolonged exposure (Woodward, 2000). NMDA receptors have also been strongly implicated in synaptic development (Medina et al., 2001) and cellular models of learning and memory such as long-term potentiation (LTP) and long-term depression (LTD) (Malenka and Bear, 2004). This raised the intriguing possibility that disruptions and subsequent adaptive changes in glutamate signaling through NMDA receptors may contribute to adaptations in brain function that produce ethanol tolerance and dependence via similar processes involved in experience-dependent plasticity.

NMDA receptors mediate the slow component of fast excitatory synaptic transmission. Native NMDA receptors consist of tetrameric combinations of various subunits coded by six different genes (Woodward, 2000). The NR1 subunit is an obligatory subunit that is a key mediator of assembly, function, trafficking and targeting of the heteromeric receptor. There are four types of NR2 subunits (NR2A-D). NR2 subunits play a regulatory role by influencing channel kinetics and trafficking, and the complexity of NMDA receptors is augmented by regional and developmental differences in expression of NR2 subunits (Watanabe et al., 1992).

Differential expression of NR2A or NR2B subunits in NMDA receptors is notable with respect to brain structures important in learning and memory. The influx of Ca^{2+} through NMDA receptors is critical for induction of synaptic modification (Hardingham and Bading, 2003), and NR2 subunit composition can determine the amount of Ca^{2+} that enters through the channel. Although NR2A-containing NMDA receptors can have higher peak currents and greater open channel

probability (Chen et al., 1999), NR2B-containing receptors have higher affinity for agonists and confer greater Ca^{2+} influx than NR2A-containing receptors due to their slower inactivation kinetics (Krupp et al., 1996). These characteristic have implicated NR2B as being particularly important in experience based synaptic plasticity. As such, organization of NR2B-containing NMDA receptors at the synapse could have significant implications for activity-dependent modification of synaptic strength.

3. NMDA-receptor trafficking and synaptic targeting

Glutamate receptors are clustered in the postsynaptic density (PSD) opposite presynaptic terminals releasing glutamate (Craig et al., 1994). This organization of the receptor is critical for efficient neurotransmission and is the product of a complex process involving trafficking and immobilization of receptors at the synapse. NMDA receptors and associated proteins are held at the PSD by members of the membrane associated guanylate kinase (MAGUK) family of scaffolding proteins (Sheng and Pak, 2000). The most thoroughly studied MAGUK is postsynaptic density protein 95 (PSD-95). Through protein–protein interactions, PSD-95 organizes the assembly of NMDA receptors, cell adhesion molecules and signaling proteins in the PSD (Fanning and Anderson, 1999).

NMDA-receptor localization at the PSD was originally considered to be relatively static. However, a number of studies have challenged this notion by demonstrating the activity-dependent regulation of synaptic NMDA-receptor number (Wenthold et al., 2003). This occurs via regulation of NMDA-receptor trafficking, a generic term for movement between different cellular domains such as the ER, Golgi, endosomes, and various microdomains on the cell surface including the synapse (Perez-Otano and Ehlers, 2005). Homeostatic enhancement of NMDA-receptor clustering at the synapse has also been observed following chronic inhibition of synaptic activity or direct inhibition of NMDA receptors (Rao and Craig, 1997). These changes are undoubtedly the result of alterations in NMDA-receptor trafficking. While the exact process by which greater numbers of NMDA receptors arrive at the synapse is unknown, protein kinase A (PKA) activity has been shown to be required (Crump et al., 2001).

4. Dendritic spines as sites of structural plasticity

Synaptic plasticity is the ability of synapses to undergo a change in strength. In what we now refer to as Hebbian plasticity (after Donald Hebb who was the first to propose the idea of activity-dependent synaptic plasticity), correlated increases in synaptic activity between pairs of neurons lead to an increase in synaptic efficacy (e.g. synaptic strengthening or potentiation), whereas reductions in activity lead to reduced synaptic efficacy (e.g. synaptic weakening of depression). Many studies have implicated modulation in the level and function of ionotropic glutamate receptors at the PSD as playing an important role in activity-dependent plasticity. As discussed in greater detail in a subsequent section, homeostatic plasticity is a type of higher-order plasticity that functions to counter the destabilizing effects of Hebbian plasticity on neural networks.

