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AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine

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Abstract

This review focuses on cocaine-induced postsynaptic plasticity in the nucleus accumbens (NAc) involving changes in AMPA receptor (AMPA) transmission. First, fundamental properties of AMPAR in the NAc are reviewed. Then, we provide a detailed and critical analysis of literature demonstrating alterations in AMPAR transmission in association with behavioral sensitization to cocaine and cocaine self-administration. We conclude that cocaine exposure leads to changes in AMPAR transmission that depend on many factors including whether exposure is contingent or non-contingent, the duration of withdrawal, and whether extinction training has occurred. The relationship between changes in AMPAR transmission and responding to cocaine or cocaine-paired cues can also be affected by these variables. However, after prolonged withdrawal in the absence of extinction training, our findings and others lead us to propose that AMPAR transmission is enhanced, resulting in stronger responding to drug-paired cues. Finally, many results indicate that the state of synaptic transmission in the NAc after cocaine exposure is associated with impairment of AMPAR-dependent plasticity. This may contribute to a broad range of addiction-related behavioral changes.

Keywords

AMPA receptors; behavioral sensitization; cocaine; glutamate; incubation; nucleus accumbens; plasticity; reinstatement; self-administration

1. Introduction

1.1 Why focus on AMPAR transmission in the nucleus accumbens?

The nucleus accumbens (NAc) occupies a key position in the neural circuitry of motivation and reward (Kelley, 2004) and alterations in this circuitry are believed to underlie drug addiction (Kalivas and Volkow, 2005; Everitt and Robbins, 2005). The majority of NAc neurons (~90%) are medium spiny GABA neurons (MSN; Meredith and Totterdell, 1999). MSN receive glutamate inputs from cortical and limbic regions important for regulating motivated behaviors, including drug seeking, and their projections influence motor regions important for the execution of motivated behaviors (Groenewegen et al, 1999; Kelley, 1999). Based on this connectivity and functional studies, Mogenson (1987) suggested that the NAc

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translates “...the motivational determinants of behavior...into actions”. Indeed, in many common experimental situations, the the final common pathway for drug seeking involves glutamate inputs terminating on MSN in the NAc (Kalivas and McFarland, 2003; Kalivas and Volkow, 2005), although motor circuitry involving the dorsal striatum becomes more important as drug use becomes habitual (Everitt and Robbins, 2005). This review will focus specifically on cocaine-induced alterations in glutamate transmission and plasticity involving α -amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPA) in the NAc.

Glutamate inputs acutely excite NAc MSN primarily by activating AMPAR (Pennartz et al., 1990; Hu and White, 1996) and results in many animal models of addiction indicate that AMPAR activation in the NAc is necessary for drug seeking. Thus, intra-NAc infusion of AMPAR antagonists blocks cue-induced cocaine seeking after withdrawal (¹Conrad et al., 2008), cocaine seeking under second-order schedules of reinforcement (²Di Ciano and Everitt, 2001; ³Di Ciano and Everitt, 2004), cue-induced reinstatement (⁴Bäckström and Hyttiä, 2007), and cocaine-primed reinstatement (⁵Cornish and Kalivas, 2000; ⁶Famous et al., 2008). Cocaine-primed reinstatement is also blocked by intra-NAc injection of antisense oligonucleotides directed against the AMPAR subunit GluR1 (⁷Ping et al., 2008). Conversely, infusion of AMPA into the NAc reinstates cocaine seeking after extinction (⁸Cornish et al., 1999; ⁹Cornish and Kalivas, 2000; ¹⁰Suto et al., 2004; ¹¹Kruzich and Xi, 2006; ¹²Ping et al., 2008). Together, these studies indicate that AMPAR transmission in both core and shell can play a role in cocaine seeking, although core is more important for cue-controlled cocaine seeking, whereas blocking AMPAR transmission in either core or shell prevents cocaine-primed reinstatement (see footnotes for placement of injection cannula in each study; Meredith et al., 2008 for a review of anatomical and functional distinctions between core and shell; and Section 3 for information about animal models).

An important feature of many animal models of addiction is that behavioral responses to cocaine and cocaine-associated cues are strengthened in animals with prior cocaine experience. We hypothesize that this occurs, in part, because additional AMPAR are added to excitatory synapses onto NAc MSN. The addition of AMPAR to MSN synapses will prime these neurons to respond more robustly to glutamate released by cortical and limbic inputs in response to drug and drug-associated cues. This enhanced responding will promote cocaine seeking and affect subsequent cocaine-induced plasticity in the NAc. Our hypothesis is based in part on evidence that AMPAR trafficking into synapses underlies increased synaptic strength in many forms of LTP (Section 2), although we are not equating drug experience with LTP (see Section 7). One major shortcoming of this hypothesis is that it does not take into account the heterogeneity of both NAc MSN and glutamate afferents to the NAc (see Section 1.2). Nevertheless, it provides a useful theoretical framework for interpreting the literature and designing future experiments.

This review will begin by describing fundamental features of AMPAR transmission in the NAc (Section 2) and animal models used for studies of cocaine-induced AMPAR plasticity (Section 3). Then, we will critically evaluate the literature related to our hypothesis, focusing on sensitization to cocaine (Section 4) and cocaine self-administration (Section 5). We will also discuss the effects of cocaine re-exposure after contingent and non-contingent cocaine administration (Section 6) and the effect of cocaine-induced AMPAR adaptations on the ability to elicit subsequent synaptic plasticity in the NAc (Section 7). We conclude with a brief comparison of cocaine and amphetamine (Section 8).

¹effective in core, shell not tested

²effective in core but not shell

³mixed core/shell placements

⁴effective in both core and shell

⁵placements mainly in core

In light of the central role of AMPAR in many forms of experience-dependent plasticity (Kessels and Malinow, 2009), cocaine-induced alterations in AMPAR function would be expected to have widespread consequences by disrupting the normal way that behavior is shaped by experiences related to drugs or natural rewards. Here, we focus on the hypothesis that enhanced AMPAR transmission in the NAc contributes to the pathologically strengthened incentive-motivational properties of drugs and drug-paired cues that characterizes addiction (Robinson and Berridge, 1993; 2001; 2008).

1.2 Integration of our postsynaptic AMPAR-based hypothesis with other ideas

A large number of transmitters and signaling pathways have been implicated in addiction-related plasticity (e.g., Thomas et al., 2008). Our hypothesis (AMPA upregulation contributes to enhanced drug seeking) is narrowly focused on postsynaptic changes in NAc neurons. This piece of the puzzle deserves careful attention based on enormous precedent for a critical role of postsynaptic AMPAR transmission in synaptic plasticity throughout the brain. We hope that a comprehensive review of the AMPAR-related literature will facilitate its integration with studies focusing on DA transmission (Self, 2004; Anderson and Pierce, 2005) as well as other adaptations that regulate the excitability of MSN. Among these other adaptations, two will be discussed briefly because they are expected to work in concert with synaptic AMPAR changes to determine the output of the NAc after repeated cocaine exposure.

First, basal extracellular glutamate levels in the NAc are decreased after both experimenter- and self-administered cocaine due to reduced activity of the cystine-glutamate antiporter (Kalivas, 2009). This has been observed at short (1 day) and long (3 week) withdrawal times (e.g., Miguens et al., 2008; Baker et al., 2003). A major consequence of the reduction of extracellular glutamate levels is loss of glutamate tone on presynaptic metabotropic glutamate receptors (mGluR2/3) that normally exert a “braking” effect on synaptic glutamate release. Restoring or preventing the decrease in extracellular glutamate levels restores normal regulation of glutamate transmission and decreases cocaine-primed reinstatement of drug seeking (as well as other cocaine-related behaviors), in concert with normalization of many cocaine-induced neuroadaptations in the NAc (Baker et al., 2003; Madayag et al., 2007; Moussawi et al., 2009; Kalivas, 2009). It is notable that all of these studies have been conducted in the core subregion. More recent work has also implicated the glial glutamate transporter GLT-1 in cocaine-induced dysregulation of glutamate transmission (Sari et al., 2009; Knackstedt et al., 2010).

Second, both experimenter- and self-administered cocaine decrease the intrinsic excitability of MSN in the NAc through effects on voltage-sensitive ion channels (Zhang et al., 1998, 2002; Hu et al., 2004, 2005; Dong et al., 2006; Kourrich and Thomas, 2009; Ishikawa et al., 2009; Mu et al., 2010). Most of these studies sampled core and shell during the first several days after discontinuing experimenter-administered cocaine (Zhang et al., 1998, 2002; Hu et al., 2004, 2005), but some results suggest different effects in core and shell (Kourrich and Thomas, 2009). Decreased intrinsic excitability in NAc shell persists for at least 3 weeks after experimenter-administered cocaine (Ishikawa et al., 2009; Kourrich and Thomas, 2009) but is less persistent if cocaine is self-administered (Mu et al., 2010). Together, intrinsic excitability and synaptic strength determine the net output of NAc neurons, so it is important to understand whether changes in these two parameters are interdependent (Ishikawa et al., 2009). Some possible relationships between presynaptic and postsynaptic adaptations are discussed briefly in Section 4.2.

It is notable that AMPAR upregulation, decreased intrinsic excitability and decreased extracellular glutamate levels are probably “global” changes affecting many or all MSN (with caveats about core/shell differences noted above). A role for global NAc plasticity is not incompatible with the abnormal response of human addicts to a broad spectrum of stimuli.

However, many recent results emphasize the importance of subpopulations of MSN (“functional ensembles”; Pennartz et al., 1994) that exhibit heterogeneous responses to drugs and drug-paired cues, as well as natural rewards and aversive stimuli, probably due to input-specific regulation (O'Donnell, 2003; Peoples et al., 2007; Goto and Grace, 2008; Mattson et al., 2008; Carelzon and Thomas, 2009; Koya et al., 2009; Wheeler and Carelli, 2009). Hypotheses must be developed to explain how global changes interact with ensemble-specific changes to selectively enhance particular behaviors (e.g., cocaine-related behaviors). One idea, based on the ability of DA to gate the activation of NAc neurons by glutamate inputs (Nicola et al., 2000; O'Donnell, 2003; Owesson-White et al., 2009), is that DA and other inputs select the population of MSN that is activated in a particular behavioral context, and then global adaptations (e.g., cocaine-induced AMPAR upregulation) influence the strength of that activation and perhaps broaden the population of MSN that is affected (see Section 5.4 for more discussion).

2. AMPAR properties in the NAc

2.1 AMPAR and their role in neuronal plasticity

AMPA mediate the majority of excitatory transmission in the brain. Furthermore, changes in synaptic strength in many forms of plasticity are mediated by AMPAR trafficking in and out of synapses (Carroll et al., 2001; Malinow and Malenka, 2002; Song and Haganir, 2002; Bredt and Nicoll, 2003; Shepherd and Haganir, 2007; Derkach et al., 2007). AMPAR assemble as tetramers (dimers of dimers) from GluR1-4 subunits (Cull-Candy et al., 2006; Greger and Esteban, 2007). Landmark co-immunoprecipitation (co-IP) studies using adult rat hippocampus (CA1/CA2) as starting material indicated that most AMPAR are GluR1/2 or GluR2/3 (Wentholt et al., 1996). However, co-IP studies assess all AMPAR in the cell and their interpretation is therefore complicated by several factors including the presence of partially assembled AMPAR in the biosynthetic pathway (Greger et al., 2003). A recent study, conducted in hippocampal slices from young mice, used a single cell-genetic approach combined with electrophysiological recordings to determine the subunit composition of AMPAR in synapses on CA1 pyramidal neurons and in extrasynaptic regions of the soma (Lu et al., 2009b). This study revealed that GluR1/2 receptors comprised ~81% of the synaptic AMPAR and ~95% of extrasynaptic somatic AMPAR, whereas GluR2/3 receptors represented ~16% of the synaptic pool and were absent from the extrasynaptic pool.

Resolving the subtypes of AMPAR present in particular neurons and synapses is important because subunit composition plays an important role in determining AMPAR functional properties and trafficking rules. According to the leading theory, AMPAR composed of subunits with short C-termini (e.g., GluR2/3) cycle constitutively in and out of synapses, while AMPAR containing a subunit with a long C-terminus (e.g., GluR1) are inserted into synapses in an activity-dependent manner (Malinow, 2003). This may reflect differences in regulatory phosphorylation events and protein-protein interactions between subunits with a long C-terminal tail, such as GluR1, and those with shorter tails (GluR2 and GluR3) (Song and Haganir, 2002; Derkach et al., 2007). AMPAR trafficking and function are also regulated by two classes of AMPAR auxiliary subunits, transmembrane AMPA receptor regulatory proteins, or TARPs, and cornichon proteins (Tigaret and Choquet, 2009).

A subpopulation of AMPAR lacks the GluR2 subunit (e.g., homomeric GluR1 or GluR3, and GluR1/3) (Cull-Candy et al., 2006; Liu and Zukin, 2007; Isaac et al., 2007). These AMPAR exhibit several unique properties: permeability to Ca^{2+} resulting in higher conductance, inward rectification due to voltage-dependent block by polyamines, and sensitivity to certain antagonists (e.g., 1-naphthylacetylperimine; Napsm) that do not block GluR2-containing AMPAR. Co-IP studies revealed a small number (<10% of total) of GluR2-lacking AMPAR in adult rat hippocampus (Wentholt et al., 1996), although they do not appear to contribute

significantly to synaptic transmission onto CA1 pyramidal neurons under normal conditions (Lu et al., 2009b; see next section for discussion of GluR2-lacking AMPAR in the NAc). In contrast, GluR2-lacking AMPAR are present under normal conditions in some other synapses (e.g., parallel fiber synapses onto cerebellar stellate cells; Liu and Cull-Candy, 2000).

Recently, there has been tremendous interest in the role of GluR2-lacking AMPAR in synaptic plasticity. Due to their higher conductance, the insertion or removal of only a few of these receptors leads to robust changes in synaptic strength (Liu and Cull-Candy, 2000; Plant et al., 2006; Guire et al., 2008). Due to their Ca^{2+} permeability, their synaptic insertion alters the properties of excitatory transmission and also influences subsequent plasticity (Thiagarajan et al., 2007). However, the mechanisms that selectively regulate the trafficking of GluR2-lacking AMPAR are poorly understood.

Most information about AMPAR plasticity comes from studies of the hippocampus. However, as it becomes clear that AMPAR plasticity is important in addiction, it is important to study AMPAR properties in addiction-related brain regions. Available information about AMPAR subtypes and AMPAR trafficking in the NAc is summarized in the next two sections.

2.2 What types of AMPAR are present in the NAc of drug-naïve rats and mice?

We have conducted quantitative co-IP studies identical to those of Wenthold et al. (1996) using membrane preparations from the NAc (core + shell), dorsal striatum, prefrontal cortex and hippocampus of untreated adult rats (P60-70) (Reimers et al., submitted). This section will focus on the NAc, although similar results were found in the other brain regions, as well as in the NAc of rats that self-administered saline (Conrad et al., 2008). In agreement with Wenthold et al. (1996), our results indicated a large population of GluR1/2 receptors. Thus, ~90% of the GluR1 in the NAc of untreated adult rats is physically associated with GluR2 or GluR3. The majority of this represents GluR1/2, although a small population of GluR1/3 receptors also exists. These results are consistent with prior data indicating that most GluR1-containing AMPAR in the striatum and other forebrain regions also contain GluR2 or GluR3 (Bernard et al., 1997; Gold et al., 1997). Homomeric GluR1 receptors may also exist, based on detection of a small amount of GluR1 (~7% of total) that was not immunoprecipitated by antibodies to other AMPAR subunits, but this could also represent GluR1 monomers or dimers in the AMPAR biosynthetic pathway. We also found a substantial amount of GluR2 (~53% of total) that is not physically associated with GluR1. However, analysis of GluR2 assembly state using blue native polyacrylamide gel electrophoresis suggested that a substantial portion of this “left-over” GluR2 represents GluR2 dimers or monomers, rather than GluR2 present in tetrameric GluR2/3 receptors. In contrast, relatively more GluR1 was present in tetramers. Our findings are consistent with prior work showing that GluR2 in cultured neurons and rat brain is largely retained in the ER in dimer form whereas GluR1 is mainly found in tetrameric receptors (Greger et al., 2003). If these findings are taken into account when interpreting the co-IP data, these data fall into line with recent results indicating a predominant role for GluR1/2 receptors at hippocampal CA1 synapses (Lu et al., 2009b; see previous section). However, a predominant role for GluR1/2 receptors in the NAc does not rule out a role for GluR2/3 or GluR1/3 receptors. Our protein crosslinking studies indicate that GluR3 is expressed on the cell surface in the NAc (Boudreau et al., 2007) and this measure is altered by cocaine self-administration (Conrad et al., 2008). GluR4 is not present in medium spiny neurons (Bernard et al., 1997; Stefani et al., 1998).

Co-IP results described above (Reimers et al., submitted), as well as earlier results (Boudreau et al., 2007), indicate the existence of a small population of GluR2-lacking AMPAR in the adult rat NAc, consisting of GluR1/3 and perhaps homomeric GluR1 receptors. In agreement with these biochemical results, whole-cell patch clamp recordings in the NAc core of adult rats with previous saline self-administration experience found linear AMPAR current-voltage

relationships and only a small reduction (~5%) in the evoked excitatory postsynaptic current (EPSC) amplitude after bath application of Naspm (a selective antagonist of GluR2-lacking AMPAR; Conrad et al., 2008). Similarly, two electrophysiological studies in the NAc shell of juvenile mice given saline injections found linear current-voltage relationships (Kourrich et al., 2007, P24-28; Mameli et al., 2009; P16-35). Taken together, these studies indicate that GluR2-lacking AMPAR do not contribute substantially to synaptic transmission in drug-naïve rodents. However, another study in the NAc shell of adult mice (no treatment; P56-70) observed inwardly rectifying currents and a 25% reduction in evoked AMPAR EPSC amplitude by Naspm (Campioni et al., 2009) consistent with the presence of functional GluR2-lacking synaptic AMPAR. Differences in species, age and the procedures used may contribute to the divergence. Age of the animal is a particularly important variable in studies of GluR2-lacking AMPAR because their expression in principal neurons is highest early in development (e.g., Kumar et al., 2002; Ho et al., 2007). This appears to hold for the NAc as well. Thus, a substantial number of GluR2-lacking AMPAR are present in MSN in primary NAc cultures prepared from P1 rats (Sun and Wolf, 2009), but not in the adult rat NAc (above). Interestingly, studies of dorsal striatal MSN of young rats (P15-19) indicate that GluR2-lacking AMPAR provide an important source of Ca^{2+} entry for MSN even though they contribute only a relatively small amount to excitatory synaptic currents (Carter and Sabatini, 2004). The contribution of Ca^{2+} entry through these receptors in the adult NAc has yet to be examined.

