Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis

William A. Carlezon, Jr.,* Mark J. Thomas

Behavioral Genetics Laboratory, Department of Psychiatry, Harvard Medical School, McLean Hospital, MRC 217, 115 Mill Street, Belmont, MA 02478, USA

Department of Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA

Department of Psychology, University of Minnesota, Minneapolis, MN 55455, USA

Article history:
Received 21 May 2008
Received in revised form 25 June 2008
Accepted 29 June 2008

Keywords:
Behavior
Molecular biology
Electrophysiology
Addiction
Model

1. Introduction

The biological basis of mood-related states such as reward and aversion is not understood. Classical formulations of these states implicate the mesocorticolimbic system, comprising brain areas including the NAc, VTA, and PFC, in reward (Bozarth and Wise, 1981; Goeders and Smith, 1983; Wise and Rompré, 1989). Other brain areas, including the amygdala, periaqueductal gray, and the locus coeruleus, are often implicated in aversion (Aghajanian, 1978; Phillips and LePiane, 1980; Bozarth and Wise, 1983). However, the notion that certain brain areas narrowly and rigidly mediate reward or aversion is becoming archaic. The development of increasingly sophisticated tools and methodologies has enabled new approaches that provide evidence for effects that previously would have been difficult (if not impossible) to detect. As one example from our own work, we have found that a prominent neuroadaptation triggered in the NAc by exposure to drugs of abuse (activation of the transcription factor CREB) contributes to depressive-like and aversive states in rodents (Carlezon et al., 2005). Other work suggests that changes in the activity of dopaminergic neurons in the VTA – which provides inputs to the NAc that are integrated with glutamatergic inputs from areas such as the PFC, AMG, and HIP – can also encode both rewarding and aversive states (Liu et al., in press).

In this review, we will focus on the role of the NAc in simple states of reward and aversion. The role of NAc activity in more complex states such as drug-craving and drug-seeking is beyond the scope of this review, since these states depend upon experience-dependent neuroadaptations and do not easily map onto basic conceptualizations of rewarding and aversive states. An improved understanding of the neurobiology of mood states will facilitate the development of well-tolerated medications that treat and prevent addiction and other conditions (e.g., mood disorders) associated with dysregulation of brain motivation systems.

*Corresponding author.
E-mail address: bcarlezon@mclean.harvard.edu (W.A. Carlezon Jr.).
alcohol and opiates – except that addicts often report aversive effects and discontinue treatment (Weiss 2004). Methods to predict rewarding or aversive responses in normal and addicted brains would accelerate the pace of drug discovery, medication development, and recovery from addiction. Here we review evidence for the simple working hypothesis that rewarding and aversive states are encoded by the activity of NAc medium spiny GABAergic neurons.

2. The NAc

The NAc comprises the ventral components of the striatum. It is widely accepted that there are two major functional components of the NAc, the core and the shell, which are characterized by differential inputs and outputs (see Zahn, 1999; Kelley, 2004; Surmeier et al., 2007). Recent formulations further divide these two components into additional subregions (including the cone and the intermediate zone of the NAc shell) (Todtenkopf and Stellar, 2000).

As in the dorsal striatum, GABA-containing medium spiny neurons (MSNs) make up the vast majority (~90–95%) of cells in the NAc, with the remaining cells being cholinergic and GABAergic interneurons (Merched, 1999). Striatal regions contain subpopulations of these MSNs: those of so-called “direct” and “indirect” pathways (Gerfen et al., 1990; Surmeier et al., 2007). The MSNs of the direct pathway predominantly co-express dopamine D1-like receptors and the endogenous opioid peptide dynorphin, and project directly back to the midbrain (substantia nigra/VTA). In contrast, the MSNs of the indirect pathway predominantly co-express dopamine D2-like receptors and the endogenous opioid peptide enkephalin, and project indirectly to the midbrain via areas including the ventral pallidum and the subthalamic nucleus. Traditional formulations posit that dopamine actions at D1-like receptors, which are coupled to the G-protein Gs (stimulatory) and associated with activation of adenylate cyclase, tend to excite the MSNs of the direct pathway (Albin et al., 1989; Surmeier et al., 2007). Elevated activity of these cells would be expected to provide increased GABAergic and dynorphin (an endogenous ligand at k-opioid receptors) input to the mesolimbic system and negative feedback on midbrain dopamine cells. In contrast, dopamine actions at D2-like receptors, which are coupled to Gi (inhibitory) and associated with inhibition of adenylate cyclase, tend to inhibit the MSNs of the indirect pathway (Albin et al., 1989; Surmeier et al., 2007). Inhibition of these cells would be expected to reduce GABAergic and enkephalin (an endogenous ligand at δ-opioid receptors) input to the ventral pallidum, a region that normally inhibits subthalamic cells that activate inhibitory inputs to the thalamus. Through multiple synaptic connections, inhibition of the indirect pathway at the level of the NAc would ultimately activate the thalamus (see Kelley, 2004).