Dendritic spines are tiny microprotuberances that contain extremely high concentrations of filamentous actin (F-actin) (Nimchinsky et al., 2002), and an individual neuron may possess thousands of spines. While spines are structurally amorphous, a typical mature spine has a rounded head connected to the dendrite by a thin neck. The space within the spine head averages less than 1 μm^3 in volume and contains the PSD and its associated signal molecules. In addition to providing a site of synaptic contact, spines compartmentalize signaling networks and events associated with synaptic activity. Most notably is their ability to restrict the diffusion of Ca^{2+} from the spine to the dendritic shaft and adjacent spines during synaptic activity (Korkotian and Segal, 2000). Furthermore, the dynamics of this compartmentalization is highly influenced by the spine morphology. Thus, a mature spine with a broad, rounded head and thin neck, is able to restrict diffusion of calcium from the spine into the dendritic shaft much more efficiently in comparison to an immature spine with a small head and broad neck. Therefore, variability in spine morphology is significant with respect to synaptic strength. Finally, dendritic spines are not fixed structures but can undergo rapid changes in size and shape, and can disappear and reappear over time (Holtmaat et al., 2005). These plastic properties of spines, commonly referred to as “spine dynamics”, are the products of modifications of the spine actin cytoskeleton (Dunaevsky et al., 1999; Lendvai et al., 2000).

Glutamate receptors, have been shown to play an important role in regulating spine dynamics (McKinney et al., 1999) via the influx of extracellular Ca^{2+} (Brunig et al., 2004). The influence of activity on spine morphology suggests that spine shape reflects the previous activity of individual synapses, and implies these structures are a major locus for information storage in the brain (Van Rossum and Hanisch, 1999). NMDA receptors appear to be particularly important regulators of dendritic spine actin and, therefore, spine formation and morphology (Carlisle and Kennedy, 2005; Lamprecht and LeDoux, 2004). The mechanisms by which this takes place are complex and not yet fully described, but have been shown to vary depending on experimental conditions. For example, NMDA-receptor activity has been observed to stabilize spines by reducing actin turnover (Fischer et al., 2000; Star et al., 2002), but can also cause the collapse of spines during excessive activation (Halpain et al., 1998). With respects to synapse formation, NMDA-receptor activity induces the rapid formation of filopodia, which are believed to be precursors of spines (Maletic-Savatic et al., 1999). NMDA receptors may also allow spines to “sniff out” and grow towards sources of glutamate, thereby facilitating the establishment of appropriate contact with a presynaptic membrane prior to synapse maturation (Matus, 2001). All of these actions place NMDA receptors as ideal candidates for mediating structural modifications in the brain that reflect learning and memory.

4.1. Dendritic spines and learning

The relationship between learning and memory and dendritic spines is often studied using hippocampal long-term potentiation

(LTP). LTP, a well characterized form of activity-dependent plasticity, is a widely accepted cellular model of learning and memory. Though it has been difficult to reach a consensus on whether LTP is associated with increases in either spine number or size, live-time observations with multi-photon imaging have provided some insight into these events (Yuste and Bonhoeffer, 2001). This work has shown that LTP can increase spine number via either a *de novo* process or through bifurcation of existing spines (Toni et al., 1999). Similar techniques have shown that spines often shrink during long-term depression (LTD) (Zhou et al., 2004). While evidence of the formation of new spines as a consequence of learning *in vivo* is controversial, decreases in spine number in sensory deprived mice and mice with mutations that result in deficits in spines can be overcome by environmental enrichment that also reverses deficits in learning and memory (Nimchinsky et al., 2002; Rampon et al., 2000). Increased numbers of spines have also been identified in the hippocampus following hippocampal-dependent memory tasks (Leuner et al., 2003).

