Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use

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Drug addiction is a neuropsychiatric disorder that marks the end stage of a progression beginning with recreational drug taking but culminating in habitual and compulsive drug use. This progression is considered to reflect transitions among multiple neural loci. Dopamine neurotransmission in the ventromedial striatum (VMS) is pivotal in the control of initial drug use, but emerging evidence indicates that once drug use is well established, its control is dominated by the dorsolateral striatum (DLS). In the current work, we conducted longitudinal neurochemical recordings to ascertain the spatiotemporal profile of striatal dopamine release and to investigate how it changes during the period from initial to established drug use. Dopamine release was detected using fast-scan cyclic voltammetry simultaneously in the VMS and DLS of rats bearing indwelling i.v. catheters over the course of 3 wk of cocaine self-administration. We found that phasic dopamine release in DLS emerged progressively during drug taking over the course of weeks, a period during which VMS dopamine signaling declined. This emergent dopamine signaling in the DLS mediated discriminated behavior to obtain drug but did not promote escalated or compulsive drug use. We also demonstrate that this recruitment of dopamine signaling in the DLS is dependent on antecedent activity in VMS circuitry. Thus, the current findings identify a striatal hierarchy that is instantiated during the expression of established responses to obtain cocaine.

Drug use often begins as a recreational behavior driven by the rewarding properties of the abused drug. However, addiction is characterized by habitual and compulsive drug use in which other factors, such as withdrawal symptoms, stress, and drug-associated conditioned stimuli (CS), also contribute to the motivation to consume drugs, and drug taking becomes increasingly prioritized over other behaviors (1). A wealth of evidence shows that the mesolimbic dopamine projection from the ventral tegmental area to the ventromedial striatum (VMS) is central to drug reinforcement (2). The ambient concentration of dopamine in the VMS is increased when animals self-administer drugs of abuse, including cocaine (3), and animals maintain this elevated dopamine level by regulating their rate of responding for drug (4). In addition, with repeated pairing of environmental stimuli with the drug, these CS also gain the propensity to elicit changes in dopamine concentration in the VMS (5–8); and even though these phasic neurochemical responses last only a few seconds, they are capable of controlling drug-seeking and -taking behavior (5). Together, these results implicate dopamine release in the VMS as a critical substrate in the control of drug use (2, 3, 9).

However, the progression of drug taking beyond recreational use is thought to reflect the engagement of different psychological processes mediated within several neural loci (10, 11), with a particular emphasis on the incorporation of the sensorimotor (dorsolateral) striatum (DLS) in the control of established drug-seeking behavior (10, 12). Specifically, dopamine transmission in the DLS has been linked to habitual CS-elicited reward seeking (13) and therefore may play an important role in the development of habitual and compulsive seeking of drugs (14–16). However, it is not known whether the encoding of drug-related actions or stimuli by phasic dopamine changes as drug-taking behavior advances from recreational drug use or whether this coding extends beyond the VMS to other parts of the striatum. In support of generalized signaling properties of dopamine across striatal regions, reward-associated cues produce transient increases in the firing rate of dopamine neurons throughout midbrain nuclei where the projection targets collectively encompass the entire striatum (17). However, evidence for this "global" signaling scheme from neurochemical recordings within the striatum itself is lacking. In fact, recent studies with natural rewards have challenged the concept of uniform phasic dopamine signaling throughout the striatum, instead reporting dopamine release in the VMS in response to natural rewards and associated cues but little or no dopamine release in the DLS (18, 19).

Therefore, to gain a fuller comprehension of the neural substrates underlying the development of drug abuse, we assessed the spatiotemporal dynamics of phasic dopamine release across the striatum during the progression of the early stages of drug taking by conducting neurochemical recordings in the VMS and DLS simultaneously and repeatedly over multiple sessions of cocaine self-administration (3 wk) in rats. We complemented these measurements with pharmacological and lesion approaches to investigate the behavioral function of DLS dopamine signaling and its relationship to that in the VMS, respectively.

Results

Male Wistar rats with chronically implanted microsensors (20) in the VMS and DLS (see Fig. S1 for histological verification of electrode placement) and indwelling i.v. catheters were trained to self-administer cocaine during daily 1-h sessions in a chamber equipped with two nose-poke ports (Fig. 1*A*). A nose poke into the active port elicited an infusion of cocaine (0.5 mg/kg body weight per infusion) and a 20-s presentation of a light/tone CS on a FR-1 schedule of reinforcement (Fig. 1*B*). Responses in the second (inactive) nose-poke port or in the active port during CS presentation (time-out) were without programmed consequence. Cocaine-reinforced responding remained relatively stable over 3 wk with only a modest increase in intake that did not reach significance [$n = 18$; $F_{(2,\ 34)} = 1.682$, $P = 0.201$; Fig. 1 *C* and *D*], whereas inactive and time-out responding (i.e., nonreinforced responding) diminished significantly [$F_{(2,\ 34)} = 5.075$, $P = 0.012$; Fig. 1 *C* and *E*]. Consequently, the ratio of reinforced to total responses (the efficiency of responding) was significantly greater in the second and third weeks than in the first week [$F_{(2,\ 34)} = 16.803$, $P < 0.001$; Fig. 1*F*].

Drug Cue-Induced Phasic Dopamine Release in the VMS Is Present Early in Cocaine Self-Administration. To characterize the long-term dynamics of dopamine transmission, longitudinal neurochemical recordings were carried out using fast-scan cyclic voltammetry. In the first week of self-administration, there was a significant phasic increase in extracellular dopamine concentration in the VMS following active responses ($P = 0.002$; Fig. 2*A* and Fig. S2). This increase produced an average change in dopamine concentration over the 7 s following the response of 7.77 ± 1.69 nM, with a mean

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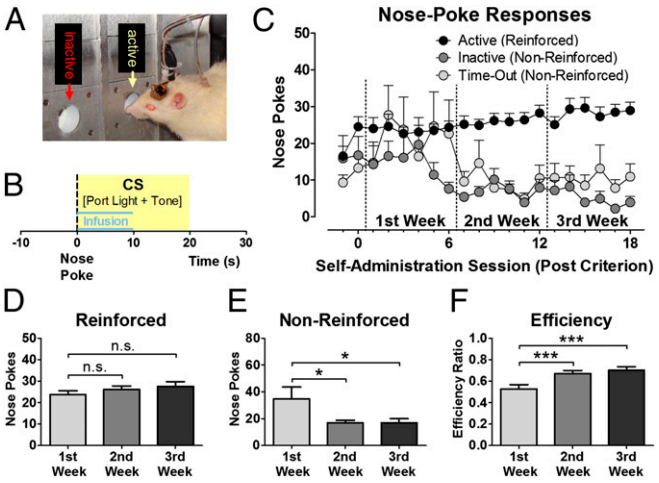

Fig. 1. Drug-taking behavior over the course of weeks. (*A*) Depiction of a rat connected to voltammetric recording equipment and infusion pump for i.v. delivery of cocaine during an approach to the active nose-poke port in the operant chamber. (*B*) A nose poke (dashed line) into the active port elicits an infusion of cocaine (0.5 mg/kg per infusion) and the presentation of a CS (yellow box) during a 20-s time-out. (*C*) Nose pokes into the active port, inactive port, and during the time-out period over 20 d of self-administration (n = 18). (*D*) The number of reinforced nose pokes did not change significantly across weeks, whereas the number of nonreinforced responses decreased (*E*), and the ratio of reinforced over total number of nose pokes (efficiency) increased (*F*) in the second and third weeks compared with the first week. *$P < 0.05$, ***$P < 0.001$; n.s., not significant.

peak of 13.47 ± 2.16 nM occurring 2.45 ± 0.26 s after the response and returning to baseline at 7.41 ± 0.28 s. These kinetics are similar to those reported in previous studies following a comparable amount of training (5–8), and the concentration matches those from recordings in the VMS with unbiased recording site selection (8), as in the current study (*SI Discussion* and Fig. S3). This pattern of activation continued into the second and third weeks ($P < 0.01$; Fig. 2*A* and Fig. S2) but diminished in amplitude with an average change in dopamine concentration of 5.96 ± 0.84 in the second week and 2.99 ± 0.85 nM in the third week [main effect of week: $F_{(2,44)} = 5.176$, $P = 0.010$; Fig. 2*B*]. In contrast, no significant change in dopamine concentration was detected following inactive nose pokes in either the first or the second and third week [main effect of inactive poke: $F_{(1,160)} = 1.392$, $P = 0.240$; Fig. 2*C*], indicating that the neurochemical signal was not simply a result of the motor response. However, noncontingent CS presentation alone was sufficient to elicit a significant VMS dopamine signal [$t_{(17)} = -2.361$, $P = 0.030$; Fig. 2*D*] that was similar in magnitude and duration to the signal following contingent CS presentation [$R = 0.92$; $P < 0.001$].

