Preface

Drugs of Abuse: Neurological Reviews and Protocols is intended to provide insightful reviews of key current topics and, particularly, state-of-the-art methods for examining drug actions in their various neuroanatomical, neurochemical, neurophysiological, neuropharmacological, and molecular perspectives. The book should prove particularly useful to newcomers (graduate students and technicians) in this field, as well as to those established scientists (neuroscientists, biochemists, and molecular biologists) intending to pursue new careers or directions in the study of drugs. The book's protocols cover a wide variety of coherent methods for gathering information on quantitative changes in proteins and mRNAs at both tissue and cellular levels. Inducible gene expression in striatal neurons has been a hot topic over the last decade. Alterations in gene expression for a wide range of proteins in the striatum have been investigated in response to drug administration. Altered expression of given mRNAs and their product proteins constitutes essential molecular steps in the development of neuroplasticity related to long-term addictive properties of drugs of abuse. With the multiple labeling methods that are also described in the book, gene expression can be detected in a chemically identified cell phenotype; the expression of multiple genes of interest can be detected in a single cell simultaneously. Hundreds or thousands of gene expression products can today be detected in one experimental setup using the powerful systematic cDNA macroarray or microarray screening technology. Moreover, protocols useful in analyzing the functional roles of genes and proteins (e.g., viral-mediated gene transfer, knockout mice, and antisense strategy) are also included. Also important here is the inclusion of studies on the release kinetics of striatal dopamine, a prime brain transmitter that such psychostimulants as cocaine and amphetamine interact with, using an in vivo microdialysis or real-time voltammetry technique. This study will also expand to include the quantitative measurement of other neurotransmitters (such as acetylcholine) because increasing evidence for the role of this transmitter in the control of drug actions has emerged. The properties of drugs have also been recently linked to the activity of adult neural stem and progenitor cells in the forebrain. Therefore, a timely review and two protocol chapters describing an immunohistochemical method to examine cellular proliferation and differentiation in the adult rodent

brain, along with a culture method to grow viable neural progenitors, are also provided in the book.

A further feature of *Drugs of Abuse: Neurological Reviews and Protocols* is the introduction of primary neural culture preparation for studies on intracellular signaling pathways, gene expression, and so on. These cultures provide a relatively purified and easily controlled model for the investigation of cellular events related to drug's actions. Analysis of DNA binding activity in specific sites of DNA promotor regions is now possible with an electrophoretic mobility-shift assay in the cell culture tissue, in addition to striatal tissue from living brain. It can be anticipated that the usefulness of the neural culture model will undoubtedly help expand cellular and molecular research into drugs of abuse.

The chapters in *Drugs of Abuse: Neurological Reviews and Protocols* follow the format of previous volumes in the Methods in Molecular Medicine series. All chapters have been contributed by scientists with considerable experience in the protocols covered, and each protocol has been thoroughly tried and successfully tested in the respective contributor's laboratory. In each article, a final section of Notes has proven to be particularly helpful because many of the tricks of the trade are provided there; I recommend reading them thoroughly whenever troubleshooting is necessary. Illustrative data have also been included as frequently as possible so that the reader will have an opportunity to compare well-documented data with their own results from the first run of a protocol. Good luck!

John Q. Wang

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Effects of Psychomotor Stimulants on Glutamate Receptor Expression

Marina E. Wolf

1. Introduction: Addiction as a Form of Glutamate-Dependent Plasticity

It is increasingly well accepted that addiction can be viewed as a form of neuronal plasticity, even as a type of very powerful, albeit maladaptive, learning. On a behavioral level, this can be conceptualized as the transition from experimentation to compulsive drug-seeking behavior. This view of addiction has been strengthened by many recent studies demonstrating commonalities between mechanisms underlying learning and addiction. Both are associated with changes in gene expression, phosphorylation and phosphatase cascades, neurotrophin signaling, altered dendritic morphology, and activity-dependent forms of plasticity such as long-term potentiation (LTP) and long-term depression (LTD) ***(1**,**2)***. Through these mechanisms, drugs of abuse are proposed to strengthen or weaken activity in pathways related to motivation and reward. This in turn may produce behavioral changes that drive compulsive drug-seeking behavior in addiction, including sensitization of incentive-motivational effects of drugs, enhanced ability of drug-conditioned stimuli to control behavior, and loss of inhibitory control mechanisms that normally govern reward-seeking behavior ***(3**,**4)***.