In addition to unidirectional changes during Hebbian plasticity, dendritic spines can also be modified through homeostatic responses. In fact, spine morphology is subject to bidirectional activity-dependent remodeling (Harris, 1999), and chronic inhibition of synaptic activity dramatically increases spine number and size (Kirov and Harris, 1999). Consistent with these observations, the equilibrium of F-actin is also bidirectionally regulated by the frequency of synaptic activity (Okamoto et al., 2004). While the relationship between homeostatic enhancements in synaptic NMDA-receptor clustering and dendritic spines has not been previously investigated, the number of NMDA receptors in a spine is proportional to its size (Noguchi et al., 2005). Thus, events that promote greater synaptic clustering of NMDA receptors could impact spine number and morphology through NMDA-receptor-dependent mechanisms.

5. Activity-dependent targeting of NMDA receptors to the synapse as a mechanism of homeostatic plasticity

As discussed above, Hebbian mechanisms of plasticity are thought to play an important role in modifying neuronal circuitry in response to changes in activity. However, if considered as an isolated phenomenon, this form of plasticity would be particularly destabilizing over time since increases or decreases in activity would ultimately drive neuronal activity towards runaway excitation or depression, respectively. Thus, homeostatic plasticity has been referred to as a higher-order form of plasticity that is thought to impart stability to neuronal circuits (Turrigiano and Nelson, 2004). By dynamically scaling synaptic strength, homeostatic processes prevent neuronal circuits from becoming hyper- or hypoactive in the face of an ongoing destabilizing force such as ethanol. While acute ethanol may disrupt Hebbian-based plastic events, continued ethanol exposure engages homeostatic processes to stabilize the neuronal network. Consistent with this, ethanol-induced increases in NMDA-receptor activity is a homeostatic response that restores normal glutamatergic tone that otherwise would promote the collapse of the network if left unopposed. One aspect of homeostatic adaptation to chronic ethanol may be a change in the number of NMDA receptors located at the synapse via altered trafficking of these receptors between specific locations.

To examine the effects of prolonged ethanol exposure on the subcellular localization of NMDA receptors, we used a combination of immunohistochemistry of NMDA receptors and confocal microscopy in cultured hippocampal neurons (Carpenter-Hyland et al., 2004). We observed that chronic ethanol (4-day 50 mM or 8-day 25 mM) enhanced the clustering of dendritic NMDA receptors both in terms of size and density of clusters. To differentiate synaptic from extrasynaptic populations of NMDA receptors, the presynaptic marker protein synapsin was labeled in conjunction with the NR1 subunit. Analysis of NR1/synapsin cluster colocalization revealed that the increase in NMDA-receptor cluster size and density was restricted to the synaptic receptor pool with no changes observed in the extrasynaptic pool. These changes could be blocked by co-application of low-dose NMDA or PKA inhibitors with ethanol, and were reversed following prolonged withdrawal produced by allowing ethanol to slowly evaporate from the culture media.

To determine the functional correlate of increased synaptic NMDA-receptor clustering following chronic ethanol, we utilized electrophysiological procedures that distinguish synaptic from extrasynaptic NMDA-receptor currents. As predicted by the imaging studies, chronic ethanol exposure was associated with an increase in synaptic NMDA currents without changing either synaptic AMPA currents or altering extrasynaptic NMDA currents (Carpenter-Hyland et al., 2004). Also as predicted from the imaging studies, ethanol-induced change in synaptic NMDA currents was reversed following ethanol withdrawal. Finally, the increased density and function of synaptic NMDA receptors following chronic ethanol exposure enhance neuronal excitability as reflected by increases in the number and amplitude of spontaneous NMDA- and AMPA-mediated excitatory postsynaptic currents observed immediately following ethanol washout.

In addition to imparting distinct kinetic properties, NR2 subunits appear to differentially modulate the synaptic localization of NMDA receptors (Barria and Malinow, 2002). Ethanol-induced changes in the synaptic localization of the NR1 subunit immunoreactivity were mirrored by nearly identical changes in the synaptic immunoreactivity of the NR2B subunit (Carpenter-Hyland et al., 2004). The movement of NR2B-containing receptors into the synapse is significant since extrasynaptic NR2B-containing receptors may serve as a pool for enhancement of synaptic NMDA-receptor numbers in response to reductions in synaptic activity (Tovar and Westbrook, 1999; Tovar and Westbrook, 2002). Importantly, studies have suggested that NR2B-containing receptors may underlie enhanced plasticity of the young brain compared to the adult brain (Tang et al., 1999). The NR2B subunit has also been implicated in neuroadaptive changes in the central amygdala and hippocampus in response to ethanol exposure (Miyakawa et al., 1997; Roberto et al., 2004; Yaka et al., 2003b). Given the importance of NR2B subunits in experience-dependent plasticity, the ability of prolonged ethanol to increase their synaptic localization suggests a mechanism for ethanol-induced changes in synaptic plasticity similar to learning and memory processes.