As discussed in Sections 4 and 5, GluR2-lacking AMPAR are detected at NAc synapses after certain types of cocaine exposure. Co-IP and protein crosslinking results suggest that both homomeric GluR1 and GluR1/3 receptors may contribute to this effect (Conrad et al., 2008).

2.3 AMPAR trafficking in cultured NAc neurons

Properties of LTP and LTD in the NAc have been reviewed previously (Kauer and Malenka, 2007). Here we will focus specifically on the contribution of AMPAR trafficking to synaptic plasticity. To study AMPAR trafficking in MSN, we began by using “pure” NAc cultures, prepared by dissociating the NAc from P1 rats (Chao et al., 2002a,b; Mangiavacchi and Wolf, 2004a,b). However, these cultures do not contain glutamate neurons and therefore do not contain glutamate synapses. Therefore, to study AMPAR synaptic trafficking, we developed a co-culture system in which prefrontal cortex (PFC) neurons from enhanced cyan fluorescent protein-expressing mice are co-cultured with NAc neurons from P1 rats. The PFC neurons restore excitatory synapses onto the MSN, but the two cell types can be distinguished because the PFC neurons are fluorescent (Sun et al., 2008).

Using these culture systems, we have shown that AMPAR trafficking in NAc MSN is very similar to AMPAR trafficking in better characterized neuronal populations such as hippocampal pyramidal neurons (Section 2.1). As in other cell types, AMPAR in the NAc constitutively cycle on and off the cell surface (Mangiavacchi and Wolf, 2004a,b). Brief exposure to glutamate, AMPA, NMDA or a group 1 mGluR agonist (3,5-dihydroxyphenylglycine) elicits rapid AMPAR internalization that may contribute to some forms of LTD in the NAc (Mangiavacchi and Wolf, 2004b). In addition, GluR1-containing AMPAR undergo activity-dependent synaptic insertion in a two-step process. First AMPAR are externalized onto the cell surface at extrasynaptic sites, a process that is accelerated by protein kinase A (PKA) activation (most likely via phosphorylation of GluR1 itself at serine 845). These extrasynaptic AMPAR are then translocated into synapses in response to subsequent NMDA receptor and Ca^{2+} -calmodulin-dependent protein kinase (CAMK) activation (Chao et al., 2002a,b; Mangiavacchi and Wolf, 2004a; Sun et al., 2008). Support for this two-step mechanism also exists for other cell types (Passafaro et al., 2001; Esteban et al., 2003; Sun et al., 2005; Gao et al., 2006; Oh et al., 2006; Man et al., 2007).

NAc MSN also exhibit bidirectional synaptic scaling (Sun and Wolf, 2009). Synaptic scaling is a form of homeostatic plasticity in which prolonged activity blockade leads to enhanced excitatory synaptic transmission, while prolonged increases in activity produce the opposite effect (Turrigiano and Nelson, 2004; Thiagarajan et al., 2007; Turrigiano, 2008). This is thought to stabilize the activity of neurons and neuronal circuits. For example, synaptic scaling may prevent synapses from becoming unresponsive due to ceiling effects after repeated LTP. Many mechanisms contribute to expression of synaptic scaling. However, the major postsynaptic component involves AMPAR accumulation after chronic activity blockade and AMPAR removal after prolonged activation (Turrigiano and Nelson, 2004). Our studies in cultured NAc MSN demonstrated bidirectional synaptic scaling involving GluR1/2-containing AMPAR. Furthermore, we showed that increased AMPAR surface expression after prolonged activity blockade requires protein synthesis and is occluded by inhibition of the ubiquitin-proteasome system (Sun and Wolf, 2009). While LTP and LTD may be important in the initiation of addiction-related plasticity and in rapid responses to drug re-exposure, synaptic scaling is a logical candidate for mediating slowly-developing and global forms of plasticity during drug withdrawal. We have suggested (Boudreau and Wolf, 2005; Boudreau et al., 2007) that either decreased activity of glutamate inputs to the NAc (Goldstein and Volkow, 2002; Porrino et al., 2007) or decreased intrinsic membrane excitability of MSN (Section 1.2) after repeated cocaine treatment could elicit synaptic scaling in the NAc. This offers one possible explanation for increased AMPAR surface expression after withdrawal from cocaine (for more discussion, see Section 4.2 and Sun and Wolf, 2009).

There are two general ways that drugs may modulate activity-dependent plasticity at excitatory synapses. First, drugs may act at the circuit level to influence the activation of glutamate pathways and thus influence activity-dependent plasticity at synapses made by these pathways. Second, drugs may act directly on synapses that undergo activity-dependent plasticity by targeting transmitter systems that normally regulate these plasticity mechanisms. These two possibilities need not be mutually exclusive and the interplay between the two may change over the course of repeated drug administration and withdrawal. For cocaine, this direct mechanism is likely to involve blockade of the DA transporter, leading to elevation of extracellular DA levels and activation of DA receptors. In the NAc, as in several other regions important in addiction, DA receptors are well positioned anatomically to regulate synaptic plasticity at excitatory synapses onto MSN. The anatomical substrate for this is the synaptic triad consisting of DA and glutamate inputs that converge onto common spines of MSN (Sesack et al., 2003).

To explore this direct mechanism, we studied the effect of DA receptor stimulation on AMPAR trafficking in cultured NAc neurons and found strong evidence for direct modulatory effects (Chao et al., 2002a; Mangiavacchi and Wolf, 2004a; Sun et al., 2008). Most strikingly, we found that brief D1 receptor stimulation (5-15 min), through a PKA-dependent mechanism, enhances the rate of AMPAR externalization at extrasynaptic sites on MSN (Sun et al., 2008), as well as other cell types (Sun et al., 2005; Gao et al., 2006; but see Gao and Wolf, 2007). This increases the size of the extrasynaptic AMPAR pool and thus facilitates AMPAR synaptic incorporation in response to subsequent NMDAR stimulation. We speculate that this is one mechanism by which DA release may promote plasticity of reward-related learning (Sun et al., 2005; 2008; Gao et al., 2006), a concept discussed in more detail elsewhere (Wolf et al., 2004; Wolf, in press; see also Jay, 2003; O'Donnell, 2003; Lisman and Grace, 2005). Interestingly, norepinephrine may exert similar effects (e.g., Hu et al., 2007). In hippocampal neurons, D1 receptor stimulation also increases GluR1 surface expression through a mechanism dependent on local protein synthesis (Smith et al., 2005). Finally, although this section focuses on AMPAR in cultured NAc MSN, it should be noted that DA receptors also exert rapid modulatory effects on NMDAR trafficking in the dorsal striatum (e.g., Dunah and Standaert, 2001; Hallett et al., 2006) and other brain regions (e.g., Gao and Wolf, 2008).

In studies designed to model the effect of repeated cocaine exposure, we found that repeated DA treatment of PFC/NAc co-cultures leads to a time-dependent and persistent upregulation of GluR1 and GluR2 surface expression by MSN that occludes subsequent synaptic scaling induced by activity blockade (Sun et al., 2008; Sun and Wolf, 2009). This could suggest impairment of synaptic scaling after drug withdrawal, although it should be emphasized that this occlusion was demonstrated 4 days after discontinuing repeated DA treatment and therefore, if extrapolated to animal models, is only relevant to very short withdrawal times. In fact, a temporary occlusion of synaptic scaling could help explain why withdrawal-dependent changes in AMPAR transmission take time to develop (Conrad et al., 2008). On the other hand, a different kind of homeostatic plasticity, in which MSN adjust their membrane excitability to functionally compensate for changes in the level of excitatory synaptic input, shows a prolonged impairment in the NAc after repeated cocaine exposure (Ishikawa et al., 2009).

Studies of AMPAR trafficking in NAc cultures are useful because trafficking mechanisms can be readily characterized, and this information can be used to guide hypotheses regarding in vivo plasticity. However, primary neuronal cultures have obvious limitations. They do not reproduce complex in vivo circuitry and they cannot be used to study long-term effects of drug exposure because cultured neurons begin to deteriorate soon after they develop extensive synaptic connections. Furthermore, the neurons in primary cultures are young neurons. This is a serious concern because plasticity mechanisms (McCutcheon and Marinelli, 2009) and the properties of AMPAR transmission in the NAc (Zhang and Warren, 2008; Kasanetz and Manzoni, 2009) both change during development. The same concern applies to the use of juvenile rodents in studies of cocaine-related plasticity.

2.4 Summary

AMPA subunit composition has been studied in the NAc of untreated adult rats (Boudreau et al., 2007; Reimers et al., submitted), cocaine-sensitized rats (Boudreau et al., 2007) and rats with saline or cocaine self-administration experience (Conrad et al., 2008). Our results suggest that AMPAR subunit composition in the NAc of untreated adult rats is similar to that of other brain regions, including hippocampus. GluR1/2 receptors play the major role, while the contribution of GluR2/3 receptors is uncertain. There is also a small population of GluR2-lacking AMPAR. However, GluR2-lacking AMPAR do not contribute significantly to synaptic transmission in the NAc core of saline-treated adult rats (~5% of synaptic currents; Conrad et al., 2008).

To understand AMPAR trafficking in the NAc, we have studied primary cultures of NAc neurons (Chao et al., 2002^{a,b}; Mangiavacchi and Wolf, 2004a,b; Sun et al., 2008; Sun and Wolf, 2009). We found that trafficking “rules” were similar to those described previously in hippocampal neurons. Furthermore, we found that DA receptor activation modulates both rapid AMPAR plasticity (activity-dependent synaptic insertion; Sun et al., 2008) and slowly developing homeostatic plasticity (synaptic scaling; Sun and Wolf, 2009) in cultured NAc neurons. Cocaine, which increases extracellular DA levels and thus promotes DA receptor stimulation, may tap into these mechanisms to alter synaptic plasticity in the intact brain. For example, the ability of brief DA receptor stimulation to facilitate AMPAR synaptic incorporation may help explain DA’s ability to facilitate learning about drugs and drug-related cues during repeated cocaine exposure (see Wolf, in press for more discussion) and may also explain some rapid responses to cocaine re-exposure (Section 6.5). Furthermore, results in cultured NAc neurons suggest that in vivo cocaine exposure may produce long-term alterations in AMPAR transmission at least in part by producing long-term changes in DA receptor signaling. However, this is a complicated issue (see Section 4.2 for a brief discussion).

There are many caveats associated with extrapolating from drug effects in neuronal cultures and young neurons to their in vivo effects in the adult brain. However, results obtained in these

preparations regarding fundamental properties of AMPAR transmission are valuable to the design and interpretation of the in vivo studies that are the focus of the remainder of this review. In the sections that follow, evidence will be presented that changes in AMPAR distribution and subunit composition play an important role in behavioral plasticity resulting from in vivo cocaine exposure.

3. Behavioral procedures considered in this review

3.1 Sensitization

The repeated administration of a psychostimulant drug like cocaine produces an enduring hypersensitivity to its psychomotor activating effects, a phenomenon known as psychomotor sensitization (Segal, 1975; Robinson and Becker, 1986). Psychomotor activity includes a variety of behaviors including locomotion and patterned, repetitive movements (stereotyped behaviors) such as head and limb movements, sniffing and rearing. These behaviors occur to varying degrees depending on a number of factors including the dose of drug administered and previous drug exposure (Post and Rose, 1976; Segal and Kuczenski, 1987; Crombag et al., 1999). Psychomotor sensitization refers to an increased sensitivity to the psychomotor activating effects of the drug that can manifest in a number of different ways depending on the dose of drug used, testing environment, time since last injection, and other factors. For example, the presence of psychomotor sensitization can be indicated by a progressive increase in locomotor activity in response to repeated injections of the same dose of drug, but can also be indicated by the emergence of stereotyped behaviors, or changes in their nature, intensity and timing (Segal and Kuczenski, 1987). Although stereotyped behaviors are most often associated with responding to moderate to high amphetamine doses, they also occur after repeated cocaine exposure (Post and Rose, 1976; Ferrario et al, 2005; Knackstedt and Kalivas, 2007) and are indicative of a stronger response to drug. As the intensity and frequency of stereotyped behaviors increases, locomotor activity may decrease, making it difficult to assess psychomotor sensitization by locomotor activity measures alone (see Robinson, 2009 for discussion).

Psychomotor sensitization is associated with a number of neurobehavioral adaptations implicated in addiction including sensitization of the incentive-motivational properties of drug and cues paired with drug (Robinson and Berridge, 1993;2001;2008). For example, rats that are pretreated with psychostimulant drugs resulting in psychomotor sensitization will subsequently acquire psychostimulant self-administration more readily (Piazza et al., 1989; Horger et al., 1990), work harder to obtain drug under a progressive ratio schedule of reinforcement (Mendrek et al., 1998; Lorrain et al., 2000), and more readily develop addiction-like behaviors such as escalation of drug intake (Ferrario and Robinson, 2007). Thus, the relatively simple psychomotor sensitization model is quite useful for studying plasticity relevant to addiction because neuroadaptations that accompany psychomotor sensitization overlap, at least in part, with those that underlie enhanced motivation for drug. Furthermore, psychomotor sensitization is very persistent, lasting for months up to one year after the last drug exposure (Paulson et al., 1991) and can be enhanced after drug-free periods (Robinson and Becker, 1987). Thus, neuroadaptations initiated by a sensitizing drug regimen may play a role in the persistent risk of relapse that marks addiction (Robinson and Berridge, 1993, Vanderschuren and Pierce, 2009).

In most studies of psychomotor sensitization, repeated injections of drug are given by the experimenter either in the home cage or a novel test environment (see Robinson and Berridge, 2001 and Badiani and Robinson, 2004 for discussion of differential effects of cocaine given in a home versus novel environment). This form of drug administration is referred to as “non-contingent” because drug administration is not dependent upon the animal’s behavior. However, psychomotor sensitization also develops in response to drug exposure that occurs in a contingent manner, i.e., as a result of drug self-administration (e.g., Hooks et al., 1994;

Phillips and Di Ciano, 1996; Zapata et al., 2003; Ferrario et al., 2005; Knackstedt and Kalivas, 2007).

3.2. Cocaine self-administration

In cocaine self-administration procedures, drug administration is dependent on the rat's response, such as a nose poke or lever press. Responding on one lever (active) results in intravenous drug delivery whereas responding on a second lever (inactive) has no consequences. Self-administration procedures differ widely in a variety of ways including: whether or not training to respond to obtain food proceeds drug self-administration, the dose of drug available, the duration of access to drug (see below), the number of responses required to obtain drug, whether or not cues are paired with drug delivery, or whether or not the number of attainable infusions is limited. Thus, depending on the parameters used, self-administration procedures can be useful for assessing the reinforcing and motivational properties of a drug or drug paired cues, and for studying neuroadaptations associated with drug taking and/or addiction-like behaviors. However, differences in self-administration experiments (especially total drug intake and experience with the self-administration procedure and drug-associated cues) make it complicated to integrate findings between labs. Below we will briefly describe a few self-administration models that are relevant to this review.

Relapse Models—Relapse to drug taking after a drug-free period is a common aspect of addiction in humans and is often instigated by stress or exposure to cues previously associated with the drug. In rodent models, the ability of a drug, stress, or previously drug-paired cues to instigate drug seeking behavior is often assessed with reinstatement procedures. In a typical reinstatement procedure, self-administration is initially established and then rats undergo extinction training. During extinction, responding on the previously active lever no longer results in drug delivery. Lack of drug availability during extinction training leads to a decrease (extinction) of the behavioral response (e.g., lever pressing) that originally delivered the drug. The renewal (reinstatement) of responding is then induced (primed) by one of the same stimuli that often triggers relapse in humans: a stressor (stress-induced reinstatement), a cue previously associated with drug (cue-induced reinstatement), or re-exposure to the drug itself (drug-primed reinstatement). Reinstatement in response to these different stimuli involves distinct but overlapping neuronal mechanisms and circuits (Kalivas and McFarland, 2003; Feltenstein and See, 2008). Importantly, drug is not available during reinstatement, but responding often results in a presentation of the previously drug-paired cue. Although this procedure does not directly mimic the human condition, it has provided insight into the neuroadaptations that underlie the ability of these stimuli to reinstate and maintain drug seeking behavior (Shaham et al 2000; Shaham et al 2003; Kalivas and McFarland, 2003; See, 2005; Schmidt et al., 2005). However, as discussed in Section 5.7, extinction training is a form of learning associated with its own effects on AMPAR transmission. Thus, the use of extinction training in some animal studies but not others may help to explain certain discrepancies in the AMPAR literature.

Forced abstinence (withdrawal)—In human addicts, drug taking is often terminated by hospitalization or incarceration, although it frequently resumes after the individual is released. In these forced abstinence situations, no extinction training takes place. For brevity, we will refer to forced abstinence as withdrawal (i.e., removal of drug). In rodent models of this nature (see Reichel and Bevins, 2009), animals are allowed to self-administer drug for a period of time and are then left undisturbed, usually in their home cages, for days to weeks with no drug available. The effect of withdrawal on drug seeking behavior can then be assessed by re-exposing the rats to the self-administration procedure without drug available (i.e., under extinction conditions) in the presence or absence of drug-paired cues. In this situation,

responding on the previously active lever provides a measure of drug seeking and possibly “craving” (Lu et al., 2004a).

