JOURNAL OF THE EXPERIMENTAL ANALYSIS OF BEHAVIOR 2005, **84,** 653–666 NUMBER 3 (NOVEMBER)

NEURONAL SUBSTRATES OF RELAPSE TO COCAINE-SEEKING BEHAVIOR: ROLE OF PREFRONTAL CORTEX

GEORGE V. REBEC AND WENLIN SUN

INDIANA UNIVERSITY

The return to drug seeking, even after prolonged periods of abstinence, is a defining feature of cocaine addiction. The neural circuitry underlying relapse has been identified in neuropharmacological studies of experimental animals, typically rats, and supported in brain imaging studies of human addicts. Although the nucleus accumbens (NAcc), which has long been implicated in goal-directed behavior, plays a critical role in this circuit, the prefrontal cortex (PFC) appears to process the events that directly trigger relapse: exposure to acute stress, cues previously associated with the drug, and the drug itself. In this paper, we review animal models of relapse and what they have revealed about the mechanisms underlying the involvement of the NAcc and PFC in cocaine-seeking behavior. We also present electrophysiological data from PFC illustrating how the hedonic, motor, motivational, and reinforcing effects of cocaine can be analyzed at the neuronal level. Our preliminary findings suggest a role for PFC in processing information related to cocaine seeking but not the hedonic effects of the drug. Further use of this recording technology can help dissect the functions of PFC and other components of the neural circuitry underlying relapse.

Key words: cocaine seeking, prefrontal cortex (PFC), nucleus accumbens (NAcc), drug self-administration, reinstatement, single-unit, electrophysiology, rat

Repeated use of amphetamine, cocaine, and related psychomotor stimulants can elicit long-lasting changes in behavior. Evidence of this effect in human addicts is a high rate of relapse, which often occurs months or years after the last use. In fact, relapse *prevention* is perhaps the single biggest challenge to successful treatment of stimulant addiction. Reaching this goal requires an understanding of the neurobiological substrates of addictive behavior. Excellent progress has come from research on the brain circuits underlying the reinstatement of cocaine seeking in experimental animals, typically rats (for review, see Kalivas & McFarland, 2003). In this report, we describe the reinstatement model and what it has revealed about the role of prefrontal cortex (PFC) in relapse to cocaine seeking. Although most research on the brain substrates of reinstatement has focused on the nucleus accumbens (NAcc), which has long been linked to appetitive behavior and arousal (LeMoal, 1995), increasing evidence implicates the PFC as the final common pathway by which the stimuli that trigger reinstatement converge to drive behavior (Kalivas & Nakamura, 1999).

At the neuronal level, however, relatively little information is available on how the PFC processes information related to relapse. A powerful tool that can be used to address this issue is extracellular, single-unit recording from awake, unrestrained animals. Not only is the neuronal circuitry intact and functioning normally in this preparation, but with chronically implanted micro-wires, neuronal activity can be recorded when animals are actively seeking and taking the drug during self-administration training as well as during subsequent reinstatement testing. This technology combined with select manipulations of the behavioral protocol can be used to reveal how individual neurons in key brain structures process drug-related information. For example, do these structures process information related to the cues that signal the drug, the drug itself, or the motor sequence required to obtain the drug? To address these questions, we have begun to apply this approach to the study of the relation of PFC circuitry and activity to the reinstatement of cocaine seeking. Our preliminary data, summarized near the end of this paper, lead to interesting predictions about the role of this brain region in relapse to cocaine-seeking behavior.

This research was supported, in part, by NIH (DA02451 and DA012964). We also acknowledge the assistance of Paul E. Langley and Faye Caylor.

Address correspondence to George V. Rebec, Indiana University, 1101 E. Tenth Street., Bloomington, Indiana 47405-7007 (e-mail: rebec@indiana.edu).

doi: 10.1901/jeab.2005.105-04

A BEHAVIORAL MODEL OF REINSTATEMENT OF COCAINE SEEKING

The reinstatement of drug-seeking behavior in rats is widely used as a model of relapse in human addicts. In this model, rats are first trained to self-administer drugs by pressing a lever for an intravenous (IV) drug infusion in an operant conditioning chamber. After this behavior is eliminated by extinction training, rats are tested for their ability to reinstate drug-seeking behavior (lever pressing) in response to a priming stimulus: for example, cues previously paired with drug infusion (cue priming) or acute, non-contingent exposure to the drug itself (drug priming). Although human addicts typically do not experience formal extinction training, drug cues and drug re-exposure are widely believed to precipitate a return to drug use, suggesting that the model has good predictive validity (Shalev, Grimm, & Shaham, 2002). In fact, a third condition that can reliably reinstate drug seeking in human addicts, exposure to some stressors, also is effective in experimental animals (Lu, Shepard, Hall, & Shaham, 2003).

Of course, the relationship of the model to clinical realities is not perfect (Bergman & Katz, 1998; Everitt & Robbins, 2000; Marlatt, 1996), and more prospective clinical data are needed to confirm the model's predictive validity (Katz & Higgins, 2003). However, the study of reinstatement can have clinical value even if it does not completely model relapse (Epstein & Preston, 2003). Clinical neuroimaging studies, for example, implicate the same brain circuitry in addictive behavior as biochemical data obtained from experimental animals (Kalivas & Nakamura, 1999). Thus, despite the need for more clinical investigations, the reinstatement model provides a reasonable basis for studying the neuronal mechanisms underlying relapse to drug-seeking behavior.

Several variations of the model have emerged over the years (Shalev et al., 2002). Perhaps the most widely used is the between-session method in which training for drug self-administration, extinction of the drug-reinforced behavior, and reinstatement tests are conducted on different daily sessions (Davis & Smith, 1976). This is the preferred method for inducing relapse long after the phase of acute drug withdrawal has passed. The between-session method also nicely captures two key characteristics of human addiction: relatively high levels of drug exposure and the need to expend some effort to obtain the drug. With this method, however, only a limited number of cue-primed reinstatement tests are possible.

For repeated cue-primed reinstatement testing, a within-sessions method is preferred in which drug self-administration, extinction, and reinstatement testing occur within a single daily session (de Wit & Stewart, 1981). This method, however, does not mimic the conditions of long-term relapse. In fact, rats are never completely drug free at the time of testing. To avoid this problem, extinction and reinstatement testing occur on the same day but after one or more days of drug withdrawal in what has become known as the between-within method (Tran-Nguyen et al., 1998). It is not yet clear, however, that this variation allows for multiple reinstatement tests. Interestingly, the between-sessions design appears adequate for testing cocaine-primed reinstatement; our own studies indicate good responding in rats tested on six or more occasions.

Other animal models have been developed to study the neural substrates of drug-seeking behavior including conditioned-place-preference (Mueller & Stewart, 2000) and runway models of reinstatement (Ettenberg, 1990). In these models, animals are tested in a drug-free state, which minimizes motor-confounding effects. In both cases, however, the amount of drug used during training is much less than that used in a self-administration paradigm. It also is relevant that drugs are passively injected in the conditioned-place-preference model, and passive injections cannot be expected to induce the same neurochemical effects as drugs administered actively via self-administration (Hemby, Co, Koves, Smith, & Dworkin, 1997). Further, neither the conditioned-place-preference nor the runway model allows for measuring repeated operant behavior, which is an integral part of an addict's drug-response pattern. In fact, operant behaviors can develop into low-level habits (stimulus-response connections), which may automatically produce high rates of relapse (McFarland & Kalivas, 2001). Thus, the between-sessions method, with its parallel to human addictive behavior and its ability to permit at least some reinstatement tests, seems the best suited for studying neural mechanisms of addiction.