An open question is how drugs of abuse, which initially target monoamine receptors, are able to influence mechanisms of synaptic plasticity. Glutamate is a key transmitter for synaptic plasticity, and many neuronal pathways implicated in addiction are glutamatergic ***(4)***. Historically, studies of behavioral sensitization, a well-established animal model for addiction, were important in directing drug addiction research toward glutamate ***(5)***. Behavioral sensitization

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refers to the progressive enhancement of species-specific behavioral responses that occurs during repeated drug administration and persists even after long periods of withdrawal. Although most studies have measured sensitization of locomotor activity, sensitization also occurs to the reinforcing effects of psychomotor stimulants. Behavioral sensitization is influenced by the same factors that influence addiction (stress, conditioning, and drug priming), and is accompanied by profound cellular and molecular adaptations in the neuronal circuits that are fundamentally involved in normal motivated behavior as well as addiction. Like addiction, it is extremely persistent. Robinson and Berridge ***(6,7)*** have argued for an incentive-sensitization view of addiction, which holds that repeated drug administration sensitizes the neuronal systems involved in drug "wanting" rather than drug "liking."

It is now acknowledged that the development of sensitization requires glutamate transmission in the midbrain, where dopamine (DA) cell bodies are located, whereas its maintenance and expression are associated with profound changes in glutamate transmission in limbic and cortical brain regions that receive dopaminergic innervation. To understand the role of glutamate transmission in sensitization, many studies have examined drug effects on glutamate transmission in these brain regions. This review focuses on cocaine and amphetamine effects on glutamate receptor expression in the midbrain (ventral tegmental area [VTA] and substantia nigra), the striatal complex (nucleus accumbens [NAc] and dorsal striatum), and the prefrontal cortex (PFC). Recent studies are emphasized, with the goal of updating a comprehensive review published 4 yr ago ***(8)***.

2. Effects of Psychomotor Stimulants on Glutamate Receptor Expression in the VTA and Substantia Nigra

2.1. Role of the VTA in Behavioral Sensitization

Many lines of evidence have suggested that the development of behavioral sensitization is associated with an increase in excitatory drive to VTA DA neurons ***(8)***. This provided the impetus for examining whether glutamate transmission is enhanced in the VTA during the early phase of drug withdrawal. The first evidence to support this hypothesis came from in vivo single-unit recording studies demonstrating that VTA DA neurons recorded from cocaine- or amphetamine-sensitized animals were more responsive to the excitatory effects of iontophoretic glutamate ***(9)***. A subsequent study showed that increased responsiveness was selective for α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (there was no change in sensitivity to *N*-methyl-D-aspartate [NMDA] or a metabotropic glutamate receptor agonist) and transient, present 3 but not 10–14 d after discontinuing repeated drug adminis-

tration ***(10)***. Recently, we have shown that AMPA receptor supersensitivity can also be demonstrated in microdialysis experiments, by monitoring the ability of intra-VTA AMPA to activate VTA DA neurons and thus increase DA levels in the ipsilateral NAc. Using dual-probe microdialysis, we found that intra-VTA administration of a low dose of AMPA produced significantly greater DA efflux in the NAc of amphetamine-treated rats ***(11)***. This augmented response was transient (present 3 but not 10–14 d after the last injection) and specific for AMPA, as intra-VTA NMDA administration produced a trend toward increased NAc DA levels that did not differ between groups. Thus, both microdialysis and in vivo electrophysiological data suggest an enhancement of AMPA receptor transmission onto VTA DA neurons during the early phase of drug withdrawal. An increase in glutamate receptor expression would provide a simple explanation for such findings. For this and other reasons, a number of studies have examined the effect of repeated drug administration on glutamate receptor expression in VTA. Studies on glutamate receptor expression in the substantia nigra are also considered. Although the substantia nigra has received less attention in recent years than the VTA, it exhibits similar drug-induced adaptations and is also implicated in the development of sensitization ***(8)***.

2.2. Results in the VTA and Substantia Nigra

Using Western blotting, Nestler and colleagues found increased GluR1 levels in the VTA of rats killed 16–18 h after discontinuation of repeated cocaine, morphine, ethanol, or stress paradigms ***(12,13)***. Increased GluR1 was not observed in the substantia nigra after repeated cocaine or morphine treatment ***(12)***. The substantia nigra was not examined in stress studies ***(12)***, but after repeated ethanol administration, there was a greater increase in GluR1 in the substantia nigra than in the VTA ***(13)***. Repeated cocaine also increased NR1 in VTA but had no effect on GluR2, NR2A/B, or GluR6/7 ***(12)***. Churchill et al. ***(14)*** treated rats with saline or cocaine for 7 d (15 mg/kg on d 1 and 7, 30 mg/kg on d 2–6), measuring locomotor activity after the first and last injections; those rats that showed >20% increase in locomotor activity were defined as sensitized. Then, protein levels of glutamate receptor subunits were determined by Western blotting 24 h or 3 wk after daily injections were discontinued. In agreement with results of Fitzgerald et al. ***(12)***, Churchill et al. ***(14)*** found increased GluR1 and NR1 levels in the VTA of rats killed 1 d but not 3 wk after discontinuation of this different cocaine regimen. Interestingly, this was observed only in those cocaine-treated rats that developed sensitization. GluR2/3 was not measured after 1 d but was unaltered after 3 wk ***(14)***.