Like neurons throughout the brain, MSNs also express glutamate-sensitive AMPA and NMDA receptors. These receptors enable glutamate inputs from brain areas such as AMG, HIP, and deep (infralimbic) layers of the PFC (O’Donnell and Grace, 1995; Kelley 2004; Grace et al., 2007) to activate NAc MSNs. Dopamine and glutamate inputs can influence one another: for example, stimulation of D1-like receptors can trigger phosphorylation of glutamate (AMPA and NMDA) receptor subunits, thereby regulating their surface expression and subunit composition (Snyder et al., 2000; Chao et al., 2002; Mangiavacchi and Wolf, 2004; Chartoff et al., 2006; Hallett et al., 2006; Sun et al., 2008). Thus the NAc is involved in a complex integration of excitatory glutamate inputs, sometimes excitatory dopamine (D1-like) inputs, and sometimes inhibitory dopamine (D2-like) inputs. Considering that VTA tends to have a uniform response-activation to both rewarding (e.g., morphine; see Di Chiara and Imperato, 1988; Leone et al., 1991; Johnson and North, 1992) and aversive (Dunn, 1988; Herman et al., 1988; Kalivas and Duffy, 1989; McFarland et al., 2004) stimuli, the ability of the NAc to integrate these excitatory and inhibitory signals downstream of mesolimbic dopamine neurons likely plays a key role in attaching valence and regulating mood.

3. Role of the NAc in rewarding states

It is well accepted that the NAc plays a key role in reward. Theories about its role in motivation have been a critical element in our understanding of addiction (e.g., Wise and Bozarth, 1987; Wise and Rompré, 1989). There are three primary lines of evidence implicating the NAc in reward, involving pharmacological, molecular, and electrophysiological approaches.

3.1. Pharmacological evidence

It is well established that drugs of abuse (Di Chiara and Imperato, 1988) and natural rewards (Fibiger et al., 1992; Pfaus, 1999; Kelley, 2004) have the common action of elevating extracellular concentrations of dopamine in the NAc. Moreover, lesions of the NAc reduce the rewarding effects of stimulants and opiates (Roberts et al., 1980; Kelsey et al., 1989). Pharmacology studies in rats (e.g., Caine et al., 1999) and monkeys (e.g., Caine et al., 2000) suggest that D2-like receptor function plays a critical role in reward. However, studies involving the direct microinfusion of drugs into the NAc have provided the strongest evidence for its role in rewarding states. For example, rats will self-administer the dopamine releasing agent amphetamine directly into the NAc (Hoebel et al., 1983), demonstrating the reinforcing effects elevating extracellular dopamine in this region. Rats will also self-administer the dopamine reuptake inhibitor cocaine into the NAc, although this effect is surprisingly weak in comparison to that reported with amphetamine (Carlezon et al., 1995). This observation has led to speculation that the rewarding effects of cocaine are mediated outside the NAc, in areas including the olfactory tubercle (Ikemoto, 2003). However, rats will avidly self-administer the dopamine reuptake inhibitor nomifensine into the NAc (Carlezon et al., 1995), suggesting that the local anesthetic properties of cocaine complicate studies in which the drug is applied directly to neurons. Co-infusion of the dopamine D2-selective antagonist sulpiride attenuates intracranial self-administration of nomifensine, demonstrating a key role for D2-like receptors in the rewarding effects intra-NAc microinfusions of this drug. When considered together with evidence from a variety of other studies (for review, see Wise and Rompré, 1989), these studies are entirely consistent with theories prevailing in the 1980s that dopamine actions in the NAc play a necessary and sufficient role in reward and motivation.

While there is little controversy that dopamine actions in the NAc are sufficient for reward, other work began to challenge the notion that they are necessary. For example, rats will self-administer morphine directly into the NAc (Olds, 1982), away from the trigger zone (the VTA) in which the drug acts to elevate extracellular dopamine in the NAc (Leone et al., 1991; Johnson and North, 1992). Considering that μ- and δ-opioid receptors are located directly on NAc MSNs (Mansour et al., 1995), these data were the first to suggest that reward can be triggered by events occurring in parallel with (or downstream of) those triggered by dopamine. Rats will also self-administer phenylcyclidine (PCP), a complex drug that is a dopamine reuptake inhibitor and a non-competitive NMDA antagonist, directly into the NAc (Carlezon and Wise, 1996). Two lines of evidence suggest that this effect is not dopamine-dependent. First, intracranial self-administration of PCP is not affected by co-infusion of the dopamine D2-selective antagonist sulpiride; and second, rats will self-administer other non-competitive (MK-801) or competitive (CPP) NMDA antagonists with no direct effects on dopamine systems directly into the NAc (Carlezon and Wise, 1996). These data provided early evidence that blockade of NMDA receptors in the NAc would accelerate the pace of drug discovery, medication development, and recovery from addiction.
is sufficient for reward and, by extension, reward can be dopamine-independent. Blockade of NMDA receptors would be expected to produce an overall reduction in the excitability of NAC MSNs without affecting baseline excitatory input mediated by AMPA receptors (Uchimura et al., 1989; Pennartz et al., 1990). Importantly, rats also self-administered NMDA antagonists into deep layers of the PFC (Carlezon and Wise, 1996), which project directly to the NAc (see Kelley, 2004) and have been conceptualized as a part of an inhibitory (STOP!) motivational circuit (Childress, 2006). When considered together, these studies provide two critical pieces of evidence that have played a prominent role in the formulation of our current working hypothesis: first, that dopamine-dependent reward is attenuated by blockade of D2-like receptors, which are inhibitory receptors expressed predominately in the NAc on the MSNs of the indirect pathway; and second, that events that would be expected to reduce the overall excitability of the NAc (e.g., stimulation of G coupled opioid receptors, reduced stimulation of excitatory NMDA receptors, reduced excitatory input) are sufficient for reward. This interpretation led to the development of a model of reward in which the critical event is reduced activation of MSNs in the NAc (Carlezon and Wise, 1996).

