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Excessive cocaine use results from decreased phasic dopamine signaling in the striatum

Ingo Willuhn[1–3], Lauren M Burgeno[1,2], Peter A Groblewski[2] & Paul E M Phillips[1,2]

Drug addiction is a neuropsychiatric disorder marked by escalating drug use. Dopamine neurotransmission in the ventromedial striatum (VMS) mediates acute reinforcing effects of abused drugs, but with protracted use the dorsolateral striatum is thought to assume control over drug seeking. We measured striatal dopamine release during a cocaine self-administration regimen that produced escalation of drug taking in rats. Surprisingly, we found that phasic dopamine decreased in both regions as the rate of cocaine intake increased, with the decrement in dopamine in the VMS significantly correlated with the rate of escalation. Administration of the dopamine precursor L-DOPA at a dose that replenished dopamine signaling in the VMS reversed escalation, thereby demonstrating a causal relationship between diminished dopamine transmission and excessive drug use. Together these data provide mechanistic and therapeutic insight into the excessive drug intake that emerges following protracted use.

Drug abuse is closely linked to the release of dopamine in the striatum[1,2]. However, drug use–related changes in dopamine neurotransmission vary in duration and subregion[3–5]. Slow increases in the extracellular concentration of dopamine in the VMS, stimulated by many drugs of abuse, including cocaine[6], are assumed to reflect the reinforcing properties of drugs[7], as animals regulate their rate of cocaine self-administration to maintain an elevated ambient dopamine concentration[8]. Within the VMS, overlapping putative roles of dopamine signaling in the core and shell subregions of the nucleus accumbens have been reported, but with an emphasis on the shell for mediating primary drug reward and the core for acting as a substrate for conditioned reinforcement[1]. Indeed, phasic dopamine release in the nucleus accumbens core, lasting for a few seconds, is conditioned to presentation of environmental stimuli that have been repeatedly paired with the drug[9–12] and is capable of controlling drug seeking and taking[9]. The encoding of such conditioned stimuli by dopamine release is also found in sensorimotor aspects of the striatum (dorsolateral striatum, DLS)[13], a striatal subregion that has been linked to the development of habitual and compulsive drug seeking[14–16]. Thus, the progression of drug taking beyond recreational use is considered to reflect the engagement of dopamine signaling in different striatal subregions[1,17], with an emphasis of shift from the limbic (VMS) to the sensorimotor (DLS) striatum during the development of established drug-seeking behavior[1,18]. However, it is not known whether encoding of drug-related actions or stimuli by phasic dopamine changes as moderate drug-taking behavior escalates.

Rodent models that are deemed to best capture the transition from moderate drug use to addiction use protracted access to the drug[19,20], such as extending access from 1 h (short access, ShA) to 6 h (long access, LgA) per day for a period of weeks[21]. Such a drug self-administration regimen is capable of producing escalated[21] and compulsive[22] drug seeking, among other cardinal symptoms that characterize substance dependence in humans[23]. Here we tested how LgA to cocaine affects the regional dynamics of phasic dopamine signaling in the striatum, previously characterized during stable ShA drug use[13], to gain a better comprehension of the neurobiological mechanisms underlying escalation of drug use.

RESULTS

Phasic dopamine in the striatum diminishes during LgA

Male Wistar rats with indwelling intravenous catheters were trained to self-administer cocaine during daily ShA sessions and, after learning this operant behavior, were switched to LgA sessions in chambers equipped with two nose-poke ports. A nose poke into the active port elicited an infusion of cocaine (0.5 mg per kg body weight per infusion) and 20-s presentation of a light-tone stimulus on a fixed-interval (FI) 20 schedule of reinforcement. Responses in the second (inactive) nose-poke port, or in the active port during stimulus presentation (20-s time-out), were without programmed consequence. For purposes of reporting, nose-poke responses in the active port outside the time-out period (that is, those that elicited a cocaine infusion) are referred to as "active nose pokes" and those in the inactive port outside the time-out period as "inactive nose pokes." The number of active nose pokes significantly exceeded inactive nose pokes (main effect of nose-poke port, $F_{1,23} = 383.2$, $P < 0.001$; **Fig. 1**) during each week ($P < 0.001$). After the switch from ShA to LgA, cocaine intake significantly increased over time (main effect of week, $F_{3,69} = 25.50$, $P < 0.001$; **Fig. 1**), as consistently reported by many others[24].

To assess the long-term dynamics of dopamine transmission, longitudinal neurochemical recordings were carried out simultaneously in the nucleus accumbens core of the VMS and in the DLS at chronically implanted microsensors[25] using fast-scan cyclic voltammetry (see **Supplementary Fig. 1** for histological verification of electrode placement). In the first week of LgA, we observed a transient increase

[1]Department of Psychiatry & Behavioral Sciences, University of Washington, Seattle, Washington, USA. [2]Department of Pharmacology, University of Washington, Seattle, Washington, USA. [3]Present address: Netherlands Institute for Neuroscience, and Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. Correspondence should be addressed to I.W. (ingo.willuhn@gmail.com).

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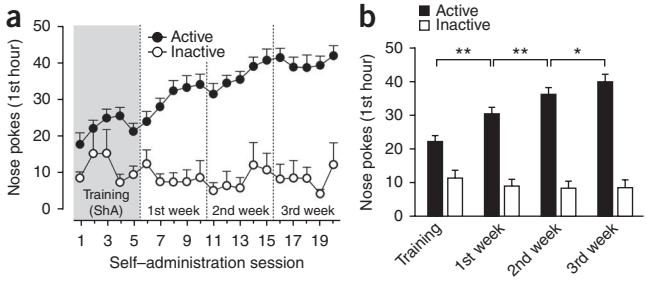

Figure 1 Escalation of drug taking over the course of weeks. (**a**) Nose pokes into the active (filled circles) and inactive (open circles) ports (excluding pokes inside the time-out period) over 5 d of ShA training (gray background) and the first hour of 15 d of LgA (white background) cocaine self-administration ($n = 24$ rats). (**b**) The number of active nose pokes (filled bars) increased significantly across weeks (training versus first week, $P = 0.006$; first versus second week, $P = 0.003$; second versus third week, $P = 0.013$), whereas the number of inactive responses (open bars) remained stable. Data are mean + s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$, *post hoc* two-sided *t*-tests following two-way ANOVA.

in extracellular dopamine concentration in VMS following active responses ($P < 0.001$; **Fig. 2a**). This pattern of activation declined during LgA; dopamine release in the third week was significantly smaller than in the first ($P < 0.001$) and second ($P = 0.030$) weeks (main effect of week, $F_{2,72} = 10.23$, $P < 0.001$; **Fig. 2b**). Phasic dopamine release in the DLS emerged in the second week ($P = 0.006$; **Fig. 2c**) but was absent in the third week of LgA (main effect of week, $F_{2,51} = 3.474$, $P = 0.039$; active poke × week interaction, $F_{2,51} = 4.021$, $P = 0.024$; **Fig. 2c**,**d**). These data show that phasic dopamine signals in VMS and DLS emerge sequentially at different stages of drug taking, similarly to what we reported for a ShA regimen[13]. However, this signaling diminished in both regions over the course of LgA, a period over which it is known that the pharmacokinetics of intravenously administered cocaine do not change[26,27].

Decrement in VMS dopamine correlates with drug intake

To test the relationship between the loss of dopamine signaling and escalation of drug consumption, we took advantage of individual differences in susceptibility to escalation of drug self-administration during the LgA regimen by separating animals into two groups depending on whether they exhibited significant escalation as based on linear regression of drug consumption over LgA sessions or not (**Fig. 3a**,**b**). Validation of this separation of animals demonstrated that non-escalated rats showed no significant increase in active nose pokes over the course of 3 weeks of LgA (main effect of week, $F_{2,18} = 0.633$, $P = 0.542$; **Fig. 3b**, left), whereas escalated rats increased their intake significantly (main effect of week, $F_{2,26} = 14.83$, $P < 0.001$; **Fig. 3b**, right; intake × week interaction, $F_{2,44} = 4.674$, $P = 0.014$), making more active nose pokes than non-escalated animals during the third LgA week ($t_{22} = 2.307$, $P = 0.031$; **Fig. 3b**). Notably, escalated animals displayed an increased motivation to obtain cocaine, as demonstrated in a progressive-ratio task ($P = 0.028$; **Supplementary Fig. 2**). In escalated rats, there was a significant decline in dopamine release in the VMS (main effect of week, $F_{2,51} = 15.51$, $P < 0.001$; **Fig. 3c**, right, and **Supplementary Fig. 3a**). However, VMS dopamine release was stable in non-escalated rats (main effect of week, $F_{2,18} = 0.057$, $P = 0.945$; **Fig. 3c**, left, and **Supplementary Fig. 4a**), conferring significantly more phasic dopamine in the third week than in escalated rats (main effect of intake, $F_{1,69} = 6.444$, $P = 0.013$; **Fig. 3d**, left; intake × week interaction, $F_{1,70} = 4.303$, $P = 0.042$). This difference in dopamine release between escalated and non-escalated rats was evident throughout the entire 6 h of self-administration ($t_{43} = 2.599$, $P = 0.013$). This difference did not result from a general decline in dopamine function in escalated animals, as dopamine release following non-contingent, experimenter-induced infusions of cocaine did not differ between non-escalated and escalated animals ($P = 0.605$; **Supplementary Fig. 5a**).