An important finding is that, in rats, cue-induced cocaine seeking increases during the first weeks to months of withdrawal (Neisewander et al., 2000; Grimm et al., 2001; Lu et al., 2004a,b; Sorge and Stewart, 2005). This phenomenon is termed “incubation” of cocaine craving (Grimm et al., 2001). Incubation is relevant to the observation that vulnerability to cue-induced craving and relapse in human users may increase after a period of abstinence (Gawin and Kleber, 1986). In one well characterized incubation regimen (Lu et al., 2004a,b; Lu et al., 2005), rats are allowed to self-administer cocaine for 6 h/day for 10 days and each drug infusion is paired with a discrete cue. After different withdrawal periods (days to months), cue-induced cocaine seeking is assessed under extinction conditions: lever pressing results in presentation of the previously cocaine-paired cue, but no drug is available. Testing under extinction conditions after this cocaine regimen has revealed similar responding to the last self-administration session on withdrawal day (WD) 1, a progressive increase during the first month of withdrawal, high responding after 3 months, and intermediate responding after 6 months of withdrawal (Lu et al., 2004a,b). In rats trained for only 2 h/day, incubation is less robust, indicating that it is influenced by drug intake (Lu et al., 2004a; see also Sorge and Stewart, 2005). In contrast to cue-induced drug seeking, cocaine-primed seeking is not enhanced after withdrawal (Lu et al., 2004b). This indicates that the plasticity underlying incubation is related to learned associations between cocaine and a discrete cue paired with delivery of the drug. Incubation of cue-induced craving similarly occurs after withdrawal from other drugs of abuse (heroin, ethanol, and methamphetamine) and sucrose, making results in this model of potentially broad significance (Shalev et al., 2001; Lu et al., 2004a).

Shaham, Hope, Lu and colleagues have identified a number of neuroadaptations in several brain regions that are associated with and help to mediate the incubation of cocaine craving (Grimm et al., 2003; Lu et al., 2004c; 2005; 2007a; 2009a; Koya et al., 2009). In Section 5.3, we describe our findings on the role of NAc AMPAR transmission in the incubation of cocaine craving model, and briefly discuss integration of the AMPAR results with these other reports.

Limited access versus Extended Access—Self-administration experiments can be subdivided in terms of duration of access to drug. In limited access procedures, drug is generally available for 1–3 h per session for a week or two. Extended access procedures utilize longer sessions (e.g., 6 h per day) or a greater number of short sessions. Rats given extended access to self-administration exhibit a number of behaviors that are enhanced relative to animals given limited access to the drug. For example, extended access self-administration is associated with an escalation in cocaine intake (Ahmed and Koob, 1998), increased motivation for cocaine as assessed by break point (Paterson and Markou, 2003; Deroche-Gamonet et al., 2004), continued pursuit of cocaine despite adverse consequences (Vanderschuren and Everitt, 2004) and enhanced drug-induced and cue-induced reinstatement (Ahmed and Cador, 2006; Kippin et al., 2006) compared to limited access procedures. Thus, extended access self-administration procedures may more closely model the compulsive drug taking characteristic of addiction, whereas limited access procedures appear to more accurately model drug taking behavior. Of course, not all extended access regimens produce the same behavioral changes due to variations between procedures.

3.3 Role of sensitization in drug self-administration experiments

As noted in Section 3.1, it is clear that psychomotor sensitization develops during cocaine self-administration. However, the degree to which it is evident during or after a particular regimen depends on how it is measured (for example, assessing both stereotypy and locomotion may be required; Ferrario et al., 2005) as well as many other aspects of drug exposure (see

Knackstedt and Kalivas, 2007 for discussion). Although the significance of psychomotor sensitization is a controversial topic (Robinson and Berridge, 2008; Vanderschuren and Pierce, 2009), there are some important areas of agreement: psychomotor sensitization develops rapidly (during the first few drug exposures), it is very persistent (lasting up to one year after some regimens), and it may be augmented after a prolonged drug free period. Thus, neuroadaptations that accompany psychomotor sensitization may have at least two important roles in addiction. First, they may contribute to initial learning about drugs and drug-related cues during the induction of sensitization; and, if rats are sensitized with non-contingent injections and subsequently trained to self-administer drug, prior sensitization may enhance learning about drug self-administration and thus explain faster acquisition of drug self-administration in “pre-sensitized” rodents (Piazza et al., 1989; Horgers et al., 1990; Section 3.1). Second, these adaptations may contribute significantly to the persistent vulnerability to relapse that continues even after a drug-free period.

In the sections below, evidence will be presented that changes in AMPAR distribution and subunit composition play an important role in behavioral plasticity resulting from in vivo cocaine exposure. Section 4 will consider sensitization; unless otherwise specified, the term “cocaine-sensitized” will be used to refer to rats showing sensitized cocaine-induced locomotor activity after repeated cocaine treatment. Section 5 will consider cocaine self-administration models.

4. AMPAR adaptations accompanying sensitization to cocaine

4.1 AMPAR surface and synaptic expression in the NAc after withdrawal from a sensitizing regimen of cocaine

To test the hypothesis that AMPAR trafficking is associated with locomotor sensitization, we developed a protein crosslinking assay that enables the detection of changes in cell surface and intracellular protein pools resulting from in vivo treatments. This assay uses BS³, a bi-functional crosslinking reagent that does not cross cell membranes and therefore crosslinks cell surface proteins into high molecular weight aggregates, whereas intracellular proteins are unmodified. Surface and intracellular glutamate receptor pools can then be separated using SDS-PAGE and quantified by immunoblotting. The BS³ assay is a useful tool because it assesses the functional pool of AMPAR on the cell surface, whereas analysis of non-crosslinked homogenates measures AMPAR protein independent of its location in the cell.

Our initial study using this assay found that GluR1 and GluR2/3 antibodies detected increased cell surface expression of AMPAR in the NAc (combined core/shell dissection) of adult rats that developed locomotor sensitization in response to repeated cocaine injections, whereas this was not observed in cocaine-exposed rats that failed to sensitize (see Section 4.3 for more discussion of the relationship between AMPAR upregulation and locomotor sensitization). Compared to saline controls, AMPAR surface expression in the NAc of cocaine-sensitized rats was unchanged on withdrawal day (WD) 1 but significantly increased on WD21 (Boudreau and Wolf, 2005). Our subsequent studies examined other withdrawal times and found increased GluR1 surface expression on WD7 and WD14 (Boudreau et al., 2007; 2009; Ferrario et al., 2010). Together, these results indicate that AMPAR surface expression increases sometime during the first week of withdrawal and then persists at least through WD21. Consistent with these biochemical results, cocaine-sensitized rats tested after 2-3 weeks of withdrawal showed a greater locomotor response to infusion of AMPA into either NAc core or shell (Pierce et al., 1996) or medial core (Bell and Kalivas, 1996; Bachtell and Self, 2008).

In some of our studies, we used BS³ crosslinking to analyze surface expression of GluR2 and GluR3 as well as GluR1 in the NAc of cocaine-sensitized rats. Our results indicated that GluR1/2-containing AMPAR are the major AMPAR population showing increased surface

expression on WD14-21 (Boudreau and Wolf, 2005; Ferrario et al., 2010) but it is possible that GluR2-lacking AMPAR contribute significantly at longer withdrawal times (Mameli et al., 2009; see also Boudreau et al., 2007 and Section 4.5). An important question is whether the increase in surface AMPAR is due to their redistribution from intracellular pools or to new protein synthesis. Further analysis of intracellular and total GluR1 levels indicated that both mechanisms could potentially be contributing (Boudreau et al., 2007).

A limitation of the protein crosslinking assay is that it does not distinguish synaptic and extrasynaptic receptors (although some support for a synaptic location of new surface AMPAR was provided in Boudreau and Wolf, 2005). However, electrophysiological studies demonstrated a 40% increase in the AMPA/NMDA ratio at excitatory synapses in the NAc shell of cocaine-sensitized mice on WD10-14 (Kourrich et al., 2007). Changes in the AMPA/NMDA ratio can be due to changes in the function or number of either AMPAR or NMDAR. Kourrich et al. (2007) presented evidence that the increased AMPA/NMDA ratio on WD10-14 was attributable at least in part to increased AMPAR function or number, whereas cocaine-induced changes in NMDAR function were not detected at synapses containing both AMPAR and NMDAR. Thus it is likely that the increased AMPA/NMDA ratio after cocaine withdrawal is attributable to the increased AMPAR surface expression detected with protein crosslinking. It should be noted that the electrophysiological studies were conducted in shell and the protein crosslinking studies were conducted with a combined core/shell dissection. It will be important in the future to directly compare core and shell regions using both approaches. Furthermore, with respect to the AMPA/NMDA ratio, other contributing factors cannot be ruled out, such as additional changes that would strengthen AMPAR function (e.g., phosphorylation; Shepherd and Huganir, 2007; Derkach et al., 2007).

Other biochemical studies provide further support for a withdrawal-dependent increase in synaptic AMPAR levels in the NAc of cocaine-sensitized rats. Ghasemzadeh et al. (2009a) found increased GluR1 and GluR2 levels in synaptosomal membrane fractions from the NAc of sensitized rats on WD21 but not WD1; the effect was present in both core and shell. Schumann and Yaka (2009) similarly found increased GluR1 in a NAc synaptosomal membrane fraction (combined core/shell dissection) on WD21. They did not assess GluR2. Whereas both GluR1 and GluR2 were increased in studies of AMPAR cell surface or synaptosomal membrane levels (above), two studies measuring total tissue levels found increased GluR1 but not GluR2 or GluR2/3 immunoreactivity after 1-3 weeks, but not 1 day, of withdrawal (Churchill et al., 1999; Scheggi et al., 2002). Together, the biochemical and electrophysiological results indicate that AMPAR upregulation in sensitized rats probably occurs in both core and shell and that GluR1/2 containing AMPAR are most affected.

Interestingly, in addition to elevated AMPA/NMDA ratios, Kourrich et al. (2007) also observed an increase in the frequency of miniature AMPAR EPSCs in the NAc of sensitized mice on WD10-14. This suggests a presynaptic alteration (i.e., an increase in release probability). However, this potential change was not confirmed by a different presynaptic measure, that is, the paired-pulse ratio did not differ between saline and cocaine groups. Alternatively, the increase in frequency may reflect generation of new synaptic connections, potentially mediated by the increase in the number of dendritic spines observed in cocaine-sensitized animals, although this remains to be directly tested (for details regarding the populations of dendritic spines affected by cocaine, see Robinson and Kolb, 2004; Lee et al., 2006; Shen et al., 2009).

Whereas the AMPA/NMDA ratio in the NAc shell was increased on WD10-14 (Kourrich et al., 2007), it was decreased below control levels on WD1 (Kourrich et al., 2007; Mameli et al., 2009) and this occluded the induction of low frequency stimulation-induced LTD (Mameli et al., 2009). Together with our protein crosslinking studies which demonstrated increased AMPAR surface expression on WD7-21 but not on WD1 (above), this clearly indicates that a

period of withdrawal is required in order for increased AMPAR transmission to develop. However, why was AMPAR surface expression unchanged on WD1 whereas the AMPA/NMDA ratio in the NAc shell was decreased? This could reflect the fact that AMPAR surface expression was measured in a combined core/shell dissection, as well as differences in species, age or sensitivity of techniques. However, another possibility is that the decreased AMPA/NMDA ratio on WD1 is due in part to increased NMDAR function or number. As noted by Thomas et al. (2001) in the first study on AMPA/NMDA ratios in cocaine-sensitized mice, the AMPA/NMDA ratio assesses NMDAR at synapses that contain both AMPAR and NMDAR, so the possibility of a change in NMDAR transmission at silent synapses, which contain NMDAR but not AMPAR, remains open. Indeed, a recent study found that silent synapses are generated in the NAc during repeated cocaine treatment (peak increase after the third injection) and are significantly elevated on WD1-2, although they dissipate after further withdrawal such that only small non-significant increases are detected on WD7 and WD14 (Huang et al., 2009). The generation of silent synapses by cocaine injections was demonstrated in both P30-32 and adult rats (~P65). Thus, enhanced NMDAR transmission, due to new silent synapses, may contribute to the decreased AMPA/NMDA ratio on WD1. Another interesting idea is that the creation of silent synapses enhances the capacity of NAc synapses to undergo plasticity and recruit AMPAR (Huang et al., 2009). Perhaps AMPAR upregulation after 1-3 weeks of withdrawal (Boudreau and Wolf, 2005; Boudreau et al., 2007, 2009; Ferrario et al., 2010; Kourrich et al., 2007) involves AMPAR addition to these silent synapses; this would explain the decline of silent synapses during long-term withdrawal (Huang et al., 2009). For discussion of other studies showing altered NMDAR expression in the NAc of cocaine-sensitized rats, see Ferrario et al. (2010).

In addition to withdrawal-dependent changes, AMPAR transmission in cocaine-sensitized rodents is altered by acute re-exposure to cocaine. Thus, while AMPAR surface expression and the AMPA/NMDA ratio are increased on WD7-21 in the NAc of sensitized rodents, administering a cocaine challenge on WD10-14 leads to a decrease in these measures 24 h later (Thomas et al., 2001; Boudreau et al., 2007; Kourrich et al., 2007; Ferrario et al., 2010). This is discussed further in Section 6.1.

4.2 What mechanisms produce AMPAR upregulation after withdrawal from a sensitizing cocaine regimen?

A clear answer to this question does not exist. However, positive correlations have been found between GluR1 surface/intracellular ratios and mitogen-activated protein kinase (MAPK) signaling in the NAc of sensitized rats (Boudreau et al., 2007; Boudreau et al., 2009; see also Schumann and Yaka, 2009). This is intriguing since MAPK activity has been implicated in AMPAR synaptic insertion during LTP (Zhu et al., 2002). Withdrawal-dependent changes in CaMKII and PKA signaling pathways may also contribute (Boudreau et al., 2009). However, all of our results are based on correlations and do not prove a causal relationship.

A broader issue is whether the AMPAR upregulation occurring in the NAc of sensitized rats is produced by mechanisms similar to LTP. This would imply heightened activity of excitatory pathways to the NAc during early withdrawal (which has not been tested). However, AMPAR upregulation appears to occur in most or all MSN, based on electrophysiological observations (Dr. Mark Thomas, personal communication) and the ability to demonstrate robust biochemical changes in tissue homogenates (e.g., Boudreau and Wolf, 2005). The NAc receives glutamate inputs from multiple brain regions, so if LTP-like mechanisms are responsible for AMPAR upregulation, inputs that synapse on most or all MSN must be involved. Furthermore, if it is LTP, the potentiation must persist for weeks.

Another possibility, suggested previously (Boudreau and Wolf, 2005; Boudreau et al., 2007), is that AMPAR upregulation represents synaptic scaling, a form of homeostatic plasticity in

which decreased activity of excitatory inputs leads to the accumulation of AMPAR at excitatory synapses (Section 2.3). Synaptic scaling could be triggered by cocaine-induced hypoactivity of cortical regions that send glutamate projections to the NAc, although it should be noted that the evidence for cocaine-induced cortical hypoactivity comes primarily from studies in which cocaine was self-administered (Porrino et al., 2007; see Sun and Wolf, 2009 for more discussion). Alternatively, a homeostatic increase in postsynaptic AMPAR levels could occur in response to the cocaine-induced decrease in the intrinsic excitability of MSN (Section 2.1). It is not clear whether the cocaine-induced decrease in extracellular glutamate levels that has been found using microdialysis (Section 2.1) represents another potential trigger for AMPAR upregulation. Decreasing extracellular glutamate levels could have the paradoxical effect of increasing synaptic glutamate transmission in the NAc, due to decreased glutamate tone on mGluR2/3 receptors that inhibit synaptic glutamate release (Moran et al., 2005; Kalivas, 2009). This might oppose compensatory increases in postsynaptic AMPAR levels, although this opposing effect would be less significant if afferent inputs to the NAc are hypoactive during cocaine withdrawal (see above). However, in this case, the decreased afferent activity, not the decreased extracellular glutamate levels, would represent the trigger for scaling up of postsynaptic AMPAR.

As described in Section 2.3, acute D1 receptor stimulation rapidly increases AMPAR surface expression and facilitates AMPAR synaptic incorporation in cultured NAc neurons, while repeated DA treatment produces more persistent AMPAR upregulation. This raises the possibility that a similar postsynaptic interaction between D1 and AMPA receptors could explain AMPAR upregulation in the NAc of cocaine-sensitized rats. However, this presumes that DA transmission in the NAc is elevated (via presynaptic or postsynaptic mechanisms or both) during the first week of withdrawal (the time when AMPAR upregulation occurs; Section 4.1). Indeed, there are reports of supersensitive D1 receptor responses in the NAc of cocaine-sensitized rats, but the endpoint studied was enhanced ability of D1 receptor stimulation to inhibit MSN activity driven by AMPAR EPSC in brain slices (Beurrier and Malenka, 2002; recordings 24 h after cocaine challenge) or iontophoretic glutamate application in vivo (Henry and White, 1991; 1995; D1 receptor supersensitivity demonstrated on WD1, WD7 and WD30). The former effect was linked to enhanced responsiveness of presynaptic D1 receptors that inhibit glutamate release (Beurrier and Malenka, 2002), so it is not relevant to the postsynaptic mechanism in question. With respect to the latter studies (Henry and White, 1991; 1995), the mechanism underlying D1 receptor supersensitivity is unclear (see Pierce and Kalivas, 1997) and it could simply reflect decreased intrinsic excitability of MSN. On the presynaptic side, it is well known that the DA efflux elicited by a challenge injection of a psychostimulant drug is augmented after 2-3 weeks of withdrawal from repeated cocaine injections, but different studies have reported increases, decreases or no change in basal DA levels in the NAc during the first few days after discontinuing cocaine injections (Chefer and Shippenberg, 2002 and references therein). Another concern is that cocaine and amphetamine have many common effects on DA transmission, but exert very different effects on AMPAR levels (Section 8). A comprehensive consideration of the role of DA/glutamate interactions in mediating cocaine-induced neuroadaptations is beyond the scope of this review.

Section 5.3 describes evidence for addition of GluR2-lacking AMPAR to NAc synapses after prolonged withdrawal from extended access cocaine self-administration. In some respects, possibilities related to mechanisms underlying this effect are similar to those discussed above for AMPAR upregulation after experimenter administered cocaine, since both contingent and non-contingent cocaine exposure are associated with decreased extracellular glutamate levels and decreased intrinsic excitability of MSN (Section 2.1). However, the duration of decreased intrinsic excitability is shorter after contingent cocaine exposure (Mu et al., 2010). Furthermore, there are expected to be differences in the activity of afferent pathways to MSN following contingent versus non-contingent cocaine (Jacobs et al., 2003). At the synaptic level,

additional differences may exist that are related to the type of AMPAR that undergoes upregulation after contingent versus non-contingent cocaine administration (Section 4.5). Indeed, molecular mechanisms have been identified that selectively regulate the access of GluR2-lacking AMPAR to synapses or regulate their exchange with GluR2-containing AMPAR (e.g., Liu and Cull-Candy, 2005; Gardner et al., 2005; Guire et al., 2008; He et al., 2009).