The ability of neurons to differentially alter the localization and trafficking of NMDA receptors between spatially resolved and functionally distinct membrane domains is an elegant and dynamic means for adapting to reductions in synaptic signaling produced by ethanol. It is also consistent with the emerging concept that, at least at the level of the synapse, normal experience-dependent plastic processes in the brain are adversely co-opted by alcohol and other drugs of abuse. In addition, the selective targeting of NMDA receptors to the synapse in response to prolonged ethanol exposure appears to be distinct from adaptations involved in acute tolerance to ethanol. In mice, acute ethanol exposure induces the rapid tyrosine phosphorylation of NR2B in the hippocampus (Yaka et al., 2003a). This phosphorylation is most likely mediated via activation of Fyn tyrosine kinase since enhanced phosphorylation was not observed in Fyn-deficient mice (Miyakawa et al., 1997). Furthermore, Fyn-deficient mice exhibit enhanced sensitivity to the sedative effects of ethanol and do not develop acute tolerance (Yaka et al., 2003b). While changes in the tyrosine phosphorylation state of the NMDA receptor may directly alter channel activity, it also appears to underlie changes in their surface expression via endocytotic/exocytotic processes (Dunah et al., 2004). In the adult hippocampus, LTP is associated with increased tyrosine phosphorylation of the NR2B subunit and increased NMDA-receptor surface expression (Rostas et al., 1996). It has also been reported in the nucleus accumbens that acute tolerance to ethanol involves alterations in serine phosphorylation of the NR1 subunit (Maldve et al., 2002). We suggest that while rapid adaptive responses to acute ethanol involve phosphorylation-dependent changes in channel conductance and/or surface expression, long lasting changes that occur in response to chronic ethanol involve alterations in their synaptic localization.

6. A structural basis for homeostatic changes in synaptic function following alcohol exposure

Cellular models of experience-dependent synaptic plasticity have shown that changes in the subcellular localization of glutamate receptors can be accompanied by molecular reorganization of the PSD and alterations in spine morphology and/or density (Segal, 2005). Drugs of abuse have also been observed to produce changes in dendritic spines that may correlate with various behavioral aspects of addiction (Robinson and Kolb, 2004). Ethanol has been shown to produce spine changes in the hippocampus following chronic exposure. This includes an increase in the proportion of wide and stubby spines in addition to a reduction in the proportion of thin and mushroom shaped spines (Lescaudron et al., 1989; Tarelo-Acuna et al., 2000). However, ethanol's effects on dendritic spines in animal models are difficult to interpret due to confounds such as nutritional status, cell death, developmental disruptions and/or methodological complications associated with the chronic administration of ethanol to rodents (Chandler, 2003). If structural changes to dendritic spines do in fact represent the engagement of physiological mechanisms of learning by ethanol, identification of underlying mechanisms could be a significant step in attempts to limit or reverse the long-term effects of this drug on memory and motivation systems of the CNS.

To explore the potential relationship between enhanced synaptic NMDA-receptor targeting and structural adaptations in response to prolonged ethanol exposure, we recently examined the effects of a 4-day 50 mM ethanol exposure on the subcellular organization of the synaptic scaffolding protein PSD-95 and dendritic spine morphology in cultured hippocampal neurons (Carpenter-Hyland and Chandler, 2006). Our results indicate that chronic ethanol exposure induced synaptic targeting of NMDA receptors is associated with synaptic clustering of PSD-95. In addition, chronic ethanol also produced enlargements in F-actin clusters, but only those that colocalized with PSD-95 (i.e., no change in F-actin clusters that were not colocalized with PSD-95). This suggests that chronic ethanol exposure leads to an increase in spine size (Fig. 1). Similar to increases in synaptic NMDA receptors, the increases in PSD-95 at the synapse and enlargement of dendritic spines by ethanol occurred through activity-dependent processes. Taken together, these observations provide further evidence that chronic ethanol engages homeostatic responses to modify synaptic efficacy.