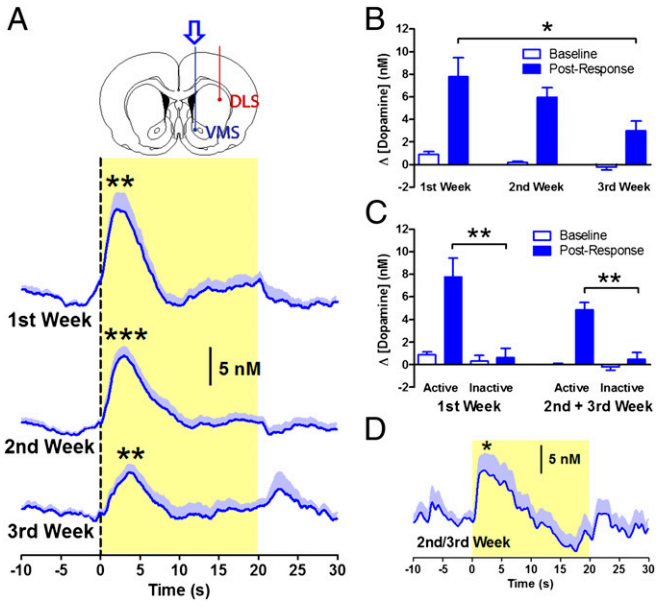

Fig. 2. Dopamine signaling in the VMS over the course of weeks. (*A*) Phasic dopamine release in the VMS following responses into the active nose-poke port was observed during all 3 wk of cocaine self-administration (n = 10). (*B*) Dopamine signals decreased in amplitude over the course of 3 wk. (*C*) Dopamine signals following responses into the active nose-poke port were larger than signals following inactive responses. (*D*) Noncontingent delivery of the CS induced dopamine release. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Phasic Dopamine Signaling in the DLS Develops over the Course of Weeks. Measurements in DLS revealed phasic dopamine release, similar to that in the VMS, in the second and third weeks of self-administration, with an average change in dopamine concentration of 3.10 ± 0.70 and 2.24 ± 0.38 nM, respectively ($P < 0.001$; Fig. 3*A*). However, such signaling was absent in the DLS during the first week (0.14 ± 0.50 nM; $P = 0.298$; Fig. 3*A*), demonstrating that phasic dopamine release in the DLS emerges over the course of drug taking [main effect of week: $F_{(2,62)} = 8.843$, $P < 0.001$; active poke × week interaction: $F_{(2,62)} = 6.468$, $P = 0.003$; Fig. 3*B* and Fig. S2]; that is, the long-term dynamics are in the opposite direction of those in the VMS [nose poke × week × region interaction: $F_{(2,\ 106)} = 5.505$, $P = 0.005$; Figs. S2 and S4]. Nonetheless, as in the VMS, the signal in the DLS was not elicited by the motor response [main effect of inactive poke: $F_{(1,193)} = 2.238$, $P = 0.136$; Fig. 3*C*] but increased following CS presentation [$t_{(17)} = -3.083$, $P = 0.007$; Fig. 3*D*; $R = 0.91$; $P < 0.001$]. These data demonstrate that phasic dopamine signals in the DLS and the VMS are elicited by the same drug-associated stimuli, but the signals emerge at a later stage of drug taking in the DLS, at a time when the VMS dopamine signal actually is decreasing.

Dopamine Receptors in the DLS Are Necessary for Discriminated Responses to Obtain Cocaine. To test the causal relationship between these neurochemical and behavioral observations, dopamine signaling was manipulated by bilateral infusion (see Fig. S5 for histological verification of cannula placement) of the nonspecific dopamine receptor antagonist alpha-flupenthixol into the DLS of additional groups of animals (n = 32; Fig. 4). In one group, flupenthixol and vehicle were infused on counterbalanced days in the first week of cocaine self-administration, corresponding to an early time point before the onset of CS-associated DLS signaling. A second group of animals was infused in the third week, corresponding to the later time point when DLS dopamine signals were present. The temporal pattern of the responses assessed in these animals (Fig. 4 *A*–*C*) was similar to that observed in the previous cohort (Fig. 1 *D*–*F*). Specifically, the rate of reinforced nose pokes remained stable over time [$F_{(2,\ 141)} = 1.092$, $P = 0.338$; Fig. 4*A*)], but the rate of nonreinforced nose pokes decreased significantly over the weeks of self-administration [$F_{(2,\ 141)} = 4.155$, $P = 0.018$; Fig. 4*B*], producing an increase in response efficiency across this period [$F_{(2,\ 141)} = 7.843$, $P < 0.001$; Fig. 4*C*]. Intra-DLS infusion of flupenthixol resulted in an increase in cocaine intake (reinforced nose pokes) at both the early and late time points ($P < 0.05$ vs. vehicle; Fig. 4*D*), suggesting that DLS dopamine may contribute to the reinforcing properties of cocaine, as is consistent with previous reports (21, 22). Importantly, this effect therefore is not attributable to the CS-associated phasic dopamine signal, which was present at the late time point but not the early time point. In contrast to the effect on reinforced responding at both time points, the average number of nonreinforced responses was increased after the late infusion ($P = 0.024$; Fig. 4*E*) but not after the early infusion ($P = 0.970$). Accordingly, the nose-poke efficiency was decreased after the intracerebral administration of flupenthixol at the late ($P = 0.004$; Fig. 4*F*) but not the early

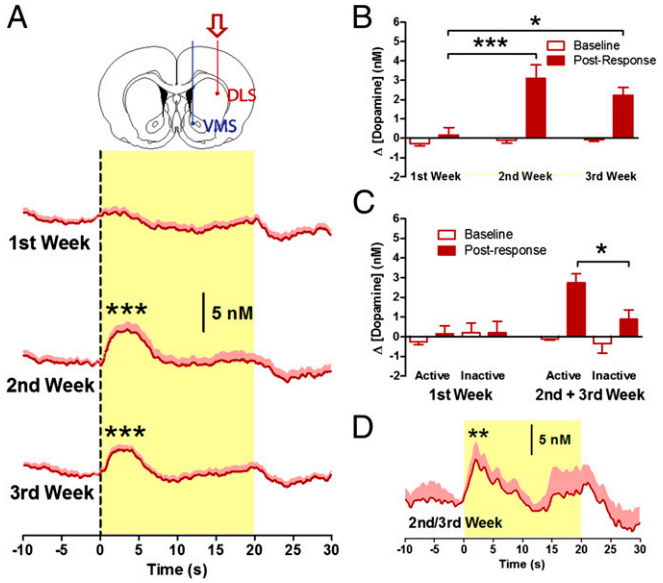

Fig. 3. Dopamine signaling in DLS over the course of weeks. (*A*) Phasic dopamine release in the DLS following responses into the active nose-poke port was observed during the second and third weeks of cocaine self-administration ($n = 15$). (*B*) Dopamine signals in the second and third weeks were greater in amplitude than those in the first week. (*C*) Dopamine signals following responses into the active nose-poke port were larger than signals following inactive responses during the second and third weeks but not during the first week. (*D*) Noncontingent delivery of the CS induced dopamine release. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

($P = 0.762$) time point [drug × time-point interaction: $F_{(1, 27)} = 7.482$, $P = 0.011$]. These data show that the gain in efficiency as measured by discriminated drug-taking responses between the first and third weeks of cocaine self-administration was reversed by the infusion of flupenthixol into the DLS, indicating that emergent dopamine signaling in the DLS is necessary for the improved action selection of drug-taking behavior.