Ultimately, elucidating the mechanisms that lead to AMPAR upregulation will require an understanding of time-dependent changes during cocaine withdrawal in the level of activity of glutamate and DA afferents that synapse on NAc neurons. Clearly this is an area that requires further investigation.

4.3 Is AMPAR upregulation in the NAc required for sensitization of cocaine's locomotor activating effects?

Multiple lines of evidence indicate that locomotor sensitization and AMPAR upregulation are closely associated after 2-3 weeks of withdrawal. Using a regimen that produces locomotor sensitization in ~40% of the rats, we found that those rats that developed locomotor sensitization exhibited significantly increased GluR1 and GluR2/3 surface/intracellular ratios on WD21, whereas rats that failed to sensitize did not (Boudreau and Wolf, 2005; combined core/shell dissection). Furthermore, the magnitude of locomotor sensitization was positively correlated with the GluR1 and GluR2/3 surface/intracellular ratios (Boudreau and Wolf, 2005; Boudreau et al., 2009). Similarly, rats that developed locomotor sensitization, but not those that failed to sensitize, showed an enhanced locomotor response to AMPA infusion into the NAc after 2-3 weeks of withdrawal (Pierce et al., 1996, core and shell; Bell and Kalivas, 1996, medial core), likely due to the increase in AMPAR surface expression. In more recent studies, we used a regimen that produces locomotor sensitization in all rats and found a statistically significant increase in GluR1 and GluR2 surface/intracellular ratios in the total population of cocaine-treated rats on WD14 (Ferrario et al., 2010; combined core/shell dissection).

While these results show a close association between AMPAR upregulation and locomotor sensitization, they do not address the question of whether AMPAR upregulation is responsible for sensitized locomotor activating properties of cocaine. Addressing causality requires distinguishing between two distinct questions: 1) Is NAc AMPAR transmission required for the expression of locomotor sensitization? 2) Is enhanced NAc AMPAR transmission required for the expression of locomotor sensitization? With regard to the first question, given that AMPAR constitute the major excitatory driving force to NAc neurons (Section 1), it is likely that any NAc-mediated behavior (including expression of locomotor sensitization) would ultimately be impaired by sufficient blockade of AMPAR transmission. In fact, a landmark study (Pierce et al., 1996) found that injection of the AMPAR antagonist CNQX into the NAc core (shell was not tested) prior to cocaine challenge on WD21 nearly eliminated the "sensitized" portion of the locomotor response in sensitized rats, but did not alter cocaine-induced locomotor activity in non-sensitized rats. Infusion of CNQX into the NAc core also blocked expression of locomotor sensitization elicited with a regimen in which all cocaine injections were paired with the test environment (Bell et al., 2000). These results show that AMPAR transmission is required for the expression of locomotor sensitization. They leave open the question of whether even higher doses of CNQX, leading to more complete or more widespread AMPAR blockade, would inhibit cocaine-induced locomotor activity in previously drug-naïve rats.

A distinct issue (question 2) is whether the expression of locomotor sensitization requires an increase in postsynaptic AMPAR levels. Several lines of evidence argue against a simple relationship between these phenomena:

1. In contrast to results described above, we did not observe a decrease in the expression of locomotor sensitization when the same CNQX doses were infused into the NAc core prior to a challenge injection of cocaine (Ferrario et al., 2010). CNQX is a competitive antagonist, so its efficacy might be influenced by procedural differences that alter the level of cocaine-induced glutamate release in the NAc. Other differences between the studies (e.g., withdrawal time) may also contribute to the discrepancy (see Ferrario et al., 2010 for more discussion) and it is notable that none of the CNQX studies targeted the shell (but see Todtenkopf et al., 2002). Nevertheless, given that CNQX certainly produced some reduction of AMPAR occupancy, our results suggest that one can reduce AMPAR occupancy in the core without affecting the magnitude of the sensitized locomotor response.
2. Along the same lines, two studies have shown that decreasing the surface expression of endogenous AMPAR in the NAc does not reduce the expression of locomotor sensitization. Both studies took advantage of the ability of a challenge injection of cocaine to decrease NAc AMPAR surface expression 24 h later (see Section 6.1 for more discussion). We showed that administering a challenge injection of cocaine on WD14, resulting in decreased AMPAR surface expression 24 h later on WD15, did not alter the locomotor response to a second cocaine challenge on WD15 (Ferrario et al., 2010). Similarly, Bachtell and Self (2008) challenged cocaine-sensitized rats with cocaine and then, 24 h later, measured the locomotor response to infusion of AMPA into the medial core or the locomotor response to a second cocaine challenge. The response to AMPA was reduced 24 h after the initial cocaine exposure, consistent with decreased AMPAR surface expression. In contrast, the locomotor response to cocaine was actually enhanced compared to the first cocaine challenge 24 h earlier. Taken together, these data suggest that although cocaine challenge after withdrawal from a sensitizing cocaine regimen does decrease AMPAR surface expression (probably in both core and shell), this decrease does not prevent cocaine from eliciting a sensitized locomotor response.
3. A temporal dissociation exists between the development of locomotor sensitization (which occurs over the course of repeated cocaine injections, i.e., prior to WD1) and the development of increased AMPAR surface expression (which is not yet detected on WD1) (Boudreau and Wolf, 2005). Other biochemical studies have similarly observed no change in NAc AMPAR expression on WD1 in NAc core or shell (Fitzgerald et al., 1996; Churchill et al., 1999; Ghasemzadeh et al., 2009a), while the AMPA/NMDA ratio is decreased in the shell on WD1 (Kourrich et al., 2007; Mameli et al., 2009; see Section 4.1 for more discussion). These results show that locomotor sensitization can be expressed prior to AMPAR upregulation. They do not rule out the possibility that AMPAR upregulation underlies locomotor sensitization at longer withdrawal times, although results outlined in (1) and (2) above suggest that reducing AMPAR transmission at these withdrawal times does not necessarily reduce the expression of locomotor sensitization.
4. Self and colleagues explored the effect of transient viral-mediated over-expression of wild-type GluR1, a pore-dead GluR1 mutant, or a control vector in the NAc core during withdrawal from a sensitizing regimen of cocaine (Bachtell et al., 2008). The pore-dead GluR1 contains a single point mutation in the pore region and therefore reduces AMPAR currents. All groups were challenged with cocaine on WD7 and all showed locomotor sensitization relative to treatment day 1. However, the magnitude of sensitization was smaller in rats over-expressing wild-type GluR1 as compared to controls, and greater in those over-expressing pore-dead GluR1. These results are complicated to interpret because effects of GluR1 over-expression are being superimposed on endogenous AMPAR plasticity during withdrawal. Nevertheless,

these results do not support the idea that AMPAR upregulation underlies locomotor sensitization. Furthermore, in one of their control studies, Bachtell et al. (2008) over-expressed wild-type GluR1, pore-dead GluR1, or control vector in the NAc core of naïve rats. The wild-type GluR1 group might be expected to mimic AMPAR upregulation after cocaine withdrawal and thus create a “pre-sensitized state”, yet the three groups showed no difference in locomotor activity when injected with cocaine for the first time. Thus, there is not a simple relationship between AMPAR upregulation and the expression of locomotor sensitization. It should be noted that only core was evaluated in the studies described here, although both core and shell were studied in related experiments in which over-expression occurred during cocaine treatment (Section 4.6). See Section 5.6 for more discussion of the consequences of viral-mediated over-expression of GluR1 and caveats associated with this approach.

4.4 Does AMPAR upregulation in the NAc contribute to sensitization of cocaine’s incentive motivational properties?

As mentioned in Section 3.1, the sensitization produced by repeated exposure to experimenter-administered and self-administered cocaine has multiple facets. Sensitization of drug-induced locomotion or stereotypy is commonly measured, but sensitization also occurs to the incentive motivational properties of drugs and drug-paired cues. For example, prior treatment with psychostimulant drugs resulting in psychomotor sensitization enhances the subsequent motivation to obtain drug in self-administration experiments (Vezina, 2004). As summarized in Section 3.3, it is reasonable to propose that incentive sensitization contributes to subsequent learning about drugs and drug-related cues, and to the vulnerability to cue-induced relapse that persists long after drug use is discontinued. We suggest that these effects – and in particular, the vulnerability to cue-induced relapse – are related to the AMPAR upregulation that we have described after 1-3 weeks of withdrawal from cocaine regimens that produce psychomotor sensitization (Boudreau and Wolf, 2005; Boudreau et al., 2007, 2009; Ferrario et al., 2010). While direct tests of this hypothesis are lacking, it has two types of indirect support. First, all studies conducted in intact animals are consistent with a positive relationship between the level of AMPAR transmission in the NAc and motivation to seek cocaine (Suto et al., 2004; Conrad et al., 2008; Anderson et al., 2008), although different conclusions have been drawn from studies of cocaine self-administration in animals with deletion or over-expression of GluR1 (Sutton et al., 2003; Mead et al., 2007; Bachtell et al., 2008; see Section 5.6). Second, it has been convincingly demonstrated, in a variety of models, that cocaine seeking depends on glutamate release, from PFC or limbic afferents to the NAc, leading to activation of AMPAR on NAc neurons (Cornish and Kalivas, 2000; Vorel et al., 2001; Di Ciano and Everitt, 2001, 2004; Park et al., 2002; McFarland et al., 2003; Hayes et al., 2003; Ito et al., 2004; Di Ciano et al., 2007; Bäckström and Hyytiä, 2007; Conrad et al., 2008; Famous et al., 2008; Ping et al., 2008; Suto et al., 2009; Sari et al., 2009). Thus, it makes sense that cocaine seeking would be enhanced by AMPAR upregulation on NAc neurons.

In their original formulation of the incentive sensitization hypothesis, Robinson and Berridge (1993) suggested that common neuroadaptations (in the mesocorticolimbic DA system and others) enable sensitization of cocaine’s motor activating and incentive-motivational effects. Indeed, recent work has provided evidence for overlapping mechanisms, both dopaminergic and non-dopaminergic (Vezina et al., 2002; Suto et al., 2004; Kim et al., 2005; Taylor et al., 2007; Loweth et al., 2010). However, it is important to keep in mind that while locomotor sensitization is indicative of the presence of neuroadaptations associated with enhanced motivation for drug, the neuroadaptations underlying these motivational changes need not be identical to those underlying expression of the sensitized locomotor response. The evidence presented in the previous paragraph and in Section 4.3 suggests that the level of postsynaptic AMPAR transmission in the NAc may be directly involved in setting the “gain” on incentive

motivation and less directly involved in determining the magnitude of locomotor sensitization. This idea receives some support from a study comparing cocaine-induced behaviors in wild-type and GluR1 knockout mice (Dong et al., 2004). Although the knockout mice developed locomotor sensitization, they failed to exhibit a conditioned locomotor response when placed in a context previously paired with a single cocaine injection, and they did not exhibit conditioned place preference (CPP) to cocaine, leading the authors to conclude that they have deficits related to “the attribution of incentive value to drug-associated cues” (Dong et al., 2004). A subsequent study showed that the same GluR1 knockout mice did develop CPP when a stronger pairing protocol was used (Mead et al., 2005), which may suggest that GluR1 contributes to but is not essential for the development of CPP. When interpreting these studies, it must be recalled that GluR1 was deleted throughout development, raising the possibility of compensation, and that GluR1 was deleted throughout the brain, raising the possibility of offsetting or interacting effects in different brain regions.

4.5. What type of AMPAR upregulates in the NAc of sensitized rats?

At withdrawal times of 1–3 weeks, biochemical results summarized in Section 4.1 indicate that GluR1/2-containing AMPAR are the major population that exhibits increased surface expression in the NAc (combined core/shell dissection) of cocaine-sensitized rats. Likewise, electrophysiological results suggest that GluR2-containing AMPAR are responsible for the increased AMPA/NMDA ratio observed in the NAc shell of sensitized mice on WD10–14 (Kourrich et al., 2007). However, some of our data indicated the possibility of a very minor role for GluR2-lacking AMPAR on WD14–21 (Boudreau et al., 2007). More recently, Mameli et al. (2009) found inwardly rectifying AMPAR ESPC, the electrophysiological signature of GluR2-lacking AMPAR, in the NAc shell of mice after more prolonged withdrawal (WD35) from 10 daily i.p. cocaine injections. In the same study, they found GluR2-lacking AMPAR in the NAc shell on WD35 after extended access cocaine self-administration. We had previously reported GluR2-lacking AMPAR in the NAc core after prolonged withdrawal from extended access cocaine self-administration in rats (Conrad et al., 2008; see Section 5.3).

Together, these results could suggest a two-stage process for strengthening excitatory synapses in the NAc of cocaine-sensitized rats: perhaps during the first three weeks of withdrawal this is accomplished primarily by the addition of GluR1/2-containing AMPAR (Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007; Ferrario et al., 2010), whereas, after more protracted withdrawal, GluR2-lacking AMPAR are added to NAc synapses (Mameli et al., 2009). Furthermore, results of Mameli et al. (2009) may suggest that non-contingent and contingent cocaine self-administration ultimately have the same effect (synaptic incorporation of GluR2-lacking AMPAR) provided that sufficiently long withdrawal times are examined. This degree of similarity would not necessarily have been predicted, because neurotransmission in addiction-related neuronal circuits is differently engaged by contingent versus non-contingent cocaine administration (Jacobs et al., 2003). Greater cocaine exposure in contingent compared to non-contingent studies, and a different route of administration, are other potentially significant variables.

The possible interpretations outlined above rest on combining results obtained in juvenile and adult rodents. For sensitization studies, Kourrich et al. (2007) and Mameli et al. (2009) administered cocaine to juvenile mice (P24–28 and P16–35, respectively), whereas we began with adult rats (P60–70). For studies of long withdrawal from extended access cocaine self-administration, adult animals were used (Conrad et al., 2008, P60–70 rats; Mameli et al., 2009, 7–8 month-old mice). As discussed in Section 2.2, GluR2-lacking AMPAR are relatively abundant in the young brain, e.g., in MSN in primary NAc cultures prepared from P1 rats (Sun and Wolf, 2009), whereas they are a minority population in the adult rat NAc. It will be important to determine if a sensitizing cocaine regimen administered during adulthood leads

to the formation of GluR2-lacking AMPAR after protracted withdrawal, whether this occurs in rats, and whether there are core-shell differences.

4.6 Effects of over-expressing AMPAR subunits during repeated cocaine treatment

Prior sections focused on alterations in AMPAR levels in the NAc after cocaine withdrawal. Bachtell et al. (2008) tested the effects of over-expressing wild-type or pore-dead GluR1 in the NAc core or shell during repeated cocaine treatment. Locomotor sensitization was assessed 7 days later. In the core, wild-type GluR1 had no effect on initial sensitivity to cocaine (injection day 1) but decreased the magnitude of locomotor sensitization detected in response to cocaine challenge on WD7. Pore-dead GluR1 increased the initial response to cocaine and, while the locomotor response on the WD7 test was higher than in control rats (HSV-LacZ injected), the proportional increase between injection day 1 and WD7 was similar in both groups. In the shell, wild-type GluR1 had no effect on initial sensitivity to cocaine but prevented significant sensitization, while pore-dead GluR1 had the same enhancing effect as found in the core. These findings show that interfering with endogenous AMPAR transmission in the NAc during cocaine exposure can alter subsequent plasticity, but they do not address issues related to the significance of AMPAR plasticity after withdrawal from a sensitizing drug regimen. See Section 5.6 for more discussion of the consequences of GluR1 over-expression and caveats associated with this approach.

4.7 Summary

AMPA surface expression in the rat NAc increases during the first week of withdrawal from a sensitizing regimen of cocaine and remains elevated for at least 21 days (Boudreau and Wolf, 2005; Boudreau et al., 2007, 2009; Ferrario et al., 2010; combined core/shell dissection). The AMPA/NMDA ratio in the mouse NAc shell is also increased on WD10-14 (Kourrich et al., 2007). If the biochemical and electrophysiological results are reflecting the same phenomenon, it suggests that at least a portion of the additional surface AMPARs are located at synapses and that AMPAR upregulation probably occurs in both core and shell. Interestingly, AMPAR upregulation appears to occur in most or all MSN (Section 4.2). GluR1/2-containing receptors are responsible for most or all AMPAR upregulation during the first 3 weeks of withdrawal (Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007), although a recent study found GluR2-lacking AMPAR in the NAc shell of sensitized mice on WD35 (Mameli et al., 2009). Further studies are required to understand the contributions of different AMPAR subtypes in sensitized rodents.

The functional significance of increased AMPAR surface expression in the NAc of sensitized rodents remains to be determined. While many results suggest a positive correlation between AMPAR upregulation and expression of locomotor sensitization, the two phenomenon can be dissociated (Boudreau and Wolf, 2005; Kourrich et al., 2007; Bachtell and Self, 2008; Bachtell et al., 2008; Ferrario et al., 2010). We hypothesize that enhanced AMPAR surface expression is more directly related to the ability of cocaine pretreatment to produce enhanced motivation for drug and drug-paired cues. For example, it is well known that non-contingent cocaine treatment leading to psychomotor sensitization subsequently enhances drug taking and drug seeking behavior. This can be described as sensitization of cocaine's incentive motivational properties. We suggest that the AMPAR upregulation accompanying locomotor sensitization increases the responsiveness of MSN to glutamate inputs. This in turn may facilitate learning about drugs and drug-paired cues during subsequent training in contingent models such that motivation for both is increased. In addition, it may produce a long-lasting increase in the responsiveness of MSN to relapse-inducing activation of glutamate inputs during abstinence. Of course, it is very unlikely that a single neuronal adaptation, such as enhanced AMPAR surface expression, is responsible for the complex changes in glutamate transmission observed in the NAc of sensitized rats. For example, locomotor sensitization is also accompanied by

altered presynaptic glutamate transmission and decreased intrinsic excitability of MSNs (Section 1.2) as well as changes in DA transmission (Section 4.2). Understanding inter-relationships between these changes and the AMPAR adaptations reviewed here is an important challenge for the future.