NEUROANATOMICAL AND NEUROCHEMICAL SUBSTRATES OF REINSTATEMENT

The brain circuitry implicated in the reinstatement of drug-seeking behavior overlaps with many of the structures known to process information related to both movement and reward (Heimer, Alheid, & Zahm, 1993). These structures form the so-called motive circuit, which includes PFC and its downstream target, the NAcc. Both receive dopamine (DA) innervation from the ventral tegmental area (VTA) via the mesocorticolimbic DA pathway. At one time, this pathway was considered the neural substrate of reward but now has assumed a more complex role that includes reward prediction (Schultz, 1998), reward salience (Comoli et al., 2003; Horvitz, 2000; Robinson & Berridge, 2000), and the appetitive, as opposed to consummatory, aspects of reward (Ikemoto & Panksepp, 1999; Salamone, 1996). Most importantly, the release of DA in NAcc and PFC appears to be a critical mechanism by which drugs of abuse, including psychomotor stimulants, gain control over behavioral output (DiChiara, 1998; Wang & McGinty, 1999; White & Kalivas, 1998; Wise, 1998). To appreciate how DA and other transmitters may underlie cocaine-seeking behavior, it is important to understand the basic anatomic organization of the NAcc and PFC and the evidence linking these structures to reinstatement.

Role of the NAcc

Anatomically, the NAcc is divided into several sub-regions. Three have been identified in the rat: core, shell, and rostral pole (Gronenwegen, Wright, Beijer, & Voorn, 1999; Zahm & Brog, 1992). Each receives excitatory glutamate (GLU) projections from corticolimbic structures including the basolateral amygdala (BLA) and the ventral subiculum (vSUB) of the hippocampus as well as the PFC. These inputs, however, are not distributed homogeneously. PFC input to shell, for example, arises from ventral or infralimbic regions, whereas core receives PFC input primarily from dorsal prelimbic and adjacent areas (Brog, Salyapongse, Deutch, & Zahm, 1993). Core and shell also differ in their downstream targets. Output from the core closely resembles basal ganglia circuitry in that the main targets are ventral pallidum, substantia nigra, and subthalamic nucleus (Zahm & Heimer, 1993; Zahm, 1999). Projections from these targets, moreover, travel to motor thalamus and then to cortical motor areas. The shell, in contrast, sends axons mainly to lateral hypothalamus and VTA. In addition, although the shell projects to ventral pallidum, the pattern of innervation does not overlap with that from core. Thus, the NAcc is in a strategic position to integrate corticolimbic information for motor output (LeMoal & Simon, 1991; Mogenson, Jones, & Yim, 1980).

NAcc Core. Consistent with this conclusion, the NAcc appears to play a role in both cue- and drug-primed reinstatement. For drug priming, an active NAcc core seems essential in that temporary inactivation of core blocks reinstatement induced by cocaine (McFarland & Kalivas, 2001). The reinstatement response, moreover, appears to depend on GLU rather than DA transmission. Stimulation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid) subtype of GLU receptor in core, for example, reinstates cocaine-seeking behavior, and this effect cannot be explained by a depolarization-induced inhibition of core activity because temporary inactivation of core fails to reinstate cocaine seeking (Cornish, Duffy, & Kalivas, 1999). Furthermore, infusion of an AMPA antagonist in core blocks cocaine-primed reinstatement (Cornish & Kalivas, 2000). The NMDA (N-methyl-D-aspartate) receptor subtype also may participate in cocaine-primed reinstatement but the evidence for this role is weak. For example, intracore infusion of an NMDA agonist increases responding on both the drug-paired and inactive levers suggesting a general activating effect on behavior. Furthermore, direct application of an NMDA antagonist in core fails to block cocaine-primed reinstatement (Cornish & Kalivas, 2000).

Although activation of core DA receptors can reinstate cocaine-seeking behavior (Cornish & Kalivas, 2000; Self et al., 1998), cocaine-primed reinstatement persists after blockade of these receptors with fluphenazine, a nonselective DA antagonist (Cornish & Kalivas, 2000). It also is interesting that intra-core fluphenazine blocks DA- but not AMPA-induced cocaine seeking, whereas an AMPA antagonist in core blocks both DA- and AMPA-induced reinstatement responding. Mi-

crodialysis studies further support GLU involvement. Thus, cocaine-primed reinstatement is accompanied by increased GLU in the core of rats with a cocaine self-administration history but not in yoked groups receiving passive cocaine or saline; core DA, however, increases in all groups (Baker et al., 2003; McFarland, Lapish, & Kalivas, 2003). Collectively, the data suggest that GLU activation of core AMPA receptors plays a key role in cocaine-primed reinstatement.

The role of the core in cue-primed reinstatement is less clear. Although DiCiano and Everitt (2001) reported that cue-primed drug-seeking behavior is impaired by an intracore infusion of an AMPA antagonist, the animals in this study were trained on a second-order reinforcement schedule, which may not involve the same neural substrates as reinstatement testing. In addition, core inactivation fails to block cocaine seeking primed by the presentation of a cocaine-related cue (Grimm & See, 2000). Cue-primed reinstatement, moreover, does not activate core neurons selectively; in fact, these units respond indiscriminately to both cocaine-predictive and neutral stimuli (Ghitza, Fabbricatore, Prokopenko, Pawlak, & West, 2003).

NAcc Shell. Although the shell has been implicated in both cue- and drug-primed reinstatement, a clear picture of shell involvement has not yet emerged. In cocaine-primed reinstatement, for example, shell inactivation fails to block responding (McFarland et al., 2003), but there is evidence that cocaine-priming requires both DA and GLU mechanisms in shell (Anderson, Bari, & Pierce, 2003; Park et al., 2002). Cue-priming appears to require shell GLU. Extinction training of rats with a cocaine self-administration history enhances expression of the GluR1 sub-unit of the AMPA receptor in shell, and this effect is negatively correlated with cue-primed reinstatement (Sutton et al., 2003). This report also showed that over-expression of the GluR1 and GluR2 sub-units facilitates extinction of cocaine-, but not sucrose-seeking behavior. Thus, shell activation may inhibit drug relapse. A recent electrophysiological analysis, however, indicates that shell activation is associated with cocaine-predictive cues in a reinstatement paradigm in which rats were withdrawn from cocaine self-administration for several weeks (Ghitza et al., 2003). Unfortunately, there was no extinction training in this study making it difficult to predict how shell units will respond to cue-primed reinstatement.

Role of the PFC

Emerging evidence suggests that PFC is the common final pathway underlying relapse triggered by stress, conditioned stimuli, and drugs. Clinical neuroimaging studies show that both cocaine and cocaine-paired cues activate PFC in cocaine addicts (Breiter et al., 1997; Childress et al., 1999; Grant et al., 1996). The same studies also show a positive correlation between the self-reported craving induced by either drugs or drug-related cues and metabolic activation in specific regions of PFC such as the dorsolateral prefrontal, orbito-prefrontal, anterior cingulate cortex (ACC), and insula. These data, moreover, are generally consistent with reports from animal studies that cocaine-seeking behavior in rats is associated with changes in Fos, an immediate early gene, and γ protein kinase C, a plasticity-regulated gene, in PFC (Ciccocioppo, Sanna, & Weiss, 2001; Neisewander et al., 2000; Thomas & Everitt, 2001).

Neuropharmacological investigations of animal reinstatement models also implicate PFC, particularly dorsal PFC, which includes prelimbic cortex (PLC) and area 1 (Cg1) of ACC, in cocaine-seeking behavior. For example, inactivation of dorsal PFC, but not infralimbic cortex (ILC), blocks cue-induced (McLaughlin & See, 2003), cocaine-primed (McFarland & Kalivas, 2001) and stress-induced (Capriles, Rodaros, Sorge, & Stewart, 2003) reinstatement. Furthermore, cocaine-primed reinstatement involves increased GLU output from dorsal PFC to NAcc core. Cocaine-priming, for example, increases GLU in core but not when PFC is inactivated (Baker et al., 2003; McFarland et al., 2003), and blockade of core AMPA receptors decreases cocaine-primed reinstatement (Cornish et al., 1999; Cornish & Kalivas, 2000).