Development of Phasic Dopamine Signaling in the DLS Depends on the VMS. A salient feature of the current findings and those of others (21) is the progressive onset of function in the DLS during drug use. This progressive involvement of the DLS in drug seeking has been linked to circuitry that connects the VMS to the DLS by a serial disconnection study that demonstrated that the development of advanced cue-controlled drug-seeking behavior is dependent on intact VMS circuitry (23). Therefore, to test whether the later-emerging phasic dopamine signal in DLS reported in the present study was dependent upon antecedent activity in the VMS circuitry, we mimicked a disconnection of the VMS from DLS on one side of the brain with a unilateral excitotoxic lesion of the nucleus accumbens core (VMS) by infusing quinolinic acid before training (23), leaving the other side intact. Voltammetric microsensors were implanted bilaterally in the DLS ($n = 17$), permitting within-subject comparison of emergent DLS dopamine transmission between hemispheres, one hemisphere having an intact and the other a lesioned VMS (see Fig. S6 for histological verification of lesion and electrode placement). Cocaine intake was similar to that in nonlesioned animals (Fig. S7), as is consistent with previous findings (23). Also, similar to nonlesioned animals (Fig. 3), active nose-poke responses evoked significant dopamine release in the DLS contralateral to the lesion in the second and third weeks (1.81 ± 0.23 and 1.77 ± 0.22 nM; $P < 0.01$; Fig. 5*A*) but not in the first week (-0.19 ± 0.49; $P = 0.778$; Fig. 5*A*). However, in the hemisphere ipsilateral to the VMS lesion, there were no significant changes in dopamine release compared with baseline at any time point of cocaine self-administration, with an average change in dopamine concentration of 0.85 ± 0.38, 0.84 ± 0.22, and 0.92 ± 0.23 nM in weeks 1–3, respectively ($P > 0.05$; Fig. 5*B*). Thus, phasic dopamine signals evolved over the 3 wk of self-administration contralateral [main effect of active poke: $F_{(1, 63)} = 19.386$, $P < 0.001$; main effect of week: $F_{(2, 63)} = 15.294$, $P < 0.001$; active poke × week interaction: $F_{(2, 63)} = 19.386$, $P = 0.048$; Fig. 5*C* and Fig. S8] but not ipsilateral [main effect of week: $F_{(2, 43)} = 0.001$, $P = 0.999$; Fig. 5*C*] to the lesion, conferring significantly different patterns of dopamine release in the two hemispheres [brain region × week interaction: $F_{(2, 106)} = 7.204$, $P < 0.001$; Fig. 5*C*)] Similarly, noncontingent delivery of the CS induced significant dopamine release ($P = 0.040$; Fig. 5*D*) contralateral but not ipsilateral to the VMS lesion ($P = 0.761$; Fig. 5*E* and Fig. S9). Importantly, during periods in the recording sessions that were free of operant behavior and CS presentations, the magnitude of "spontaneous" dopamine release in the DLS was similar ipsilateral and contralateral to VMS lesion (Fig. S10). Furthermore, the magnitude of DLS signals measured in the first study (Fig. 3*A*) and in the DLS contralateral to the lesion (Fig. 5*A*) were not significantly different [main effect of brain region: $F_{(1,125)} = 0.851$; $P = 0.358$]. Therefore, the VMS lesion did not produce a general suppression of dopamine transmission in the DLS but had a selective effect on task-related signaling. These results demonstrate that neural activity in VMS is required for the development of CS-elicited dopamine signaling in the DLS that regulates the efficiency, or automaticity, of drug-taking responses.

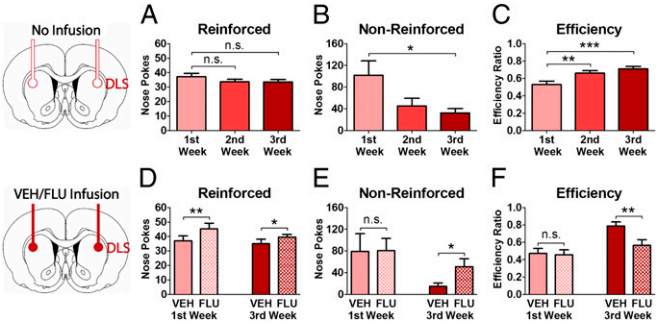

Fig. 4. Blockade of dopamine receptors in the DLS disrupts discriminated drug-taking behavior. (*A*–*C*) The rate of reinforced nose pokes remained stable across weeks (*A*), but the rate of nonreinforced nose pokes was decreased (*B*), and response efficiency increased (*C*) during the second and third weeks compared with the first week. (*D*) Infusion of flupenthixol (FLU) into the DLS produced an increase in reinforced nose pokes in both the first ($n = 16$) and the third weeks ($n = 16$). (*E*) The average number of nonreinforced responses was increased after flupenthixol only during the third but not during the first week. (*F*) Consequently, response efficiency was decreased after flupenthixol at the late but not at the early time point. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; VEH, vehicle.

Discussion

Spatiotemporal Changes in Striatal Dopamine Signaling. Drug self-administration studies in animals have revealed neuroadaptations in functional markers that progress from the VMS to encompass the DLS over the course of drug use (12). To test whether there are complementary changes in phasic dopamine transmission, we carried out longitudinal subsecond dopamine measurements simultaneously in the VMS and DLS during the establishment of drug taking in rats. We observed phasic dopamine release in both the VMS and DLS following the operant response for drug during the course of our study in which the VMS signal declined and the DLS signal emerged during the progression of drug taking. Despite these differences in temporal profiles, phasic dopamine release encoded similar information in the VMS and DLS. In both regions, it was elicited selectively by active and not by inactive nose pokes, indicating that the signal was not simply related to the motoric action of making a response. Instead, we hypothesized that dopamine release was a result of successful completion of the response to obtain cocaine (signaled by the CS). This notion was

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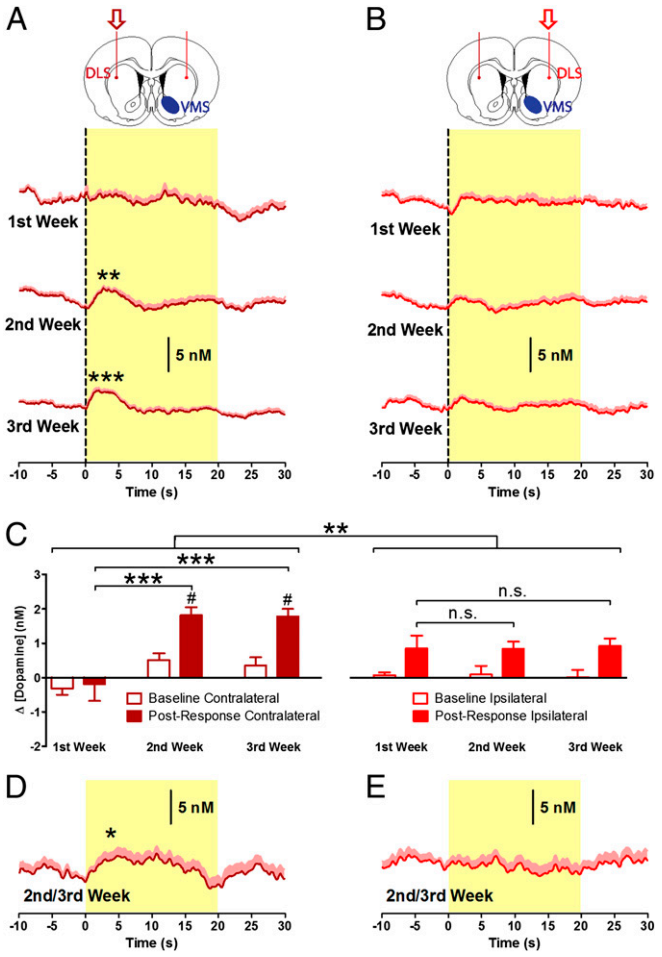

Fig. 5. VMS lesion prevents development of phasic dopamine signaling in the DLS. (*A*) Phasic dopamine release was observed in the DLS contralateral to the unilateral lesion of the VMS following responses into the active nose-poke port during the second and third weeks of cocaine self-administration ($n = 17$). (*B*) Dopamine release in the ipsilateral DLS was not significantly increased in any week ($n = 11$). (*C*) In the contralateral DLS, phasic signaling in the second and third weeks was larger in amplitude than signals detected in the first week (*Left*), whereas signals did not change in amplitude across weeks in the ipsilateral DLS (*Right*). Emergence of such signaling had significantly different patterns of dopamine release between hemispheres. A direct post hoc comparison between ipsilateral and contralateral hemispheres showed greater dopamine release in the contralateral hemisphere in the second and third weeks of training but not in the first week ($^{\#}P < 0.05$). (*D* and *E*) Noncontingent delivery of the CS consistently induced DLS dopamine release contralateral (*D*) but not ipsilateral (*E*) to the VMS lesion. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

supported, because noncontingent presentation of the CS alone was sufficient to recapitulate dopamine release following an active response in both regions, similar to that reported previously (5) for a time point equivalent to the first week of training in the present study. Drug-associated CS are integral to drug use, guide the acquisition and maintenance of drug taking, and increasingly assume control over behavior to the extent of triggering the resumption of drug taking even after long periods of abstinence (24, 25). Thus, the current findings reveal a process by which drug-associated stimuli gain access to sensorimotor circuitry with repeated drug use. Interestingly, the emergent sensorimotor signal generally was smaller than that in the VMS, even when drug use was established. This observation is notable because the density of dopamine terminals (26), tissue content (27), and capacity for release (27, 28) are greater in the DLS than in the VMS and suggest that the phasic dopamine responses use less of the available "bandwidth" for encoding of drug cues in the DLS than in the VMS. Similarly, the long-term effect of prior cocaine exposure on the processing of stimuli associated with natural reinforcers is not uniform across these two regions. Instead of increasing processing in both the VMS and DLS, cocaine reduces the degree and flexibility of cue-evoked neuronal firing in VMS while enhancing firing in DLS, with effects in the DLS being relatively weak compared with those in the VMS (29).