In contrast to the maintained phasic dopamine release in the VMS of non-escalating rats, we previously reported that there was a decrease in dopamine release in animals that had undergone 3 weeks of ShA of only 1 h per daily session[13]. Therefore, we carried out further analyses on the data obtained from these ShA rats to permit a detailed characterization of the relationship between dopamine function and drug intake across animals who had undergone ShA or LgA cocaine self-administration. While there was not a significant escalation of the mean drug consumption across animals during ShA, there were individual differences, with a subset of animals (6 of 16) exhibiting significant escalation of drug intake over 3 weeks of ShA cocaine self-administration. Interestingly, VMS phasic dopamine in the third week of ShA cocaine self-administration in the group of animals who maintained stable drug consumption (that is, did not exhibit

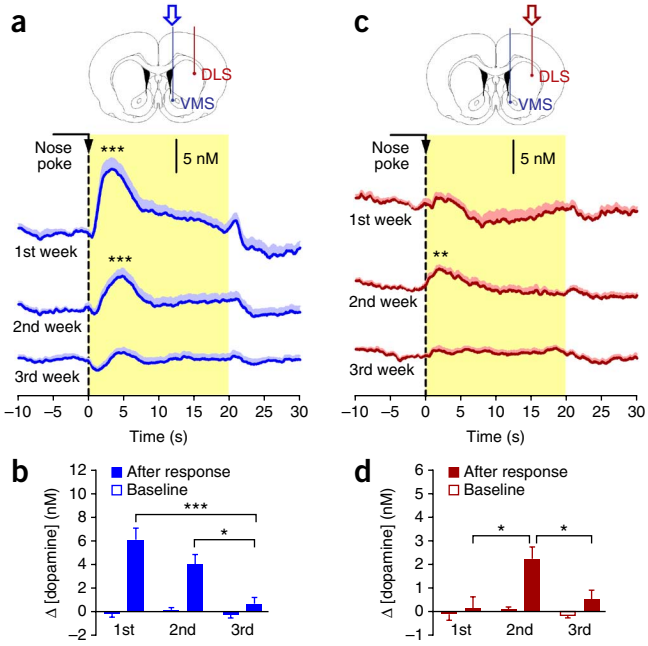

Figure 2 Dopamine signaling in VMS and DLS over the course of weeks. A nose poke (dashed line) into the active port elicited an infusion of cocaine (0.5 mg/kg per infusion) paired with the presentation of an audiovisual stimulus (yellow box) during a 20-s time out. (**a**) Phasic dopamine release in VMS following active nose-poke responses was observed during the first and second, but not third, weeks of LgA cocaine self-administration (first hour; $n = 18$ electrodes). (**b**) The average amplitude of dopamine release decreased over the course of weeks (first versus third week, $P < 0.001$; second versus third week, $P = 0.030$). (**c**) Phasic dopamine release in DLS following active nose-poke responses was observed during the second week of cocaine self-administration only (first hour; $n = 18$ electrodes). (**d**) Dopamine releases in the second week were greater in amplitude than those in the first and third weeks (first versus second week, $P = 0.014$; second versus third week, $P = 0.020$). Data are mean + s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, *post hoc* two-sided *t*-tests following two-way ANOVA.

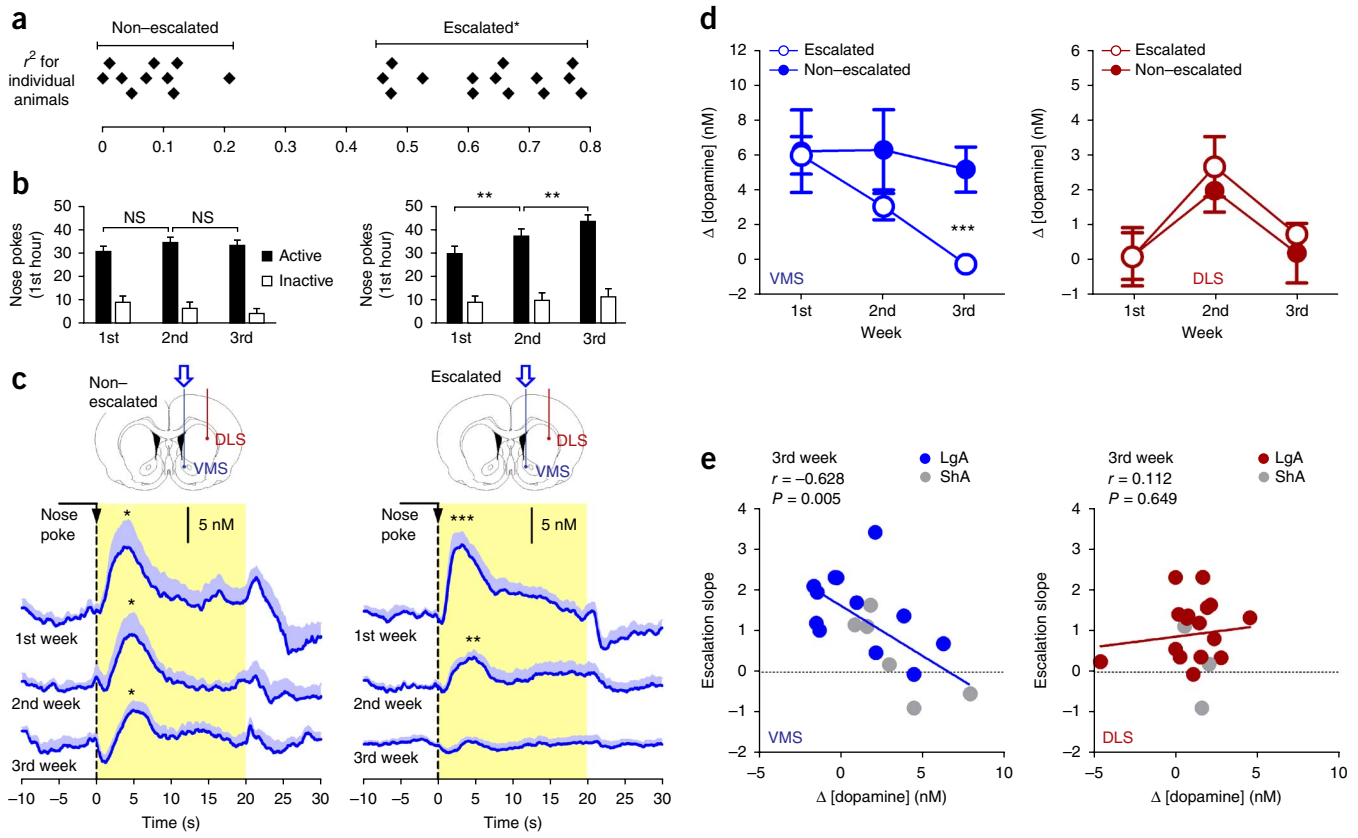

Figure 3 Individual differences in drug-taking behavior and striatal dopamine signaling. (**a**) A linear regression between the number of active nose pokes per session and the number of days of self-administration revealed a non-escalated ($P > 0.05$; $n = 10$ rats) and an escalated ($P < 0.05$; $n = 14$ rats) population of rats. (**b**) Non-escalated animals showed no significant increase (NS) in cocaine intake over the course of LgA (left; first versus second week, $P = 0.956$; second versus third week, $P = 0.338$), whereas escalated rats increased their intake significantly (right; first versus second week, $P = 0.009$; second versus third week, $P = 0.008$). (**c**) Phasic dopamine release in VMS of non-escalated animals (left) following active nose-poke responses was observed during all three weeks of cocaine self-administration ($P = 0.039$, 0.034 and 0.048, respectively), whereas release in VMS of escalated rats (right) was observed during the first ($P < 0.001$) and second ($P = 0.006$), but not third ($P = 0.754$), weeks. (**d**) Consequently, VMS dopamine release was significantly different between non-escalated and escalated animals during the third week (left). DLS dopamine signaling did not differ between non-escalated and escalated animals at any time point (right). (**e**) A significant relationship between the slope of escalation and dopamine release was detected in VMS, but not in DLS (ShA (gray circles) and LgA (colored circles) rats pooled). Data are mean + s.e.m. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, *post hoc* two-sided *t*-tests following ANOVA.

significant escalation) was not significantly different from that of non-escalated animals in the third week of LgA ($P = 0.741$; **Supplementary Fig. 5b**). Animals that escalated their drug intake under ShA conditions exhibited lower rates of drug consumption (32.7 ± 3.9 versus 43.9 ± 3.1 infusions in the first hour, $P = 0.017$) and less attenuated dopamine release ($P = 0.049$; **Supplementary Fig. 5b**) than animals that escalated their intake under LgA conditions. Nonetheless, there was a nonsignificant trend for decreased VMS dopamine compared to that in their non-escalating counterparts ($P = 0.094$) and no significant interaction for dopamine release over time between ShA and LgA escalating rats (no intake × regimen interaction, $F_{1,57} = 0.111$, $P = 0.740$; **Supplementary Fig. 5b**). Given these individual differences, we carried out regression analysis across all of the ShA and LgA rats to test for a direct relationship between dopamine levels and the degree of escalation, and found a significant negative correlation (ShA and LgA rats pooled together, $r = -0.628$, $P = 0.005$), with greatest escalation in animals that had the lowest dopamine release in week 3 (**Fig. 3e**, left). Therefore, the attenuation of dopamine signaling in the VMS predicted escalation of drug self-administration across LgA and ShA drug-access regimens. These data highlight the finding that the germane factor for changes in dopamine release is whether animals escalate or not, rather than the self-administration regimen they have been exposed to *per se*. Likewise, we find that, across all rats, escalation is a significant predictor of increased motivation for cocaine ($P = 0.037$; **Supplementary Fig. 6a**), but LgA versus ShA regimen is not, as assessed in a progressive ratio schedule ($P = 0.340$; **Supplementary Fig. 6b**).