Glutamate receptor activity has been shown to acutely regulate clustering of PSD-95 in synapses (El-Husseini and Bredt, 2002), and dispersal of PSD-95 in response to increases in NMDA-receptor activity may serve as a dampening mechanism for feedback regulation of receptor-activated signaling events. Conversely, chronic blockade of synaptic activity may allow for synaptic accumulation of PSD-95. Such a process could be an important step in homeostatic adaptations as additional

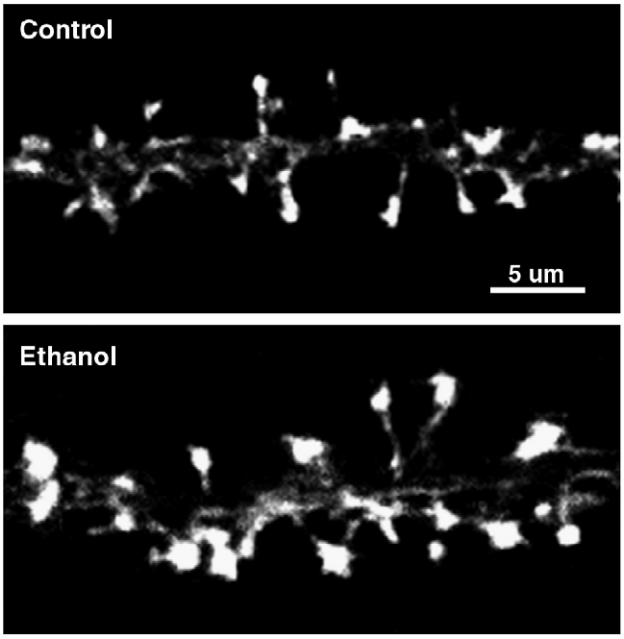

Fig. 1. Chronic ethanol exposure enhances the size of dendritic spines. Cultured hippocampal neurons were exposed to 50 mM ethanol for 4 days. Cultures were then stained for F-actin and imaged using confocal microscopy. Spines were clearly visible as regions of intense F-actin staining protruding from the dendritic shaft. Ethanol treatment significantly enlarged the spines on many neurons while only slightly increasing their number.

scaffolding could enhance the synaptic retention of NMDA receptors (Lin et al., 2004) and alter their lateral mobility dynamics in the cellular membrane (Choquet and Triller, 2003). As such, increasing synaptic levels of PSD-95 may allow for additional scaffolding of NMDA receptors at the PSD. Up-regulation of PSD-95 expression has been associated with the maintenance of the late phase of LTP (Williams et al., 2003), and similar processes could be engaged in long-term homeostatic adaptations *in vivo* to ethanol. Indirect evidence supporting the need for enhanced PSD-95 clustering in the synapse that supports enhanced synaptic efficacy may be inferred from observations that PSD-95 expression is down-regulated in the striatum following long-term exposure to psychostimulants (Yao et al., 2004). The potential relationship between PSD-95 expression and development of psychostimulant sensitization is also supported by enhanced cocaine sensitization in PSD-95 knock-out mice (Yao et al., 2004). These changes could reflect attempts of striatal circuits to dampen psychostimulant-induced plasticity, and implicate PSD-95 in adaptations to chronic exposure to addictive drugs (Roche et al., 2001).