Overall, our data identify the spatiotemporal pattern of phasic dopamine release in the striatum during the establishment of drug-taking behavior. The gradual decline in VMS dopamine signaling is somewhat surprising in the context of models postulating that the amount of dopamine release in response to drug cues, specifically in the nucleus accumbens, increases over repeated drug administration as these cues undergo incentive sensitization (9). In contrast, the emergence of phasic dopamine signaling in the DLS provides further empirical support for current theories postulating the engagement of an increasing number of brain regions with prolonged drug use (10–12, 16).

Dopamine Signaling in the Sensorimotor Striatum Emerges Before Compulsive Drug Abuse. The observed spatiotemporal dynamics of striatal dopamine signaling illustrate the progressive engagement of brain systems with persistent drug self-administration. It has been suggested that each of the stages in the series of transitions from goal-directed to habitual and eventually to compulsive responding for drug is associated with specific brain systems that are recruited progressively (10). Indeed, the DLS comes to exert more dominant control over drug seeking during the course of drug use (21, 30) as drug taking becomes maintained by drug-associated stimuli (10, 12, 16). Although we have demonstrated that phasic dopamine release does indeed develop at a later stage of drug use in the DLS than in the VMS, the training regimen used typically is not sufficient to produce compulsive responding or the significant escalation of drug intake that emerges following extended or long-access training in drug self-administration (31, 32). Thus, our data demonstrate that the engagement of DLS dopamine, which is thought to be linked closely to stimulus–response processing (13), is not sufficient to account for the loss of control over drug intake characteristic of addiction, underlining the important dissociation between the habitual and compulsive stages of drug taking and their neural substrates (33). In fact, the behavioral measure that most closely correlated with the emergence of phasic dopamine release in the DLS was the efficiency of response, that is, the number of active nose-poke responses as a proportion of the total number of responses (including time-out responses and responses in the inactive port). This increase in response efficiency between the first and third weeks of self-administration was reversed by dopamine-receptor antagonism in the DLS, whereas this treatment had no effect on efficiency in the first week, before phasic dopamine signaling in the DLS had emerged. In contrast, cocaine intake (reinforced nose pokes) was increased by the antagonist in both the first and third weeks, suggesting that this effect likely is not associated with the phasic modality of dopamine signaling time-locked to drug taking, and therefore tonic dopamine signaling may be implicated. This notion is consistent with the work of others indicating a role for DLS dopamine in mediating the reinforcing properties of cocaine (21, 22). Therefore, rather than contributing to escalated or compulsive responding, the progressive recruitment of DLS phasic dopamine promotes the refinement of behavior toward reinforced actions, as operant responding for the drug becomes more reliably discriminated over the course of weeks in the absence of escalated drug intake.

Although DLS dopamine appears to suppress nonreinforced responses, it was not observed around these actions. Instead, it seems that the feedback collected from reinforced responses promotes exclusivity (i.e., actions that are not associated with a DLS dopamine signal are not maintained). Although this inference may appear elaborate, it is consistent with the idea that the striatum does not generate movement itself but rather promotes focused selection of available actions by simultaneously and focally

removing the inhibition of specific actions and acting broadly by inhibiting rivaling/conflicting motor mechanisms that otherwise would interfere with the desired action (34). Consistent with our findings, dorsal striatal circuits serve to evaluate behavior and to exploit optimal behaviors following initial behavioral variability during trial-and-error learning (exploration) (35) as an integral part of the sensorimotor domain of the basal-ganglia network mediating action sequencing as well as selection/inhibition of competing motor programs (34, 36). Thus, our findings suggest that the observed changes in DLS dopamine signaling (i.e., task representation in DLS circuits) might facilitate a switch from exploring the availability of drug rewards present in the environment to exploiting this environment.

Addiction often is described as a disorder of brain memory systems. The DLS is considered to be a critical locus for procedural learning (37), with dopamine acting as a neurotransmitter that induces plasticity to enable the formation of long-lasting network changes. Brain regions that mediate the evolving discrimination of drug cues and drug taking are potentially of great interest in the identification of neural systems underlying addiction. Our data suggest that the observed behavioral refinement may represent an amplified focus on drug-related behaviors that causes the prioritization of drug taking over behavior not reinforced by drug, a development also observed in drug addiction (1). Thus, although the efficiency of drug taking does not itself imply compulsive or addiction-like behavior, monitoring response discrimination may prove useful in the investigation of abuse-related behaviors comparable to a period when drug abusers narrow their behavioral repertoire to actions that prioritize the intake of drugs over other actions. Taken together, these data demonstrate a mechanism involving sequential recruitment of phasic dopamine transmission in the striatum in the dynamically changing neural control over drug intake even before compulsive use emerges.

A Hierarchy for Recruiting Dopamine in Different Striatal Modules. Limbic circuits that converge on the VMS have been hypothesized to affect and enable sensorimotor circuits, thus functioning as a gateway for limbic structures to reach motor systems (38). Sensorimotor aspects of the striatum are thought to contribute to facilitating automatic execution of motor acts or to implementing habits by building up individual motor acts to coherent chunks of performance units (36). We investigated interactions between motivational and sensorimotor networks within the striatum during drug self-administration using the combination of a unilateral VMS lesion and bilateral electrochemical recordings in DLS. This approach enabled the study of dopamine neurotransmission simultaneously in intact and disrupted basal ganglia circuits during the same trial of the same animal and thus in the same motivational state. Our data provide functional evidence supporting an interaction between limbic and motor networks in the development of discriminated responses to obtain cocaine, in which the VMS, which receives limbic inputs, enables dopamine signaling in the sensorimotor DLS.

Previous support for a role of serial circuitry that connects the VMS and DLS comes from a study that combined lesioning of the VMS on one side of the brain and antagonism of dopamine receptors in the contralateral DLS, thereby functionally disconnecting serial interactions between these striatal domains on both sides of the brain (23). Although either manipulation on its own was without effect, the combined procedures selectively decreased cocaine seeking in extensively trained rats but not in rats that had undergone only moderate training (23). Together with our study, these findings underline the functional significance of the network interaction between the VMS and the DLS in drug-related behavior. Specifically, they indicate that this circuit is used for multiple, related processes in the procurement of drugs, both in prioritization of drug-taking behavior and the exploitation of a drug environment in animals with a moderate drug history (present study) and in energizing and driving drug-seeking behavior in an environment where the drug is not readily available in animals with an extended drug-taking history (33). Therefore, the hierarchical recruitment of striatal subregions for dopamine-mediated control of behavior may signify an overarching organizing principle throughout the stages of drug use to enable representation of drug cues in DLS.

There has been a long-standing debate on how interactions between limbic and motor systems are implemented. On the level of basal ganglia circuitry, a potential anatomical substrate for this interaction of striatal modules is the interconnectivity between striatal projection neurons and the dopaminergic midbrain. Nauta et al. (39) discovered that VMS neurons, which receive dopaminergic afferents from the ventral tegmental area, send axons to the substantia nigra, which provides a dopaminergic projection to the dorsal striatum. This connectivity later was found to display an elaborate spiraling organization with several striato-nigro-striatal loops spanning from the limbic VMS to the sensorimotor DLS (40). However, other pathways also channel information from VMS to DLS via the midbrain (41–43). Irrespective of anatomical pathway, the demonstration of a striatal hierarchy in the control of dopamine transmission provides important insight into how neurotransmission within neural circuits regulating behavior is shaped over prolonged drug use.

Conclusions

Overall, the present data offer insight into neurobiological processes that establish drug-taking behavior. It demonstrates that phasic dopamine signaling in the striatum is dynamic and region specific, emerging sequentially in the VMS and then in the DLS in the early stages of drug use. We ascertained that the progression from limbic to sensorimotor regions of the striatum requires intact VMS circuitry. This hierarchical control enables drug-associated stimuli to access the brain systems implicated in the development of a drug-taking habit.