DA Modulation. The involvement of PFC in cocaine-seeking behavior appears to require DA input from the VTA. Micro-infusions of the nonselective DA antagonists flupenthixol or fluphenazine into PFC block cocaine-primed reinstatement, whereas microinfusions of cocaine, amphetamine, or DA into this area reinstate cocaine-seeking behavior (McFarland & Kalivas, 2001; Park et al., 2002). Together, these data suggest that activation of DA

receptors in PFC is critical for cocaine-seeking behavior. Furthermore, preliminary data show that blockade of ionotropic GLU receptors in the VTA block cocaine-primed reinstatement in a dose dependent fashion, suggesting that cocaine-seeking behavior depends on increased DA input in PFC derived from the enhanced activity of DA neurons in the VTA (Sun, Akins, Mattingly, & Rebec, in press). Consistent with this view, we recently investigated the relative involvement of PFC D1- and D2-like receptors in cocaine seeking and found that blockade of D1-like receptors selectively attenuated cocaine-primed reinstatement without affecting food-seeking behavior, whereas both types of responding were impaired by blockade of D2-like receptors (Sun & Rebec, 2005).

It appears, therefore, that D1-like receptors play a specific role in cocaine seeking. Our results contrast with evidence (Capriles et al., 2003) that microinjection of either D1- or D2-like receptor antagonists into PFC fails to block cocaine-primed reinstatement. A comparison is difficult, however, because apart from differences in reinforcement schedules, reinstatement paradigms, and the cocaine self-administration dose, Capriles et al. (2003) tested only one dose of each DA antagonist. In fact, they used a dose of SCH 23390 which was below any of our tested doses and thus may have been too low to observe an effect. Recently, the Kalivas group (Bowers et al., 2004) showed that repeated cocaine exposure in either a self-administration or sensitization paradigm up-regulated the expression of AGS3, a member of the activator-of-G-protein-signaling family in rat PFC. This protein disrupts the interaction between $G_{i\alpha}$ and $G_{\beta\gamma}$ subunits of the G protein to decrease signal transduction related to $G_{i\alpha}$ Furthermore, prevention of this up-regulation with anti-sense oligonucleotides blocks cocaine-primed reinstatement or sensitization. Because D1- and D2-like receptors are coupled with Gs and Gi proteins respectively, the authors suggest that up-regulation of PFC ASG3 may preferentially increase signaling through D1-like receptors. This hypothesis is consistent with our findings that D1-like receptors are specifically involved in cocaine-primed reinstatement (Sun & Rebec, 2005).

Because cocaine-primed reinstatement depends on increased GLU input from dorsal PFC to NAcc (Baker et al., 2003; McFarland et al., 2003) as well as DA input to PFC (McFarland & Kalivas, 2001; Park et al., 2002), it is reasonable to suggest that an increase in PFC DA after cocaine priming may enhance the activity of PFC output neurons and thereby drive drug-seeking behavior. Although there is no convincing evidence that DA strongly excites PFC output neurons, it appears to modulate such activity. For example, the resting membrane potential of PFC output neurons alternates between an ''up'' state, which is favorable for impulse generation, and a ''down'' state, which is not (Lewis & O'Donnell, 2000; Peters, Lewis, & O'Donnell, 2000).

DA appears to switch these neurons from the ''down'' to the ''up'' state and to increase resident time in the ''up'' state (Lavin & Grace, 2001; Lewis & O'Donnell, 2000). In fact, the ''up'' state is synchronized with VTA activity (Peters, Barnhardt, & O'Donnell, 2004). It also is interesting that the D1-like receptor may be responsible for these effects. Activation of D1- but not D2-like receptors, for example, increases membrane excitability of PFC output neurons (Gonzalez-Islas & Hablitz, 2003). In fact, it has been suggested that activation of D1-like receptors in PFC may underlie the sustained network activity that allows for focused behavior (Durstewitz & Seamans, 2002).

Hypo- or hyperfrontality?. Abnormal functioning of PFC has been reported in cocaine addicts. One line of work suggests that PFC hypofunction may underlie compulsive drug-seeking behavior (Jentsch & Taylor, 1999; J. H. Lee, Telang, Springer, & Volkow, 2003). In contrast, clinical imaging (Breiter et al., 1997; Childress et al., 1999; Grant et al., 1996) and animal studies consistently indicate that drug-seeking behavior requires activation of PFC (Baker et al., 2003; Capriles et al., 2003; McFarland & Kalivas, 2001; McFarland et al., 2003; McLaughlin & See, 2003; Volkow et al., 2005). As Kalivas and McFarland (2003) point out, the finding from the animal literature ''that activation of anterior cingulate is a common feature of cocaine-, cue- and stress-primed reinstatement may seem incongruous with the [clinical] literature showing that enduring hypofrontality (e.g. deficient activation of the prefrontal cortex by cognitive challenges) may be a feature of cocaine

addiction'' (p. 51). It is conceivable, however, that hypofrontality provides a low level of background activity from which PFC activation appears enhanced (Bowers et al., 2003). This increased signal-to-noise ratio may be critical for compulsive drug-seeking behavior.

ELECTROPHYSIOLOGY OF COCAINE SEEKING

Early electrophysiological studies of NAcc in behaving animals focused on responses for natural reinforcers in monkeys (Apicella, Ljungberg, Scarnati, & Schultz, 1991; Schultz, Apicella, Scarnati, & Ljungberg, 1992). The data showed that distinct populations of NAcc neurons increased activity in response to reward delivery. Other NAcc neurons responded just prior to reward onset, suggesting a role in expectation of reward. Similar results have been obtained from rats. Groups of NAcc neurons in core and shell, for example, respond during behavior aimed at obtaining water reinforcement (Carelli & Deadwyler, 1994; Carelli, Ijames, & Crumling, 2000). Some neurons increase activity immediately before and others either increase or decrease activity immediately after the reinforced response. Comparable firing patterns have been observed in NAcc during operant responses for food or sucrose reward (R. S. Lee, Koob, & Henriksen, 1998; Roop, Hollander, & Carelli, 2002). It appears, therefore, that NAcc neurons encode the initiation, execution, and completion of goal-directed responding (Carelli, 2002).

Ample evidence also implicates NAcc neurons in drug reinforcement. Populations of these cells show patterned changes in firing that either occur within seconds of the reinforced response (immediately before or after a lever press for IV cocaine) or that persist for several minutes during the period between IV infusions (Carelli & Deadwyler, 1994, 1996; Carelli, King, Hampson, & Deadwyler, 1993; Chang, Sawyer, Lee, & Woodward, 1994; Nicola & Deadwyler, 2000; Peoples, Uzwiak, Gee, & West, 1997; Peoples & West, 1996; Uzwiak, Guyette, West, & Peoples, 1997). The period immediately before the lever press for IV cocaine is likely related to drug-seeking behavior and a subset of NAcc neurons show increases or decreases in activity. Interestingly, these anticipatory neuronal responses are blocked by systemic injection of a D1 DA receptor antagonist (Nicola & Deadwyler, 2000). In line with this observation, DA neurons in VTA increase firing in response to cues that signal reward availability (Schultz, 2000).

Thus, anticipatory firing, given the role of DA in cue-priming, may be a neuronal correlate of primed reinstatement (Kalivas & McFarland, 2003). This hypothesis, however, is inconsistent with data that anticipatory firing is not changed in rats exposed to a cue or drug prime during a within-session reinstatement paradigm (Carelli & Ijames, 2000). In a study of cue-evoked unit activity after a period of abstinence from cocaine, shell neurons, unlike those in core, continue to process cocaine-related stimuli (Ghitza et al., 2003).