Other pharmacological evidence supports this theory, and implicates calcium (Ca ) and its second messenger functions. Activated NMDA receptors gate Ca , an intracellular signaling molecule that can affect membrane depolarization, neurotransmitter release, signal transduction, and gene regulation (see Carlezon and Nestler, 2002; Carlezon et al., 2005). Microinjection of the L-type Ca antagonist diltiazem directly into the NAc increases the rewarding effects of cocaine (Chartoff et al., 2006). The mechanisms by which diltiazem-induced alterations in Ca influx affect reward are unknown. One possibility is that blockade of Ca influx through voltage-operated L-type channels reduces the firing rate of neurons within the ventral NAc (Cooper and White, 2000). It is important to note, however, that diltiazem alone was not rewarding, at least at the doses tested in these studies. This might indicate that baseline levels of Ca influx via L-type channels within the NAc are normally low, and difficult to reduce further. A related possibility is that microinjection of diltiazem reduces aversive actions of cocaine that are mediated within the NAc, unmasking reward. For example, activity of the transcription factor cAMP response element binding protein (CREB) within the NAc is associated with aversive states and reductions in cocaine reward (Pliaakis et al., 2001; Nestler and Carlezon, 2006). The activation of CREB depends on phosphorylation, which can occur via activation of L-type Ca channels (Rajadhyaksha et al., 1999). Phosphorylated CREB can induce expression of dynorphin, a neuropeptide that might contribute to aversive states via activation of k-opioid receptors in the NAc (for review, see Carlezon et al., 2005). The potential role of intr-NAC Ca in regulating rewarding and aversive states is a common theme in our work that will be explained in greater detail below.

3.2. Molecular evidence

Mice lacking dopamine D2-like receptors have reduced sensitivity to the rewarding effects of cocaine (Welter et al., 2007). Ablation of D2-like receptors also reduces the rewarding effects of morphine (Maldonado et al., 1997) – presumably by reducing the ability of the drug to stimulate dopamine via VTA mechanisms (Leone et al., 1991; Johnson and North, 1992) – and lateral hypothalamic brain stimulation (Elmer et al., 2005). One interpretation of these findings is that loss of D2-like receptors in the NAc reduces the ability of dopamine to inhibit the indirect pathway, a putative mechanism of reward. These findings, when combined with evidence that human addicts have reduced dopamine D2-like receptor binding in the NAc, suggest that this receptor plays an essential role in encoding reward (Volkow et al., 2007).

Other advances in molecular biology have enabled the detection of neuroadapative responses to drugs of abuse and the ability to mimic such changes in discrete brain areas to examine their significance. One such change is in the expression of AMPA-type glutamate receptors, which are expressed ubiquitously in the brain and composed of various combinations of the receptor subunits GluR1–4 (Hollmann et al., 1991; Malinow and Malenka, 2002). Drugs of abuse can alter GluR expression in the NAc. For example, repeated intermittent exposure to cocaine elevates GluR1 expression in the NAc (Churchill et al., 1999). Furthermore, GluR2 expression is elevated in the NAc of mice engineered to express ΔFosB, a neuroadaptation linked with increased sensitivity to drugs of abuse (Kelz et al., 1999). Studies in which viral vectors were used to elevate GluR1 selectively in the NAc indicate that this neuroadaptation tends to make cocaine aversive in place conditioning tests, whereas elevated GluR2 in the NAc increases cocaine reward (Kelz et al., 1999). Potential explanations for this pattern of findings likely involve Ca and its effect on neuronal activity and intracellular signaling. Increased GluR1 expression favors formation of GluR1–homomeric (or GluR1–GluR3 heteromeric) AMPARs, which are Ca permeable (Hollmann et al., 1991; Malinow and Malenka, 2002). In contrast, GluR2 contains a motif that prevents Ca influx; thus increased expression of GluR2 would favor formation of GluR2-containing Ca impermeable AMPARs (and theoretically decrease the number of Ca-permeable AMPARs). Thus GluR2-containing AMPARs have physiological properties that render them functionally distinct from those lacking this subunit, particularly with respect to their interactions with Ca (Fig. 1).