In contrast to that in the VMS, response-contingent dopamine release in the DLS did not differ between escalated and non-escalated LgA animals (main effect of intake, $F_{1,48} = 0.472$, $P = 0.496$; **Fig. 3d**, right, and **Supplementary Figs. 3b** and **4b**), nor was there a significant relationship between the slope of escalation and dopamine release across animals that underwent ShA or LgA cocaine self-administration ($r = -0.112$, $P = 0.649$; **Fig. 3e**, right). Thus, whereas dopamine in the VMS correlated with the escalation of drug taking, we did not observe a similar correlation in the DLS, a brain region that has been widely associated with extended drug self-administration[1,14,16,18].

L-DOPA restores VMS dopamine and de-escalates drug taking

Given this provocative correlation between neurochemistry and behavior, we hypothesized that the decline in phasic dopamine signaling was causal in producing escalation of drug taking, in a manner

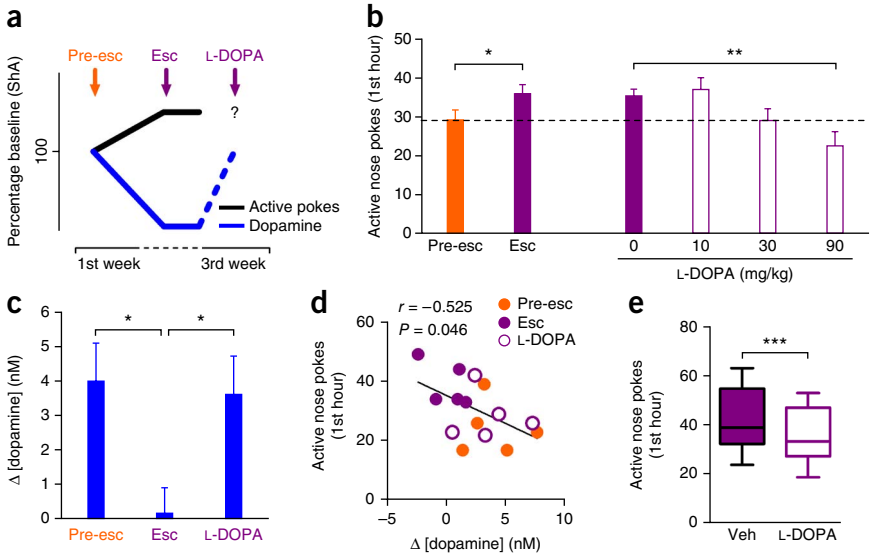

Figure 4 L-DOPA decreases escalated drug intake by replenishing VMS dopamine release. (**a**) Schematic of findings posing the question of whether L-DOPA will normalize LgA-induced escalation of drug-taking behavior by correcting the observed neurochemical deficit. Esc, escalation. (**b**) Escalated rats received an intravenous injection of the dopamine precursor L-DOPA (0, 10, 30, and 90 mg/kg) and DOPA decarboxylase inhibitor benserazide (2 mg/kg) 30 min before a self-administration session during the third week of LgA. (**c**,**d**) Administration of L-DOPA (30 mg/kg) and benserazide (2 mg/kg) restored escalation-related decremented phasic dopamine release in the VMS following active nose-poke responses. (**e**) L-DOPA infused into the VMS of escalated rats was effective at reducing drug consumption. Veh, vehicle control. Data are mean + s.e.m. $^{*}P < 0.05$, $^{***}P < 0.001$, two-sided *t*-tests or *post hoc* Newman-Keuls. $^{**}P < 0.01$, one-way ANOVA.

akin to the increase in drug taking produced by dopamine-receptor antagonists[28–30], and so restoring it would produce a reversal in escalation (**Fig. 4a**). Therefore, we treated escalated animals ($P = 0.024$; **Fig. 4b**) with L-3,4-dihydroxyphenylalanine (L-DOPA) before session start to increase phasic dopamine release[31]. L-DOPA dose-dependently (0, 10, 30 and 90 mg/kg, intravenous) decreased cocaine intake (main effect of L-DOPA, $F_{3,53} = 5.053$, $P = 0.004$; **Fig. 4b**), with 30 mg/kg returning intake to the pre-escalated level. Notably, the 30 mg/kg dose of L-DOPA was sufficient to completely restore phasic dopamine signaling in the VMS (pre-escalated versus escalated, $P = 0.027$; escalated versus L-DOPA, $P = 0.026$; see **Supplementary Fig. 7** for recording sites) during drug taking ($F_{2,8} = 6.316$, $P = 0.023$; **Fig. 4c**), an effect also observed for the full 6 h of self-administration ($F_{2,8} = 7.610$, $P = 0.0141$). Thus, the amount of phasic dopamine release in the VMS predicted the amount of drug intake during a cocaine self-administration session ($r = -0.525$, $P = 0.046$; **Fig. 4d**). This behavioral effect of L-DOPA cannot be explained by changes in the pharmacological response to cocaine, as the slow concentration changes in VMS dopamine following contingent drug infusion were not altered by L-DOPA treatment and, in fact, did not differ between pre-escalation, escalated and escalated L-DOPA–treated states ($F_{2,8} = 0.020$, $P = 0.980$; **Supplementary Fig. 8**). Furthermore, the effect of L-DOPA on drug consumption was also observed when L-DOPA was locally infused into the VMS (see **Supplementary Fig. 9** for infusion sites) of escalated rats before a session ($t_7 = 6.517$, $P < 0.001$; **Fig. 4e**). Taken together, this set of studies demonstrates that a single dose of L-DOPA administered before drug access is effective in restoring dopamine signaling and normalizing cocaine use to the pre-escalated state.

We next tested whether the use of L-DOPA would be effective at reducing escalated drug consumption in longer-term dosing regimens, more relevant to clinical applications. First, we conducted experiments introducing repeated infusion of L-DOPA on consecutive days during the induction of escalation. Animals were trained to stably self-administer cocaine and then either switched to LgA or remained on ShA, during which time they were injected with L-DOPA (30 mg/kg, intravenous) or saline before each session for 2 weeks (**Fig. 5a**). L-DOPA significantly affected drug intake in a regimen-specific manner (main effect of treatment, $F_{1,53} = 9.297$, $P = 0.004$; main effect of

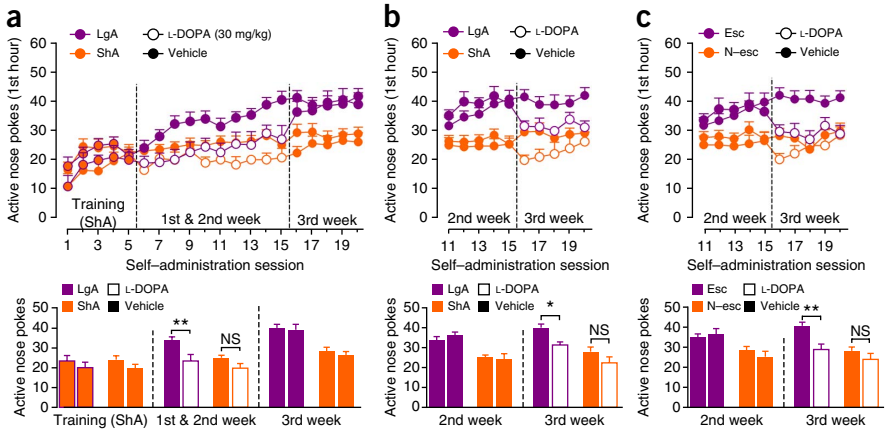

Figure 5 L-DOPA prevents and reverses the escalation of drug intake. (**a**) Animals were trained to self-administer cocaine (ShA) and subsequently either switched to LgA or remained on ShA ($n = 57$ rats). Rats that received an intravenous injection of the dopamine precursor L-DOPA (30 mg/kg) before each LgA session during the first and second weeks (open purple circles) did not escalate their drug intake (first hour) compared to animals that received vehicle (filled purple circles). Upon cessation of L-DOPA treatment (third week), we observed no differences between these LgA groups. L-DOPA-induced changes in drug intake of ShA rats (orange circles) were not significant. (**b**) In another experiment, animals were trained to self-administer cocaine (ShA) and subsequently were either switched to LgA or remained on ShA ($n = 55$ rats). LgA-trained animals (purple circles) showed a significant increase in cocaine use compared to ShA-trained animals (orange circles) during the second week. During the third week, a subset of rats was treated with L-DOPA. In LgA animals (open purple circles), L-DOPA treatment decreased escalated cocaine intake. In ShA animals (open orange circles), L-DOPA treatment did not yield a significant change. (**c**) The differential effect of L-DOPA on active nose pokes (**b**) was more robust when animals were divided into escalated and non-escalated groups, instead of ShA and LgA. Data are mean + s.e.m. NS, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; *post hoc* two-sided *t*-tests following two-way ANOVA.