Neuronal response patterns in PFC to cocaine reward closely resemble those obtained from NAcc (Chang, Sawyer, Paris, Kirillov, & Woodward, 1997; Woodward, Chang, Janak, Azarov, & Anstrom, 2000). In fact, Chang, Janak, and Woodward (2000) report a close correlation between anticipatory firing in NAcc and PFC to lever presses for IV cocaine. Interestingly, the NAcc response typically preceded the change in PFC activity suggesting that the ability of NAcc neurons to process cocaine-related information is not dependent on PFC input. The relation of PFC neuronal responses to the stimuli that trigger relapse, however, remains to be established. To understand the role of PFC in reinstatement, it first is important to understand PFC operations during drug-taking behavior. Thus, we have begun a series of studies to determine how PFC neurons process information related to cocaine self-administration.

PFC Neurons and Cocaine Self-administration

Although there is evidence that PFC is involved in reward seeking as well as motor planning and other relevant behaviors (Brown & Bowman, 2002; Cardinal, Parkinson, Hall, & Everitt, 2002), few studies have focused on the self-administration task. Chang et al. (1997, 2000) reported anticipatory changes in neuronal firing frequency in medial PFC of rats lever pressing for cocaine, but training did not include a discrete CS making it difficult to relate these results to any subsequent assessment of reinstatement-related activity. The

authors, moreover, sampled neurons from what appears to be a large dorso-ventral area, even through the PFC is neither structurally nor functionally homogeneous. In rodents, the PFC includes dorsal and ventral anterior cingulate (ACC), which have been designated Cg1 and Cg2, respectively, pre-limbic (PLC), infra-limbic (IFC), orbital, and angular insular cortices (Kolb & Tees, 1990). Each area, moreover, has distinct patterns of connectivity, which regulates different behavioral functions (Cardinal et al., 2002).

Although relating rodent cortical areas to those in primates requires caution (Brown & Bowman, 2002; Porrino & Lyons, 2000; Ulyings, Groenewegen, & Kolb, 2003), it appears that dorsal PFC, which includes the interface between Cg1 and PLC, is crucial for both cue-related and drug-primed reinstatement of drug-seeking behavior (McFarland & Kalivas, 2001). In human addicts, for example, intensity of craving correlates with metabolic activity in ACC (Breiter et al., 1997; Volkow et al., 1999). ACC units in primates, moreover, change the level of cue-related responding with reward expectancy (Shidara & Richmond, 2002). In rats, metabolic activity in the ACC/PLC sub-region is activated by both a cue- and a drug-prime stimulus (Neisewander et al., 2000). Thus, it is important to assess how ACC/PLC neurons are involved in cocaine self-administration, and our preliminary results provide interesting insight.

METHOD

Male, Sprague-Dawley rats (300–350 g), approved for use by the Institutional Animal Care and Use Committee, were anesthetized and prepared for subsequent single-unit recording as previously described (Gulley, Kosobud, & Rebec, 2002). Two electrode assemblies were installed bilaterally in Cg1-PLC (2.7 mm anterior and 0.6-0.8 mm lateral to bregma, and 3.5–4.5 mm ventral to skull surface) according to the coordinates of Paxinos and Watson (1998). Each assembly consisted of cylindrical bundles of eight microwires (Formvar-insulated stainless steel, 25 μm diameter) and a stainless steel ground wire. All animals were allowed at least five days of post-surgical recovery.

Behavioral testing occurred in standard operant chambers (30L X 24W X 21H cm), one wall of which was equipped with a glass lever (5 cm wide), two cue lights (1 W), and a food cup. The lever was located 9 cm above the grid floor in the middle of the wall. The cue lights were located 11 cm above the grid floor and spaced 21 cm apart. The food cup was located below the lever. A houselight was located on the back wall outside the chamber. Also outside the chamber were a programmable audio speaker, food dispenser, and fluid pump. Each chamber was housed in a sound- and light-attenuating cubicle equipped with a fan to mask extraneous noise. Pre-testing dietary restriction, which brought the animals to 85% of their free-feeding weight, was used to increase motivation for operant responding.

After animals achieved a relatively stable rate of responding for food (45-mg sucrose pellet) on a fixed-ratio 5 (FR 5) schedule of reinforcement (i.e., 60 food pellets in 30 min), they were trained to press the lever for cocaine in a daily 2-hr session using a modified FR-5 schedule. The first response of the session resulted in IV injection of 0.25 mg cocaine in a volume of 0.05 ml physiological saline accompanied by a compound CS (cue), which consisted of the onset of the two cue lights and a 4-s tone. Delivery of the CS was followed by a 16-s timeout signaled by illumination of the houselight. During the cocaine injection and timeout, which together last for 20 s, responding was recorded but had no programmed consequences. After the first cocaine injection, subsequent injections and CS presentations were delivered on an FR-5 schedule (Sun & Rebec, 2003). The session ended after 2 hr or 30 injections of cocaine, whichever occurred first.

Unit recording began after at least 10 days of training when cocaine self-administration stabilized (i.e., the number of cocaine injections varied by $< 20\%$ on 3 consecutive training sessions). We analyzed peri-event time histograms (PETHs) in relation to cue onset (i.e., the reinforced lever press), and neuronal firing was assessed before and after the event. Raster displays were examined for non-reinforced lever presses, which were evaluated separately. Because unit activity is likely to be variable early in the self-administration session as rats load-up on cocaine, PETH analysis began with the fifth reinforced lever press.

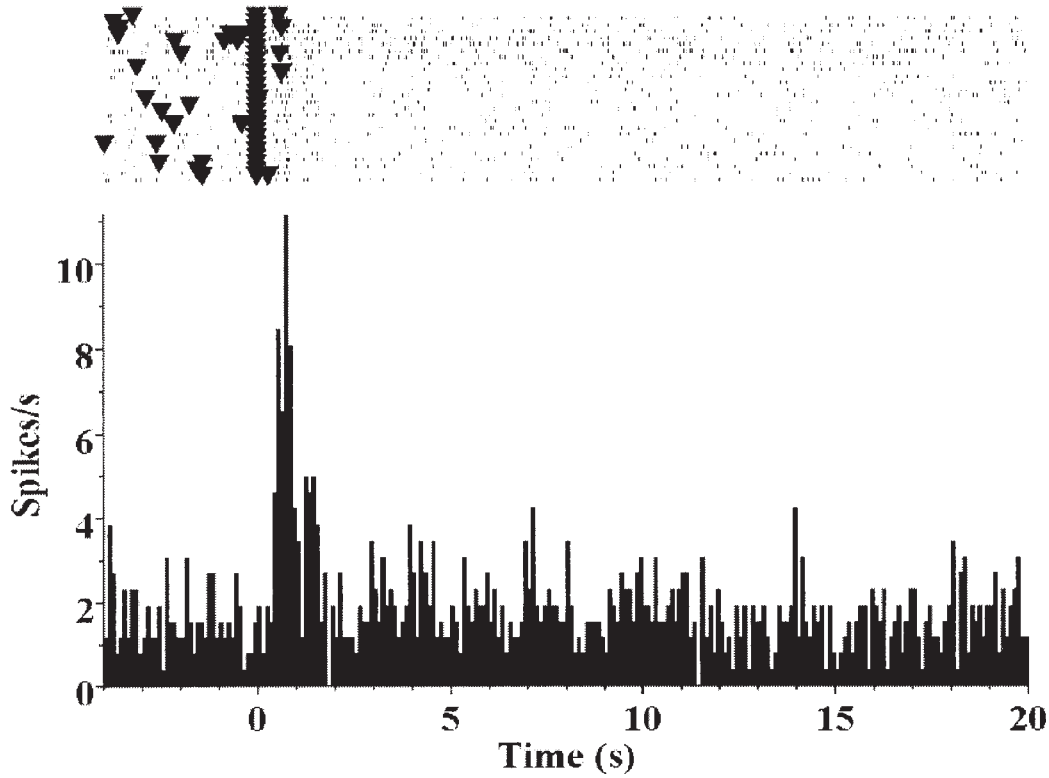

Fig. 1. Cue-related activation of neuronal activity in PFC (Cg1–PLC) of one rat during cocaine self-administration. The upper part of the figure is a raster display in which each dot represents a spike from the recorded neuron and each row represents an individual trial. Black triangles indicate lever presses; lever presses that occur at Time 0 trigger cue presentation and activation of the pump for cocaine infusion. The PETH in the lower part of the figure represents the average firing rate around the time of cue onset. The Y axis depicts firing rate (spikes /s); bin size is 100 ms. Note the increase in firing rate that begins within 500 ms of cue onset and that persists for ~700 ms.