These early studies involved place conditioning studies, which generally require repeated exposure to drugs of abuse and presumably involve cycles of reward and aversion (withdrawal). More recent studies examined how alterations in GluR expression modeling those acquired through repeated drug exposure affect intracranial self-stimulation (ICSS), an operant task in which the magnitude of the reinforcer (brain stimulation reward) is precisely controlled (Wise, 1996). Elevated expression of GluR1 in NAc shell increases ICSS thresholds, whereas elevated GluR2 decreases them (Todtenkopf et al., 2006). The effect of GluR2 on ICSS is qualitatively similar to that caused by drugs of abuse (Wise, 1996), suggesting...
that it reflects increases in the rewarding impact of the stimulation. In contrast, the effect of GluR1 is qualitatively similar to that caused by prodepressive treatments including drug withdrawal (Markou et al., 1994) and κ-opioid receptor agonists (Pfeiffer et al., 1986; Wadenberg, 2003; Todtenkopf et al., 2004; Carlezon et al., 2006), suggesting that it reflects decreases in the rewarding impact of the stimulation. These findings indicate that elevated expression of GluR1 and GluR2 in NAc shell have markedly different consequences on motivated behavior. Moreover, they confirm previous observations that elevated GluR1 and GluR2 expression in NAc shell have opposite effects in cocaine place conditioning studies (Kelz et al., 1999), and extend the generalizability of these effects to behaviors that are not motivated by drugs of abuse. Perhaps most importantly, they provide more evidence to implicate Ca\(^{2+}\) flux within the NAc in reduced reward or elevated aversion. Because Ca\(^{2+}\) plays a role in both neuronal depolarization and gene regulation, alterations in GluR expression and AMPAR subunit composition in NAc shell likely initiate physiological and molecular responses, which presumably interact to alter motivation. Again, the mechanisms by which Ca\(^{2+}\) signal transduction might trigger genes involved in aversive states are described in detail below.

3.3. Electrophysiological evidence

Several lines of electrophysiological investigation support the idea that decreases in NAc firing may be related to reward. First, rewarding stimuli produce NAc inhibitions in vivo. Second, neurobiological manipulations that specifically promote inhibition of NAc firing appear to enhance rewarding effects of stimuli. Third, the inhibition of NAc GABAergic MSNs can disinhibit downstream structures such as the ventral pallidum to produce signals related to the hedonic qualities of stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. During self-administration of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.

The most substantial line of investigation involves studies of NAc single-unit activity in rodent paradigms where a wide variety of drug and non-drug rewards are delivered. A consistent finding across these studies is that the most commonly observed pattern of firing modulation is a transient inhibition. This has been observed during self-administration of many different types of rewarding stimuli. Each of these lines of investigation will be addressed in turn.
Although hypertonic saline solutions are typically aversive taste stimuli, in salt-deprived humans or experimental animals their palatability is increased. Both behavioral measures of positive hedonic response (i.e. facial taste reactivity measures) and increases in palidal neuron firing occurred in response to a hypertonic saline taste stimulus in sodium-deprived animals, but not in animals maintained on a normal diet. Thus, increased firing of palidal neurons, downstream targets of NAc efferents, appears to encode a key feature of reward. Of course, it is possible that other inputs to palidal neurons could contribute to these reward-related firing patterns. However, recent studies have indicated a strong relationship between the ability of μ-opioid receptor activation (a factor which is known to inhibit MSN firing) in discrete regions of the NAc shell to drive increases in behavioral response to a hedonic stimulus and its ability to activate c-fos in discrete regions of ventral pallidum (Smith and Berridge, 2007). This apparently tight coupling between NAc and palidial "hedonic hotspots" is an intriguing new phenomenon that is just beginning to be explored.

4. Role of the NAc in aversive states

The fact that the NAc also plays a role in aversion is sometimes underappreciated. Pharmacological treatments have been used to demonstrate aversion after NAc manipulations. In addition, molecular approaches have demonstrated that exposure to drugs of abuse and stress cause common neuroadaptations that can trigger signs (including anhedonia, dysphoria) that characterize depressive illness (Nestler and Carlezon, 2006), which is often co-morbid with addiction and involves dysregulated motivation.

4.1. Pharmacological evidence

Some of the earliest evidence that NAc plays a role in aversive states came from studies involving opioid receptor antagonists. Microinjections of a wide-spectrum opioid receptor antagonist (methyl-naloxononium) into the NAc of opiate-dependent rats establish conditioned place aversions (Stinus et al., 1990). In opiate-dependent rats, precipitated withdrawal can induce immediate-early genes and transcription factors in the NAc (Gracy et al., 2001; Chartoff et al., 2006), suggesting activation of MSNs. Selective κ-opioid agonists, which mimic the effects of the endogenous κ-opioid ligand dynorphin, also produce aversive states. Microinjections of a κ-opioid agonist into the NAc cause conditioned place aversions (Bals-Kubik et al., 1993) and elevate ICSS thresholds (Chen et al., in press). Inhibitory (G<sub>i</sub>-coupled) κ-opioid receptors are localized on the terminals of VTA dopamine inputs to the NAc (Swingos et al., 1999), where they regulate local dopamine release. As such, they are often in apposition to μ- and δ-opioid receptors (Mansour et al., 1995), and stimulation produces the opposite effects of agonists at these other receptors in behavioral tests. Indeed, extracellular concentrations of dopamine are reduced in the NAc by systemic (Di Chiara and Imperato, 1988; Carlezon et al., 2006) or local microinfusions of κ-opioid agonist (Donzanti et al., 1992; Spanagel et al., 1992). Decreased function of midbrain dopamine systems has been associated with depressive states including anhedonia in rodents (Wise, 1982) and dysphoria in humans (Mizrachi et al., 2007). Thus one path to aversion appears to be reduced dopamine input to the NAc, which would reduce the stimulation of inhibitory dopamine D2-like receptors that seem critical for reward (Carlezon and Wise, 1996).