regimen, $F_{1,53}$ = 5.968, P = 0.018; **Fig. 5a**), with decreased cocaine intake in LgA animals (P = 0.004) but not ShA animals (P = 0.170; **Fig. 5a**), and without effect on inactive nose pokes (LgA, P = 0.202; ShA, P = 0.101; data not shown). Therefore, the L-DOPA treatment was effective at preventing escalation of drug consumption during LgA. However, upon treatment cessation, this effect did not endure (P = 0.789; **Fig. 5a**). Second, we repeatedly administered L-DOPA on consecutive days in animals with established escalated drug consumption. Animals were trained to stably self-administer cocaine and subsequently were either switched to LgA or remained on ShA for 3 weeks. These animals were then treated with L-DOPA or saline before self-administration sessions in the third week (**Fig. 5b**). LgA-trained animals showed a significant increase in cocaine use during the first 2 weeks as compared to ShA-trained animals (main effect of regimen, $F_{1,51}$ = 15.71, P < 0.001; data not shown). L-DOPA treatment produced a regimen-specific effect (main effect of treatment, $F_{1,51}$ = 5.303, P = 0.025; main effect of regimen, $F_{1,51}$ = 11.88, P = 0.001; **Fig. 5b**), decreasing cocaine intake in LgA animals (P = 0.048) but not ShA animals (P = 0.210; **Fig. 5b**) without affecting inactive responding (LgA, P = 0.641; ShA, P = 0.664). Notably, the differential effect of L-DOPA on active nose pokes was more robust when animals were divided into escalated and non-escalated groups, instead of ShA and LgA (escalated animals, P = 0.005; non-escalated animals, P = 0.421; **Fig. 5c**), indicating that L-DOPA reduced escalated cocaine intake preferentially rather than affecting drug consumption *per se*, an interaction that developed over days (intake × treatment (day 1) interaction, $F_{1,51}$ = 0.562, P = 0.457; but intake × treatment (day 5) interaction, $F_{1,51}$ = 4.091, P = 0.048). These differences between escalated and non-escalated subpopulations, as well as the de-escalating effects of acute and chronically administered L-DOPA, were also observed across all 6 h of self-administration (**Supplementary Fig. 10**). Together these findings demonstrate that phasic dopamine release decreases in animals that escalate their cocaine intake and that restoring it with repeated administration of the dopamine precursor L-DOPA prevents and reverses this escalation, providing evidence that decreased dopamine drives escalation of drug self-administration.

DISCUSSION

Here we investigated phasic dopamine release in the VMS and DLS during escalation of drug intake, a phenomenon that models a key diagnostic criterion for drug dependence[21,23]. Our findings demonstrate that escalation is associated with decreased dopamine signaling in both the VMS and DLS, with the decrement in dopamine in the VMS significantly correlated with the rate of escalation. This effect appears to be selective for phasic dopamine, as we did not observe comparable changes in tonic dopamine in the present study, in previous work using the same regimen in rats[27] or in related self-administration models in nonhuman primates[32,33]. There have been several reports of reduced phasic dopamine function during drug withdrawal (tested between 18 h and 7 d from the last self-administration session), which is associated with reduced sensitivity to cocaine[34–37]. While we observed a similar reduction in the dopamine response to cocaine between ShA and LgA rats (**Supplementary Fig. 5a**), this effect did not appear to be pertinent to escalation, as the neurochemical response to non-contingent cocaine was not different between rats that escalated and those that did not (no intake × regimen interaction, $F_{1,34}$ = 1.964 P = 0.170; **Supplementary Fig. 5a**). Similarly, peak changes in tonic dopamine concentration up to 90 s after contingent cocaine, presumably due to the pharmacological actions of cocaine, did not differ between the pre-escalated and escalated state within the same animals (**Supplementary Fig. 8**). Thus, the only aspect of dopamine transmission that we observed to predict escalation of drug intake was the phasic response that occurred immediately following an active nose poke, which is a conditioned response primarily to drug-associated cues[9,11,13]. This neurochemical response diminished in animals that escalated their drug intake, which is reminiscent of a normal learning process wherein dopamine release in the VMS elicited by a reward-related stimulus decreases as that stimulus becomes temporally predicted[38,39]. However, the attenuation of dopamine release during self-administration occurred much later in the learning process than would be expected for contingency learning, long after the acquisition of established drug taking. Moreover, in animals that did not escalate their drug intake, attenuation of phasic dopamine release did not take place, even though these animals exhibited asymptotic discriminative instrumental behavior.

At face value, our observations of declining dopamine release as drug use progresses appear to be at odds with several contemporary theories of addiction. Theories focusing on drug-induced incentive sensitization processes postulate increasing reactivity of the VMS dopamine system upon repeated exposure to drugs of abuse that mediates a sensitized response to drug and cue exposure[40], a phenomenon that is specifically robust after LgA[41]. Theories on the role of aberrant learning and habit formation in drug addiction suggest that emerging dopamine signaling in the DLS increasingly assumes control over drug seeking[1,14,16]. Moreover, prominent computational models of addiction specifically implicate increased dopamine signaling to drug-associated cues as a driving force toward addiction[42,43]. Conversely, our findings appear to be more consistent with the dopamine depletion hypothesis of addiction, proposed by Dackis and Gold[44], and related opponent-process theories[21] that emphasize drug abuse–induced suppression of reward-related processes. Such suppression has been hypothesized to cause compensatory self-regulation of drug use to maintain a preferred level of drug intoxication[21]. Specifically, humans and animals compensate for lowered unit doses of cocaine with increased responding[45,46]. This process is regulated by dopamine transmission in the VMS[8], and, consequently, lowering dopamine transmission (for example, by dopamine receptor antagonism) elicits an increase in the rate of drug consumption[28,29]. Therefore, the reduction in dopamine signaling that we observed during LgA may stimulate compensatory upregulation of drug intake to achieve the preferred level of intoxication. In support of this hypothesis, the reduction of dopamine in the VMS was most pronounced in animals that exhibited greater escalation of drug taking.

Thus, we reasoned that restoring dopamine transmission would attenuate escalation. Indeed, L-DOPA administration was effective at both preventing and reversing the escalation of drug intake. Notably, the effects of L-DOPA on drug use did not endure after termination of treatment, suggesting that it did not prevent the underlying neuroadaptation. Therefore, our data indicate that escalation is mediated by a process that is manifested through a decrease in phasic dopamine during drug taking. These findings provide mechanistic information for the use of L-DOPA in the clinical treatment of psychostimulant abuse, a strategy that has had some promising, but overall mixed, outcomes in a small number of recent clinical trials[47]. Specifically, since L-DOPA reduced escalated drug use without producing abstinence, we suggest it is better suited for harm-reduction approaches and, in particular, allowing addicts to regain a degree of control over their drug use while entering behavioral therapy programs. Overall, our findings reveal a decrement in phasic dopamine release that takes place during protracted drug access that mediates the shift from recreational to uncontrolled drug use.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

I.W. and P.E.M.P. designed research, I.W., L.M.B. and P.A.G. performed research, and I.W. analyzed data; I.W. and P.E.M.P. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Adult male Wistar rats from Charles River (Hollister, CA, USA) weighing between 300 g and 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 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 voltammetry experiments, 50 animals underwent surgery, of which 29 maintained catheter patency throughout the experiments, had at least one functional and histologically verified electrode, and passed behavioral criteria (see below). For the pharmacological experiment, 28 of 32 rats that underwent catheter implantation maintained intravenous catheter patency and were used in the study. Animals were counterbalanced into experimental groups based upon their self-administration rate during ShA pre-experimental training. Sample sizes are similar to those reported in previous publications[13].