RESULTS AND DISCUSSION

Data are now available from > 40 PFC neurons recorded from 4 rats. A sizable population of units (~25%) was activated by cue onset, which occurred in conjunction with the cocaine-reinforced lever press. As shown in Figure 1, the neuronal response was brief with an onset latency of 300–500 ms. These data raise the following questions:

1. Is the transient activation related to the cue or the motor act of pressing the lever? To address this issue, the cue was omitted on a random 50% of trials in a subsequent recording session. Loss of PFC activation on these trials would rule out a role for lever pressing. We found that omitting the cue eliminated the neuronal activation in all activated units recorded to date (11/11 units) arguing against the act of lever pressing as the basis for the PFC response. The effect of cue omission on a representative PFC neuron is shown in Figure 2. Cue omission, moreover, resulted in a transient neuronal activation in response to houselight onset, which occurred 4 s after cue onset (see Figure 2, right panel), suggesting that the houselight acquired a role as secondary predictor of cocaine availability. In fact, one could argue that houselight onset provided the initial signal of cocaine availability early in self-administration training, but as animals learned the task the cue eventually took on this role.

2. Could cocaine contribute to the neuronal activation? Although IV cocaine was infused for 4 s beginning immediately after the reinforced lever press, the transient neuronal activation to cue may represent a response to the initial entry of cocaine into the brain. To assess this possibility, we omitted the cocaine injection on a random 50% of trials in another

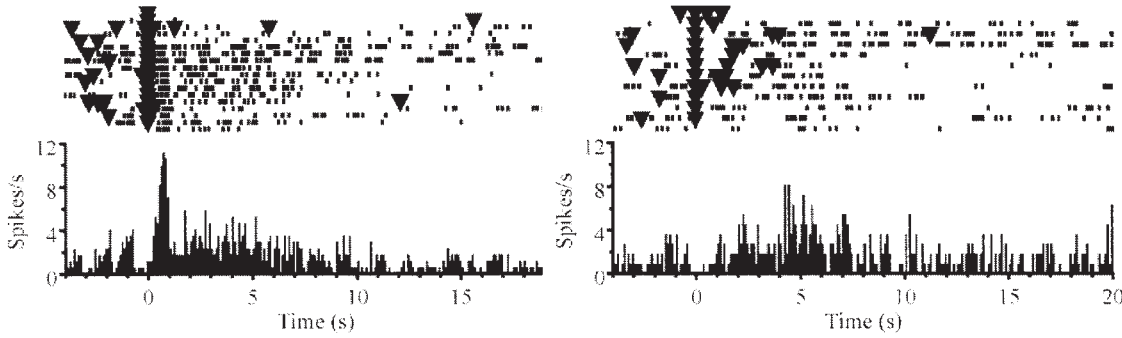

Fig. 2. PFC (Cg1–PLC) activity during a cocaine self-administration session in which the cue is omitted on a random 50% of trials. Data are presented as in Figure 1. Left: Trials with cue. Note that as in Figure 1, the transient activation of neuronal activity was within 500 ms after cue onset. Right: Trials without cue. Note the complete absence of neuronal activation during the same period after cue onset. Interestingly, a late period of activation emerged on trials without cue at the time of houselight onset (4 s after cue onset).

subset of rats. Our data show that the cue-induced activation persisted in the absence of cocaine indicating that the cocaine injection cannot explain the cue-related activation of PFC. An example of this effect is shown in Figure 3.

3. Are there other cue-related neuronal responses? Although the cue-induced activation is the most prominent response (~25% or 11/43 units) recorded to date, a smaller population (< 10% or 4/43 units) showed a transient cue-related inhibition. Like the excitation, the inhibition disappeared when the cue is randomly omitted, suggesting again that the cue and not the lever press drives the neuronal response. It is not clear, however, that the inhibition shifts to the onset of the houselight when the cue is not available since three of four inhibited units showed no houselight response under these conditions. Although further recording is required to verify this conclusion, it seems likely that excited and inhibited neurons in PFC convey different cue-related information. Previous reports of PFC excitations and inhibitions occurring several seconds before the reinforced lever press may be related to motor-task sequencing or the motivation to obtain the drug (Chang et al., 1997, 2000).

4. Is there a neuronal response to cocaine? To date, we have not found units that respond either to cocaine (beginning 2 s after cocaine injection onset and continuing throughout the 16-s period of houselight illumination) or the omission of cocaine on select trials (Figure 3). Because our analysis does not include the first few trials when animals are loading up on cocaine, it is conceivable that there is a response to the initial cocaine injection. Nevertheless, a lack of response to

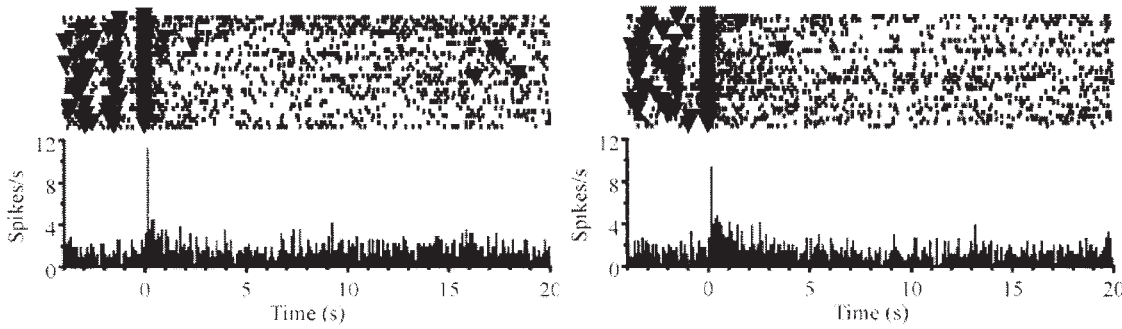

Fig. 3. PFC (Cg1–PLC) activity during a cocaine self-administration session in which the cocaine infusion is omitted on a random 50% of trials. Data are presented as in Figure 1. Left: Trials with cocaine infusion (infusion pump on from 0–4 s). Note the brief activation of unit activity almost immediately after cue onset. Right: Trials without cocaine infusion. Note the similarity of the left and right PETHs throughout the 20-s period after cue onset.