Other studies appear to confirm an important role of dopamine D2-like receptors in suppressing aversive responses. Microinjections of a dopamine D2-like antagonist into the NAc of opiate-dependent rats precipitate signs of somatic opiate withdrawal (Harris and Aston-Jones, 1994). Although the motivational effects were not measured in this study, treatments that precipitate opiate withdrawal often cause aversive states more potently than they cause somatic signs of withdrawal (Gracy et al., 2001; Chartoff et al., 2006). Interestingly, however, microinjections of a dopamine D1-like agonist into the NAc also produce somatic signs of withdrawal in opiate-dependent rats. The data demonstrate that another path to aversion is increased stimulation of excitatory dopamine D1-like receptors in rats with opiate-dependence induced neuroadaptations in the NAc. Perhaps not surprisingly, one consequence of D1-like receptor stimulation in opiate-dependent rats is phosphorylation of GluR1 (Chartoff et al., 2006), which would lead to increased surface expression of AMPA receptors on the MSNs of the direct pathway.

4.2. Molecular evidence

Exposure to drugs of abuse (Turgeon et al., 1997) and stress (Pliaakis et al., 2001) activate the transcription factor CREB in the NAc. Viral vector-induced elevation of CREB function in the NAc reduces the rewarding effects of drugs (Carlezon et al., 1998) and hypothalamic brain stimulation (Parsegian et al., 2006), indicating anhedonia-like effects. It also makes low doses of cocaine aversive (a putative sign of dysphoria), and increases immobility behavior in the forced swim test (a putative sign of "behavioral despair") (Pliaakis et al., 2001). Many of these effects can be attributed to CREB-regulated increases in dynorphin function (Carlezon et al., 1998). Indeed, κ-opioid receptor-selective agonists have effects that are qualitatively similar to those produced by elevated CREB function in the NAc, producing signs of anhedonia and dysphoria in reward models and increased immobility in the forced swim test (Bals-Kubik et al., 1993; Carlezon et al., 1998, 2006; Pliaakis et al., 2001; Mague et al., 2003). In contrast, κ-selective antagonists produce an antidepresant-like phenotype resembling that seen in animals with disrupted CREB function in the NAc (Pliaakis et al., 2001; Newton et al., 2002; Mague et al., 2003). These findings suggest that one biologically important consequence of drug- or stress-induced activation of CREB within the NAc is increased transcription of dynorphin, which triggers key signs of depression. Dynorphin effects are likely mediated via stimulation of κ-opioid receptors that act to inhibit neurotransmitter release from mesolimbic dopamine neurons, thereby reducing the activity VTA neurons, as explained above. This path to aversion appears to be reduced dopamine input to the NAc, which would produce reductions in the stimulation of inhibitory dopamine D2-like receptors that seem critical for reward (Carlezon and Wise, 1996). As explained below, there is also evidence that elevated expression of CREB in the NAc directly increases the excitability of MSNs (Dong et al., 2006) in addition to the loss of D2-regulated inhibition, raising the possibility that multiple effects contribute to the aversive responses.

Repeated exposure to drugs of abuse can elevate GluR1 expression in the NAc (Churchill et al., 1999). Viral vector-induced elevation of elevated GluR1 in the NAc increases drug aversion in place conditioning studies, an “atypical” type of drug sensitization (i.e., heightened sensitivity to the aversive rather than the rewarding aspects of cocaine). This treatment also increases ICSS thresholds (Todtenkopf et al., 2006), indicating anhedonia-like and dysphoria-like effects. Interestingly, these motivational effects are virtually identical to those caused by elevated CREB function in the NAc. These similarities raise the possibility that both effects are part of the same larger process. In one possible scenario, drug exposure might trigger changes in the expression of GluR1 in the NAc, which would lead to local increases in the surface expression of Ca<sup>2+</sup>-permeable AMPA receptors, which would increase Ca<sup>2+</sup> influx and activate CREB, leading to alterations in sodium and potassium channel expression that affect baseline and stimulated excitability of MSNs in the NAc (Carlezon and Nestler, 2002; Carlezon et al.,
place preference response that control animals show to the same exhibit a conditioned place response to a given depolarizing current pulse (Dong et al., 2006). For example, viral-mediated overexpression neurons can shift the behavioral response of a stimulus from rewarding to aversive. Although there has been little electrophysiological investigation of the hypothesis that widespread excitation of NAc neurons encodes information about aversive stimuli, the available data essentially mirror those for rewarding stimuli. First, two recent studies using aversive taste stimuli both indicate that three times as many NAc neurons respond to the stimuli with clear excitations as inhibitions (Roitman et al., 2005; Wheeler et al., 2008). Interestingly, these same studies find that units that respond to a sucrose or saccharin reward show the exact opposite profile: three times more cells with decreases in firing than those with increases. In addition, when an initially rewarding saccharin stimulus was made aversive by pairing it with the opportunity to self-administer cocaine, the predominant firing pattern of NAc units that responded to the stimulus shifted from inhibition to excitation (Wheeler et al., 2008). Thus, not only does this demonstrate that NAc may encode aversive states in firing increases, but that individual NAc neurons can track the hedonic valence of a stimulus by varying their firing-rate response to it.