In contrast, our own quantitative immunoautoradiography studies found no change in GluR1 immunoreactivity in VTA, substantia nigra, or a transitional

area after 16–24 h of withdrawal from repeated amphetamine or cocaine treatment ***(15)***. Importantly, this study ***(15)*** also failed to find a change in GluR1 immunoreactivity after 3 or 14 d of withdrawal from the same amphetamine regimen that resulted in enhanced electrophysiological ***(10)*** and neurochemical ***(11)*** responsiveness to intra-VTA AMPA at the 3-d withdrawal time. Thus, although part of the discrepancy between results from different labs may be attributable to different drug regimens, our findings suggest that increased GluR1 expression is unlikely to explain our electrophysiological or neurochemical findings of increased responsiveness to AMPA. Other possible reasons for differences between our immunoautoradiography studies and prior Western blotting studies have been discussed previously ***(15)***.

Another finding relevant to this controversy is that overexpression of GluR1 in the rostral VTA using a herpes simplex virus resulted in intensification of the locomotor stimulant and rewarding properties of morphine ***(16,17)***. Although this is an interesting finding, it does not necessarily imply that increased GluR1 expression is involved in the naturally occurring pathways that produce behavioral sensitization to morphine or psychomotor stimulants. A state resembling behavioral sensitization can be produced by a number of diverse experimental manipulations, all sharing the ability to produce brief but intense activation of VTA DA cells. These include repeated electrical stimulation of the VTA ***(18)*** or PFC ***(19)***, and pharmacological disinhibition of VTA DA cells ***(20)***.

In contrast to discrepant results at the protein level, all studies agree that mRNA levels for AMPA receptor subunits in the VTA are not altered during withdrawal from repeated amphetamine or cocaine. We found no change in GluR1 mRNA using reverse transcriptase-polymerase chain reaction (RT-PCR) in the VTA of rats killed 16–18 h after discontinuing repeated amphetamine or cocaine administration ***(15)***. Similarly, Bardo et al. ***(21)*** used RNase protection assays to quantify GluR1-4 mRNA levels in the ventral mesencephalon of rats killed 30 min after the third or tenth amphetamine injection in a repeated regimen and observed no significant changes, although behavioral sensitization was demonstrated. Ghasemzadeh et al. ***(22)*** used RT-PCR to determine mRNA levels for GluR1-4, NR1, and mGluR5 in the VTA 3 wk after discontinuing repeated cocaine or saline injections, and found no significant changes as a result of repeated cocaine treatment, although acute cocaine challenge produced a small reduction in NR1 mRNA levels in the VTA of both naïve and sensitized rats.

As noted previously, Western blotting studies have found increased NR1 levels in the VTA of rats killed 16–24 h (but not 3 wk) after discontinuing repeated cocaine administration, suggesting that the increase is transient ***(12,14)***. In contrast, using immunohistochemical methods, Loftis and Janowsky

(23) compared rats treated with repeated cocaine or saline and found significant increases in NR1 immunoreactivity in the cocaine group after 3 and 14 d of withdrawal and a trend toward an increase after 24 h. Using the same regimen as Churchill et al. ***(14)***, Ghasemzadeh et al. ***(22)*** found no significant changes in NR1 mRNA levels in VTA using RT-PCR. We used quantitative immunoautoradiography to examine NR1 expression in VTA, substantia nigra, and a transitional area in rats killed 3 or 14 d after discontinuing repeated amphetamine administration. No changes were observed after 3 d of withdrawal, whereas NR1 immunolabeling was significantly decreased in the intermediate and caudal portions of the substantia nigra, but not in other midbrain regions, after 14 d of withdrawal ***(24)***. NR1 levels in the NAc and prefrontal cortex were also decreased at this withdrawal time ***(24)***. It may be relevant to note that although NMDA receptor transmission in the VTA is required for the induction of sensitization, repeated stimulation of NMDA receptors in the VTA is not sufficient to elicit sensitization ***(25,26)***.