Experimental Procedures

Surgical Procedures. Stereotaxic surgery was performed as described previously (20). The target coordinates were 1.2 mm anterior, 3.1 mm lateral, and 4.8 mm ventral to bregma for the DLS and 1.3 mm anterior, 1.3 mm lateral, and 7.2 mm ventral to bregma for the nucleus accumbens core of the VMS. For the pharmacological experiment, guide cannulas were implanted bilaterally into the DLS. For the lesion experiment, quinolinic acid (0.09 M; 0.5 μL) was infused unilaterally into the VMS to induce an excitotoxic lesion (23). The i.v. catheters were implanted in a separate surgery.

Cocaine Self-Administration. Rats were trained to obtain cocaine following an operant response on a continuous reinforcement (FR-1) schedule in an operant chamber equipped with two nose-poke response devices. Nose-poking in the active port resulted in an i.v. infusion of cocaine (0.5 mg/kg) paired with a 20-s presentation of an audiovisual stimulus (CS). During CS presentation, a 20-s time-out was imposed during which nose poking did not result in any programmed consequences. To control for response specificity, nose-poking of the second (inactive) port was monitored. Rats were given access to cocaine for 1 h/d, 6 d/wk, for 3 wk.

Infusion of Flupenthixol into the DLS. The effects of the dopamine receptor antagonist flupenthixol (5 μg dissolved in 0.5 μL vehicle into each side; 0.5 μL/min) or vehicle on drug-taking behavior were examined in single sessions during the first or third weeks of self-administration. One group of rats received flupenthixol or vehicle in the first week of cocaine self-administration, counterbalanced on 2 d, and a separate group received counterbalanced infusions in the third week.

Voltammetric Measurements and Analysis. Electrochemical recordings (2 d/wk) using chronically implanted carbon-fiber microsensors and data analysis were carried out as described previously (20) and are described in more detail in *SI Experimental Procedures*. In brief, during each voltammetric scan (every 100 ms), the potential at the carbon-fiber electrode was ramped linearly from −0.4 V versus Ag/AgCl to +1.3 V and back at 400 V/s (total scan time, 8.5 ms). Dopamine at the surface of the electrode is oxidized during the anodic sweep to form dopamine-*o*-quinone which is reduced back to dopamine in the cathodic sweep. The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo electrolysis. The background-subtracted, time-resolved current obtained provided a chemical signature characteristic of the analyte, allowing resolution of dopamine from other substances. Dopamine was isolated from the

voltammetric signal using chemometric analysis using a standard training set (20) based on electrically stimulated dopamine release detected at chronically implanted electrodes. Dopamine concentration was estimated based on the average postimplantation sensitivity of electrodes (20), averaged over the 7 s following the operant response (postresponse) or noncontingent presentation of the CS and compared with the average concentration over the 2 s prior to the response or CS (baseline).

Statistical Analysis. Individual voltammetric recordings were averaged across session, animals, and weeks. These means then were compared using one-, two-, and three-way ANOVAs with postresponse, brain region, and week as factors. For comparison with voltammetric data, behavioral data also were binned into weeks. For the flupenthixol-infusion experiment, mean baseline values for weeks 1 and 3 during which flupenthixol was infused were computed by averaging the data over 3 d in the week during which no infusions were administered. Behavioral data were analyzed using one- and two-way ANOVAs with drug and weeks as factors. When appropriate, post hoc analyses were conducted, and *P* values were adjusted according to the Holm–Bonferroni correction method for multiple testing (44). Plots were made using Prism (GraphPad Software). All statistical analyses were carried out using SPSS, version 17.0. All data are presented as mean plus SEM.

Histological Verification of Recording Sites. On completion of experimentation, recording sites were marked with an electrolytic lesion and verified using cresyl violet staining.

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Supporting Information

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SI Experimental Procedures

Animals. Adult male Wistar rats from Charles River weighing 300–400 g were housed individually and kept on a 12-h light/12-h dark cycle (lights on at 0700) with controlled temperature and humidity and with food and water available ad libitum. All animal use was approved by the University of Washington Institutional Animal Care and Use Committee, and surgical procedures were performed under aseptic conditions. For the first voltammetry experiment, 34 animals underwent surgery; of these animals, 18 maintained catheter patency throughout the experiment, had at least one functional and histologically verified electrode, and passed behavioral criteria (see below). For the pharmacological experiment, 32 of 39 rats that underwent cannulation had histologically verified bilateral cannula placements in the dorsolateral striatum (DLS), maintained i.v. catheter patency, and were used in the study. Of the 21 rats prepared for the lesion experiment, 17 maintained catheter patency, had a histologically verified lesion of the nucleus accumbens core, had at least one functional and histologically verified electrode in the DLS, and passed behavioral criteria.

Stereotaxic Surgery. Rats were anesthetized with isoflurane and placed in a stereotaxic frame. The scalp was swabbed with alcohol and Betadine, bathed with a mixture of lidocaine (0.5 mg/kg) and bupivacaine (0.5 mg/kg), and incised to expose the cranium. Holes were drilled in the cranium, and dura mater was cleared for targeting of the DLS [1.2-mm anterior, 3.1-mm lateral, and 4.8-mm ventral to bregma (1)] and, in some animals, the nucleus accumbens core of the ventral medial striatum (VMS) (1.3-mm anterior, 1.3-mm lateral, and 7.2-mm ventral to bregma). For the first set of animals, one carbon-fiber microelectrode made in-house (2) was positioned in the nucleus accumbens core and another in the DLS, and an Ag/AgCl reference electrode was implanted in a separate part of the forebrain. For the next set of animals, guide cannulas (26 gauge; Plastics One) occluded by dummy cannulas of equal length were implanted bilaterally to target the DLS. In a final set of animals, quinolinic acid (0.09 M; 0.5 μL) was infused unilaterally into the nucleus accumbens core to induce an excitotoxic lesion (3), and carbon-fiber microelectrodes were implanted bilaterally into DLS. Electrodes and guide cannula were secured with cranioplastic cement anchored to the skull by screws. After surgery, rats were placed on an isothermal pad to maintain body temperature until ambulatory and were allowed to recover for at least 5 d. Rats were administered a long-acting, nonsteroidal anti-inflammatory, either meloxicam (1 mg/kg, s.c.) before surgery or carprofen (2 mg/kg, s.c.) just after surgery. All animals were implanted with i.v. catheters during a separate surgery.

Implantation of i.v. Catheters. Rats were anesthetized with isoflurane. Catheters were made of Silastic tubing with an outer diameter of 0.6 mm and were attached to a "hub" at one end (distal to vein insertion) (Plastics One) for connection to an infusion pump. Catheters were pushed s.c. through an incision on the back between the shoulders to the front of the body and were anchored into the right jugular vein aided by a silicon rubber bead near the proximal end of the catheter. Optimal positioning of the catheter was verified by drawing blood into it with negative pressure. The hub then was secured by a piece of Teflon mesh sutured to surrounding tissue, and incisions were closed, leaving the hub protruding from the rat's back. The catheter was flushed with a heparin solution (80 U/mL in saline) and was filled with a viscous solution of polyvinylpyrolidone (PVP) and heparin (1,000 U/mL). The catheter hub was capped with a short, crimped piece of polyethylene tubing, and the PVP solution remained in the catheter to ensure patency. After surgery, rats were allowed to recover for at least 5 d.

Cocaine Self-Administration. Self-administration sessions were conducted between 1000 and 1600 h. Rats learned to self-administer cocaine (Sigma) in a modular operant chamber (Med Associates) equipped with two nose-poke response devices (with integrated cue lights) located on adjacent panels of the same wall, a house light, and speakers to provide pure-tone and white-noise stimuli. The operant chamber was housed within a sound-attenuated outer chamber. Rats were trained to obtain cocaine following an operant response on a continuous reinforcement (FR-1) schedule. Nose-poking in the active port (the side was counterbalanced between animals) resulted in an immediate i.v. infusion of cocaine (0.5 mg/kg over about 10 s) paired with a 20-s presentation of an audiovisual stimulus [illumination of the light inside the nose poke port and tone; conditioned stimulus (CS)]. During CS presentation, a 20-s time out was imposed during which nose poking did not result in further drug infusion or any other programmed consequences. Drug availability during the session was signified by white noise and illumination of the house light. To control for response specificity, nose-poking of the second (inactive) port was monitored but was never reinforced. A criterion for inclusion in the study was five or more active responses per session on two successive sessions. The number of precriterion sessions varied among animals from none to seven sessions. After pretraining sessions, rats were given daily access to cocaine for 1 h, 6 d/wk, for 3 wk.