5. AMPAR adaptations produced by cocaine self-administration

5.1 Introduction

Many studies have found that cocaine seeking is blocked by intra-NAc infusion of AMPAR antagonists (Section 1.1). This indicates that cocaine seeking requires AMPAR transmission and suggests that enhancing AMPAR transmission could facilitate cocaine seeking in animal models of addiction. This enhancement of AMPAR transmission could occur through presynaptic and/or postsynaptic mechanisms. This section will focus on evidence for postsynaptic mechanisms involving regulation of AMPAR levels. However, if presynaptic glutamate afferents are sufficiently activated by a cue or priming injection of cocaine, sufficient AMPAR activation might be achieved regardless of whether AMPAR levels are up or down.

As described in Section 3.2, cocaine self-administration experiments can vary in many ways including the dose of drug available, whether access to self-administration is limited (e.g., 1-3 hr/day) or extended (e.g., 4-6 hr/day) and whether self-administration training is followed by extinction training or withdrawal. In the sections that follow, we will consider how these variables may interact to explain observed changes in NAc AMPAR levels after cocaine self-administration.

5.2 AMPAR adaptations after limited access cocaine self-administration

The vast majority of studies cited in Section 1.1, supporting a dependence of drug seeking on AMPAR transmission in the NAc, involved limited access cocaine self-administration, but none of these studies directly measured surface or synaptic AMPAR levels in the NAc to see if they were altered by cocaine exposure. Nevertheless, indirect evidence from the Kalivas lab suggests that AMPAR upregulation may occur in the NAc core after limited access cocaine self-administration (2h/day for 10 days). They showed that LTP (assessed with field potential recordings) was impaired in the NAc core following 3 weeks of withdrawal in home cages (Moussawi et al., 2009). This impairment could be due to dysfunction of basic cellular mechanisms that enable LTP induction, but it could also indicate that LTP has been occluded by prior potentiation of AMPAR transmission. Thus, one possible explanation for the impaired LTP observed by Moussawi et al. (2009) is that synaptic AMPAR levels were increased in the NAc core due to limited access cocaine self-administration and withdrawal, as shown previously for extended access cocaine self-administration (Conrad et al., 2008; Mameli et al., 2009; see next section). LTD was not impaired after the same cocaine self-administration regimen used in Moussawi et al. (2009) followed by 3 weeks of withdrawal in home cages (Lori Knackstedt and Peter Kalivas, personal communication). Results of Moussawi et al. (2009) are discussed further in Sections 5.7 and 7.2.

5.3. AMPAR adaptations after extended access cocaine self-administration

Most studies reviewed in this section are relevant to the incubation of cue-induced cocaine seeking that occurs during withdrawal (see Section 3.2.2). We recently tested the hypothesis that increased AMPAR levels in the NAc underlie the incubation of cue-induced cocaine seeking (Conrad et al., 2008). Rats were allowed to nose-poke to receive cocaine or saline infusions (6 h/day for 10 days). In tests for cue-induced cocaine seeking, performed under extinction conditions (i.e., no drug was available), significantly greater responding was observed on WD45 compared to WD1, confirming incubation. For biochemical studies, rats were killed on WD1 or WD45 after cocaine or saline self-administration. AMPAR distribution

in the NAc was determined using a BS³ protein crosslinking assay (described in Section 4.1). On WD1, when drug seeking was low, the cocaine group showed modest decreases in surface, intracellular and total GluR1 compared to saline controls. On WD45, when drug seeking was high, all of these measures were significantly increased in the cocaine group (cocaine-WD45) compared to saline controls or cocaine rats evaluated on WD1 (cocaine-WD1). However, GluR2 levels were unchanged by cocaine exposure, whereas GluR3 levels increased in cocaine rats at both withdrawal times compared to saline controls. An additional time-course study indicated that increased surface GluR1 in the cocaine group developed gradually, as an increase was detected on WD21 but not on WD3. All of these results were obtained using a combined core/shell dissection. We also performed an experiment in which AMPAR surface expression was compared in core and shell on WD1 and WD45 after discontinuing cocaine self-administration. The increase in surface GluR1 on WD45 was more pronounced in core than shell, so subsequent experiments (below) focused on the core. Finally, we found similar increases in surface GluR1 in rats killed after withdrawal only versus rats killed 30 min after a single test for cue-induced cocaine seeking, indicating a minimal effect of the test on AMPAR distribution in the NAc. Overall, these biochemical results suggested that GluR2-lacking AMPAR are added to excitatory synapses in the NAc in association with the incubation of cue-induced cocaine seeking.

This hypothesis was confirmed using whole-cell patch clamp recordings in MSN of the NAc core (Conrad et al., 2008). Inward rectification, a hallmark of GluR2-lacking AMPAR (Section 2.1), was observed in all MSN recorded from cocaine-WD42-47 rats but not in MSN from saline self-administering controls. Furthermore, Naspmp (a selective antagonist of GluR2-lacking AMPAR) significantly decreased the evoked EPSC amplitude only in MSN of the cocaine-WD42-47 group (Conrad et al., 2008). Subsequent studies using the same cocaine self-administration regimen detected GluR2-lacking AMPAR in the NAc core on WD30 (Kuei-Yuan Tseng, Marina Wolf and Carrie Ferrario, unpublished findings), indicating that GluR2-lacking AMPAR are present in adult rat NAc synapses for at least 17 days. The presence of GluR2-lacking AMPAR was further verified by co-IP experiments performed using NAc tissue from cocaine-WD45 rats (combined core/shell dissection). These studies suggested that some of the GluR2-lacking AMPAR may be homomeric GluR1 receptors, although GluR1/3 receptors may also contribute (Conrad et al., 2008).

MSN recorded from cocaine-WD42-47 rats also showed a change in the distribution of spontaneous EPSC (sEPSC) amplitude as a result of an increased number of high-amplitude sEPSC (Conrad et al., 2008). This is likely attributable to the addition of higher conductance GluR2-lacking AMPAR. In addition, MSN from cocaine-WD42-47 rats exhibited an increased frequency of AMPAR-mediated sEPSC (Conrad et al., 2008). This could reflect increased release probability. However, the paired-pulse ratio, another measure of release probability, did not differ between groups. Alternatively, the increased sEPSC frequency may reflect activity at putative new synaptic contacts that are suggested by increased spine density in the NAc after cocaine self-administration (Robinson and Kolb, 2004). Interestingly, increased spine density was particularly robust in the NAc core at a withdrawal time (WD30) from extended access cocaine self-administration that was associated with enhanced cue-induced cocaine seeking (Ferrario et al., 2005). At this same withdrawal time from a similar regimen, we detected GluR2-lacking AMPAR in the NAc core (see above).

To investigate the functional significance of GluR2-lacking AMPAR in the incubation model, Naspmp was infused into the NAc core of cocaine-exposed rats just prior to a test for cue-induced drug seeking (performed under extinction conditions). Naspmp had no effect on WD1, when GluR2-lacking AMPAR and incubation behavior are not present, but dramatically reduced the expression of incubated cue-induced drug seeking on WD45, indicating that it is mediated by GluR2-lacking AMPAR. The functional significance of the synaptic addition of GluR2-lacking

AMPA after prolonged withdrawal is discussed further in Section 5.4. It is intriguing and perhaps surprising that GluR2-lacking AMPAR are detected in every MSN we have recorded from after prolonged withdrawal from extended access cocaine self-administration (Conrad et al., 2008 and unpublished findings of Kuei-Yuan Tseng, Marina Wolf and Carrie Ferrario on WD30-47), just as upregulation of GluR1/2-containing AMPAR in cocaine-sensitized rats appears to occur in most or all MSN (Section 4.2). A challenge for the future is to understand how this and other “global” adaptations interact with cell-specific adaptations to determine the output of NAc neurons (see Section 1.2 and Section 5.4 for more discussion).

Regarding the decreased surface GluR1 found on WD1 (Conrad et al., 2008), it is possible that cocaine seeking was low on WD1 in part because AMPAR levels were low. Thus, the correlation between the level of AMPAR transmission and the level of cocaine seeking may be bidirectional, that is, increased AMPAR levels lead to increased seeking, whereas reductions in AMPAR surface expression are associated with less seeking. One interesting idea is that cocaine-induced activation of NAc neurons during the 10 days of cocaine self-administration produced a compensatory “scaling down” of postsynaptic AMPAR that was evident on WD1.

Consistent with our results in rats (Conrad et al., 2008), Mameli et al. (2009) detected inwardly rectifying AMPAR EPSC in the mouse NAc shell on WD35 from cocaine self-administration (4 h/day for 8 days). Furthermore, in a transgenic mouse strain in which NMDAR are deleted in DA neurons, VTA plasticity in early withdrawal was eliminated, and subsequently cue-induced cocaine seeking on WD35 was significantly reduced and inward rectification of AMPAR EPSC in NAc MSN was not observed (Mameli et al., 2009). These results further support a link between enhanced cocaine seeking and formation of GluR2-lacking AMPAR and, together with recordings of Conrad et al. (2008) in the NAc core, they suggest that GluR2-lacking AMPAR are present in both core and shell subregions after prolonged withdrawal from extended access cocaine self-administration. Results of Mameli et al. (2009) also indicate that NMDAR-dependent plasticity in VTA DA neurons during early withdrawal is required for subsequent increases in NAc AMPAR transmission and drug seeking. This extends earlier studies showing that NMDAR-dependent plasticity in the VTA is required for the development of both locomotor sensitization (e.g., Kalivas and Alesdatter, 1993; Wolf, 1998) and accompanying neuroadaptations in the NAc that persist after long withdrawals (Wolf et al., 1994; Li et al., 1999). The mechanism linking NMDAR plasticity in the VTA to subsequent adaptations in the NAc (and other VTA targets) may be a transient activation of DA cell firing (see Zhang et al., 1997; Giorgetti et al., 2001).

Two other studies have examined AMPAR expression after the same cocaine self-administration regimen used in our studies (6 h/day for 10 days followed by withdrawal in home cages). Lu et al. (2003) measured AMPAR levels in tissue homogenates and found very different results: GluR1 was increased on WD1 and WD90 (~20% over controls) but not WD30, while GluR2 was increased on WD1 and WD30 (~40% over control; there was also a trend towards increased GluR2 on WD90). A possible explanation is that our cocaine-exposed rats were compared to saline self-administering controls (Conrad et al., 2008), whereas the control group in Lu et al. (2003) self-administered sucrose. Ghasemzadeh et al. (2009b) found that GluR1 levels on WD10-12 (after 6 h/day $\times$ 14 days of cocaine self-administration) were slightly decreased in total tissue homogenates from the NAc shell of cocaine-experienced rats compared to controls that self-administered saline, whereas no differences in GluR1 levels were found in synaptosomal membrane fractions prepared from core or shell. This may indicate that GluR2-lacking AMPAR require more than 10-12 days of withdrawal to accumulate to a detectable level, consistent with GluR1 surface expression data (Conrad et al., 2008). However, as discussed in Section 5.5, the time-course of the addition of GluR2-lacking AMPAR to NAc synapses after cocaine withdrawal is most accurately determined using electrophysiological methods.

Other studies also measured GluR1 in total tissue homogenates but used different control groups. Two found no change in AMPAR protein levels in NAc tissue during the week after discontinuing cocaine self-administration compared to controls that remained in home cages throughout and therefore had no experience with the self-administration chambers or procedure (8 h/day $\times$ 15 days of self-administration followed by 5-16 h of withdrawal, Hemby et al., 2005; 4 h/day $\times$ 12 days followed by 1, 7 or 10 days of withdrawal, Sutton et al., 2003). A trend towards increased GluR1 was found in rats evaluated after 2 weeks of abstinence from binge cocaine self-administration compared with rats evaluated immediately after the binge (Tang et al., 2004).

5.4 Significance of the synaptic addition of GluR2-lacking AMPAR

GluR2-lacking AMPAR are present at very low levels in the NAc of adult drug-naïve or saline-treated rodents (Section 2.2), so their formation after prolonged withdrawal from cocaine self-administration represents a dramatic change in MSN synaptic function. As described in Section 5.3, we showed that blocking these receptors, in the NAc core, prevented the expression of incubated cocaine craving (Conrad et al., 2008). To explain this finding, we hypothesized that formation of GluR2-lacking AMPAR, which have high conductance (Cull-Candy et al., 2006; Isaac et al., 2007), enhances the responsiveness of MSN in the NAc core to glutamate inputs from cortical and limbic regions. Thus, when cocaine-associated cues are presented, activating glutamate afferents to the NAc core, the MSN respond more strongly, contributing to enhanced cocaine seeking. Electrophysiological recordings conducted in the NAc of awake rats provide some support for this hypothesis. Hollander and Carelli (2005, 2007) measured NAc neuronal activity on WD1 or WD30 following completion of cocaine self-administration training (2 h/day for 2-3 weeks). Compared to WD1, more neurons in the NAc core (but not shell) exhibited phasic activation on WD30 in response to cocaine self-administration (36% on WD30 versus 19% on WD1) (Hollander and Carelli, 2005). Furthermore, more cells exhibited a phasic response to cue presentation on WD30 than WD1 (35% versus 7%) and more of these responses were excitatory (24 excitatory, 4 inhibitory on WD30; 2 excitatory and 4 inhibitory on WD1) (Hollander and Carelli, 2007). These results suggest that withdrawal from cocaine self-administration increases the portion of NAc neurons that encode information related to cocaine or cocaine-associated cues. The strength of neuronal activation during cocaine self-administration also increased on WD30 (Hollander and Carelli, 2005; 2007). Importantly, cue-induced cocaine seeking was enhanced on WD30, demonstrating that this regimen produces incubation (Hollander and Carelli, 2005). More robust neuronal activation in the NAc core on WD30 could be due in part to the formation of GluR2-lacking AMPAR in NAc core that accompanies incubation (Conrad et al., 2008).

In a more recent study, Carelli and coworkers showed that the NAc neurons which exhibited phasic activation during cocaine self-administration were observed at locations in the NAc where rapid DA release was observed simultaneously, whereas no changes in DA release were observed in locations where nonphasic neurons were recorded (Owesson-White et al., 2009). This adds to previous evidence that DA gates the activation of NAc neurons by excitatory inputs (Nicola et al., 2000; O'Donnell, 2003). After prolonged abstinence, DA may similarly gate the phasic activation of MSN; however, MSN may be more readily activated and more neurons may be susceptible to activation, due to the presence of GluR2-lacking AMPAR. Together, these considerations may help explain the still selective but stronger and more widespread activation of NAc neurons (Hollander and Carelli, 2005, 2007) and the stronger AMPAR-dependent drug seeking (Conrad et al., 2008) observed in association with incubation of cue-induced cocaine craving.

As noted above, Hollander and Carelli (2005, 2007) observed a withdrawal-dependent increase in NAc neuronal activation in the core but not in the shell. Yet, GluR2-lacking AMPAR were

found in the mouse NAc shell at a long withdrawal time associated with high cue-induced cocaine seeking (Mameli et al., 2009). If the shell neurons have greater synaptic strength after prolonged withdrawal (Mameli et al., 2009) but do not exhibit greater activation (Hollander and Carelli, 2005,2007), perhaps the explanation is a difference in the activity of DA and glutamate inputs to core versus shell after prolonged withdrawal. This may be related to functional differences between core and shell suggested by other NAc recording studies. For example, Ghitza et al. (2003) used a tone to signal cocaine availability during self-administration training; when the tone was presented after 3-4 months of abstinence, recordings conducted immediately after tone presentation but prior to movement onset revealed selective activation of NAc shell neurons. This may suggest a preferential role for shell in processing the motivational significance of a discriminative stimulus. In contrast, recordings of Hollander and Carelli (2005,2007) were performed primarily during cocaine self-administration or cue-induced seeking; furthermore, presentation of the cue during training was dependent on the operant response. Selective plasticity in the NAc core in these latter studies is consistent with a more important role for core in cue-controlled drug seeking (e.g., Di Ciano and Everitt, 2001).

So far, we have focused on the ability of GluR2-lacking AMPAR to facilitate depolarization of NAc neurons due to their higher conductance. Another important consideration is that GluR2-lacking AMPAR add a new route of Ca^{2+} entry in MSN and directly couple AMPAR activation to Ca^{2+} -dependent signaling pathways. The persistent elevation of GluR2-lacking AMPAR will therefore produce long-lasting changes in Ca^{2+} signaling in MSN. It remains to be determined how this interacts with Ca^{2+} signaling involving NMDAR, voltage-gated channels, or intracellular stores. Another major challenge is to understand the cellular mechanisms that enable the synaptic incorporation and retention of GluR2-lacking AMPAR after cocaine withdrawal (Conrad et al., 2008; Mameli et al., 2009).

While our studies have focused on the NAc core, Shaham and colleagues have shown that neuronal activation in the central nucleus of the amygdala (Lu et al., 2005,2007) and the ventral portion of the medial prefrontal cortex (Koya et al., 2009a) is critical for the expression of incubated cocaine craving. More work is needed to understand how the three brain regions interact. Another study by these investigators showed that the incubation of cocaine seeking is accompanied by increased BDNF levels in the NAc (Grimm et al., 2003). Elevating BDNF levels in the NAc can enhance cocaine-related behavioral responses, including cocaine seeking after withdrawal (Bahi et al., 2008;Graham et al., 2007). Furthermore BDNF promotes plasticity at glutamate synapses through mechanisms that include increased synaptic delivery of GluR2-lacking AMPAR (Mokin et al., 2007;Caldeira et al., 2007). It is therefore interesting to speculate that increased BDNF levels contribute to the formation of GluR2-lacking AMPAR in the incubation model.

5.5 Relationship between GluR1 protein levels and the presence of GluR2-lacking AMPAR

Our first clue to the formation of GluR2-lacking AMPAR in the NAc after prolonged withdrawal from cocaine self-administration was an increase in GluR1 surface expression in the absence of any change in GluR2 surface expression (Conrad et al., 2008). However, it is important to point out that synaptic levels of GluR2-lacking AMPAR cannot be accurately inferred from biochemical studies of GluR1 abundance. Two examples illustrate this point.