Stereotaxic surgery. Rats were anesthetized with isoflurane, placed in a stereotaxic frame, administered the nonsteroidal anti-inflammatory carprofen (5 mg/kg, subcutaneously) and placed on an isothermal pad to maintain body temperature. 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[48]) and the nucleus accumbens core of the VMS (1.3 mm anterior, 1.3 mm lateral and 7.2 mm ventral to bregma). One carbon-fiber microelectrode made in-house[25] was positioned in the VMS and another in the DLS, and a Ag/AgCl reference electrode was implanted in a separate part of the forebrain. In a different set of animals, guide cannulas (26 gauge; Plastics One, Roanoke, VA, USA), occluded by 'dummy' cannulas of equal length, were bilaterally implanted to target the VMS. Electrodes and guide cannulas were secured with cranioplastic cement anchored to the skull by screws. Following surgery, rats were administered the long-acting, nonsteroidal anti-inflammatory carprofen (5 mg/kg, subcutaneously) and placed on an isothermal pad to maintain body temperature until ambulatory. All animals were implanted with intravenous catheters during a separate surgery 1 week later.

Implantation of intravenous catheters. Rats were anesthetized with isoflurane, administered the nonsteroidal anti-inflammatory carprofen (5 mg/kg, subcutaneously) and placed on an isothermal pad to maintain body temperature. Catheters were made of Silastic tubing with an outer diameter of 0.6 mm and attached to a hub at one end (distal to vein insertion; Plastics One, VA, USA) for connection to an infusion pump. Catheters were pushed subcutaneously through an incision on the back between the shoulders to the front of the body and 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 was then 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 then flushed with a heparin solution (80 U/ml in saline) and filled with a viscous solution of polyvinylpyrrolidone (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. Following surgery, rats were allowed to recover for at least 5 d.

Cocaine self-administration. Self-administration sessions were conducted between 0900 and 1700 h. Rats learned to self-administer cocaine (Sigma, St. Louis, MO, USA) in a modular operant chamber (Med Associates, VT, USA) equipped with two nose-poke response devices (port with integrated cue lights) located on adjacent panels of the same wall and with a house light and speakers to provide pure-tone and white-noise stimuli. The operant chamber was housed in a sound-attenuated outer chamber. Rats (3–4 months old) were trained to obtain cocaine following an operant response on an FI20 reinforcement schedule. Nose poking in the active port (side counterbalanced between animals) resulted in an immediate intravenous 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. Following pretraining sessions with a criterion of five or more active responses per session on two successive sessions for inclusion in the study, rats were given daily access to cocaine for 1 h per day (short access; ShA) for 1 week and then 6 h per day (long access; LgA) for 3 weeks (5 d per week). The number of sessions to reach criterion varied between animals from two to five sessions. Behavioral results from a previously reported control group[13] were used as a baseline to compare behavioral data from rats undergoing LgA cocaine self-administration to rats trained under a ShA regimen of an equal number of days.

Subsequent to the three ShA or LgA weeks of FI20 cocaine self-administration, a subset of rats underwent progressive-ratio testing. These sessions were identical to FI20 sessions except that animals were required to perform an increasing number of operant responses for successive infusions of cocaine during this session. The operant requirement on each trial (T) was the rounded-down integer of $1.4^{(T-1)}$ nose pokes, starting at one nose poke (that is, 1, 1, 1, 2, 3, 5, 7, 10, 14, 20, 28, 40, 56, 79, 111, 155, 217, 304, 426). This work requirement becomes so high that eventually animals stop responding and reach a 'break point'. The break point was operationally defined as the response requirement for the last earned infusion before a 30-min period during which no infusions were obtained.

L-DOPA and benserazide administration. L-DOPA (L-3,4-dihydroxyphenylalanine) was given in combination with the peripherally acting DOPA decarboxylase inhibitor benserazide to decrease peripheral breakdown of L-DOPA (both from Sigma, St. Louis, MO, USA). Both drugs were dissolved in saline and infused intravenously at a volume of 1 ml/kg body weight. L-DOPA was administered 30 min before session start at 0, 10, 30 or 90 mg/kg, whereas benserazide was given consistently at 2 mg/kg irrespective of the L-DOPA dose administered. In a first set of studies (dose response), rats were treated with L-DOPA on a single day (**Fig. 4**). None of the L-DOPA doses used inhibited general performance or caused dyskinesia. To avoid potentially confounding effects of repeated L-DOPA administration, rats were trained without L-DOPA treatment following 'L-DOPA sessions'. In a second set of studies, animals were treated with L-DOPA before each self-administration session for a period of up to 2 weeks (**Fig. 5**). In a third set of studies, rats that exhibited escalated cocaine self-administration during LgA, the effects of bilateral infusion of L-DOPA (25–50 µg dissolved in 0.5 µl ACSF into each hemisphere; 0.25 µl/min; Sigma, St. Louis, MO, USA) and ACSF into VMS on drug-taking behavior were examined. 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 10 min before session start. After the infusion, the cannulas were left in place for 2 min before removal to allow diffusion of the drug.

Voltammetric measurements and analysis. For dopamine detection by fast-scan cyclic voltammetry during experimental sessions (recordings 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, TX, USA) through an electrical swivel (Med Associates, VT, USA) 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 linearly ramped from −0.4 V versus Ag/AgCl to +1.3 V (anodic sweep) and back (cathodic sweep) at 400 V/s (8.5-ms total scan time) 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. Voltammetric data was band-pass filtered at 0.025–2,000 Hz. 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[49]. Dopamine was isolated from the voltammetric signal by chemometric analysis using a standard training set[25] based on electrically stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated on the basis of the

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average postimplantation sensitivity of electrodes[25]. Before analysis of average concentration, all data were smoothed with a five-point within-trial running average. The concentration of dopamine was averaged over 7 s (approximate duration of the observed phasic signal) following the operant response (post-response) or noncontingent presentation of the CS and was compared to the average concentration over the 2 s before the operant response (baseline). The CS was presented noncontingently during every recording sessions conducted in the second and third weeks (twice per session for 20 s each), but not during the first week to avoid interference with the associative conditioning between drug delivery and the cue during a period where this association was presumably still developing.

Statistical analysis. Individual electrochemical signals were averaged across self-administration session and then across animals and weeks to increase statistical power. Signals were compared using multivariate ANOVAs with response, brain region, cocaine intake and week as factors. For comparison with electrochemical data, behavioral data were also binned into weeks. For L-DOPA experiments, behavioral data (averaged across days if administered on consecutive days) of a respective drug treatment (no treatment, L-DOPA dose or vehicle) were analyzed using multivariate ANOVAs with drug treatment, training regimen, cocaine intake and week 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[50]. Plots were made using Prism (GraphPad Software, La Jolla, CA, USA). Statistical analyses were carried out using SPSS, version 17.0 (Chicago, IL, USA), and Prism. Data are appropriate for parametric statistical analysis. Data collection and analysis were not performed blind to the conditions of the experiments.

Histological verification of recording sites. On completion of experimentation, animals were anesthetized with an intraperitoneal injection of 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. Brains were removed and postfixed in paraformaldehyde for 24 h and then rapidly frozen in an isopentane bath, sliced on a cryostat (50-µm coronal sections, −20 °C), and stained with cresyl violet to aid visualization of anatomical structures and the electrode-induced lesion or infusion sites.

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Excessive cocaine use results from decreased phasic dopamine signaling in the striatum

Ingo Willuhn[1,2,3], Lauren M. Burgeno[1,2], Peter A. Groblewski[2], and Paul E. M. Phillips[1,2,]

[1]Department of Psychiatry & Behavioral Sciences and [2]Department of Pharmacology, University of Washington, Seattle, WA 98195, USA.

[3]Current address: Netherlands Institute for Neuroscience, and Department of Psychiatry, Academic Medical Center, University of Amsterdam, 1105BA Amsterdam, The Netherlands