2.3. Summary: VTA and Substantia Nigra

As reviewed in **Subheading 2.1.**, both neurochemical and electrophysiological studies suggest that there is an enhancement in the responsiveness of VTA DA neurons to the excitatory effects of AMPA shortly after discontinuing repeated psychostimulant administration. An increase in AMPA receptor expression in the VTA would provide a simple explanation for these results. However, although Western blotting studies have found increased GluR1 and NR1 levels in the VTA shortly after cocaine administration is discontinued, this is not observed with immunoautoradiography following either cocaine or amphetamine administration (see ***15*** for discussion). More importantly, after the same drug regimens and withdrawal times that are associated with increased responsiveness of VTA DA neurons to AMPA, no changes in GluR1 are observed. Thus, although considerable evidence suggests that enhanced responsiveness of VTA DA neurons to AMPA is closely linked to the induction of sensitization, the mechanisms are likely to be more complex than a generalized increase in GluR1 expression within the VTA.

As LTP is expressed as a potentiation of AMPA receptor transmission, an alternative explanation is that sensitization is accompanied by LTP-like changes that increase the efficiency of glutamate transmission in the VTA. Although LTP appears to involve insertion of AMPA receptor subunits into synaptic sites ***(27)***, there is no evidence that this is accompanied by increases in total cellular expression of AMPA receptor subunits. Supporting the involvement of LTP in the development of sensitization, a single systemic injection of cocaine to mice (sufficient to elicit behavioral sensitization) produced LTP in midbrain DA neurons ***(28)***. The mechanisms responsible are probably complex.

DA-releasing stimulants could promote LTP by decreasing the opposing influence of LTD, as D2 receptor activation inhibits LTD in midbrain slices ***(29,30)***. Psychostimulant-induced increases in VTA glutamate levels may also promote LTP ***(31,32)***. Of course, mechanisms unrelated to LTP may also contribute to increased excitability of VTA DA neurons, including inhibition of mGluR-mediated inhibitory postsynaptic potentials (IPSPs) ***(33,34)***. Finally, it should be noted that glutamate transmission in the VTA may be influenced by drug-induced alterations in other transmitter systems. Mechanisms that may contribute to sensitization-related plasticity in the VTA have been reviewed elsewhere ***(2,35)***.

An interesting future direction is to study sensitization in transgenic mice with alterations in glutamate receptors or signaling pathways implicated in LTP. Chiamulera et al. ***(36)*** reported that mGluR5 knockout mice do not exhibit locomotor activation when injected with acute cocaine, and do not acquire cocaine self-administration. Mao et al. ***(37)*** found that mGluR1 knockout mice have augmented locomotor responses to amphetamine, perhaps due to impaired mobilization of inhibitory dynorphin systems that normally regulate responses to amphetamine. Using GluR1 knockout mice, Vekovischeva et al. ***(38)*** found that sensitization was normal when mice received repeated morphine injections in the same environment in which they were ultimately tested (context-dependent sensitization) but did not develop when the repeated treatment was given in home cages (context-independent sensitization), whereas wild-type mice developed sensitization under both conditions. Although all of these results are potentially important, it is hard to draw firm conclusions because of the possibility of altered neuronal development in glutamate receptor deficient mice.

3. Effect of Psychomotor Stimulants on Glutamate Receptor Expression in the Nucleus Accumbens and Dorsal Striatum

3.1. Role of the NAc and Striatum in Behavioral Sensitization

The NAc occupies a key position in the neural circuitry of motivation and reward. Not surprisingly, it is also critical for behavioral sensitization. While psychostimulants act in the midbrain to trigger the development of sensitization, drug actions in the NAc lead to the expression of a sensitized response. Accordingly, the VTA is associated with transient cellular adaptations during the early withdrawal period, while the NAc is the site of more persistent adaptations (*see* **refs. *10*** and ***39***). The output neurons of the NAc, medium spiny γ-aminobutyric acid (GABA) neurons, are regulated by convergent DA and glutamate inputs, although the nature of the interaction between DA and glutamate is complex and remains controversial ***(40)***. Repeated psychostimulant administration leads to profound changes in both DA and glutamate trans-

mission in the NAc ***(8,39)***, and many recent studies have demonstrated that glutamate transmission in the NAc plays a critical role in drug-seeking behavior ***(4)***. Therefore, many groups have examined the effects of psychostimulants on glutamate receptor expression in the NAc, as well as in the dorsal striatum. The dorsal striatum exhibits many of the same drug-induced adaptations as the NAc, although the NAc has received much more attention in recent years ***(8)***.