Second, molecular genetic manipulations of synaptic and intrinsic membrane properties that increase the excitability of NAc neurons can shift the behavioral response of a stimulus from rewarding to aversive. For example, viral-mediated overexpression of CREB in NAc produces an increase in neuronal excitability in MSNs as indicated by an increase in the number of spikes in response to a given depolarizing current pulse (Dong et al., 2006). Under these conditions of enhanced NAc excitability, animals exhibit a conditioned place aversion to cocaine, rather than the place preference response that control animals show to the same dose (Pliakas et al., 2001). In addition, they exhibit increased depressive-like behaviors in forced swim test (Pliakas et al., 2001) and learned-helplessness paradigm (Newton et al., 2002). Another molecular manipulation that produces a similar behavioral phenotype is the overexpression of the AMPAR subunit GluR1 in NAc (Kelz et al., 1999; Todtenkopf et al., 2006). Although it is has not yet been confirmed by electrophysiological study, this GluR1 overexpression is likely to produce an enhancement of synaptic excitability in NAC MSNs. Not only may this occur through the insertion of additional AMPARs in the membrane in general, but the abundance of GluR1 could potentially lead to the formation of GluR1-homomeric receptors, which are known to have a larger single-channel conductance (Swanson et al., 1997) and thus contribute even further to enhanced excitability.

Third, if NAc firing is elevated during aversive conditions, downstream targets should be suppressed via GABA release from MSNs during these conditions as well. Ventral pallidal unit recordings show very low firing rates following oral infusion of hypertonic saline – a taste stimulus that under normal physiological circumstances is aversive (Tindell et al., 2006). Although clearly more work with aversive stimuli of different modalities is needed to make any firm conclusions, the present data are consistent with the possibility that enhanced firing of NAc neurons during aversive conditions may suppress pallidal neuron firing as part of the process of encoding the unpleasant nature of a stimulus.

4.3. Electrophysiological evidence

4.3.1. Electrophysiological correlates of NAc firing

According to this model (Fig. 2), NAc neurons tonically inhibit reward-related processes. Under normal circumstances, excitatory influences mediated by glutamate actions at AMPA and NMDA receptors or dopamine actions at D1-like receptors are balanced by inhibitory dopamine actions at D2-like receptors. Treatments that would be expected to reduce activity in the NAc – including cocaine (Peoples et al., 2007), morphine (Olds 1982), NMDA antagonists (Carlezon and Wise, 1996), L-type Ca²⁺ antagonists (Chartoff et al., 2006), palatable food (Wheeler et al., 2008) and expression of dominant-negative CREB (Dong et al., 2006) – have reward-related effects because they reduce the inhibitory influence of the NAc on downstream reward pathways. In contrast, treatments that activate the NAc by amplifying glutamatergic inputs (e.g., elevated expression of GluR1; Todtenkopf et al., 2006), altering ion channel function (e.g., elevated expression of CREB: Dong et al., 2006), reducing inhibitory dopamine inputs to D2-like cells (e.g., k-opioid receptor agonists), or blocking inhibitory µ- or δ-opioid receptors (West and Wise, 1988; Weiss, 2004) are perceived as aversive because they increase the inhibitory influence of the NAc on downstream reward pathways. Interestingly, stimuli such as drugs of abuse may induce homeostatic (or allostatic) neuroadaptations that persist beyond the treatment and cause baseline shifts in mood. Such shifts may be useful in explaining co-morbidity of addiction and psychiatric illness (Kessler et al., 1997): repeated exposure to drugs that reduce the activity of NAc neurons might induce compensatory neuroadaptations that render the system more excitable during abstinence (leading to conditions characterized by anhedonia or dysphoria), whereas repeated exposure to stimuli (e.g., stress) that activate the NAc might induce compensatory neuroadaptations that render the system more susceptible to the inhibitory actions of drugs of abuse, increasing their appeal. This working hypothesis is testable through a variety of increasingly sophisticated approaches.

4.1. Testing the hypothesis with electrophysiology

One caveat to the inhibition–reward hypothesis is that widespread and prolonged inhibition of NAc firing, as in inactivation or lesion studies, does not appear to produce rewarding effects (e.g., Yun et al., 2004b). This raises the possibility that it is not inhibition of the NAc, per se, that encodes reward but rather the transitions from normal basal firing rates to lower rates that occur when rewarding stimuli are present. Prolonged inhibition may degrade the dynamic information normally encoded in the transient depressions of NAc firing.

Electrophysiology-based tests of the predictions of this hypothesis fall into two basic categories. The first category involves manipulating an animal’s behavioral state to produce sustained changes in responsivity to rewarding stimuli followed by testing for electrophysiological correlates of this altered reward state. For example, the early withdrawal state from chronic exposure to psychostimulants is characterized by anhedonia and lack of responsiveness to natural rewarding stimuli. What would the inhibition–reward hypothesis predict about the electrophysiological status of NAc neurons during this state? The major prediction is that NAc neurons would exhibit decreases in the activity suppression normally produced by exposure to a rewarding stimulus (e.g., sucrose). To our knowledge, this has not yet been investigated. Possible mechanisms for such a decrease in inhibition, should it occur, might include overall increases in neuronal excitability produced by any combination of changes in intrinsic excitability (e.g., increased Na⁺ or Ca²⁺ currents, decreased K⁺ currents) or synaptic transmission (e.g., decreases in glutamatergic or increases in GABAergic transmission). On the other hand, the available data on NAc MSN excitability during early psychostimulant withdrawal suggest that it is actually decreased during this phase (Zhang et al., 1998; Hu et al. 2004; Dong et al., 2006; Kourrich et al., 2007). As
noted above, it is possible that a prolonged depression in excitability may degrade reward-related information contained in transient firing inhibitions, perhaps by creating a "floor" effect and reducing the magnitude of these inhibitions. This possibility remains to be tested.