Activity-dependent changes in dendritic spine morphology produced by ethanol also appear to reflect homeostatic enhancement of synaptic NMDA-receptor clustering. In addition, these changes in spine morphology may also represent the capacity of ethanol to engage molecular mechanism of learning and memory. Inappropriate learning may be one of the most persistent aspects of drug addiction and associated maladaptive behaviors (Kelley, 2004). The persistence of these behaviors may be reflected in altered patterns of synaptic connectivity. This proposed relationship has been supported by observations of changes in spine density in the nucleus accumbens and prefrontal cortex of rats exposed to various psychostimulants that develop in parallel with drug sensitization (Robinson and Kolb, 2004). Greater levels of F-actin (but not total actin) have also been identified in the nucleus accumbens following both acute exposure to and withdrawal from repeated cocaine exposure (Toda et al., 2006)).

While both ethanol and psychostimulants produce structural changes to dendritic spines, they differ in that ethanol primarily enhances dendritic spine size, with only minor changes in spine density. While both types of changes may be produced by ethanol and cocaine, selective changes in spine size could be particularly important with respect to strengthening and stabilizing drug induced changes in synaptic connectivity within the addiction neurocircuitry. Evidence for this includes observations that large spines appear to be stable over time while small spines are much more transient (Holtmaat et al., 2005). As such, enhancement in spine number may reflect either transient modifications or early stages of lasting changes in synaptic architecture. Consistent with this, late phases of LTP have been shown to involve enlargement in spine size but no change in spine number (Popov et al., 2004). Although speculative, spine enlargement might reflect a process that promotes stabilization of existing neurocircuitry. One potential consequence of this would be to limit future capacity for synaptic modification. Thus, increasing dendritic spine size could be one factor associated with the persistent and relapsing nature of drug addiction.

7. Glutamatergic neurotransmission and enhanced vulnerability of the adolescent brain to addiction

In light of the biological relationship between addiction and learning, heightened adaptability in the adolescent brain may underlie increased vulnerability of adolescents and young adults to addictive processes. At the neurocircuitry level, this heightened vulnerability may be reflective of the plasticity of NMDA receptors and dendritic spines within the addiction circuit.

7.1. Differential role of NR2B- and NR2A-containing NMDA receptors in plasticity

Early in brain development, synapses predominantly express NR2B-containing NMDA receptors (Jin et al., 1997). As development proceeds, NR2A-containing NMDA receptors are added to synapses in a manner dependent on synaptic NMDA-receptor activity (Barria and Malinow, 2002). As NR2A-containing NMDA receptors have a smaller window in which the receptor can promote synaptic plasticity, subunit replacement during development has been linked with synapse stabilization and reduced learning with age. It also suggests that the NR2B subunit is important in promoting learning. This hypothesis is consistent with reductions in expression of NR2B being correlated with a shortening of the NMDA-mediated EPSCs (Okabe et al., 1998).

The role of experience in influencing the ratio of NR2A/NR2B-containing receptors has been examined in several different models of synaptic plasticity and development. With respect to LTP, the timing of expression appears important as NR2A can be up-regulated following short-term LTP while NR2B changes are particularly long-lived and correlate with the persistence of LTP over days (Williams et al., 1998). This NMDA-receptor subunit substitution has been shown to play a role developmentally where experience and critical periods are related to synaptic NR2A/NR2B levels in visual plasticity (Chen et al., 2000). The involvement of the NR2B subunit in learning has also been demonstrated in mice with altered levels of NR2B. While over-expression of the NR2B subunit produces mice with improved memory and greater LTP (Tang et al., 1999), knock-out mice that lack the NR2B subunit exhibit learning and memory deficits and reduced LTP (Clayton et al., 2002). An interesting question that remains to be addressed is whether enhanced expression of the NR2B subunit increases vulnerability to addictive drugs similar to the enhanced vulnerability of the adolescent brain.

The differential role of NR2A- and NR2B-containing NMDA receptors in learning appears to be a product of their spatial localization and mobility. Unlike NR2A-containing receptors which are predominantly synaptic, NR2B-containing NMDA receptors exist in both synaptic and extrasynaptic pools (Tovar and Westbrook, 1999). These extrasynaptic NR2B-containing NMDA receptors can migrate into synapses in response to selective blockade of synaptic receptors (Tovar and Westbrook, 2002). They also undergo greater cycling between intracellular and membrane surface locations than do NR2A-containing NMDA receptors (Lavezzari et al., 2004), and the

scaffolding of NR2B-containing receptors with PSD-95 has been observed to determine the propensity for NMDA-receptor internalization (Lavezzari et al., 2003). The presence of a large, rapid cycling extrasynaptic pool of NR2B-containing receptors with the capacity to move into and out of synapses fits with NR2B subunit expression determining the capacity of neurons to make homeostatic adjustments to changes in activity levels.