3.2. Results in the NAc and Striatum

We have measured glutamate receptor subunit mRNA levels and immunoreactivity in rats treated for 5 d with 5 mg/kg of amphetamine or saline and perfused 3 or 14 d after the last injection. For AMPA receptor subunits, quantitative *in situ* hybridization studies showed no changes in GluR1-3 mRNA levels in the NAc after 3 d, but decreases in GluR1 and GluR2 mRNA levels were observed after 14 d ***(41)***. Parallel changes were observed at the protein level using quantative immunoautoradiography ***(42)***. Similarly, mRNA and protein levels for NR1 in the NAc were not altered by repeated amphetamine at the 3-d withdrawal time, but both were significantly decreased after 14 d of withdrawal ***(24)***. The decreased levels of GluR1, GluR2, and NR1 subunits in amphetamine-treated rats may be functionally significant. Single-unit recording studies performed in the NAc of rats treated with the same amphetamine regimen, or a sensitizing regimen of cocaine, revealed that NAc neurons recorded from drug-treated rats were subsensitive to glutamate as compared to NAc neurons from saline-pretreated rats ***(9)***. Follow-up studies showed that NAc neurons were also subsensitive to NMDA and AMPA but not a metabotropic glutamate receptor agonist (Hu and White, *unpublished observations*). However, the correspondence is not perfect. The decreases in glutamate receptor subunit expression were observed only after 14 d of withdrawal, whereas electrophysiological subsensitivity was observed after both 3 and 14 days of withdrawal. Perhaps other mechanisms account for subsensitivity at the early withdrawal time (*see* **ref. *43***). Another problem is that NAc neurons recorded from repeated cocaine treated rats also show electrophysiological subsensitivity to glutamate agonists (*see* previous discussion in this subheading), but most studies report increased glutamate receptor expression after long withdrawals from repeated cocaine administration (*see* following portions of this subheading).

Similar to our results showing no changes in glutamate receptor subunit expression in the NAc 3 d after discontinuing repeated amphetamine, Fitzgerald et al. ***(12)*** found no change in NAc levels of GluR1, GluR2, NR1, NR2A/B, GluR6/7, and KA-2 subunit proteins (measured by Western blotting) 16–18 h

after withdrawal from repeated cocaine treatment. However, alterations are observed at later withdrawal times, and they differ from those produced by amphetamine. Churchill et al. ***(14)*** used Western blotting to determine protein levels of glutamate receptor subunits 24 h or 3 wk after discontinuing daily cocaine or saline injections (*see* **Subheading 2.2.** for more details). After 24 h, there were no changes in GluR1 or NMDAR1 levels in the NAc, consistent with the findings of Fitzgerald et al. ***(12)***. However, after 3 wk, sensitized rats (but not cocaine-treated rats that failed to sensitize) showed a significant increase in GluR1 levels in the NAc compared to saline-treated rats. When saline-treated rats were compared to all cocaine rats (sensitized + nonsensitized), there was a trend toward increased NMDAR1 in the NAc after repeated cocaine, but this was actually more pronounced in nonsensitized rats. GluR2/3 was not changed in the NAc at either withdrawal time. Dorsal striatum was analyzed only after 3 wk of withdrawal; there were no changes in GluR1, GluR2/3, or NR1. Likewise, these subunits were unchanged in prefrontal cortex or VTA after 3 wk of withdrawal, although increases in GluR1 and NR1 were found in VTA of sensitized rats 24 h after discontinuing cocaine (*see* **Subheading 2.2.**).

Interestingly, the changes in protein levels found by Churchill et al. ***(14)*** were not paralleled by changes at the mRNA level. Ghasemzadeh et al. ***(22)*** used *in situ* hybridization histochemistry and RT-PCR to quantify glutamate receptor subunit mRNA levels 3 wk after discontinuing the same regimen of cocaine or saline injections used by Churchill et al. ***(14)***. Twenty-four hours before decapitation, half the rats in each group were challenged with saline and half with cocaine. In NAc, acute cocaine decreased mRNA levels for GluR3, GluR4, and NR1, while repeated cocaine also decreased GluR3 mRNA and increased mGluR5 mRNA. The only significant effect in dorsolateral striatum was decreased NR1 mRNA after acute cocaine. The VTA and PFC were also evaluated (see **Subheadings 2.2.** and **4.2.**). Because of the complexity of the design, the reader should consult the article for an in-depth discussion of interactions between chronic cocaine treatment and acute challenge, and interesting trends that were apparent in some groups.