First, we ran 4 cohorts of “incubation” rats (over the course of 2 years) in which AMPAR subunit levels were compared between saline and cocaine self-administering groups on WD45 using a BS³ crosslinking assay (Conrad et al., 2008 and unpublished results). The increase in total GluR1 protein in the cocaine-WD45 group was always significant, but the magnitude varied widely between cohorts (from 141% to 280% of control) even though all cohorts exhibited similar cocaine intake. In a recent study, we compared GluR1 levels in NAc

synaptosomal membrane fractions from identical experimental groups and found only a 25% increase (statistically significant) in the cocaine-WD45 rats. However, in electrophysiological studies of MSN from the NAc core, we have found inwardly rectifying, Naspm-sensitive AMPAR currents in every MSN recorded after prolonged withdrawal from cocaine self-administration. This applies to cohorts exhibiting both large and small GluR1 increases, including the cohort that exhibited only a very modest GluR1 increase in synaptosomal membrane fractions (Kuei-Yuan Tseng, Marina Wolf, and Carrie Ferrario, unpublished observations). Thus, incubation of cocaine craving is associated with varying increases in GluR1 levels, but is always associated with synaptic incorporation of GluR2-lacking AMPAR.

An elegant study by Guire et al. (2008) also illustrates that synaptic strengthening due to GluR2-lacking AMPAR need not require dramatic changes in AMPAR subunit expression. These investigators studied AMPAR synaptic delivery in hippocampal neurons during LTP induced by theta burst stimulation. Their findings indicated that addition of a very small number of GluR2-lacking AMPAR (less than 5% of existing synaptic AMPAR) can fully account for the 80% increase in synaptic strength resulting from LTP. Thus, a very small change in synaptic GluR1 abundance, well below the detection limits of biochemical techniques, can be associated with a functionally significant change in AMPAR transmission.

5.6 Results that question the relationship between AMPAR upregulation and enhanced cocaine seeking

Although studies reviewed above suggest that increased NAc AMPAR levels lead to increased cocaine seeking, the opposite conclusion was reached by studies that either deleted or over-expressed AMPAR subunits. Mead et al. (2007) trained wild-type and GluR1 knock-out mice to self-administer cocaine (8 sessions, 16 h each; each session consisted of alternating 2 h periods in which the levers were available or retracted). No differences in acquisition or intake were found. However, in a subsequent test for conditioned reinforcement, in which mice had the opportunity to perform a novel operant response to obtain a presentation of the conditioned stimulus (CS) previously paired with cocaine administration, GluR1 knock-out mice showed no selective responding for the CS on WD1 but a heightened response compared to wild-type mice when tested again on WD65. After extinction on WD2, the GluR1 knock-out mice showed greater spontaneous recovery of the extinguished response on WD3 but no difference in cue-induced reinstatement; when tested again on WD66, they showed enhanced cue-induced reinstatement compared to their own WD3 (indicating that incubation of cocaine craving occurs in these mice). In fact, “incubation” in GluR1 knock-out mice was even greater than that observed in wild-type mice (Mead et al., 2007). These results are interesting but there are two caveats associated with their interpretation. First, GluR1 was deleted throughout development (and drug taking), raising the possibility of compensation. Second, GluR1 was deleted throughout the brain, raising the possibility of off-setting or interacting effects in different brain regions. The same GluR1 knockout mice were used for studies discussed in Section 4.4 (Dong et al., 2004; Mead et al., 2005).

The complexity of the relationship between AMPAR levels and motivation for cocaine is also illustrated by two studies by Self and colleagues (Sutton et al., 2003; Bachtell et al., 2008). The first study found that extinction training, which decreases cocaine seeking, was associated with AMPAR upregulation in the NAc (Sutton et al., 2003). These results are discussed in more detail in Section 5.7. In the second study, Bachtell et al. (2008) studied the effects of viral-mediated over-expression of GluR1 in the NAc in sensitization and cocaine self-administration models. As described in Section 4.3, their sensitization studies suggested an inverse relationship between AMPAR levels in the NAc core during withdrawal from repeated non-contingent cocaine injections and the magnitude of locomotor sensitization. Related studies in which they over-expressed GluR1 during non-contingent cocaine treatment, in either

core or shell, are described in Section 4.6. In their self-administration studies, Bachtell et al. (2008) followed cocaine self-administration (4 h/day for 15 days) with 7 days of withdrawal and 6 days of extinction training. Then, wild-type or pore-dead GluR1 was over-expressed in the NAc core for several days prior to testing for cue-induced or cocaine-primed reinstatement. Cue-induced reinstatement was not altered, but cocaine-primed reinstatement was decreased by wild-type GluR1 and markedly increased by pore-dead GluR1, leading the authors to conclude that there is an inverse relationship between basal AMPAR function in the NAc core and vulnerability to drug-induced relapse (Bachtell et al., 2008). In addition, experiments were performed in which over-expression of wild-type or pore-dead GluR1 in the NAc core was restricted to the period of cocaine self-administration (10 h/day for 5 days, 4 h/day for 10 days). Cocaine intake was not affected by either treatment. After 7 days of withdrawal (during which time wild-type and pore-dead GluR1 over-expression declined), rats underwent extinction training and reinstatement tests. Prior wild-type GluR1 over-expression reduced cocaine seeking responses during the initial extinction test and produced a marked decrease in cocaine-primed reinstatement, but had no effect on cue-induced reinstatement. Prior pore-dead GluR1 over-expression had no effect on extinction, enhanced cocaine-primed reinstatement, and did not alter cue-induced reinstatement (Bachtell et al., 2008). These effects are the same as those produced when over-expression occurred after cocaine self-administration (above). Together, these results suggest that interfering with endogenous AMPAR transmission during or after cocaine exposure can affect endogenous AMPAR plasticity and functional outcome. Interestingly, in the sensitization and self-administration experiments conducted by Bachtell et al. (2008), bidirectional changes in responding to cocaine after over-expression of wild-type or pore-dead GluR1 were paralleled by changes in the response to D2 receptor stimulation. These results suggest an interaction between basal AMPAR tone and D2 receptor sensitivity.

There are a number of general caveats regarding viral-mediated GluR1 over-expression. The number of MSN affected by the manipulation is unknown and may be limited. The cellular location of new GluR1 is also unclear, but it cannot be assumed to be synaptic (see Shi et al., 1999). Over-expression of GluR1 could also alter the normal profile of AMPAR subunit composition, or influence the availability of AMPAR-interacting proteins that regulate trafficking and function of endogenous AMPAR. While Self and colleagues confirmed that over-expression of wild-type and pore-dead GluR1 in NAc core had the predicted (opposite) effects on the locomotor response to intra-NAc AMPA infusions (Bachtell et al., 2008), this does not rule out possible confounding effects on synaptic transmission or synaptic plasticity.

5.7 Reconciling opposing evidence?

To summarize, two studies found that enhanced AMPAR transmission in the NAc was associated with enhanced cocaine seeking (Conrad et al., 2008; Mameli et al., 2009), while the opposite conclusion was reached by two studies in which AMPAR levels were upregulated by extinction training or viral-mediated over-expression (Sutton et al., 2003; Bachtell et al., 2008). As noted in Section 5.6, studies of viral-mediated AMPAR over-expression are associated with several caveats, whereas studies of endogenous AMPAR (Conrad et al., 2008; Mameli et al., 2009) are more straightforward to interpret. Nevertheless, it is important to attempt to understand the basis for different findings in these studies.

Some differences may be related to whether cue-induced cocaine seeking or cocaine-primed reinstatement was assessed. Thus, Bachtell et al. (2008; core) observed an inverse relationship between GluR1 function and cocaine-primed reinstatement, but cue-induced reinstatement was not altered. Results of Conrad et al (2008; core) and Mameli et al. (2009; shell) indicate a positive relationship between GluR1 levels and cue-induced cocaine seeking, but these studies did not examine cocaine-primed reinstatement. Ultimately, however, responding elicited by cues and a priming injection of cocaine should both rely on AMPAR transmission in the NAc

(Section 1.1), so it is surprising that increased GluR1 levels should have opposite consequences in the two models. As discussed in more detail below, core-shell differences do not provide a clear solution to this and other discrepancies.

In reconciling the four studies cited in the first sentence of this section, a critical variable is the use of extinction training by Bachtell et al. (2008) and Sutton et al. (2003), but not in the other two studies. Extinction training inhibits cocaine seeking through the learning of new contextual relationships (Self et al., 2004). Therefore it is not surprising that extinction training exerts its own effects on NAc AMPAR levels. Two studies found increased AMPAR expression in the NAc after extinction training (Sutton et al., 2003; Ghasemzadeh et al., 2009b). In the first study, after cocaine self-administration (4 h/day for 12 days) and 1 week of extinction training, GluR1 and GluR2/3 protein levels were increased in the NAc shell (but not core). The increases persisted for at least 3 days but were no longer evident after 12 days. Elevated levels of GluR1 (but not GluR2/3) were correlated with extinction of cocaine seeking and resistance to cue-induced reinstatement. Conversely, viral-mediated over-expression of GluR1 or GluR2 in the NAc shell before extinction decreased responding during the first extinction session and produced more rapid extinction of cocaine seeking (Sutton et al., 2003). In contrast to the increases in endogenous GluR1 and GluR2/3 observed after extinction training, rats withdrawn in home cages did not show significant changes in AMPAR subunit levels, although the longest withdrawal time examined was 10 days (Sutton et al., 2003). The second study found similar but not identical results (Ghasemzadeh et al., 2009b). GluR1 levels were increased in NAc synaptosomal membrane fractions prepared from either core or shell when extinction training followed cocaine self-administration, but not when rats were withdrawn in their home cages for the same period (10-12 days). Only trends were found in total tissue homogenates. It is important to note that the home cage withdrawal periods used in these studies (10-12 days) were significantly shorter than the withdrawal times associated with synaptic addition of GluR2-lacking AMPAR in the incubation model (30-47 days based on electrophysiological recordings; Conrad et al., 2008; Mameli et al., 2009; Kuei-Yuan Tseng, Marina Wolf, and Carrie Ferrario, unpublished observations).

Importantly, the fact that AMPAR are upregulated in the NAc after prolonged withdrawal (Conrad et al., 2008; Mameli et al., 2009), extinction training (Sutton et al., 2003) and viral-mediated over-expression (Bachtell et al., 2008) does not mean that NAc MSN are in identical states following these three manipulations. For example, LTD in the NAc is impaired when cocaine self-administration is followed by ~3 weeks of extinction training (Moussawi et al., 2009) but not when it is followed by ~3 weeks of withdrawal in home cages (Lori Knackstedt and Peter Kalivas, personal communication). On a behavioral level, cue-induced cocaine seeking is enhanced after withdrawal (Conrad et al., 2008; Mameli et al., 2009), whereas cue-induced reinstatement is decreased after extinction training (Sutton et al., 2003) and unchanged after viral-mediated GluR1 over-expression (Bachtell et al., 2008). Each of these three conditions is certainly associated with a distinct set of other neuroadaptations that must interact with elevated AMPAR levels to promote the different functional outcomes. It is also possible that AMPAR upregulation occurs in different cell populations in the three conditions. Whereas our results indicate that GluR2-lacking AMPAR are added to all MSN after prolonged withdrawal (Section 5.3), subpopulations of MSN may be affected by extinction training or viral expression. There may also be differences in subcellular localization; for example, upregulated endogenous GluR1 and over-expressed GluR1 may traffic differently. In addition, different types of AMPAR may undergo upregulation in the different conditions. Whereas GluR2-lacking AMPAR are increased after prolonged withdrawal from cocaine self-administration (Conrad et al., 2008), both GluR1 and GluR2/3 increased when cocaine self-administration was closely followed by extinction training (Sutton et al., 2003). In comparing Bachtell et al. (2008) and Sutton et al. (2003), even though both studies used cocaine self-administration followed by extinction training, the former superimposed “exogenous”

AMPA plasticity (viral-mediated over-expression) upon the endogenous AMPAR plasticity evoked by cocaine self-administration and extinction training. All of these considerations may help explain the different functional outcomes.

As noted above, core-shell differences do not explain the complex literature considered in this section. Thus, AMPAR upregulation in both core (Conrad et al., 2008) and shell (Mameli et al., 2009) has been associated with enhanced cue-induced cocaine seeking in the incubation model. Viral-mediated GluR1 over-expression in both core (Bachtell et al., 2008) and shell (Sutton et al., 2003) leads to reduced cocaine seeking. One study found that extinction training upregulates GluR1 selectively in shell (Sutton et al., 2003) while another found GluR1 upregulation in both core and shell (Ghasemzadeh et al., 2009b). AMPA infusion into core or shell elicits cocaine seeking, although studies with AMPAR antagonists suggest that AMPAR transmission in core is more important for cue-induced or cue-maintained cocaine seeking whereas AMPAR blockade in either core or shell decreases cocaine-primed reinstatement (Section 1.1).

An interesting suggestion is that over-expression of pore-dead GluR1 after cocaine exposure enhances the expression of locomotor sensitization and cocaine-primed reinstatement because it produces a generalized decrease in the intrinsic excitability of NAc neurons, whereas the opposite effects of wild-type GluR1 on sensitization and reinstatement are due to increased intrinsic excitability (Bachtell et al., 2008). Prior studies have shown that NAc MSN from cocaine-sensitized rodents exhibit decreased intrinsic excitability due to cocaine's effects on voltage-gated channels (Section 1.2). Furthermore, decreasing intrinsic excitability of MSN (by over-expressing a potassium channel) is sufficient to increase the locomotor response to cocaine (Dong et al., 2006), and a rat strain with low MSN excitability shows an enhanced locomotor response to cocaine and enhanced cocaine self-administration (Mu et al., 2010). By analogy, decreasing MSN excitability through a different route (over-expression of pore-dead GluR1) might produce the same behavioral phenotype (Bachtell et al., 2008). The mechanism by which decreased MSN excitability promotes cocaine-related behaviors remains an open question (see Hu and Kalivas, 2006; Peoples et al., 2007) and a role for a homeostatic increase in synaptic strength triggered by decreased intrinsic excitability cannot be ruled out.

5.8 Summary

Prolonged withdrawal from extended access cocaine self-administration is associated with a time-dependent enhancement of AMPAR transmission in the NAc that results from addition of GluR2-lacking AMPAR to excitatory synapses onto MSN (Conrad et al., 2008; Mameli et al., 2009). These GluR2-lacking AMPAR mediate the enhanced cue-induced cocaine craving observed after prolonged withdrawal, presumably by facilitating the activation of MSN by glutamate inputs to the NAc (Conrad et al., 2008). Indirect evidence suggests that NAc AMPAR may also be upregulated after 3 weeks of withdrawal from limited access cocaine self-administration (Moussawi et al., 2009). These results suggest a positive relationship between NAc AMPAR levels and cocaine seeking.

On the other hand, studies in which GluR1 was genetically deleted or over-expressed in the NAc suggest an inverse relationship between AMPAR levels in the NAc and cocaine seeking (Sutton et al., 2003; Mead et al., 2007; Bachtell et al., 2008). These results are difficult to reconcile with studies demonstrating a requirement for NAc AMPAR transmission in a variety of cocaine seeking models (Section 1.1). Many considerations are important when attempting to integrate these disparate findings. One important variable is the use of extinction training in some studies but not others. Extinction training in itself is a form of learning and is associated with AMPAR upregulation in the NAc (Sutton et al., 2003; Ghasemzadeh et al., 2009b). When viral-mediated AMPAR over-expression is performed following cocaine self-administration and extinction training, it means that dual forces (over-expression and extinction training) are

influencing AMPAR transmission, and the net effect of these dual forces are overlaid on the effects of cocaine itself (and withdrawal). Perhaps the most important point is that even though AMPAR upregulation occurs in association with incubation, extinction, and viral-mediated over-expression, the functional consequences are quite different. There are many possible explanations for this, including additional neuroadaptations associated with each of the three conditions that interact differently with AMPAR upregulation. Finally, we note that core-shell differences do not provide a satisfactory explanation for this complex literature.

6. Effect of cocaine re-exposure on AMPAR surface and synaptic levels in the NAc: locomotor sensitization and cocaine self-administration studies

6.1 Cocaine challenge reverses AMPAR upregulation in cocaine-sensitized rodents

When we published our results demonstrating increased AMPAR surface expression in the NAc of sensitized rats on WD21 (Boudreau and Wolf, 2005), they appeared to conflict with a prior study demonstrating a decreased AMPA/NMDA ratio in the NAc shell of cocaine-sensitized mice on WD10-14 (Thomas et al., 2001). The decreased AMPA/NMDA ratio was attributable, at least in part, to decreased AMPAR number or function; altered NMDAR function was not detected (Thomas et al., 2001). The results of Thomas and colleagues were often interpreted as indicating an association between locomotor sensitization and a persistent LTD-like state in the NAc. It is now understood that the discrepancy between the two studies was due to an important procedural difference. In Boudreau and Wolf (2005), we evaluated rats after withdrawal in home cages. However, Thomas et al. (2001) administered a cocaine challenge to both saline- and cocaine-pretreated mice on WD10-14 (forming Sal-Coc and Coc-Coc groups) and conducted recordings 24 h later. Subsequent studies directly comparing “withdrawal only” versus “withdrawal plus challenge” groups found that AMPAR surface expression and the AMPA/NMDA ratio are increased after ~2 weeks of withdrawal, but these effects are reversed 24 h after cocaine challenge (Boudreau et al., 2007; Kourrich et al., 2007).

Kourrich et al. (2007) recorded exclusively in shell, establishing that bidirectional plasticity occurs in shell. However, results in the core are more complex. We observed a significant decrease in AMPAR surface expression 24 h after cocaine challenge using a combined core/shell dissection that includes a substantial amount of core tissue, suggesting that bidirectional plasticity also occurs in core (Boudreau et al., 2007; Ferrario et al., 2010). Furthermore, Bachtell and Self (2008) demonstrated that cocaine challenge to sensitized rats (Coc-Coc) resulted, 24 h later, in decreased sensitivity to AMPA infusion into the medial core, consistent with internalization of AMPAR in the core. However, Thomas et al. (2001) found that AMPA/NMDA ratios in the shell were significantly lower in Coc-Coc compared to Sal-Coc groups, whereas AMPA/NMDA ratios in the core did not differ between these groups. This could be interpreted to suggest that cocaine challenge produced AMPAR internalization only in the shell. However, AMPA/NMDA ratios in the core of drug naïve mice were not determined by Thomas et al. (2001). Therefore it remains possible that cocaine challenge decreased the AMPA/NMDA ratio in the core in both pretreatment groups, but to a greater extent in cocaine pretreated mice (assuming they began from an elevated level), bringing the NAc core AMPA/NMDA ratio to the same level in the Coc-Coc and Sal-Coc groups.