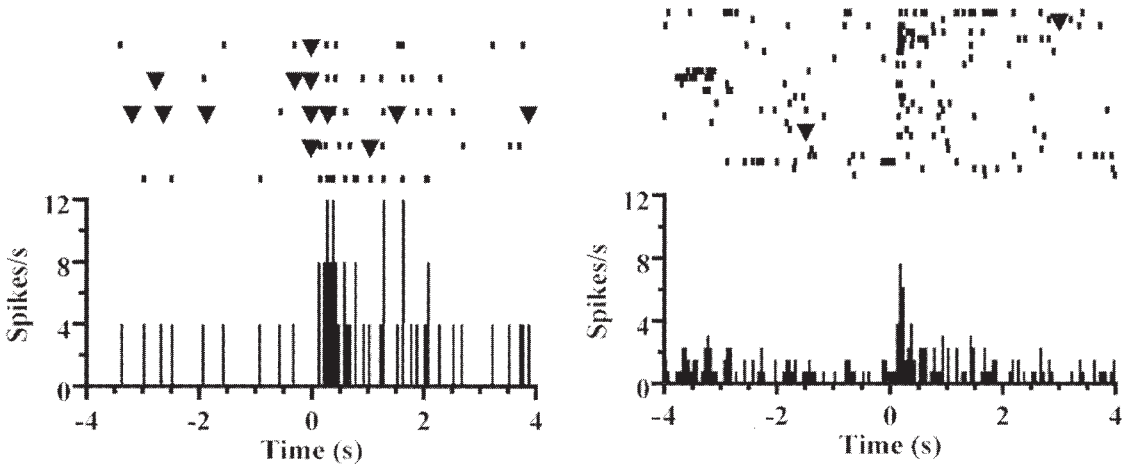

Fig. 4. PFC (Cg1–PLC) activity during conditioned (left) and cue-primed (right) reinstatement. Data are presented as in Figure 1, but bin size is 50 ms. In both graphs, transient, cue-related activation is evident within 500 ms after cue onset. Over the course of both reinstatement sessions, lever pressing (black triangles) exceeded that during extinction by 4 times (left; a total of 36 lever presses; 13 are shown) and 12 times (right; a total of 20 lever presses; one is shown). During conditioned reinstatement, cue presentation follows an FR-5 schedule.

each cocaine injection or its omission as the session progresses makes it unlikely that PFC processes the pharmacological effects of cocaine.

Taken together, our preliminary findings indicate cue-related activation of PFC neurons during cocaine self-administration. An analysis of PFC activity after IV cocaine indicates no consistent change in neuronal activity. It appears, therefore, that PFC neurons do not encode cocaine reward but rather the availability of cocaine signaled by the transient cue response.

PFC Neurons and Cue-related Reinstatement

To determine if PFC activation is important for cue-induced reinstatement, we recorded from the same animals during reinstatement testing. The cue was presented in one of two paradigms: cue-priming, in which the cue was presented noncontingently at regular intervals, and conditioned reinstatement in which after an initial presentation of the cue, each subsequent presentation was contingent on an FR-5 schedule of lever pressing. Although in the latter case the contingent cue serves as a secondary reinforcer, this situation closely mimics relapse in human addicts such that in searching for drug the addict encounters cues that predict drug availability. The cue-priming paradigm, in contrast, allows us to focus exclusively on the role of the cue in initiating relapse. A cue-related activation of dorsal PFC units in both paradigms would strongly support a role for PFC in cue processing for both the initiation (cue priming) and maintenance (conditioned reinstatement) of drug seeking.

After one week of extinction training in which lever presses were recorded but had no programmed consequences, responding fell to below 20% of that recorded during cocaine self-administration, and reinstatement testing began the next day. Each reinstatement session lasted for 60 min. In the cue-priming paradigm, the CS used in self-administration training was presented noncontingently at 2-min intervals. PETHs were analyzed in relation to the noncontingent cue. In the conditioned-reinstatement paradigm, the cue was first presented noncontingently to generate lever pressing and then contingent on a FR-5 schedule thereafter. Separate animals were tested in each paradigm.

Data that we have obtained from both paradigms strongly suggest that the cue drives cocaine-seeking behavior. Our recordings indicate, for example, a population of PFC neurons (10/41) that show transient activation characterized by short (~500 ms) onset latency in response to either non-contingent or contingent cue. Representative examples are presented in Figure 4. Thus, during either

conditioned reinstatement, when cue presentation is contingent on the lever press (Figure 4, left), or cue priming, when the cue is presented noncontingently at regular intervals throughout the session (Figure 4, right), PFC is activated. These results also argue against the neuronal response as an indicator of secondary reinforcement since noncontingent cue presentation was just as effective as contingent cue in activating PFC.

SUMMARY AND CONCLUSION

Neuropharmacological studies of the reinstatement of drug-seeking behavior in animals have provided important insight into the neural circuitry underlying relapse in human addicts. It is clear, for example, that the mesocorticolimbic DA system is critically involved and that increased glutamate input from PFC to the NAcc constitutes what appears to be the final common pathway for the relapse response. At present, however, the functional involvement of these structures in drug-seeking behavior is not well understood. Do they process the motivational effects of conditioned cues and drug priming or the motor effects? Do these structures have any role to play in drug-related hedonia or reinforcement? Electrophysiological recordings from behaving animals can begin to shed light on these issues.

For example, the lack of responding of neurons in dorsal PFC to self-administered cocaine indicates that this brain region does not process the hedonic effects of cocaine. Activation of these neurons, however, appears to play an important role in drug-seeking behavior. The anticipatory response of NAcc neurons before lever pressing, moreover, suggests a role either in the motivation or the motor programming to obtain drug. It would be useful, therefore, to extend our recordings to NAcc to understand how this brain region interacts with PFC to process drug-related information in the relapse to cocaine-seeking behavior. NAcc neurons are believed to modulate their firing rate to drug and natural reward (Carelli, 2002), but this process is highly plastic and dependent on input from sub-cortical circuits as well as PFC input.

Electrophysiological data also can shed light on how the adaptive molecular changes induced by repeated drug exposure alter behavior by changing the firing properties of individual circuit neurons. Another advantage is the opportunity to track how information flows in the underlying neural circuit. With simultaneous multiple-site recordings, for example, it is possible to assess correlated neuronal responding to a specific operant event, and by calculating the latency of each neuronal response, inferences can be made about how one brain region influences another. This type of information will clarify how individual circuit neurons cooperate with each other to drive drug-seeking behavior. Thus, the application of electrophysiological techniques to neuropharmacological studies of reinstatement is likely to become a powerful tool for dissecting the functions of the neural circuits underlying relapse.

REFERENCES

Anderson, S. M., Bari, A. A., & Pierce, R. C. (2003). Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. *Psychopharmacology (Berl), 168*, 132–138.

Apicella, P., Ljungberg, T., Scarnati, E., & Schultz, W. (1991). Responses to reward in monkey dorsal and ventral striatum. *Experimental Brain Research, 85*, 491–500.

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X. C., Toda, S., & Kalivas, P. W. (2003). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nature Neuroscience, 6*, 743–749.

Bergman, J., & Katz, J. L. (1998). Behavioral pharmacology of cocaine and the determinants of abuse liability. In S. T. Higgins & J. L. Katz (Eds.), *Cocaine abuse: Behavior, pharmacology, and clinical applications* (pp. 51–79). San Diego: Academic Press.

Bowers, M. S., Lake, R. W., McFarland, K., Peterson, Y. K., Lanier, S. M., Lapish, C. C., & Kalivas, P. W. (2003). AGS3: A G-Protein regulator of addiction-associated behaviors. *Annals of the New York Academy of Science, 1003*, 356–357.

Bowers, M. S., McFarland, K., Lake, R. W., Peterson, Y. K., Lapish, C. C., Gregory, M. L., Lanier, S. M., & Kalivas, P. W. (2004). Activator of G protein signaling 3: A gatekeeper of cocaine sensitization and drug seeking. *Neuron, 42*, 269–281.

Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., Goodman, J. M., Kantor, H. L., Gastfriend, D. R., Riorden, J. P., Mathew, R. T., Rosen, B. R., & Hyman, S. E. (1997). Acute effects of cocaine on human brain activity and emotion. *Neuron, 19*, 591–611.

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the accumbens part of the rat ventral

striatum—immunohistochemical detection of retrogradely transported fluoro-gold. *Journal of Comparative Neurology, 338*, 255–278.