Scheggi et al. ***(44)*** used Western blotting to measure glutamate receptor subunits after administering 40 mg/kg of cocaine every other day over 14 d, testing for sensitization after 10 d of withdrawal, and killing the rats 1 wk after the test for sensitization. In NAc, significant increases in GluR1, NR1, and NR2B (but not GluR2 or NR2A) were found in sensitized rats. The changes in GluR1 and NR1 are in agreement with those reported by Churchill et al. ***(14)***. In hippocampus, only the NR2B subunit was significantly elevated although there was a trend toward increased NR1 (26% increase). In the PFC, small increases (~20%) were observed for NR1 and NR2B, but these were not significant, and

there was no change in GluR1. All of these changes were blocked if MK-801 was continuously infused (s.c., via osmotic minipumps) during cocaine administration, a treatment that also blocked development of sensitization, suggesting they are linked to sensitization.

Chronic cocaine treatment leads to accumulation in some NAc neurons of stable isoforms of the transcription factor ΔFosB, so Kelz et al. ***(45)*** used transgenic mice in which ΔFosB was induced in a subset of NAc neurons to model chronic cocaine treatment. These mice showed increased responsiveness to rewarding and locomotor-activating effects of cocaine, as well as increased expression of GluR2 in the NAc but not dorsal striatum. In a place conditioning test, rats that received intra-NAc injections of a recombinant herpes simplex virus vector encoding GluR2 spent more time in a cocaine-paired chamber than controls, while rats made to overexpress GluR1 spent less time in the cocaine-paired environment. Although this suggests that increased NAc levels of GluR2 may account for enhanced rewarding effects of cocaine in the ΔFosB-expressing mice, more work is needed to evaluate the relevance of these findings to the intact cocaine-treated animal.

NR2B is an interesting NMDAR subunit, as it is implicated in ethanol dependence ***(46)*** and morphine-induced conditioned place preference ***(47)***. Loftis and Janowsky ***(23)*** measured NR2B levels using immunohistochemical methods in NAc and dorsolateral neostriatum, as well as hippocampal and cortical regions (*see* **Subheading 4.2.**). Rats were treated with 20 mg/kg of cocaine × 7 d (or saline) and killed 24 h, 72 h, or 14 d after discontinuing injections. In dorsal striatum, there were no changes after 24 or 72 h, but NR2B immunolabeling was increased after 14 d. In the NAc, NR2B was decreased in shell but not core after 24 h, no changes were present after 72 h, and there were increases in core and shell after 14 d.

Several recent studies have evaluated glutamate receptor binding after repeated cocaine. Keys and Ellison ***(48)*** found a decrease in [^{3}H]AMPA binding, assessed with autoradiography, in ventral striatum, and a trend in NAc, 21 d following two exposures to cocaine administered continuously for 5 d via subcutaneous pellets. Itzhak and Martin ***(49)*** compared NMDA receptor binding in several brain regions (striatum, amygdala, and hippocampus) in rats treated for 5 d with 15 mg/kg of cocaine (a sensitizing regimen) and mice treated for the same time with a higher dose of cocaine (35 mg/kg; a regimen that resulted in kindled seizures). No changes in NMDA receptor binding were found with the sensitizing regimen, whereas binding was elevated in all regions 3 d after the high-dose regimen was discontinued, with additional alterations occurring after the expression of kindled seizures. Szumlinski et al. ***(50)*** found no changes in [^{3}H]MK-801 binding in the rat striatum after a sensitizing regimen of cocaine (five daily injections of 15 mg/kg of cocaine) and 2 wk

of withdrawal. Bhargava and Kumar ***(51)*** treated mice with a sensitizing regimen of cocaine (10 mg/kg, twice daily for 7 d). Immediately after drug treatment, [^{3}H]MK-801 binding was increased in cerebellum and spinal cord but decreased in cortex and hypothalamus. After withdrawal, binding remained decreased in cortex but other changes normalized.