Infusion of Flupenthixol into the DLS. The effects on drug-taking behavior of bilateral infusion of the nonspecific dopamine receptor antagonist flupenthixol [5 μg dissolved in 0.5 μl artificial cerebrospinal fluid (ACSF) (Sigma) into each side; 0.5 μl/min] and ACSF (vehicle) into DLS were examined in single sessions during the first or third weeks of self-administration. The dose of flupenthixol used did not inhibit general performance, as determined in pilot studies and previous studies conducted by other investigators (3, 4). To avoid potentially confounding effects of repeated flupenthixol administration, one group of rats received flupenthixol and ACSF in the first week of cocaine self-administration, counterbalanced on 2 d, and a separate group received counterbalanced infusions in the third week ($n = 16$ each). On infusion days, the dummy cannula was replaced with a 33-gauge infusion cannula that protruded 1.0 mm beyond the guide cannula. Infusions were given 5 min before session start. After the infusion, the infusion cannulas were left in place for 2 min to allow diffusion of the drug.

Voltammetric Measurements and Analysis. For dopamine detection by fast-scan cyclic voltammetry during experimental sessions (recordings were performed during two sessions per week), chronically implanted carbon-fiber microsensors were connected to a head-mounted voltammetric amplifier, interfaced with a PC-driven data-acquisition and analysis system (National Instruments) through an electrical swivel (Med Associates) that was mounted above the test chamber. Voltammetric scans were repeated every 100 ms to achieve a sampling rate of 10 Hz. During each voltammetric scan, the potential at the carbon-fiber electrode was ramped linearly from −0.4 V versus Ag/AgCl to +1.3 V and back at 400 V/s (total scan time, 8.5 ms) and held at −0.4 V between scans. When dopamine is present at the surface of the electrode, it is oxidized during the anodic sweep to form dopamine-*o*-quinone (peak

reaction detected at approximately +0.7 V), which is reduced back to dopamine in the cathodic sweep (peak reaction detected at approximately −0.3 V). The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo electrolysis. The background-subtracted, time-resolved current obtained from each scan provided a chemical signature characteristic of the analyte, allowing resolution of dopamine from other substances (5). Voltammetric data were band-pass filtered at 0.025–2,000 Hz. Dopamine was isolated from the voltammetric signal using chemometric analysis using a standard training set (2) based on electrically stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated based on the average postimplantation sensitivity of electrodes (2). Before analysis of average concentration, all data were smoothed with a five-point within-trial running average. The concentration of dopamine was averaged over the 7 s (approximate duration of the observed phasic signal) following the operant response (postresponse) or noncontingent presentation of the CS and was compared with the average concentration over the 2 s prior to the response or CS (baseline). The CS was presented noncontingently (twice per session for 20 s each) during every recording session conducted in the second and third weeks but not during the first week to avoid interference with the associative conditioning between drug delivery and the cue during the period when this association presumably was still developing.

Statistical Analysis. Individual signals were averaged across session and then across animals and weeks to increase statistical power. Averages then were compared using one-, two-, and three-way ANOVAs with response, brain region, and week as factors. For comparison with voltammetric results, behavioral data were grouped into weeks. For the flupenthixol-infusion experiment, baseline data grouped by weeks were computed by averaging the data over 3 d during the week without infusions. Behavioral data were analyzed using one- and two-way ANOVAs with drug and weeks as factors. In case of significant main effects or interactions, post hoc analyses were conducted, and *P* values were adjusted according to the Holm–Bonferroni correction method for multiple testing (6). Plots were made using Prism (GraphPad Software). All statistical analyses were carried out using SPSS, version 17.0.

Histological Verification of Recording Sites. On completion of experimentation, animals were anesthetized with i.p. ketamine (100 mg/kg) and xylazine (20 mg/kg). In animals with electrode implants, recording sites were marked with an electrolytic lesion (300 V) before transcardial perfusion with saline followed by 4% paraformaldehyde (40 g/l). Brains were removed and postfixed in paraformaldehyde for 24 h and then were rapidly frozen in an isopentane bath (~5 min), sliced on a cryostat (50-μm coronal sections, −20 °C), and stained with cresyl violet to aid in visualization of anatomical structures and the electrode-induced lesion or infusion sites.

SI Discussion

In most of the previous work that measured phasic dopamine release in the nucleus accumbens core during cocaine self-administration, recording sites were optimized for maximal stimulated dopamine release (7–10) and therefore sampled maximum dopamine responses. Only one previous study (experiment 2 in ref. 10) selected recording sites by electrophysiological recording rather than by neurochemical responses and thus provided an unbiased sample of the nucleus accumbens core, measuring the average dopamine responses across this region which exhibits neurochemical heterogeneity (11). In the current work, implantation sites were based entirely on stereotaxic coordinates without functional feedback and so also provide an unbiased sample of the nucleus accumbens core. Notably, there is remarkable concordance between these two studies with unbiased sampling in both the concentration and the dynamics of the phasic dopamine response to cocaine self-administration (Fig. S3).

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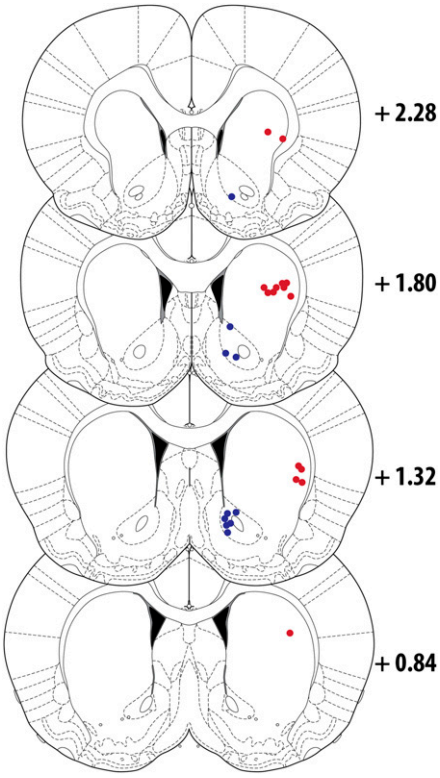

Fig. S1. Histological verification of recording sites in the VMS and DLS (first experiment). VMS recording sites (blue circles) were confirmed to be within the nucleus accumbens core, and DLS recording sites (dark red circles) were in the lateral half of the dorsal striatum. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