Brown, V. J., & Bowman, E. M. (2002). Rodent models of prefrontal cortical function. *Trends in Neuroscience, 25*, 340–343.

Capriles, N., Rodaros, D., Sorge, R. E., & Stewart, J. (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology, 168*, 66–74.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews, 26*, 321–352.

Carelli, R. M. (2002). Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. 'natural' reinforcement. *Physiology and Behavior, 76*, 379–87.

Carelli, R. M., & Deadwyler, S. A. (1994). Comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats. *Journal of Neuroscience, 14*, 7735–7746.

Carelli, R. M., & Deadwyler, S. A. (1996). Dose-dependent transitions in nucleus accumbens cell firing and behavioral responding during cocaine self-administration sessions in rats. *Journal of Pharmacological and Experimental Therapy, 277*, 385–393.

Carelli, R. M., & Ijames, S. G. (2000). Nucleus accumbens cell firing during maintenance, extinction, and reinstatement of cocaine self-administration behavior in rats. *Brain Research, 866*, 44–54.

Carelli, R. M., Ijames, S. G., & Crumling, A. J. (2000). Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus ''natural'' (water and food) reward. *Journal of Neuroscience, 20*, 4255–4266.

Carelli, R. M., King, V. C., Hampson, R. E., & Deadwyler, S. (1993). Firing patterns of nucleus accumbens neurons during cocaine self-administration in rats. *Brain Research, 626*, 14–22.

Chang, J. Y., Janak, P. H., & Woodward, D. J. (2000). Neuronal and behavioral correlations in the medial prefrontal cortex and nucleus accumbens during cocaine self-administration by rats. *Neuroscience, 99*, 433–443.

Chang, J. Y., Sawyer, S. F., Lee, R. S., & Woodward, D. J. (1994). Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats. *Journal of Neuroscience, 14*, 1224–1244.

Chang, J. Y., Sawyer, S. F., Paris, J. M., Kirillov, A., & Woodward, D. J. (1997). Single neuronal responses in medial prefrontal cortex during cocaine self-administration in freely moving rats. *Synapse, 26*, 22–35.

Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *American Journal of Psychiatry, 156*, 11–18.

Ciccocioppo, R., Sanna, P. O., & Weiss, F. (2001). Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: referal by D1 antagonists. *Proceedings of the National Academy of Sciences, 98*, 1976–1981.

Comoli, E., Coizet, V., Boyes, J., Bolam, J. P., Canteras, N. S., Quirk, R. H., Overton, P. G., & Redgrave, P. (2003). A direct projection from superior colliculus to substantia nigra for detecting salient visual events. *Nature Neuroscience, 6*, 974–980.

Cornish, J. L., Duffy, P., & Kalivas, P. W. (1999). A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. *Neuroscience, 93*, 1359–1367.

Cornish, J. L., & Kalivas, P. W. (2000). Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *Journal of Neuroscience, 20*, U11–U15.

Davis, W. M., & Smith, S. G. (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *Pavlovian Journal of Biological Science, 11*, 222–236.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl), 75*, 134–143.

Di Chiara, G. (1998). A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. *Journal of Psychopharmacology, 12*, 54–67.

Di Ciano, P., & Everitt, B. J. (2001). Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology, 25*, 341–360.

Durstewitz, D., & Seamans, J. K. (2002). The computational role of dopamine D1 receptors in working memory. *Neural Networks, 15*, 561–572.

Epstein, D. H., & Preston, K L. (2003). The reinstatement model and relapse prevention: A clinical perspective. *Psychopharmacology, 168*, 31–41.

Ettenberg, A. (1990). Haloperidol prevents the reinstatement of amphetamine-rewarded runway responding in rats. *Pharmacology, Biochemistry, and Behavior, 36*, 635–638.

Everitt, B. J., & Robbins, T. W. (2000). Second-order schedules of drug reinforcement in rats and monkeys: Measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology, 153*, 17–30.

Ghitza, U. E., Fabbricatore, A. T., Prokopenko, V., Pawlak, A. P., & West, M. O. (2003). Persistent cue-evoked activity of accumbens neurons after prolonged abstinence from self-administered cocaine. *Journal of Neuroscience, 23*, 7239–7245.

Gonzalez-Islas, C., & Hablitz, J. J. (2003). Dopamine enhances EPSCs in layer II-III pyramidal neurons in rat prefrontal cortex. *Journal of Neuroscience, 23*, 867–875.

Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Liu, X., Contoreggi, C., Phillips, R. L., Kimes, A. S., & Margolin, A. (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proceedings of the National Academy of Sciences of the United States of America, 93*, 12040–12045.

Grimm, J. W., & See, R. E. (2000). Chronic haloperidol-induced alterations in pallidal GABA and striatal D-1-mediated dopamine turnover as measured by dual probe microdialysis in rats. *Neuroscience, 100*, 507–514.

Groenewegen, H. J., Wright, C. I., Beijer, A. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences, 877*, 49–63.

Gulley, J. M., Kosobud, A. E. K., & Rebec, G. V. (2002). Behavior-related modulation of substantia nigra pars reticulata neurons in rats performing a conditioned reinforcement task. *Neuroscience, 111*, 337–349.

Heimer, L., Alheid, G. F., & Zahm, D. S. (1993). Basal forebrain organization: An anatomical framework for motor aspects of drive and motivation. In P. W. Kalivas & C. D. Barnes (Eds.), *Limbic motor circuits and neuropsychiatry* (pp. 1–32). Boca Raton: CRC Press.

Hemby, S. E., Co, C., Koves, T. R., Smith, J. E., & Dworkin, S. I. (1997). Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. *Psychopharmacology, 133*, 7–16.

Horvitz, J. C. (2000). Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience, 96*, 651–656.

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Research Reviews, 31*, 6–41.

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology, 146*, 373–390.

Kalivas, P. W., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology, 168*, 44–56.

Kalivas, P. W., & Nakamura, M. (1999). Neural systems for behavioral activation and reward. *Current Opinion in Neurobiology, 9*, 223–227.

Katz, J. L., & Higgins, S. T. (2003). The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology, 168*, 21–30.

Kolb, B., & Tees, R. C. (1990). *Cerebral cortex of the rat.* Cambridge, MA: MIT Press.

Lavin, A., & Grace, A. A. (2001). Stimulation of D1-type dopamine receptors enhances excitability in prefrontal cortical pyramidal neurons in a state-dependent manner. *Neuroscience, 104*, 335–346.

Lee, J. H., Telang, F. W., Springer, C. S., Jr., & Volkow, N. D. (2003). Abnormal brain activation to visual stimulation in cocaine abusers. *Life Science, 73*, 1953–1961.

Lee, R. S., Koob, G. F., & Henriksen, S. J. (1998). Electrophysiological responses of nucleus neurons to novelty stimuli and exploratory behavior in the awake, unrestrained rat. *Brain Research, 799*, 317–322.

LeMoal, M., & Simon, H. (1991). Mesocorticolimbic dopaminergic network: Functional and regulatory roles. *Physiological Reviews, 7*, 155–234.

LeMoal, M. (1995). Mesocorticolimbic dopaminergic neurons: Functional and regulatory roles. In F. E. Bloom & D. J. Kupfer (Eds.), *Psychopharmacology: The fourth generation of progress* (pp. 283–294). New York: Raven Press.

Lewis, B. L., & O'Donnell, P. (2000). Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cerebral Cortex, 10*, 1168–1175.

Lu, L., Shepard, J. D., Hall, F. S., & Shaham, Y. (2003). Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: A review. *Neuroscience and Biobehavioral Reviews, 27*, 457–491.

Marlatt, A. G. (1996). Models of relapse and relapse prevention: A commentary. *Experimental and Clinical Psychopharmacology, 4*, 55–60.