7.2. Heightened vulnerability of the adolescent brain to addictive drugs

Neurodevelopment during adolescence and early adulthood appears to represent a period of significantly greater vulnerability to addictive drugs. Rewiring, pruning, and shaping of neuronal circuitry continue through adolescence and into early adulthood. These changes appear to correlate temporally with changes in cognitive function. Such refinements in synaptic function and neuronal architecture are thought to represent learning-based adaptive processes important for developing an adult-like cognitive phenotype. However, this period of heightened neuroplasticity also confers greater vulnerability to the addictive actions of drugs. Importantly, continued brain development during adolescent and early adulthood is observed in brain regions of the addiction neurocircuitry, including regions associated with motivation, impulsivity, and goal-directed behaviors.

In conjunction with heightened neuroplasticity, studies also indicate that the adolescent brain of rats is more sensitive to the disruptive effects of acute ethanol on spatial learning task (Markwiese et al., 1998). This is consistent with enhanced impairments of LTP (Swartzwelder et al., 1995). Similar observations have been reported in young adult humans aged 21–24 as compared to adults aged 25–29 (Acheson et al., 1998). Furthermore, binge exposure of adolescent rats produced larger disruptions in working memory during acute ethanol challenge when tested as adults (White et al., 2000). The observations in rats of impairment in performance of tasks that involve hippocampal function are consistent with the observation in humans of reduced hippocampal volume in adolescent subjects with ethanol use disorders compared to age-match controls (De Bellis et al., 2000). This is also consistent with a number of studies indicating that the hippocampus is particularly sensitive to the disruptive effects of ethanol. In contrast to the enhanced sensitivity to the cognitive impairing actions of ethanol, adolescent rats show reduced sensitivity to the sedative and motor impairing actions of acute ethanol (Little et al., 1996; Silveri and Spear, 1998). In contrast, while acute ethanol induces greater levels of motor in-coordination in the adolescent brain, there is enhanced tolerance to an acute challenge of ethanol following prolonged exposure (Swartzwelder et al., 1998). Similarly, it was recently reported that adolescent rats exhibit enhanced acute tolerance to ethanol-induced social inhibition (Varlinskaya and Spear, 2006). Together, these observations are consistent with heightened plasticity of the adolescent brain as it relates adaptive changes in response to ethanol exposure. Further, the protracted nature of these changes is consistent with our suggestion of a structural basis of these changes at the microcircuitry level of brain wiring.

Chambers et al. (2003) outlined three primary epidemiological observations indicating that the developmental periods of adolescence and early adulthood are primary correlates of use and substance abuse disorders. 1) This developmental period is characterized by higher rates of experimentation and novelty seeking. 2) Addictive disorders in adults generally have onset in adolescence and early adulthood. 3) Early onset of substance use predicts greater severity and morbidity of substance use disorders. Of particular note is that in alcoholics, 80% of the cases began before the age of 30 and over 40% reported alcohol-related problems between the ages of 15 and 19 (Chambers et al., 2003). Our observations at the cellular level are consistent with the idea that alcohol and other drugs of abuse engage glutamatergic-based learning process that are particularly active during neurodevelopment that characterizes the adolescent brain. We suggest that homeostatic adjustments in response to the depressant effects of ethanol enhance the localization of NMDA receptors at the synapse. This may in turn promote changes in dendritic spine architecture that in a sense "hard-wires" the drug-adapted behavioral state. As such, learning-related maladaptive changes to drug exposure during the critical period of development would be persistent and resistant to change. The persistence may be further compounded in the adult brain due to its reduced plasticity compared to the adolescent brain. Thus, once addictive behaviors are established during adolescent and early adulthood via learning-based plasticity, the addictive behavior will present as recalcitrant and chronically relapsing, which are in fact, prominent features of addiction.

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