This argument presumes that cocaine challenge can decrease AMPAR surface expression in the NAc core of saline pretreated animals. Supporting this, saline pretreated rats challenged with cocaine (Sal-Coc) showed a significant decrease in AMPAR surface expression, 24 h later, compared to saline pretreated rats challenged with saline (Sal-Sal) (Ferrario et al., 2010; combined core/shell dissection). In the NAc shell, however, Sal-Coc mice showed a small (~10%) but not significant decrease in the AMPA/NMDA ratio compared to Sal-Sal mice

(Kourrich et al., 2007). Together these findings may suggest a stronger effect of cocaine challenge on AMPAR surface expression in core versus shell in saline pretreated animals. There may also be core-shell differences in the effect of acute cocaine in untreated animals (no previous injections of any type). With a combined core/shell dissection from untreated rats, we detected a small increase in AMPAR surface expression 24 h after acute cocaine exposure (Ferrario et al., submitted), whereas AMPA/NMDA ratios in the mouse NAc shell were not altered 24 h after acute cocaine exposure (Thomas et al., 2001; Kourrich et al., 2007). Of course, species and age differences could also underlie these different results. It will be important for future studies to directly compare core and shell using both protein crosslinking and electrophysiological approaches.

Another important question is whether cocaine re-exposure permanently reverses the withdrawal-dependent increases in AMPAR surface expression and AMPA/NMDA ratios observed after repeated cocaine exposure. To test this, we compared GluR1 surface expression on WD21 in cocaine-sensitized rats that received no challenge injection versus cocaine-sensitized rats that had received a cocaine challenge 7 days earlier (on WD14). AMPAR surface expression did not differ between these groups (Ferrario et al., 2010). Together with our prior results, this suggests that AMPAR in the NAc of cocaine-sensitized rats are internalized 24 h after cocaine challenge, but their surface expression recovers to levels comparable to unchallenged cocaine-sensitized rats after an additional 7 days of withdrawal. "Recovery" of AMPAR transmission after challenge has also been demonstrated using behavioral methods (Bachtell and Self, 2008; see Section 6.3 for more discussion). This persistence of AMPAR upregulation, despite temporary decreases after cocaine challenge, is consistent with a role in mediating long-lasting alterations in drug taking and seeking after exposure to sensitizing regimens of psychostimulants (Vezina, 2004).

6.2 Is AMPAR internalization necessary for the expression of locomotor sensitization?

The expression of locomotor sensitization to amphetamine was blocked when a peptide that prevents AMPAR internalization was infused into the NAc of amphetamine-sensitized rats prior to amphetamine challenge (Brebner et al., 2005). Therefore it was suggested that LTD in the NAc, occurring soon after amphetamine challenge, contributes to the expression of locomotor sensitization (Brebner et al., 2005). Results of subsequent protein crosslinking studies do not support this conclusion. AMPAR surface expression in amphetamine-sensitized rats was increased 30 min after amphetamine challenge (Tucker et al., 2008) and unchanged 24 h after an amphetamine challenge (Nelson et al., 2009) (see Section 8 for more discussion). It is possible that the crosslinking assay is not sensitive enough to detect a small degree of very rapid AMPAR internalization associated with the expression of amphetamine sensitization. Alternatively, it is possible that the peptide used by Brebner et al. (2005) disrupts locomotor sensitization by interfering with AMPAR recycling. According to this interpretation, amphetamine challenge leads to AMPAR activation on MSN, triggering the behavioral response. However after the initial activation, AMPAR internalization and subsequent recycling to the surface is necessary for overcoming AMPAR desensitization. This in turn enables a prolonged response of MSN that is sufficient for the prolonged motor behavior involved in the expression of amphetamine-induced locomotor sensitization (>1 hr).

Experiments analogous to those of Brebner et al. (2005) have not been conducted after cocaine sensitization. However, our recent findings do not support the hypothesis that rapid AMPAR internalization is required for the expression of locomotor sensitization to cocaine. We found that GluR1 surface expression in the NAc was not altered 30 min after cocaine challenge even though the rats expressed locomotor sensitization. A very slight (non-significant) decrease in GluR2/3 surface expression in the NAc was present 30 min after cocaine challenge, but this was also observed after saline challenge. Furthermore, both cocaine-sensitized rats and

repeated saline treated rats exhibited decreased GluR1 and GluR2 surface expression 24 h after a cocaine challenge (Ferrario et al., 2010). These latter results argue against a relationship between AMPAR internalization and previous cocaine history, although it could be functionally significant that AMPAR internalization occurs from a relatively higher baseline of surface expression in the cocaine-sensitized rats. In contrast to our results, Kourrich et al. (2007) found a decreased AMPA/NMDA ratio in the NAc shell 24 h after cocaine challenge in cocaine-sensitized mice but not repeated saline treated mice, although there was a small trend in the saline pretreated mice (see Section 6.1 for more discussion).

Several studies have found that intra-NAc infusion of AMPAR antagonists blocks the expression of locomotor sensitization to cocaine (Pierce et al., 1996; Bell et al., 2000), although this is not observed under all conditions (Ferrario et al., 2010; see Section 4.3). The simplest interpretation of these results is that AMPAR activation, rather than AMPAR internalization, contributes to the expression of locomotor sensitization. However, as pointed out by Bachtell et al. (2008), intra-NAc infusion of AMPAR antagonists might block the expression of locomotor sensitization not by blocking AMPAR transmission per se, but rather by preventing endogenous glutamate, released by cocaine, from producing rapid AMPAR internalization. Thus, a two-phase mechanism, in which a surge of AMPAR transmission followed by AMPAR internalization leads to expression of locomotor sensitization, cannot be ruled out (Bachtell et al., 2008). However, if this is the case, one might expect detectable AMPAR internalization 30 min after the challenge, yet this was not observed (Ferrario et al., 2010). It is clear that more work is required to understand the behavioral significance of cocaine-induced AMPAR internalization.

6.3. What causes decreased AMPAR surface expression and decreased AMPA/NMDA ratios after cocaine challenge?

As observed in other cell types, glutamate application produces rapid AMPAR internalization in cultured NAc neurons (Mangiavacchi and Wolf, 2004b). Based on this, it was hypothesized that decreased AMPAR surface expression and AMPA/NMDA ratios observed 24 h after cocaine challenge were the result of cocaine challenge acutely increasing glutamate levels in the NAc (Boudreau et al., 2007; Kourrich et al., 2007). Changes in mitogen-activated protein kinase activity were also implicated in bidirectional AMPAR plasticity (Boudreau et al., 2007), but a discussion of signaling pathways is beyond the scope of this review. The idea that glutamate triggers AMPAR internalization in cocaine-sensitized rodents is supported by in vivo results showing that a challenge injection of cocaine increases glutamate efflux in the NAc of sensitized rats, whereas only small or no effects are observed in saline pretreated controls or cocaine-exposed rats that fail to develop locomotor sensitization (e.g., Pierce et al., 1996; Reid and Berger, 1996). Similarly, cocaine challenge produces AMPAR internalization in sensitized rats but not rats that fail to sensitize (Boudreau et al., 2007).

The hypothesis that cocaine-induced glutamate release triggers AMPAR internalization in cocaine-sensitized rats was tested by Bachtell and Self (2008). After ~3 weeks of withdrawal from cocaine pretreatment, they observed that AMPA infusion into the medial core of the NAc produced an enhanced locomotor response. This increased “AMPA sensitivity” in cocaine-sensitized rats is consistent with prior results (Pierce et al., 1996; Bell and Kalivas, 1996) and is presumably mediated by the increased AMPAR surface expression described in Section 4.1. When rats were challenged with cocaine 24 h prior to intra-NAc AMPA infusion, the enhanced AMPAR sensitivity was reversed, analogous to the decrease in AMPAR surface expression produced 24 h after cocaine challenge (Section 6.1). This reversal was prevented if the AMPAR antagonist CNQX was administered along with the cocaine challenge (Bachtell and Self, 2008). This shows that AMPAR stimulation is necessary in order for a cocaine challenge to decrease AMPAR sensitivity, presumably via AMPAR internalization. As noted in Section

6.1, the decrease in AMPAR sensitivity observed 24 h after cocaine challenge was fully recovered 6 days after cocaine challenge, consistent with our studies measuring cocaine challenge induced AMPAR internalization (Ferrario et al., 2010).

However, the timing of the observed effects raises questions. In the NAc of cocaine-sensitized rats, rising glutamate levels were detected within 20 min after cocaine challenge (Pierce et al., 1996; Reid and Berger, 1996; Bell et al., 2000). Similarly, AMPAR internalization in cultured NAc neurons was detected 5 min after increasing extracellular glutamate levels (Mangiavacchi and Wolf, 2004b). These results predict a rapid decrease in AMPAR surface expression after cocaine challenge. Yet AMPAR surface expression is decreased 24 h but not 30 min after a cocaine challenge is administered to cocaine-sensitized rats (Sections 6.1 and 6.2), indicating a temporal mismatch between cocaine's effects on glutamate release and AMPAR trafficking. Furthermore, whereas cocaine challenge is less effective at increasing glutamate levels in saline pretreated rats than in sensitized rats (e.g., Pierce et al., 1996; Reid and Berger, 1996), we found that both cocaine-sensitized and saline pretreated rats show decreased AMPAR surface expression 24 h after cocaine challenge (Ferrario et al., 2010; but see Kourrich et al., 2007 and Section 6.2 regarding different results for AMPA/NMDA ratios). Perhaps there are delayed changes in glutamate release, or release of other neurotransmitters, that can account for these observations.

6.4. Effect of re-exposure to cocaine on other aspects of excitatory transmission in the MSN

In a fascinating but very complicated study, Shen et al. (2009) examined the effect of cocaine challenge (administered to rats previously given repeated saline or cocaine injections) on a number of measures related to excitatory synaptic function in the NAc core, including dendritic spine morphology, synaptic protein levels (measured in a detergent insoluble fraction enriched for PSD proteins) and field potentials evoked by in vivo stimulation of the prefrontal cortex. In the saline pretreated group, glutamate synaptic function was depressed at 120 min after cocaine injection but increased 6 h afterwards; some measures were shown to normalize after 24 h. Very different effects of cocaine challenge were observed in the cocaine pretreated group. Most striking were results obtained several hours after cocaine challenge suggesting collapse of the actin cytoskeleton, along with decreased field potentials. It is difficult to speculate about the relationship between effects of cocaine challenge on spine morphology and AMPAR trafficking, because the only time-points at which AMPAR surface expression has been measured following cocaine challenge are 30 min (no change) and 24 h (decreased) (see Sections 6.1 and 6.2). It will be important to better understand the relationship between spine and AMPAR plasticity, and the relationship of both variables to actin cycling, which plays a key role in spine plasticity and is also influenced by cocaine exposure (Toda et al., 2006). However, the available data highlight the complexity of synaptic changes occurring in response to cocaine re-exposure and suggest that it is inaccurate to describe the plasticity resulting from cocaine challenge as either LTP or LTD (see Section 7).

6.5 Cocaine-primed reinstatement is associated with rapid AMPAR trafficking

Less work has been done to characterize the effect of cocaine re-exposure on AMPAR distribution after drug self-administration, but the data so far suggest different effects compared to those observed after repeated non-contingent cocaine exposure (Sections 6.1-6.3). In a recent study of cocaine-primed reinstatement, in which rats were killed 30 min after the cocaine priming injection, Pierce and colleagues found that reinstatement of drug seeking was associated with increased cell surface GluR1 in the NAc shell, measured using BS³ protein crosslinking (Anderson et al., 2008). Furthermore, they showed that injection into the NAc shell of a peptide that interferes with GluR1 trafficking attenuated reinstatement produced by 20 mg/kg cocaine (although reinstatement produced by 10 mg/kg was not affected). These results indicate that a rapid increase in cell surface levels of GluR1-containing AMPAR may

contribute to cocaine-induced reinstatement. Other results in the paper implicated a mechanism dependent on D1 receptors, L-type Ca^{2+} channels, and CaMKII activation (Anderson et al., 2008). These findings could be related to our demonstration that D1 receptor stimulation facilitates AMPAR synaptic insertion in cultured NAc neurons (Sun et al., 2008; see Section 2.3).

Pierce and colleagues subsequently showed that cocaine-induced reinstatement led to increased GluR2 phosphorylation at serine 880 in the NAc shell 30 min after the priming injection (a trend towards an increase was found in the core). Furthermore, disruption of GluR2 trafficking in core or shell attenuated cocaine-primed reinstatement (Famous et al., 2008). The disruption was achieved using a peptide corresponding to the last 6 amino acids of the GluR2 C-terminus that interferes with GluR2-PICK1 interactions. In other cell types, serine 880 phosphorylation is associated with GluR2 internalization (Chung et al., 2000), and GluR2-PICK1 interactions are involved in exchange between GluR2-containing and GluR2-lacking AMPAR (Gardner et al., 2005; Liu and Cull-Candy, 2005). Combined with evidence for increased GluR1 surface expression 30 min after the cocaine priming injection (Anderson et al., 2008), these findings suggest that GluR1-containing AMPAR may rapidly replace GluR2-containing AMPAR on the cell surface during cocaine-induced reinstatement. Interestingly, there is also evidence for GluR2/3 internalization in the prefrontal cortex during cue-induced reinstatement of heroin seeking (Van den Oever et al., 2008).

6.6 Summary

Cocaine-sensitized rats show bidirectional AMPAR plasticity in the NAc. After 1-3 weeks of withdrawal, AMPAR surface expression and AMPA/NMDA ratios are increased. When a cocaine challenge injection is administered, this AMPAR upregulation reverses, and decreased AMPAR surface expression and AMPA/NMDA ratios are detected 24 h after the injection (Thomas et al., 2001; Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007). However, AMPAR surface expression recovers to pre-challenge levels after an additional period of withdrawal, supporting the idea that AMPAR upregulation may contribute to persistent behavioral plasticity associated with the sensitization model (Bachtell and Self, 2008; Ferrario et al., 2010). AMPAR internalization in response to cocaine challenge is likely to occur in both core and shell. It appears to require cocaine-induced glutamate release and AMPAR stimulation in the NAc (Bachtell and Self, 2008). However, there is a mismatch between the timing of cocaine-induced glutamate release, which occurs rapidly (e.g., Pierce et al., 1996), and cocaine-induced AMPAR internalization, which is detected 24 h but not 30 min after the injection (Ferrario et al., 2010).

It has been proposed that rapid AMPAR internalization after amphetamine challenge is required for the expression of locomotor sensitization to amphetamine, based on results obtained with a peptide that prevents endocytosis of GluR2-containing AMPAR (Brebner et al., 2005). As discussed in Section 8, there are substantial differences in the AMPAR plasticity elicited by cocaine and amphetamine treatment, so extrapolating these results to cocaine is unwise. However, more recent work is not consistent with the idea that AMPAR internalization mediates the expression of sensitization to either amphetamine or cocaine (Tucker et al., 2008; Ferrario et al., 2010).

As noted above, AMPAR surface expression in the NAc is decreased 24 h after cocaine injection in cocaine-sensitized rats. Saline pretreated rats challenged with cocaine also show decreased AMPAR surface expression (Ferrario et al., 2010), although the AMPA/NMDA ratio is not significantly decreased in mice treated similarly (Kourrich et al., 2007). Cocaine challenge does not alter AMPAR surface expression in cocaine-exposed rats that fail to sensitize (Boudreau et al., 2007). Surprisingly, a small increase in AMPAR surface expression is observed 24 h after drug and injection naïve rats receive their first cocaine injection (Ferrario

et al., submitted), while the AMPA/NMDA ratio is not altered in mice treated similarly (Thomas et al., 2001; Kourrich et al., 2007). Thus, the available data suggest a very complex relationship between past experiences with the injection procedure or cocaine and the AMPAR response to cocaine injection. It is notable that all our studies of AMPAR surface expression used a combined core/shell dissection, whereas AMPA/NMDA ratios were measured in the NAc shell.

After cocaine self-administration, cocaine-primed reinstatement is associated with a rapid (30 min) increase in GluR1 surface expression in the NAc shell (Anderson et al., 2008). Based on different results for the GluR2 subunit, it is possible that GluR1-containing AMPAR exchange with GluR2-containing AMPAR on the cell surface (Famous et al., 2008). Together with the non-contingent cocaine administration results summarized above, these results indicate that the AMPAR response to cocaine re-exposure depends on whether prior cocaine experience was contingent or non-contingent. Of course, other factors such as route of administration (i.p. versus i.v.) and total drug exposure may also contribute.

Finally, elegant in vivo experiments are made possible by intra-cranial injection of peptides or other reagents that interfere with AMPAR trafficking. However, reagents that interfere with acute AMPAR internalization or externalization may also affect receptor recycling mechanisms that operate over a longer time-frame to maintain synaptic AMPAR pools. This may become significant in experiments that involve a sustained behavioral response (e.g., expression of locomotor sensitization or reinstatement of cocaine seeking), which in turn requires sustained AMPAR transmission in the NAc.

7. How does repeated cocaine exposure alter subsequent synaptic plasticity in the NAc?

7.1 Sensitization

Many forms of LTP are produced by the addition of AMPAR to excitatory synapses, so the increased synaptic AMPAR levels observed in the NAc after withdrawal from repeated cocaine exposure might make it more difficult to produce further LTP. Unfortunately, no studies have addressed this possibility at the withdrawal times (7-21 days) associated with cocaine-induced increases in NAc AMPAR levels. Two studies examined earlier withdrawal times. Yao et al. (2004) found an enhanced magnitude of LTP at PFC-NAc glutamate synapses in slices prepared from adult mice 2–3 d after the last of 5 cocaine injections (core versus shell recording sites were not reported). Goto and Grace (2005) found pathway-specific effects of cocaine sensitization on synaptic plasticity in the NAc shell of adult rats. Rats were treated with cocaine for 6 days and challenged with cocaine on WD10-17 to test for locomotor sensitization. Slices were prepared 1-5 days later. Cocaine pretreatment did not affect plasticity induced by tetanic stimulation of the PFC. In contrast, tetanic stimulation of the hippocampus induced LTP in the NAc shell of saline treated rats (and modified the response to PFC stimulation), whereas these effects were absent in cocaine-sensitized rats (Goto and Grace, 2005; core was not studied after cocaine pretreatment). However, it cannot be concluded that the absence of LTP reflects a pre-existing LTP-like state due to increased AMPAR levels, because AMPAR surface expression in the NAc of sensitized rats is in transition from a challenge-induced depressed state back to an elevated state during the week following cocaine challenge (Ferrario et al., 2010; Bachtell and Self, 2008; Section 6.1). Similarly, the findings of Yao et al. (2004), on WD3-5, are difficult to relate to AMPAR levels because these levels are in transition during the first week of withdrawal from repeated cocaine treatment (Boudreau and Wolf, 2005; Kourrich et al., 2007; Boudreau et al., 2009; Section 4.1). Perhaps Yao et al. (2004) detected enhanced LTP because the NAc synapses were starting from an LTD-like state, as may be indicated by a decreased AMPA/NMDA ratio on WD1 (Kourrich et al., 2007; Mameli et al., 2009) or because

of enhanced NMDAR function during early withdrawal (Huang et al., 2009). Thus, the different findings of Yao et al. (2004) and Goto and Grace (2005) may be explained by the fact that both studies were performed when surface AMPAR levels were “in flux”, as well as by possible differences between hippocampal-NAc and PFC-NAc synapses and other procedural differences.