*Correspondence to: ingo.willuhn@gmail.com

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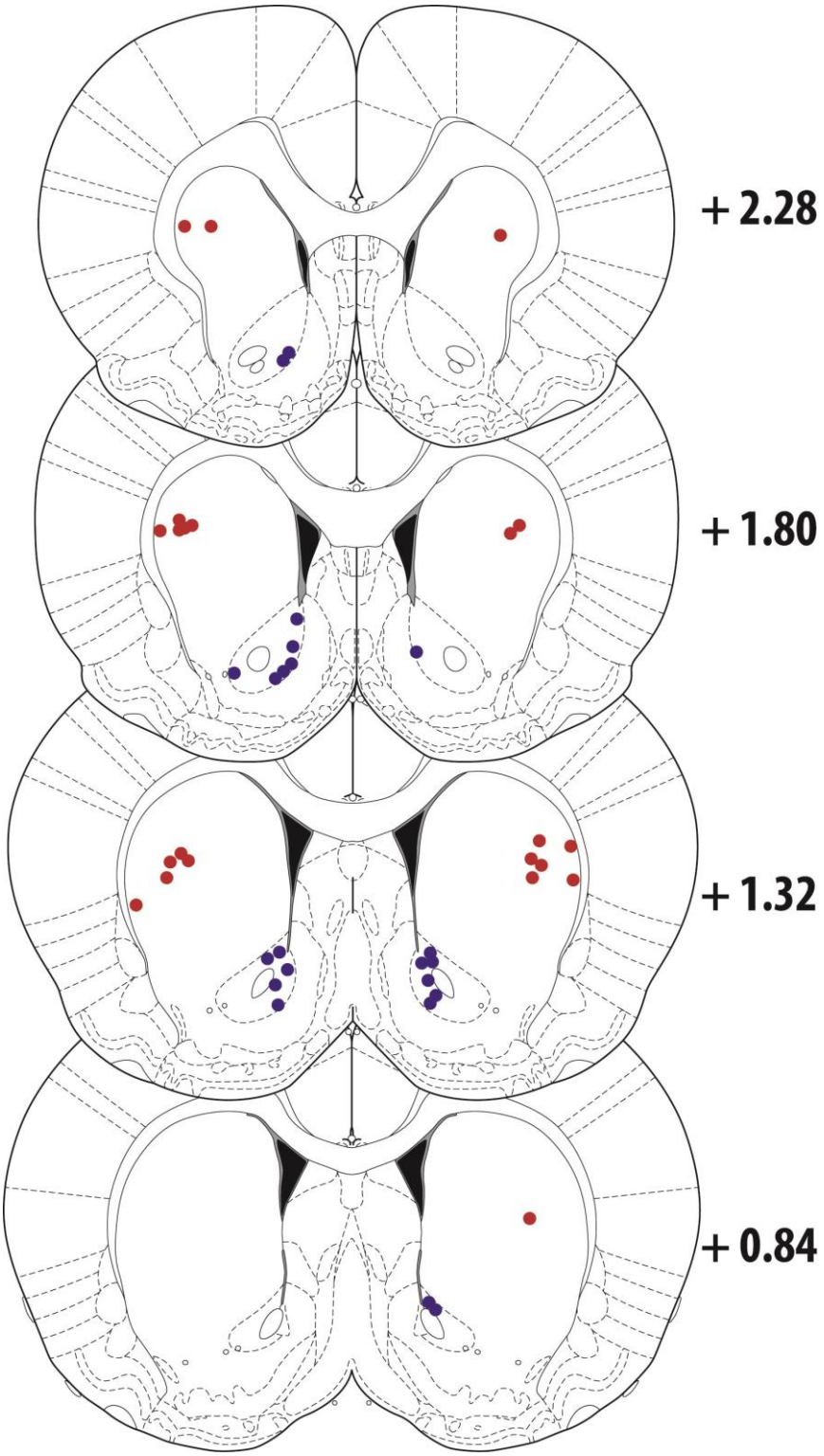
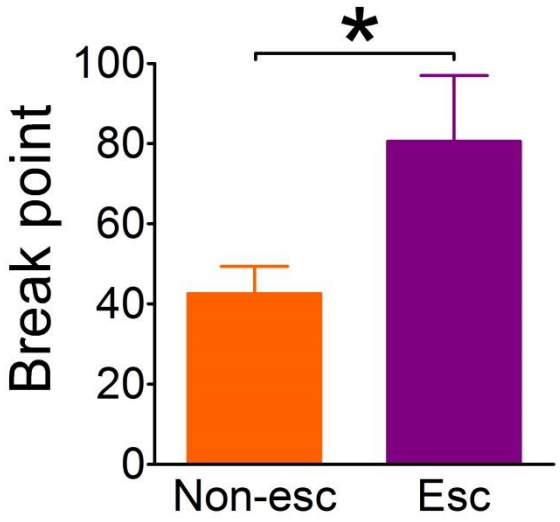
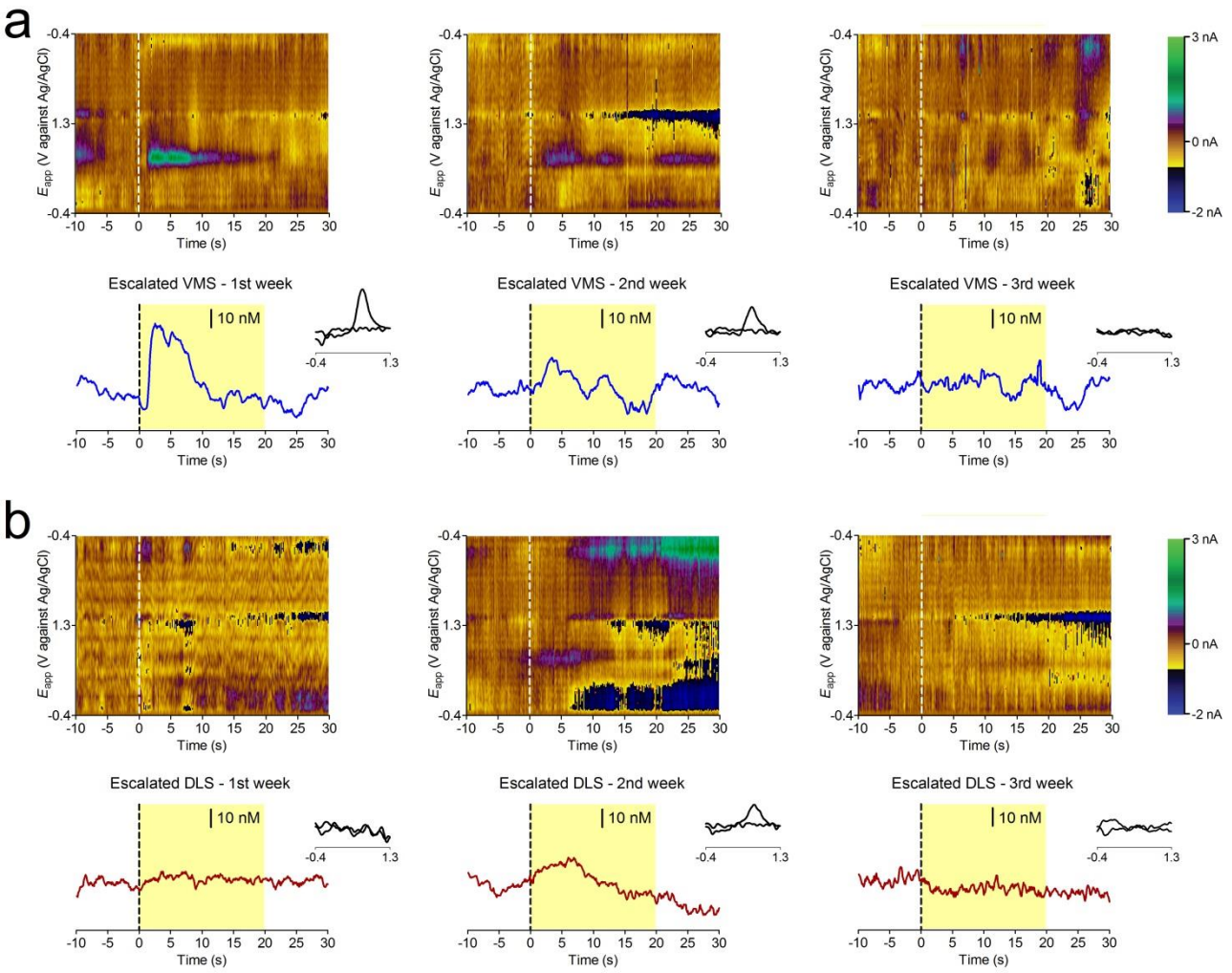


Supplementary Figure 1: **Histological verification of recording sites in VMS and DLS (1st experiment).** VMS recording sites (blue circles) were confirmed to be within the nucleus accumbens core, and DLS recording sites (red circles) were in the lateral half of the dorsal striatum. The numbers on each plate indicate distance in millimeters anterior from bregma[48].



Supplementary Figure 2: **Escalated animals display increased motivation to obtain cocaine.** Subsequent to FI20 cocaine self-administration, a subset of LgA rats (n = 19) underwent progressive-ratio testing. Progressive-ratio sessions were identical to FI20 sessions except that animals were required to perform an increasing number of operant responses for successive infusions of cocaine. The break point was operationally defined as the maximum number of responses. Average break point values are depicted (mean + SEM). Escalated rats (purple bar) displayed significantly more responses (and earned more infusions) than non-escalated animals (orange bar). *P<0.05.



Supplementary Figure 3: **Examples of phasic dopamine release in VMS and DLS associated with an individual nose poke (single trial) into the active port for animals with escalated cocaine intake.** **a**, Pseudocolor plots (top panel), dopamine traces (bottom panel), and cyclic voltammograms (inset in bottom panel) for representative current fluctuations recorded in VMS for the period 10 seconds before an operant response (dashed line), during the subsequent 20-second presentation of the CS (yellow box; includes cocaine infusion), and 10 seconds after the offset of the CS during the first (*Left*), second (*Middle*), and third (*Right*) weeks of LgA cocaine self-administration (first hour). **b**, Pseudocolor plots (top panel), dopamine traces (bottom panel), and cyclic voltammograms (inset in bottom panel) for representative current fluctuations recorded in DLS during the first (*Left*), second (*Middle*), and third (*Right*) weeks of LgA cocaine self-administration. The color plots show current changes across the applied voltages (E_{app}; *y*-axis) over time (*x*-axis).