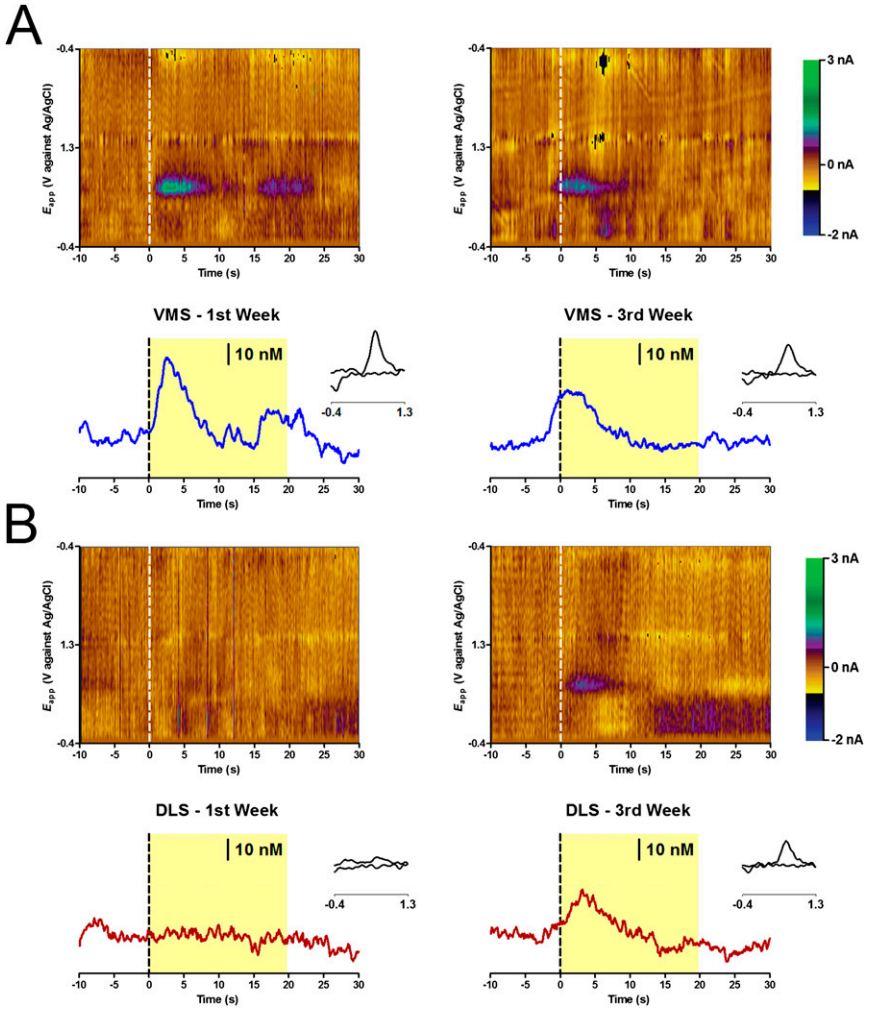

Fig. S2. Examples of phasic dopamine release in the VMS and DLS associated with an individual nose poke (single trial) into the active port. (*A*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the VMS for the period 10 s before an operant response (dashed line), during the subsequent 20-s presentation of the CS (yellow box), and 10 s after the offset of the CS during the first (*Left*) and third (*Right*) weeks of cocaine self-administration. (*B*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS during the first (*Left*) and third (*Right*) weeks of self-administration. The color plots show current changes across the applied voltages (E_{app}; *y* axis) over time (*x* axis).

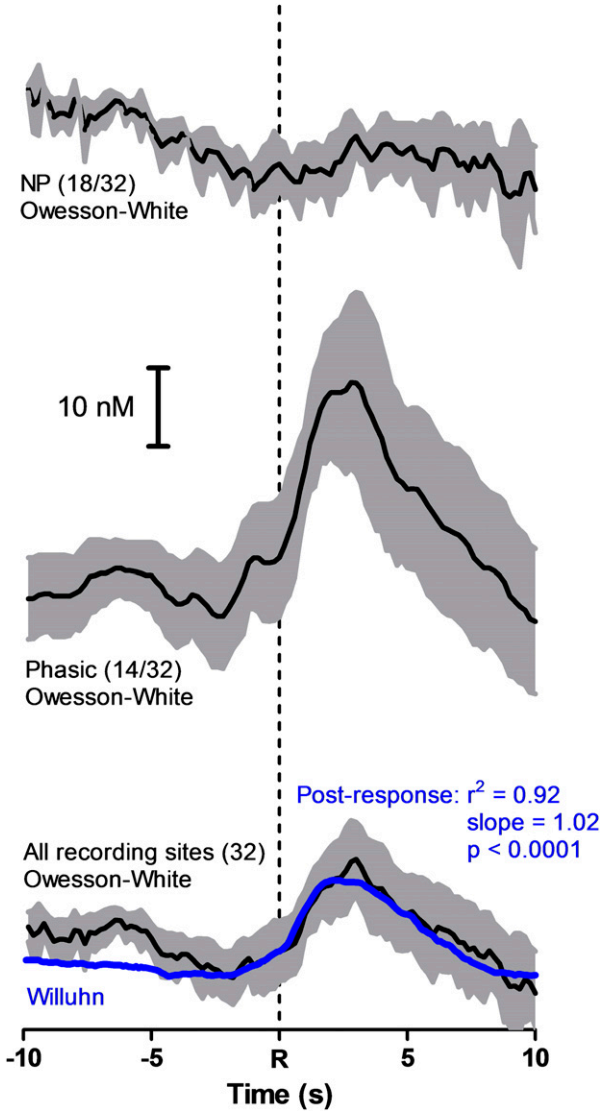

Fig. S3. Comparison of data from Owesson-White et al. (10) and the current findings. Phasic dopamine release (digitized using GetData Graph Digitizer, http://getdata-graph-digitizer.com) from recording sites adjacent to neurons whose firing rates were responsive (*Middle*) or unresponsive (*Top*) to cocaine self-administration were combined to produce a weighted average (*Bottom*). This average response was similar to the average response in the current study obtained from the nucleus accumbens core in the first week of cocaine self-administration (*Bottom*, blue trace). The phasic responses that follow an operant response (*R*) for cocaine from the two studies have concentration profiles that match closely in shape (r^2 = 0.92) and amplitude (slope = 1.02).

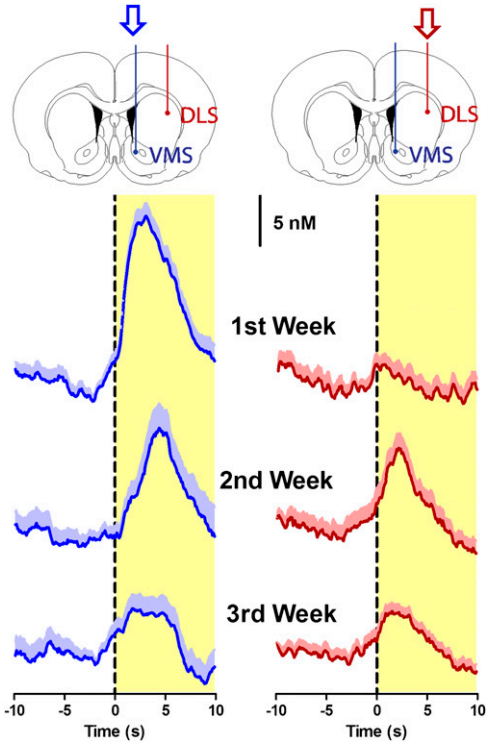

Fig. S4. Phasic dopamine signaling in the VMS and DLS for an individual animal. The pattern of average phasic dopamine release in the VMS (*Left*) and DLS (*Right*) is depicted 10 s before and 10 s after responses into the active nose-poke port across 3 wk of cocaine self-administration in an individual animal. Data are expressed as mean + SEM per week.

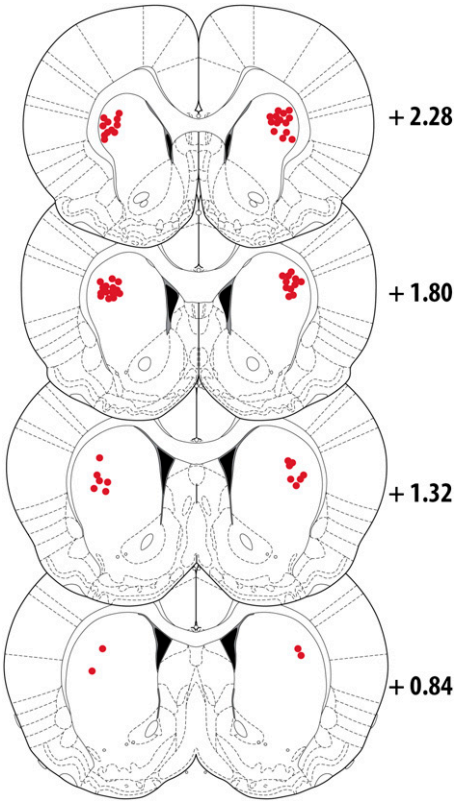

Fig. S5. Histological verification of infusion sites in the DLS (pharmacological experiment). DLS infusion sites (dark red circles) were verified to be in the lateral half of the dorsal striatum. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

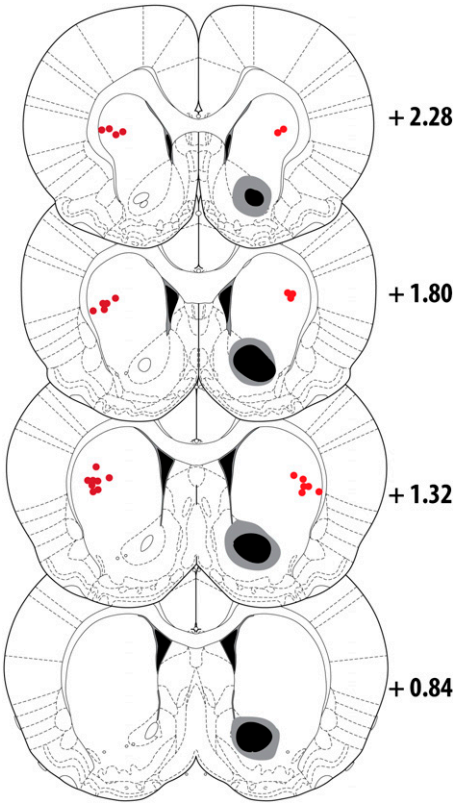

Fig. S6. Histological verification of lesion sites in VMS and recording sites in the DLS (lesion experiment). DLS recording sites were verified to be in the lateral half of the dorsal striatum, and infusions of quinolinic acid produced lesions in the nucleus accumbens core (VMS). Recording sites in the DLS ipsilateral to the lesion are shown in light red (*Right*). DLS recording sites in the contralateral hemisphere are shown in dark red (*Left*). Areas shaded in gray and black represent the largest and smallest extent of neuronal damage, respectively. The numbers on each plate indicate the distance in millimeters anterior from bregma (1).