Two other electrophysiological studies assessed AMPAR transmission in the NAc shell at later withdrawal times, when surface AMPAR levels are expected to be stably increased (WD10-14, Kourrich et al., 2007; WD35, Mameli et al., 2009). As discussed in Section 4.1, these studies found increased AMPA/NMDA ratios (Kourrich et al., 2007) and synaptic addition of GluR2-lacking AMPAR, which would strengthen synapses (Mameli et al., 2009), but they did not determine if this affected subsequent induction of LTP or LTD. In contrast to elevated AMPA/NMDA ratios after withdrawal, the AMPA/NMDA ratio in the shell of sensitized mice was decreased 24 h after a challenge injection of cocaine (the challenge injection was administered on WD10-14; Thomas et al., 2001; Kourrich et al., 2007; Section 6.1). The decreased AMPA/NMDA ratio was suggested to be due to mechanisms shared with LTD, because the magnitude of LTD that could subsequently be induced was decreased in the cocaine group (Thomas et al., 2001). Similarly, the decreased AMPA/NMDA ratio observed on WD1 in the NAc shell of sensitized mice occluded subsequent low frequency stimulation induced LTD (Mameli et al., 2009). It should be noted that cocaine-generated silent synapses may contribute to the AMPA/NMDA ratio on WD1 but probably not on WD10-14 (Huang et al., 2009; see Section 4.1 for more discussion).

7.2 Cocaine self-administration

Two groups have studied whether prior cocaine self-administration alters the ability to subsequently induce synaptic plasticity in the NAc (Martin et al., 2006; Moussawi et al., 2009). In a study using adult rats (P80-140), Martin et al. (2006) trained the rats to self-administer cocaine (2 hr/day for 14-19 days) and then recorded from NAc slices on WD1 or WD21. No extinction training occurred. On WD1, whole-cell voltage-clamp recordings showed that LTD induction was impaired in both NAc core and shell of cocaine self-administering rats compared to sham rats (passively exposed to the operant chamber), yoked cocaine rats (which received a cocaine infusion whenever a cocaine self-administering rat received an infusion), and rats that self-administered food. This could reflect an impairment of basic mechanisms required for LTD or occlusion due to a pre-existing LTD-like state. Supporting the latter possibility, another study found decreased excitatory field potential responses in the mouse NAc shell in response to stimulation of glutamate afferents soon after discontinuing cocaine self-administration (Schramm-Sapota et al., 2006) and we observed a slight decrease in GluR1 surface expression on WD1 from cocaine self-administration (Conrad et al., 2008; combined core/shell dissection). On WD21, Martin et al. (2006) found that the shell had “recovered” (robust LTD was evoked) but the core remained affected, suggesting a persistent impairment of LTD only in the core. This could reflect a pre-existing LTD-like state analogous to interpretation of WD1 data. Unfortunately, the effect of the cocaine self-administration regimen used by Martin et al. (2006) on AMPAR levels in the NAc has not been directly examined. However, after extended access cocaine self-administration (6 h/day) and a longer withdrawal time (45 days), we showed that synaptic strength was increased in the NAc core by the addition of GluR2-lacking AMPAR (Conrad et al., 2008). In Supplementary Materials from the same study, we showed a significant increase in GluR1 surface expression on WD21 (Conrad et al., 2008). If our results indicate a pre-existing LTP-like state, it should be possible to induce LTD in NAc core after our cocaine regimen, and in fact, we found this to be the case (Kuei-Yuan Tseng, Marina Wolf and Carrie Ferrario, personal communication). Together, then, these two studies (Martin et al., 2006; Conrad et al., 2008) suggest that withdrawal from cocaine self-administration can differently alter plasticity in the NAc core

depending on the cocaine self-administration regimen. The situation in the NAc shell is simpler; after >3 weeks of abstinence, AMPAR transmission is potentiated due to the addition of GluR2-lacking AMPAR to NAc synapses (Mameli et al., 2009) and the ability to elicit LTD has recovered (Martin et al., 2006), consistent with synaptic potentiation.

In the second study, Moussawi et al. (2009) stimulated the ventral prelimbic PFC and recorded AMPAR-mediated field potentials in NAc core neurons. After cocaine self-administration (2 h/day for 10 days) and ~3 weeks of extinction training, field potentials were potentiated in cocaine-self-administering rats ("cocaine rats") compared to controls (pooled yoked saline and drug naïve rats). Then, high frequency stimulation was applied to the PFCNAc pathway. LTP of field potentials was produced in control rats, but the same stimulation failed to elicit LTP in cocaine rats. An impairment of LTP induction is consistent with a preexisting potentiated state as indicated by enhanced field potentials in cocaine rats. Further supporting this, depotentiating the synapses with a strong LTD induction protocol enabled subsequent induction of LTP in the cocaine group. Moussawi et al. (2009) also observed impaired LTP in the NAc core in the cocaine group after 3 weeks of withdrawal in home cages (instead of extinction training). This could be explained by synaptic incorporation of GluR2-lacking AMPAR in the NAc core, which would strengthen synapses and thus might impair subsequent LTP, although this explanation is based on extrapolation from extended access (Conrad et al., 2008) to limited access (Moussawi et al., 2009) studies. Of course, synaptic incorporation of GluR2-containing AMPAR could also produce a pre-existing LTP-like state. In summary, limited access cocaine self-administration followed by either extinction training or withdrawal leads to impaired LTP in the NAc core, potentially related to AMPAR upregulation, although this does not mean that NAc synapses are in an identical state after extinction versus withdrawal (see Section 5.7).

Results regarding LTD are more complicated. Moussawi et al. (2009) found that it was more difficult to induce LTD in the NAc core after cocaine self-administration followed by ~3 weeks of extinction training, although this could be overcome with a stronger stimulation protocol (a 3 burst protocol induced LTD in control but not cocaine rats, whereas a 5 burst protocol induced LTD in both groups). Interestingly, however, subsequent work from this group has shown that LTD is not impaired if the same cocaine self-administration procedure is followed by 3 weeks of withdrawal in home cages rather than 3 weeks of extinction training (Lori Knackstedt and Peter Kalivas, personal communication). This fits with evidence for AMPAR upregulation (Conrad et al., 2008) and impaired LTP (Moussawi et al., 2009) in NAc core after withdrawal, both of which could be interpreted as a pre-existing LTP-like state. In such a state, LTD should be readily induced. However, it must be more complicated than this because, as noted above, Martin et al. (2006) observed impaired LTD in NAc core on WD21. Furthermore, after extinction training, Moussawi et al. (2009) observed impairment of both LTP and LTD in NAc core, which cannot be simply explained by pre-existing synaptic potentiation or depression. Thus, rather than attempt to interpret the state after cocaine exposure as akin to either LTP or LTD, it is more accurate to describe an impairment of plasticity mechanisms and to conclude that the nature of the impairment is influenced by cocaine intake, withdrawal time and NAc subregion-specific effects.

The cocaine self-administration regimen used by Moussawi et al. (2009) produces a decrease in basal extracellular glutamate levels in the NAc (see Section 1.2). By restoring glutamate levels, using N-acetylcysteine, Moussawi et al. (2009) were able to restore both LTP and LTD in rats with a history of cocaine self-administration and extinction training. This resulted from restoration of endogenous glutamate tone on mGluR2/3 and mGluR5 receptors respectively (Moussawi et al., 2009). These results suggest an important role for presynaptic dysfunction in the impairment of postsynaptic plasticity mechanisms observed after cocaine self-

administration (see Kalivas, 2009). Possible relationships between cocaine-induced presynaptic changes and AMPAR upregulation are considered briefly in Section 4.2.

7.3 Summary

Biochemical and electrophysiological results suggest a state of potentiated AMPAR transmission in the NAc of cocaine-sensitized animals after 7-35 days of withdrawal (Section 4.1). However no electrophysiological studies have examined whether induction of LTP (or LTD) is altered at these withdrawal times. Studies at earlier withdrawal times (Yao et al., 2004) or after cocaine challenge (Goto and Grace, 2005) have yielded conflicting results, perhaps because AMPAR synaptic levels are in flux during these periods (Boudreau and Wolf, 2005; Kourrich et al., 2007; Bachtell and Self, 2008; Boudreau et al., 2009; Mameli et al., 2009; Ferrario et al., 2010).

In rats studied after cocaine self-administration, WD1 is characterized by depressed AMPAR transmission (Conrad et al., 2008; Schramm-Sapota et al., 2006) and impairment of LTD (Martin et al., 2006) in core and shell. Results obtained 3 or more weeks after discontinuing cocaine self-administration will be summarized below according to the NAc subregion studied and whether extinction training was used. However, many other procedural differences (e.g., amount of drug intake) exist between these studies that may also help explain discrepant results.

Core and 3+ weeks of withdrawal—Two studies suggest potentiation of AMPAR transmission that impairs further LTP (Conrad et al., 2008; Moussawi et al., 2009). Impaired LTD is not predicted if synapses are potentiated. However, one group found impaired LTD (Martin et al., 2006) while others did not (Lori Knackstedt and Peter Kalivas, personal communication; Kuei-Yuan Tseng, Marina Wolf, and Carrie Ferrario, unpublished findings). Studies by Conrad et al. (2008) and Tseng et al. used extended access cocaine self-administration, while the other studies used limited access cocaine self-administration.

Shell and 3+ weeks of withdrawal—Two studies suggest potentiation of AMPAR transmission (Conrad et al., 2008; Mameli et al., 2009). Consistent with this, LTD can be evoked (Martin et al., 2006). LTP has not been tested.

Core and ~3 weeks of extinction—Both LTP and LTD are impaired (Moussawi et al., 2009).

Shell and extinction—Data are not available. Overall, these studies cannot be reconciled with a simple LTP- or LTD-like state after cocaine exposure. Instead, they point to a complex impairment of plasticity mechanisms due to both presynaptic and postsynaptic adaptations. The nature of the impairment depends on the cocaine self-administration regimen, whether extinction training or withdrawal was utilized, and the duration of the withdrawal period. Furthermore, there are differences between core and shell.

Significant progress has been made in characterizing presynaptic mechanisms underlying impaired plasticity in the NAc (Kalivas, 2009). In contrast, little is known about the mechanisms underlying elevation of postsynaptic AMPAR levels in the NAc after cocaine withdrawal, beyond evidence that the development of this elevation requires NMDAR-dependent plasticity in the VTA (Mameli et al., 2009; Schumann and Yaka, 2009) and its expression, at least in sensitized rats, is associated with altered MAPK signaling in the NAc (Boudreau et al., 2007, 2009; Schumann and Yaka, 2009). Testing candidate mechanisms (e.g., LTP-like versus synaptic scaling) will require an understanding of changes in the activity of glutamate afferents to the NAc during cocaine exposure and withdrawal (see Section 4.2). In light of the complex array of adaptations that occur in the NAc subsequent to repeated cocaine

exposure, including changes in gene expression, signal transduction, firing patterns and morphology (Robinson and Kolb, 2004; Kalivas and Hu, 2006; Peoples et al., 2007; McClung and Nestler, 2008; Wheeler and Carelli, 2009), it is not surprising that the state of synaptic transmission after cocaine withdrawal has proved difficult to categorize in simple terms. However, the existing results do enable predictions regarding functional consequences. For example, persistent enhancement of AMPAR transmission in the NAc after drug withdrawal would increase the reactivity of MSN to stimuli that trigger drug seeking and perhaps account for persistent vulnerability to relapse (Conrad et al., 2008), while impaired induction of subsequent plasticity could interfere with future learning, perhaps contributing to the reduced behavioral flexibility that characterizes addiction (Martin et al., 2006).

8. Cocaine and amphetamine produce different effects on AMPAR transmission

Cocaine and amphetamine exhibit cross-sensitization in locomotor activity experiments and prior exposure to one drug enhances self-administration of the other (Kalivas and Weber 1988; Pierce and Kalivas 1995; Bonate et al. 1997; Ferrario and Robinson, 2007; Liu et al. 2007). Might common AMPAR adaptations be responsible for these observations? While this is a tempting hypothesis, it is clear that amphetamine and cocaine have different effects on NAc AMPAR. This is evident from results obtained in rats tested after withdrawal from cocaine or amphetamine regimens leading to psychomotor sensitization. Whereas cocaine-sensitized rats show increased AMPAR surface and synaptic levels in the NAc (Section 4.1), protein crosslinking studies found no significant change in AMPAR surface expression in the NAc core or shell of amphetamine-sensitized rats on WD21 (Nelson et al., 2009). It remains possible that AMPAR transmission is increased in the NAc of amphetamine-sensitized rats through a different mechanism, such as enhanced presynaptic activity (Lodge and Grace, 2008) or phosphorylation of existing synaptic AMPAR (Loweth et al., 2010). For example, GluR1 phosphorylation at serine 845 increases AMPAR currents by regulating the open channel probability, while GluR1 phosphorylation at serine 831 increases AMPAR conductance in receptors containing GluR1 but not GluR2 (Shepherd and Huganir, 2007; Derkach et al., 2007). Modification of existing AMPAR, via phosphorylation or other mechanisms, could explain why amphetamine-sensitized mice show increased AMPA/NMDA ratios in the NAc (Kelly et al., 2008) whereas no changes in AMPAR surface expression are observed in amphetamine-sensitized rats (Nelson et al., 2009), although species and other differences might also contribute.

Together, these results suggest that repeated treatment with either amphetamine or cocaine may render MSN more responsive to activation by glutamate afferents, albeit due to different types of AMPAR adaptations, and that this might be responsible for aspects of cross-sensitization. In particular, they help explain an elegant study implicating AMPAR adaptations in cross-sensitization of incentive motivational effects. Vezina and colleagues pre-exposed rats to saline or non-contingent amphetamine injections (leading to locomotor sensitization) followed by a period of cocaine self-administration (Suto et al., 2004). After a short withdrawal from cocaine self-administration, the amphetamine pre-exposed rats showed an enhancement of the ability of intra-NAc AMPA infusion to reinstate cocaine seeking (infusions were made into core and shell). After a longer withdrawal, saline and amphetamine pre-exposed rats both showed this enhancement. The authors interpreted their results to suggest that the enhanced AMPA responsiveness was time-dependent, and that the enhancement at the early withdrawal was attributable to the amphetamine pre-exposure while the later enhancement was attributable to cocaine self-administration. Results summarized above suggest that amphetamine may produce its effect via modification of existing AMPAR, whereas cocaine may do so by increasing synaptic AMPAR levels. Of course, other mechanisms may also contribute.

Different AMPAR adaptations are also observed when cocaine- and amphetamine-sensitized rats are challenged with cocaine. In cocaine-sensitized rats, cocaine challenge does not significantly alter AMPAR surface expression in the NAc (combined core/shell dissection) 30 min after the challenge injection, although AMPAR surface expression is decreased 24 h after the injection (Boudreau et al., 2007; Ferrario et al., 2010). Similarly, electrophysiological studies find decreased AMPA/NMDA ratios in the NAc shell 24 h after cocaine challenge (Thomas et al., 2001; Kourrich et al., 2007). In contrast to these results, increased AMPAR surface expression in the NAc (combined core/shell dissection) was observed 30 min after an amphetamine challenge was given to amphetamine-sensitized rats (Tucker et al., 2008). This result is not consistent with the hypothesis that expression of locomotor sensitization to amphetamine requires AMPAR internalization (Brebner et al., 2005; see Section 6.2 for more discussion). Together, amphetamine results suggest that cell surface AMPAR levels are not increased in the NAc of amphetamine-sensitized rats during withdrawal (Nelson et al., 2009), although functional changes in existing AMPAR may occur (Loweth et al., 2010), but a rapid increase in cell surface AMPAR may occur shortly after rats are challenged with amphetamine (Tucker et al., 2008). In contrast, cocaine-sensitized rats have elevated AMPAR transmission during withdrawal due to increased AMPAR surface and synaptic levels (Section 4.1) and there is no evidence for further increases upon cocaine challenge (although this may occur in rats that have self-administered cocaine; Anderson et al., 2008; see Section 6.5).

Because both cocaine and amphetamine elevate extracellular DA levels, their divergent effects may argue against a major role for DA receptor stimulation in mediating AMPAR redistribution in the studies discussed above. Possible explanations for different effects of cocaine and amphetamine on AMPAR transmission in the NAc are discussed in more detail by Nelson et al. (2009).

The main conclusion to be drawn here is that cocaine and amphetamine should not be lumped together in discussing glutamate-related plasticity and, specifically, that AMPAR results from one drug cannot be extrapolated to the other.

9. Future Directions

This review focused very narrowly on the effects of cocaine on AMPAR in MSN of the NAc because the field has developed to the point where a detailed and critical review seemed warranted. However, it is important to note that we did not comprehensively address several critical issues related to this topic, including: 1) heterogeneity of NAc neurons, defined anatomically, functionally, and pharmacologically, 2) different responsiveness of NAc MSN in the upstate and the downstate, 3) cocaine-induced plasticity related to NMDA receptors and metabotropic glutamate receptors in the NAc, 4) cocaine-induced AMPAR plasticity in the dorsal striatum, which differs significantly from AMPAR plasticity in the NAc (Ferrario et al., 2010), 5) the contribution of AMPAR phosphorylation to cocaine-induced plasticity, 6) cocaine's effects on presynaptic glutamate transmission, and 7) the impact of changes in other transmitter systems, most notably the DA system, on glutamate-dependent plasticity. Hypotheses and experiments that take into account these complexities will advance our understanding of the functional significance of cocaine-induced changes in AMPAR transmission in the NAc.

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