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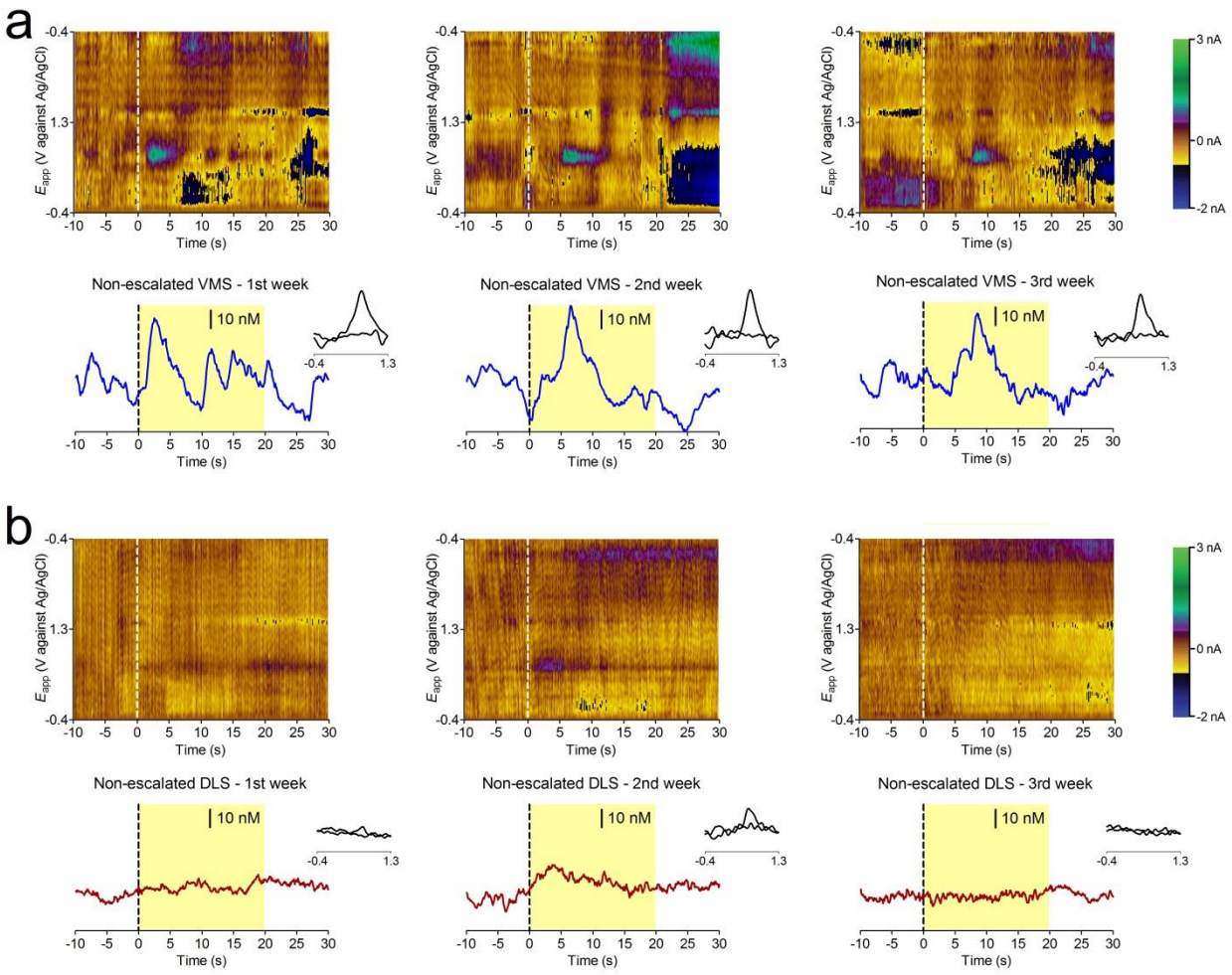

Supplementary Figure 4: **Examples of phasic dopamine release in VMS and DLS associated with an individual nose poke (single trial) into the active port for animals with non-escalated, stable cocaine intake.** **a**, Pseudocolor plots (top panel), dopamine traces (bottom panel), and cyclic voltammograms (inset in bottom panel) for representative current fluctuations recorded in VMS for the period 10 seconds before an operant response (dashed line), during the subsequent 20-second presentation of the CS (yellow box; includes cocaine infusion), and 10 seconds after the offset of the CS during the first (*Left*), second (*Middle*), and third (*Right*) weeks of LgA cocaine self-administration (first hour). **b**, Pseudocolor plots (top panel), dopamine traces (bottom panel), and cyclic voltammograms (inset in bottom panel) for representative current fluctuations recorded in DLS during the first (*Left*), second (*Middle*), and third (*Right*) weeks of LgA cocaine self-administration. The color plots show current changes across the applied voltages (E_{app}; *y*-axis) over time (*x*-axis).