Considering the apparent link between NAc and ventral pallidum in reward encoding (see above), we would predict that any excitability changes produced by sustained modulation of an animal's reward state might be particularly evident in striatopallidal/D2 neurons. Although studying the detailed physiological properties of these neurons has been difficult in the past, the recent development of a line of BAC transgenic mice that expresses GFP in these neurons (Gong et al., 2003; Lobo et al., 2006) has made it possible to visualize them in in vitro slice preparations, greatly facilitating the potential for physiological characterization of D2 cells.

The second category of electrophysiology-based tests involves using genetic engineering (see below) to alter the functional expression of key components of the cellular machinery for excitability or excitability modulation in NAc neurons. In theory, this could enable modulation of the inhibitions or excitations associated with reward or aversion, respectively, in NAc neurons. With this in mind, perhaps the most useful target molecules would be those that participate in activity-dependent modulation of neuronal excitability, rather than in maintaining basal firing rates. These targets would likely provide a better opportunity to modulate stimuli responsiveness than more general targets (e.g., Na+ channel subunits), thus enabling the evaluation of the inhibition-reward hypothesis. For example, the firing frequency of active neurons can be controlled by various ionic conductances that produce spike after-hyperpolarizations (AHPs). By targeting NAc

---

*Fig. 2.* Schema depicting a simple working hypothesis of how the nucleus accumbens (NAc) may regulate rewarding and aversive states. (a) NAc neurons tonically inhibit reward-related processes. Under normal circumstances, there is a balance between cortical (PFC, AMG) excitatory (+) influences mediated by glutamate actions at AMPA and NMDA receptors, and midbrain (VTA) inhibitory (−) influences mediated by dopamine actions at D2-like receptors. NAc neurons have low baseline rates of firing, and depolarization-mediated influx of Ca2+ through NMDA receptors and calcium channels is not sufficient to alter gene expression. (b) Depolarization of NAc neurons containing D2-like receptors and enkephalin inhibits downstream areas implicated in reward (e.g., ventral pallidum) which is encoded as aversion. It also leads to elevated Ca2+ influx, which may trigger experience-dependent neuroadaptations (e.g., activation of CREB, elevated expression of GluR1) can produce feed-forward effects that dysregulate the system. AMG, amygdala; Ca, calcium channel; Ca2+; calcium; CREB, cAMP response element binding protein; D2, dopamine D2-like receptor; DA, dopamine; ENK, enkephalin; GluR1/G2, AMPA glutamate receptor-containing GluR1 and GluR2; GABA, gamma-aminobutyric acid; GLU, glutamate; N, NMDA receptor; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.
neurons with genetic (or possibly even pharmacologic) manipulation aimed at the channels that produce AHPs, it may be possible to decrease the magnitude of aversion-related excitatory responses in these neurons and thus to test whether this physiological change correlates with reduced behavioral indices of aversion.

5.2. Testing the hypothesis with behavioral pharmacology

One of the most obvious pharmacological tests would be to determine if rats self-administer dopamine D2-like agonists directly into the NAc. Interestingly, previous work indicates that while rats self-administer combinations of D1-like and D2-like agonists into the NAc, they do not self-administer either drug component alone, at least at the doses tested (Ikemoto et al., 1997). While on the surface this finding might appear to invalidate our working hypothesis, electrophysiological evidence suggests that co-activation of D1 and D2 receptors on NAc neurons can, under some conditions, cause a reduction in their membrane excitability that is not seen in response to either agonist alone (O’Donnell and Grace, 1996). In addition, more work is needed to study the behavioral effects of intra-NAc microinfusions of GABA agonists; historically, this work has been hindered by poor solubility of benzodiazepines – which are known to be addictive (Griffiths and Ator, 1980) despite their tendency to decrease dopamine function in the NAc (Wood, 1982; Finlay et al., 1992; Muri et al., 1994) – and the relatively small number of researchers who use brain microinjection procedures together with models of reward. Still other ways of testing our hypothesis would be to study the effects of manipulations in brain areas downstream of D2 receptor-containing MSNs. Again, early evidence suggests reward is encoded by activation of the ventral pallidum, a presumed consequence of inhibition of the MSNs of the indirect pathway (Tindell et al., 2006).