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *Journal of Neuroscience, 21*, 8655–8663.

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *Journal of Neuroscience, 23*, 3531–3537.

McLaughlin, J., & See, R. E. (2003). Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned–cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacologia, 168*, 57–65.

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: Functional interface between the limbic system and the motor system. *Progress in Neurobiology, 14*, 69–97.

Mueller, D., & Stewart, J. (2000). Cocaine-induced conditioned place preference: Reinstatement by priming injections of cocaine after extinction. *Behavioural Brain Research, 115*, 39–47.

Neisewander, J. L., Baker, D. A., Fuchs, R. A., Tran-Nguyen, L. T., Palmer, A., & Marshall, J. F. (2000). Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *Journal of Neuroscience, 20*, 798–805.

Nicola, S. M., & Deadwyler, S. A. (2000). Firing rate of nucleus accumbens neurons is dopamine-dependent and reflects the timing of cocaine-seeking behavior in rats on a progressive ratio schedule of reinforcement. *Journal of Neuroscience, 20*, 5526–5537.

Park, W.-K., Bari, A. A., Jey, A. R., Anderson, S. M., Spealman, R. D., Rowlett, J. K., & Pierce, R. C. (2002). Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *Journal of Neuroscience, 22*, 2916–2925.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates.* (4th ed.). San Diego, CA: Academic Press.

Peoples, L. L., & West, M. O. (1996). Phasic firing of single neurons in the rat nucleus accumbens correlated with the timing of intravenous cocaine self-administration. *Journal of Neuroscience, 16*, 3459–3473.

Peoples, L. L., Uzwiak, A. J., Gee, F., & West, M. O. (1997). Operant behavior during sessions of intravenous cocaine infusion is necessary and sufficient for phasic filing of single nucleus accumbens neurons. *Brain Research, 757*, 280–284.

Peters, Y. M., Lewis, B. L., & O'Donnell, P. (2000). Synchronous activity in the ventral tegmental area and prefrontal cortex. *Annals of the New York Academy of Sciences, 909*, 267–269.

Peters, Y., Barnhardt, N. E., & O'Donnell, P. (2004). Prefrontal cortical up states are synchronized with ventral tegmental area activity. *Synapse, 52*, 143–152.

Porrino, L. J., & Lyons, D. (2000). Orbital and medial prefrontal cortex and psychostimulant abuse: Studies in animal models. *Cerebral Cortex, 10*, 326–333.

Robinson, T. E., & Berridge, K. C. (2000). Animal models in craving research—The psychology and neurobiology of addiction: An incentive-sensitization view. *Addiction, 95*, S91–S117.

Roop, R. G., Hollander, J. A., & Carelli, R. M. (2002). Accumbens activity during a multiple schedule for water and sucrose reinforcement in rats. *Synapse*, *43*, 223–226.

Salamone, J. D. (1996). The behavioral neurochemistry of motivation: Methodological and conceptual issues in studies of the dynamic activity of nucleus accumbens dopamine. *Journal of Neuroscience Methods*, *64*, 137–149.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology*, *80*, 1–27.

Schultz, W. (2000). Multiple reward signals in the brain. *Nature Review Neuroscience*, *1*, 199–207.

Schultz, W., Apicella, P., Scarnati, E., & Ljungberg, T. (1992). Neuronal activity in monkey ventral striatum related to the expectation of reward. *Journal of Neuroscience*, *12*, 4595–4610.

Self, D. W., Genova, L. M., Hope, B. T., Barnhart, W. J., Spencer, J. J., & Nestler, E. J. (1998). Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *Journal of Neuroscience*, *18*, 1848–1859.

Shalev, U., Grimm, J. W., & Shaham, Y. (2002). Neurobiology of relapse to heroin and cocaine seeking: A review. *Pharmacological Reviews*, *54*, 1–42.

Shidara, M., & Richmond, B. J. (May 31 2002). Anterior cingulate: Single neuronal signals related to degree of reward expectancy. *Science*, *296*, 1709–1711.

Sun, W., Akins, C. K., Mattingly, A. E., & Rebec, G. V. (in press). Ionotropic glutamate receptors in the ventral tegmental area regulate cocaine-seeking behavior in rats. *Neuropsychopharmacology*. (Available online at http://www.nature.com/npp/journal/vaop/ncurrent/abs/1300744a.html;jsessionid=1510EDA1D0752B3-D80A5B182C58CA9A4)

Sun, W. L., & Rebec, G. V. (2003). Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. *Journal of Neuroscience*, *23*, 10258–10264.

Sun, W. L., & Rebec, G. V. (2005). The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. *Psychopharmacology*, *177*, 315–323.

Sutton, M. A., Schmidt, E. F., Chol, K. H., Schad, C. A., Whisler, K., Simmons, D., Karanlan, D. A., Monteggla, L. M., Neve, R. L., & Self, D. W. (2003). Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. *Nature*, *421*, 70–75.

Thomas, K. L., & Everitt, B. J. (2001). Limbic–cortical–ventral striatal activation during retrieval of a discrete cocaine-associated stimulus: A cellular imaging study with gamma protein kinase C expression. *Journal of Neuroscience*, *21*, 2526–2535.

Tran-Nguyen, L. T. L., Fuchs, R. A., Coffey, G. P., Baker, D. A., Odell, L. E., & Neisewander, J. L. (1998). Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. *Neuropsychopharmacology*, *19*, 48–59.

Uylings, H. B. M., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioral Brain Research*, *146*, 3–17.

Uzwiak, A. J., Guyette, F. X., West, M. O., & Peoples, L. L. (1997). Neurons in accumbens subterritories of the rat: Phasic firing time-locked within seconds of intravenous cocaine self-infusion. *Brain Research*, *767*, 363–369.

Volkow, N. D., Wang, G. J., Fowler, J. S., Gatley, S. J., Logan, J., Ding, Y. S., Dewey, S. L., Hitzemann, R., Gifford, A. N., & Pappas, N. R. (1999). Blockade of striatal dopamine transporters by intravenous methylphenidate is not sufficient to induce self-reports of ''high.'' *Journal of Pharmacology and Experimental Therapeutics*, *288*, 14–20.

Volkow, N. D., Wang, G. J., Ma, Y., Fowler, J. S., Wong, C., Ding, Y. S., Mitzemann, R., Swanson, J. M., & Kalivas, P. (2005). Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: Relevance to addiction. *Journal of Neuroscience*, *25*, 3932–3939.

Wang, J. Q., & McGinty, J. F. (1999). Glutamate-dopamine interactions mediate the effects of psychostimulant drugs. *Addiction Biology*, *4*, 141–150.

White, F. J., & Kalivas, P. W. (1998). Neuroadaptations involved in amphetamine and cocaine addiction. *Drug and Alcohol Dependence*, *51*, 141–153.

Wise, R. A. (1998). Drug-activation of brain reward pathways. *Drug and Alcohol Dependence*, *51*, 13–22.

Woodward, D. J., Chang, J. Y., Janak, P., Azarov, A., & Anstrom, K. (2000). Activity patterns in mesolimbic regions in rats during operant tasks for reward. *Progress in Brain Research*, *126*, 303–322.

Zahm, D. S. (1999). Functional-anatomical implications of the nucleus accumbens core and shell subterritories. *Annals of the New York Academy of Sciences*, *877*, 113–128.

Zahm, D. S., & Brog, J. S. (1992). On the significance of subterritories in the ''accumbens'' part of the rat ventral striatum. *Neuroscience*, *50*, 751–767.

Zahm, D. S., & Heimer, L. (1993). Specificity in the efferent projections of the nucleus accumbens in the rat—comparison of the rostral pole projection patterns with those of the core and shell. *Journal of Comparative Neurology*, *327*, 220–232.

Received September 16, 2004
Final acceptance May 19, 2005