Recent studies have focused on the role of metabotropic glutamate receptors in sensitization. Mao and Wang ***(52)*** used quantitative *in situ* hybridization histochemistry to measure mRNA levels for group I mGluRs (mGluR1 and mGluR5) in the NAc and striatum in naïve and amphetamine-sensitized rats. No changes in mGluR1 or mGluR5 mRNA levels were observed in naïve rats 3 h after acute administration of amphetamine. In contrast, 3 h after the last of five daily amphetamine injections, mGluR1 mRNA levels were increased in dorsal striatum and NAc. This effect was transient, as no changes were observed after 7, 14, or 28 d of withdrawal. A different pattern was observed for mGluR5. Levels of mRNA were decreased markedly 3 h after the final amphetamine injection, and the reduction persisted at 7-, 14-, and 28-d withdrawal times. In a rare example of concordance between amphetamine and cocaine findings, Swanson et al. ***(53)*** found a small but significant reduction in mGluR5 protein levels, measured by Western blotting, in the medial NAc of rats killed 3 wk after discontinuation of repeated cocaine injections. mGluR5 is postsynaptic and can negatively modulate AMPA receptor transmission. Thus, the authors suggested that cocaine-induced decreases in mGluR5 may contribute to the potentiation of AMPA receptor-mediated behavioral responses related to drug-seeking behavior that have been reported after chronic cocaine administration ***(54,55)***. In the same study, repeated cocaine administration attenuated the ability of mGluR1 stimulation to decrease glutamate release and locomotor activity, but this was not accompanied by alterations in mGluR1 protein levels and may be attributable to altered expression of Homer1b/c, a scaffolding protein that regulates mGluR signaling ***(53)***. Increasing evidence indicates that mGluRs play an important role in behavioral responses to psychomotor stimulants ***(56)***.

A relatively unexplored question, owing primarily to technical difficulty, is whether posttranslational modification of glutamate receptors is altered after repeated drug treatment. Bibb et al. ***(57)*** found reduced peak amplitudes of AMPA/kainate-evoked currents in acutely dissociated striatal neurons from rats chronically treated with cocaine; other findings suggested that this was attributable to reduced PKA-dependent phosphorylation of GluR1.

3.3. Summary: NAc and Striatum

As discussed in **Subheading 3.1.**, considerable evidence implicates glutamate receptors in the striatal complex in persistent neuroadaptations associ-

ated with behavioral sensitization and drug-seeking behavior. There is some agreement that GluR1 and NR1 levels are not altered in the NAc after short withdrawals (1–3 d) from repeated cocaine or amphetamine administration. At longer withdrawal times (2–3 wk), cocaine-treated rats may show increases in GluR1, NR1, and NR2B, whereas amphetamine-treated rats show decreases in GluR1, GluR2, and NR1. There may also be persistent changes in the expression and function of group I mGluRs. The delayed onset of many of the reported changes in glutamate receptor expression is consistent with a role for the NAc in the long-term maintenance of sensitization and other drug-induced behavioral changes. However, it is difficult to reconcile opposite effects of cocaine and amphetamine on glutamate receptor expression with a role for these changes in the maintenance and expression of sensitization, as both drugs produce similar behavioral effects (augmented locomotor response) in sensitized rats. It should be kept in mind that the NAc contains heterogeneous populations of projection neurons and interneurons, and we do not know the phenotype of the neurons that experience changes in glutamate receptor subunit expression (e.g., ***42***,***52***). Moreover, other types of drug-induced changes may contribute importantly to the excitability of NAc neurons. For example, Zhang et al. ***(43)*** found reduced sodium currents in NAc neurons after a short withdrawal from repeated cocaine, while Thomas et al. ***(58)*** found evidence for LTD in the NAc after long-term withdrawal from cocaine. In fact, growing evidence suggests that abnormal synaptic plasticity in the NAc, triggered by chronic drug treatment, leads to dysregulation of motivation- and reward-related circuits and thereby contributes to addiction ***(2)***. It will be important to determine whether alterations in glutamate receptor expression contribute to the induction of altered plasticity, are involved in its expression, or represent compensatory responses to changes in the activity of glutamate-containing projections.

4. Effect of Psychomotor Stimulants on Glutamate Receptor Expression in the PFC and Other Cortical or Limbic Regions

4.1. Role of the Prefrontal Cortex in Behavioral Sensitization

The PFC is now acknowledged to play an important role in behavioral sensitization. Excitotoxic lesions of the PFC prevent the development of sensitization ***(59–61)*** as well as cellular changes in DA systems that are closely associated with sensitization ***(61)***. The role of PFC in the expression of behavioral sensitization in response to psychostimulant challenge is more controversial. Some evidence suggests that expression of sensitization requires glutamatergic transmission between the dorsal PFC and the NAc core ***(62)***. On the other hand, excitotoxic lesions of the PFC that are sufficient to prevent

development of sensitization do not interfere with expression ***(63,64)***. Other findings suggest that maintenance and expression of sensitization may be associated with loss of inhibitory DA tone in the PFC, leading to a loss of inhibitory control over PFC projections to subcortical regions; multiple mechanisms may contribute (e.g., ***65–69***). Less has been done to examine specifically the role of glutamate transmission in the PFC in behavioral sensitization. No studies have examined the effect of intra-PFC injection of glutamate receptor antagonists and only a few microdialysis studies have assessed glutamate release in the PFC in response to stimulants ***(70–73)***. Likewise, there have been relatively few studies on stimulant-induced alterations in glutamate receptor expression in the PFC as compared to the striatum and midbrain.