5.3. Testing the hypothesis with genetic engineering

The development of genetic engineering techniques that enable the direction of inducible or conditional mutations to specific brain areas will be an important tool with which to test our hypotheses. Mice with constitutive deletion of GluR1 (an alternative nomenclature for GluR1) show many alterations in sensitivity to drugs of abuse (Vekovischeva et al., 2001; Dong et al., 2004; Mead et al., 2005, 2007), some of which are consistent with our working hypothesis and some of which are not. The loss of GluR1 early in development could dramatically alter responsiveness to numerous types of stimuli, including drugs of abuse. In addition, these GluR1-mutant mice lack the protein throughout the brain, whereas the research reviewed here focuses on mechanisms that occur within NAc. These points are especially important because loss of GluR1 in other brain regions would be expected to have dramatic, and sometimes very different, effects on drug abuse-related behaviors. As just one example, we have shown that modulation of GluR1 function in the VTA exerts the opposite effect on drug responses compared to modulation of GluR1 in the NAc (Carlezon et al., 1997; Kelz et al., 1999). The findings in GluR1-deficient mice are not inconsistent with the combined findings from the NAc and the VTA: constitutive GluR1-mutant mice are more sensitive to the stimulant effects of morphine (an effect that could be explained by the loss of GluR1 in the NAc), but they do not develop progressive increases in responsivity to morphine (an effect that could be explained by the loss of GluR1 in the VTA) testing occurs under conditions that promote sensitization and involve additional brain regions. Accordingly, one must be cautious in assigning spatial and temporal interpretations to data from constitutive knockout mice: the literature is becoming replete with examples of proteins that have dramatically different (and sometimes opposite) effects on behavior depending upon the brain regions under study (see Carlezon et al., 2005).

Preliminary studies from mice with inducible expression of a dominant-negative form of CREB – a manipulation which reduces the excitability of NAc MSNs – are hypersensitive to the rewarding effects of cocaine while being insensitive to the aversive effects of a κ-opioid agonist (DiNieri et al., 2006). Although these findings are consistent with our working hypothesis, further studies (e.g., electrophysiology) might help to characterize the physiological basis of these effects. Regardless, an increased capacity to spatially and temporally control the expression of genes that regulate the excitability of NAc MSNs will enable progressively more sophisticated tests of our working hypothesis.

5.4. Testing the hypothesis with brain imaging

Functional brain imaging has the potential to revolutionize our understanding of the biological basis of rewarding and aversive mood states in animal models and, ultimately, people. Preliminary

---

**Fig. 3.** Intravenous infusions of the μ-opioid agonist fentanyl and the κ-opioid agonist U69,593 induce overlapping but anatomically selective blood-oxygen level-dependent functional MRI (BOLD fMRI) responses in alert male cynomolgus monkeys (N = 3). Results are from duplicate scans with each drug condition in each subject. Fentanyl induced positive BOLD fMRI responses in the caudate nucleus and bilaterally in putamen, amygdala, and insula. An equipotent dose of U69,593 induced positive BOLD fMRI responses in caudate nucleus and bilateral insula, but also induced positive responses in bilateral nucleus accumbens (NAc) and ventral striatum. Crosshairs are centered on the NAc (from Kaufman et al., in preparation; used with permission).
data from imaging studies involving alert non-human primates are providing early evidence in support of the working hypothesis described above. Intravenous administration of high doses of the κ-opioid agonist U69,593 – which belongs to a class of drugs known to cause aversion in animals (Bals-Kubik et al., 1993; Carlezon et al., 2006) and dysphoria in humans (Pfeiffer et al., 1986; Wadenberg, 2003) – causes profound increases in blood-oxygen level-dependent (BOLD) functional MRI responses in the NAc (Fig. 3: from Kaufman et al., unpublished observations; used with permission). To the extent that BOLD signal responses reflect synaptic activity, the positive BOLD response induced by U69,593 in the NAc is consistent with increased activity of MSNs, perhaps due to decreased dopamine input (Di Chiara and Imperato, 1988; Carlezon et al., 2006). In contrast, positive BOLD signal responses are conspicuously absent in the NAc after treatment with an equipotent dose of fentanyl, a highly addictive μ-opioid agonist. While these fentanyl data do not indicate inhibition of the NAc per se, absence of BOLD activity in this region is not inconsistent with our working hypothesis. Clearly, additional pharmacological and electrophysiological studies are needed to characterize the meaning of these BOLD signal changes. The development of higher magnetic field strength systems is beginning to provide early evidence in support of the working hypothesis described above. Intravenous administration of high doses of the κ-opioid agonist U50,488 on intracranial self-stimulation in rats and mice, opening the door to a more detailed understanding of BOLD signals and underlying brain function.

6. Conclusions

We propose a simple model of mood in which reward is encoded by reduced activity of NAc MSNs, whereas aversion is encoded by increased activity of MSNs, which may decrease sensitivity to natural rewards and exacerbate the addiction cycle (Volkow et al., 2007). The continued development of molecular and brain imaging techniques is establishing a research environment that is conducive to the design of studies that have the power to confirm or refute this model. Regardless, a better understanding of the molecular basis of these mood states is perpetually important and relevant, particularly as accumulated knowledge from decades of research is used to develop innovative approaches that might be used to treat and prevent addiction and other conditions (e.g., mood disorders) associated with dysregulation of motivation.

Acknowledgements

Funded by the National Institute on Drug Abuse (NIDA) grants DA012736 (to WAC) and DA019666 (to MJT) and a McKnight-Land Grant professorship (to MJT). We thank M.J. Kaufman, B. de Fredrick, and S.S. Negus for permission to cite unpublished data from their brain imaging studies in monkeys.

References

Hallett, P.J., Spoelgen, R., Hyman, B.T., Standaert, D.G., Dunah, A.W., 2006. Dopamine
Goeders, N.E., Smith, J.E., 1983. Cortical dopaminergic involvement in cocaine
Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr., F.J.,
Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma Jr., F.J.,
Gong, S., Zheng, C., Dong, M., Lusio, K., Kudnikovic, N., Schambra, U.B.,