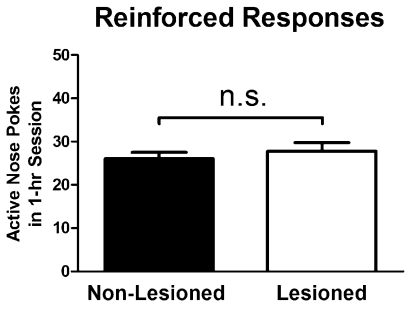

Fig. S7. Responses to obtain cocaine infusions by animals with intact VMS (first experiment) and by animals with unilateral lesion of the VMS (lesion experiment). The average total number of reinforced nose pokes (mean + SEM) did not differ significantly ($P > 0.05$) in nonlesioned rats (filled bar) and rats with unilateral lesion of the VMS (open bar). n.s., not significant.

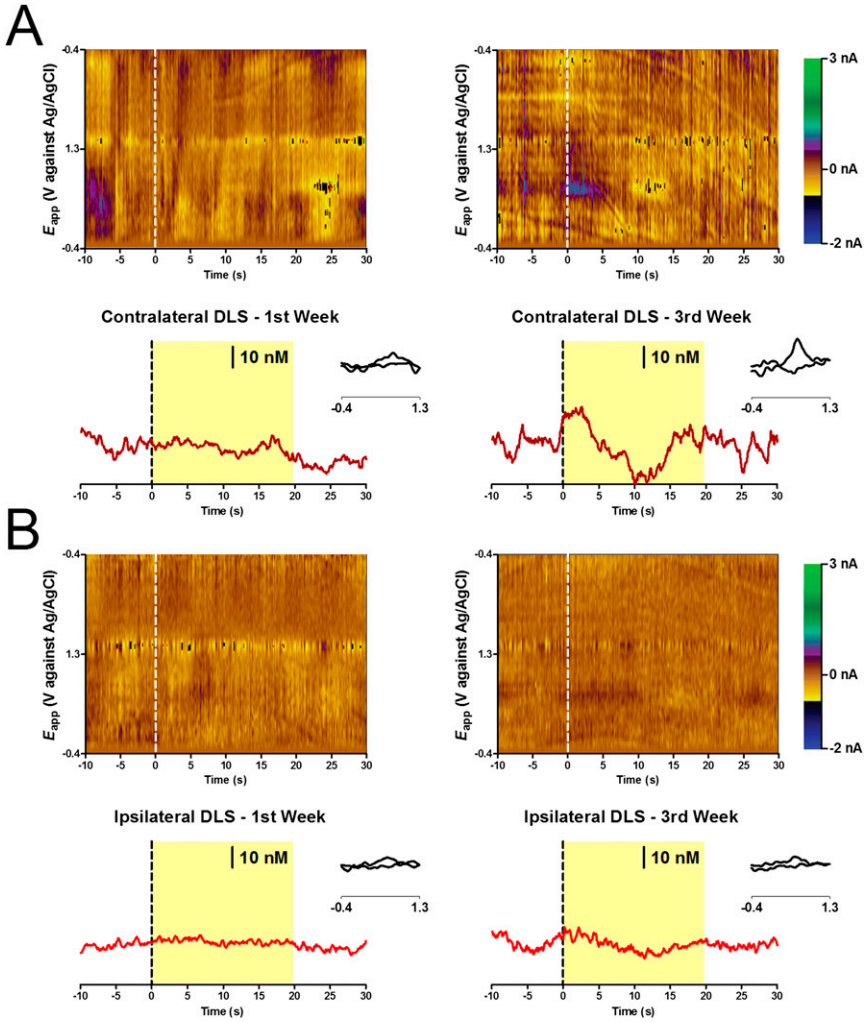

Fig. S8. Examples of phasic dopamine release in the DLS contralateral and ipsilateral to lesioned VMS associated with an individual nose poke (single trial) in the active port. (*A*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS of the hemisphere contralateral to the VMS lesion for the period 10 s before an operant response (dashed line), during the subsequent 20-s presentation of the CS (yellow box), and 10 s after the offset of the CS during the first (*Left*) and third (*Right*) weeks of cocaine self-administration. (*B*) Pseudocolor plots (*Upper*), dopamine traces (*Lower*), and cyclic voltammograms (*Insets* in lower panel) for representative current fluctuations recorded in the DLS of the hemisphere ipsilateral to the VMS lesion during the first (*Left*) and third (*Right*) weeks of self-administration. The color plots show current changes across the applied voltages (E_{app}; *y* axis) over time (*x* axis).

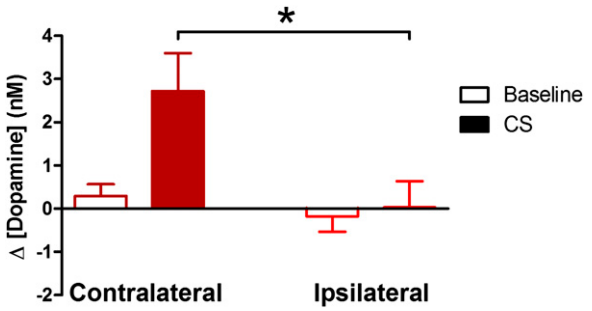

Fig. S9. CS-induced dopamine signaling in the DLS contralateral and ipsilateral to lesioned VMS (n = 17 and 11 electrodes, respectively, in 17 rats). The average phasic release of dopamine in response to the noncontingent presentation of the drug CS is greater in the DLS of the hemisphere contralateral to the VMS lesion than in the ipsilateral DLS ($P_{Holm} < 0.05$). Data are expressed as mean + SEM.

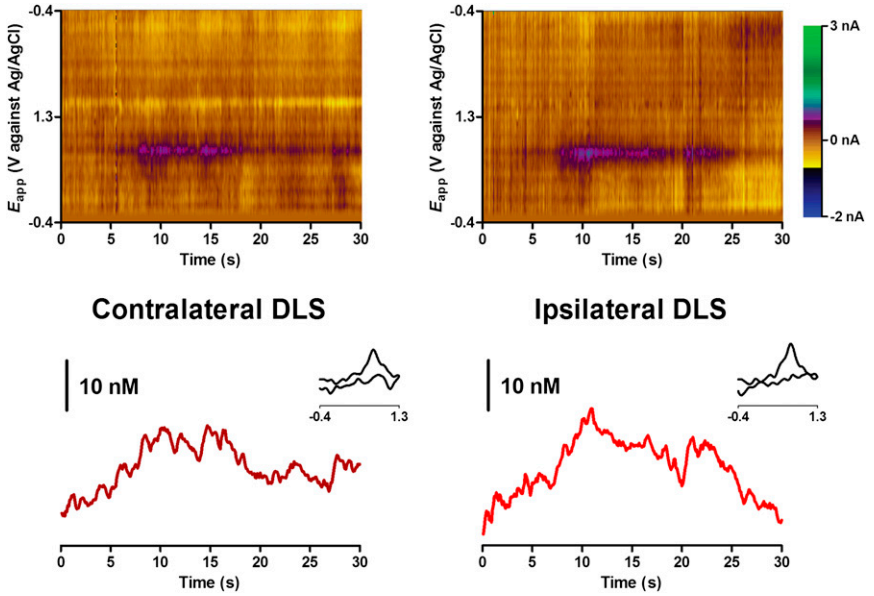

Fig. S10. Spontaneous release of dopamine in the DLS contralateral and ipsilateral to the lesioned VMS. Averaged pseudocolor plot (*Upper*), dopamine traces (*Lower*), and voltammograms (*Insets* in lower panel) for spontaneous current fluctuations (n = 7 electrodes in 4 rats) recorded in the DLS of the hemisphere contralateral (*Left*) or ipsilateral (*Right*) to the VMS lesion for a 30-s period. Dopamine release in the and ipsilateral DLS did not differ significantly [$t(11) = -0.913$, $P = 0.381$], indicating that spontaneous dopamine release (i.e.,during time periods free of operant responding and CS presentation) in the DLS is not affected by the VMS lesion.