4.2. Results in the PFC

Using quantitative *in situ* hybridization and immunoautoradiography, we found increased GluR1 mRNA and protein levels 3 d after discontinuing repeated amphetamine administration; this effect was transient, as it was not observed in rats killed after 14 d of withdrawal ***(41,42)***. This increase in GluR1 may be functionally significant, as PFC neurons recorded from amphetamine-treated rats after 3 d of withdrawal (but not 14 d of withdrawal) showed increased responsiveness to the excitatory effects of iontophoretically applied glutamate ***(67)***. In studies using the same amphetamine regimen, we found a significant decrease in NR1 mRNA levels and a trend toward decreased immunolabeling after 14 d of withdrawal, but no change after 3 d ***(24)***.

Cocaine also exerts complex effects on glutamate receptor subunit expression in the PFC. Churchill et al. ***(14)*** found no changes in PFC levels of GluR1, GluR2/3, or NMDAR1 (using Western blots) 3 wk after discontinuing a week of daily cocaine injections (see **Subheading 2.2.** for more details on this study). In another study, rats were treated with cocaine, tested for sensitization after 10 d of withdrawal, and killed 1 wk after the test for sensitization (***44***; see **Subheading 3.2.** for more details). Small increases (~20%) were observed for NR1 and NR2B in the PFC, but these were not significant, and there was no change in GluR1. Loftis and Janowsky ***(23)*** measured NR2B levels using immunohistochemical methods in VTA and NAc (*see* **Subheadings 2.2.** and **3.2.**), as well as dorsolateral neostriatum, the hippocampal formation (CA1, CA3, and dentate gyrus), and the cortex (medial frontal cortex, lateral frontal cortex, and parietal cortex). Rats were killed 24 h, 72 h, or 14 d after discontinuation of repeated cocaine or saline injections. There were no changes in the hippocampal formation following 24 or 72 h of withdrawal. Results in cortex depended on the region analyzed. For medial frontal cortex, there were

increases at all withdrawal times. For lateral frontal cortex and parietal cortex, there was no change after 24 h, but increases after 72 h and 14 d. This study also measured neuronal nitric oxide synthase, but these results will not be discussed.

4.3. Summary: PFC and Other Cortical and Limbic Regions

Glutamate receptor expression in the PFC undergoes complex changes after drug administration is discontinued that depend on the withdrawal time and probably differ between cocaine and amphetamine, at least for NR1. In general, some results suggest that AMPA receptor subunit expression changes at early withdrawal times whereas NMDA receptor subunit expression is altered after longer withdrawals. Because relatively few studies have assessed glutamate transmission in the PFC of sensitized rats using electrophysiological or neurochemical approaches, it is difficult to assess the functional significance of observed changes. An exception is the correlation between increased responsiveness of PFC neurons to glutamate ***(67)***, and increased expression of GluR1 in the PFC ***(41,42)***, after short withdrawals from repeated amphetamine administration. It will be important to conduct studies on additional brain regions implicated in addiction, such as the amygdala.

5. Conclusions

It is clear that repeated administration of cocaine or amphetamine influences glutamate receptor expression in brain regions important for behavioral sensitization and addiction. However, to date, the data obtained raise more questions than they answer. One important problem is that amphetamine and cocaine produce different patterns of changes, whereas both produce behavioral sensitization. Either there are multiple ways to achieve a sensitized state, or the changes in glutamate receptor expression are not directly associated with sensitization. The picture is made more complex by different effects at different withdrawal times, different effects with different drug regimens, and lack of agreement between laboratories using similar drug regimens. Another problem is that studies of receptor expression have been conducted at the regional level, precluding identification of the types of cells exhibiting particular alterations in glutamate receptor expression after stimulant exposure. Without such information, it is hard to predict the functional effect of these alterations at the level of neuronal circuits. For example, does the increase in GluR1 expression in the PFC after repeated amphetamine occur in pyramidal neurons or interneurons, or in a subset of one of these populations? It will be important to conduct future studies in identified cells, although this is a very challenging

undertaking. It will also be important to study the cellular mechanisms by which monoamine-releasing psychomotor stimulants influence the expression of glutamate receptors, as well as other aspects of glutamate neurotransmission.

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