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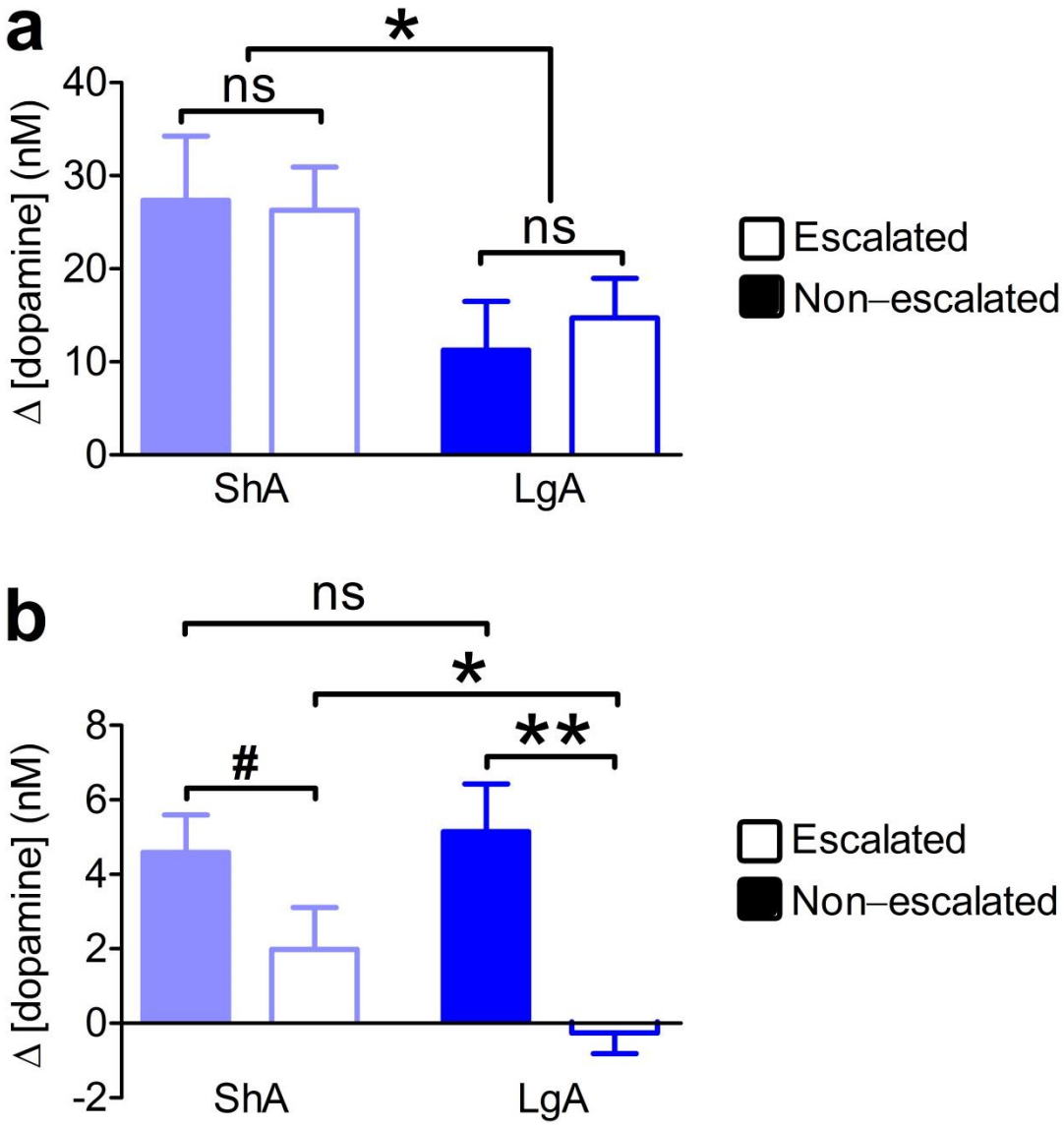
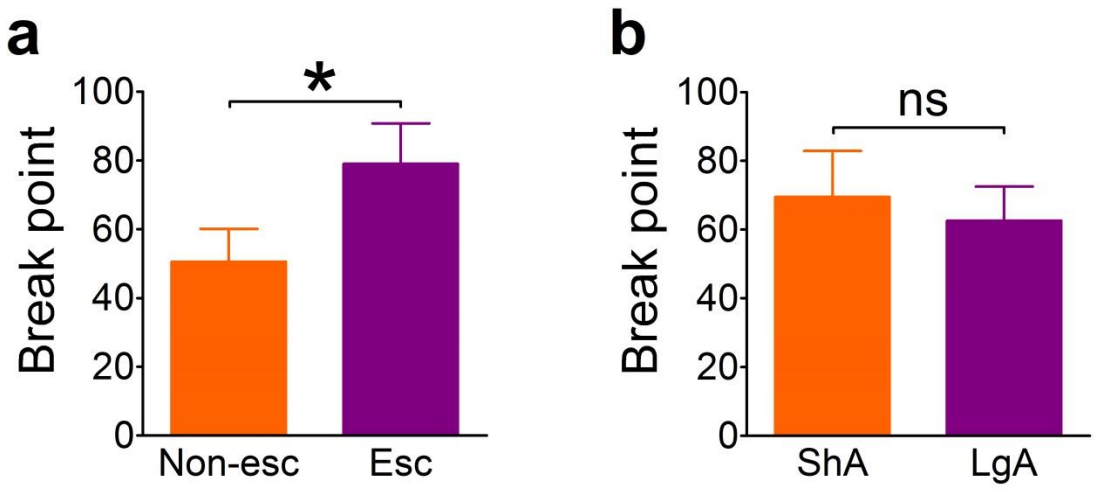
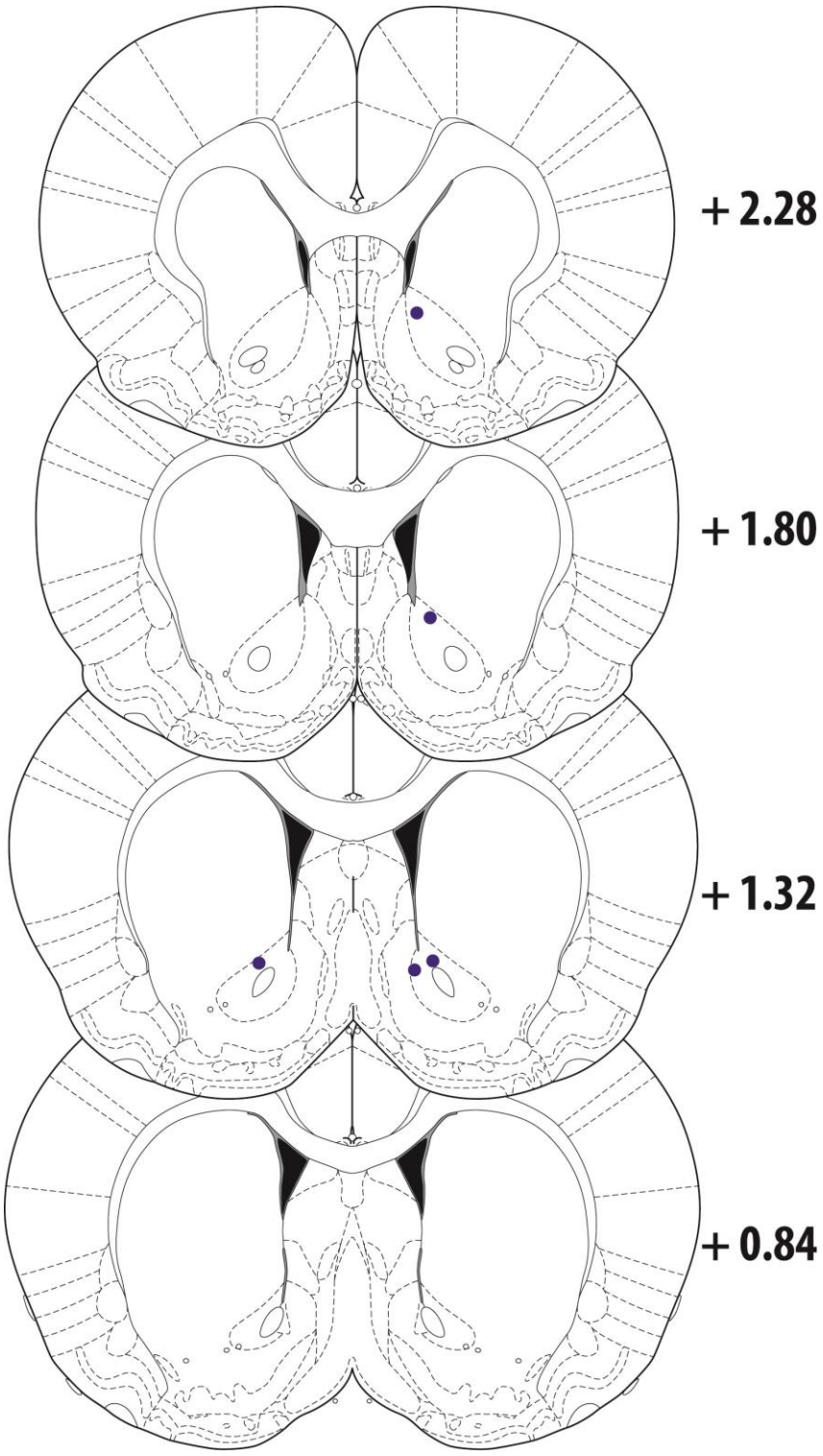


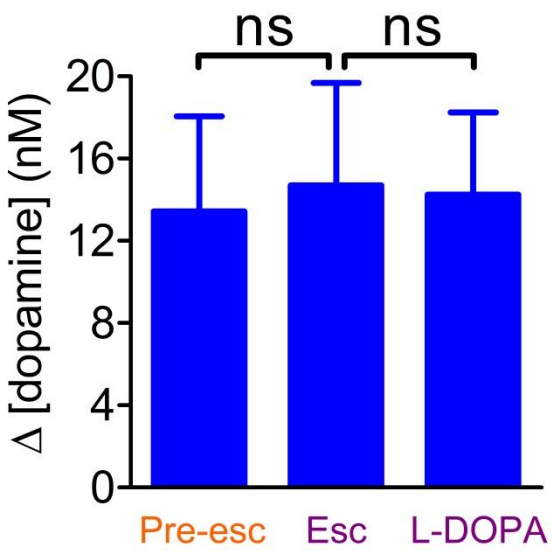
Supplementary Figure 5: **Effects of cocaine (pharmacological) and responding for cocaine delivery (behavioral) vary with access regimen (ShA/LgA) and intake pattern (Esc/Non-esc).** **a**, Average increases in extracellular concentration of dopamine in the VMS over a thirty-second period following a *non-contingent* (response-independent; no CS) intravenous infusion of cocaine (0.5 mg/kg) are depicted for non-escalated (closed bars) and escalated (open bars) animals (mean + SEM) given ShA (left) or LgA (right). Cocaine-induced dopamine release in the VMS was significantly decreased in rats given LgA compared to ShA, but release did not differ significantly between non-escalated and escalated rats ($P>0.05$). **b,** Phasic dopamine in the VMS of non-escalated animals (n = 6/16) in the third week of LgA was not different to that of non-escalated ShA rats (n = 10/16). Escalated ShA animals (n = 6/16) displayed a non-significant trend for decreased VMS dopamine compared to non-escalating ShA rats (n = 10/16); #$P = 0.094$). Escalated LgA animals exhibited less dopamine release than escalated ShA animals. Data are mean+SEM. *$P<0.05$, **$P<0.01$.



Supplementary Figure 6: **Intake pattern, but not access regimen, affects motivation to obtain cocaine.** Subsequent to FI20 cocaine self-administration, a subset of ShA and LgA rats (n = 32) underwent progressive-ratio testing. Progressive-ratio sessions were identical to FI20 sessions except that animals were required to perform an increasing number of operant responses for successive infusions of cocaine. The break point was operationally defined as the maximum number of responses. Average break point values are depicted (mean + SEM). **a**, Escalated rats (purple bar; ShA and LgA pooled) displayed significantly more responses (and earned more infusions) than non-escalated animals (orange bar; ShA and LgA). **b**, Access regimen (ShA in orange and LgA in purple) had no significant effect on the number of responses rats performed to receive an infusion of cocaine ($P>0.05$). *$P<0.05$.



Supplementary Figure 7: **Histological verification of recording sites in VMS (2nd experiment).** VMS recording sites (blue circles) were confirmed to be within the nucleus accumbens core. The numbers on each plate indicate distance in millimeters anterior from bregma[48].



Supplementary Figure 8: **Slow changes in dopamine release in the VMS following a cocaine infusion induced by an active nose-poke response.** Increases in peak concentration of extracellular dopamine in the VMS measured during LgA cocaine self-administration sessions. Measurements were conducted over 90 seconds following an infusion of cocaine induced by a nose poke into the active hole (that occurred without additional operant responses within 90 seconds following this infusion) prior to escalation (pre-esc), after escalation (esc), and after escalation with L-DOPA treatment (L-DOPA). Average changes in such "tonic" dopamine concentration did not differ significantly from each other (ns, not significant; $P > 0.05$). Data are mean+SEM.

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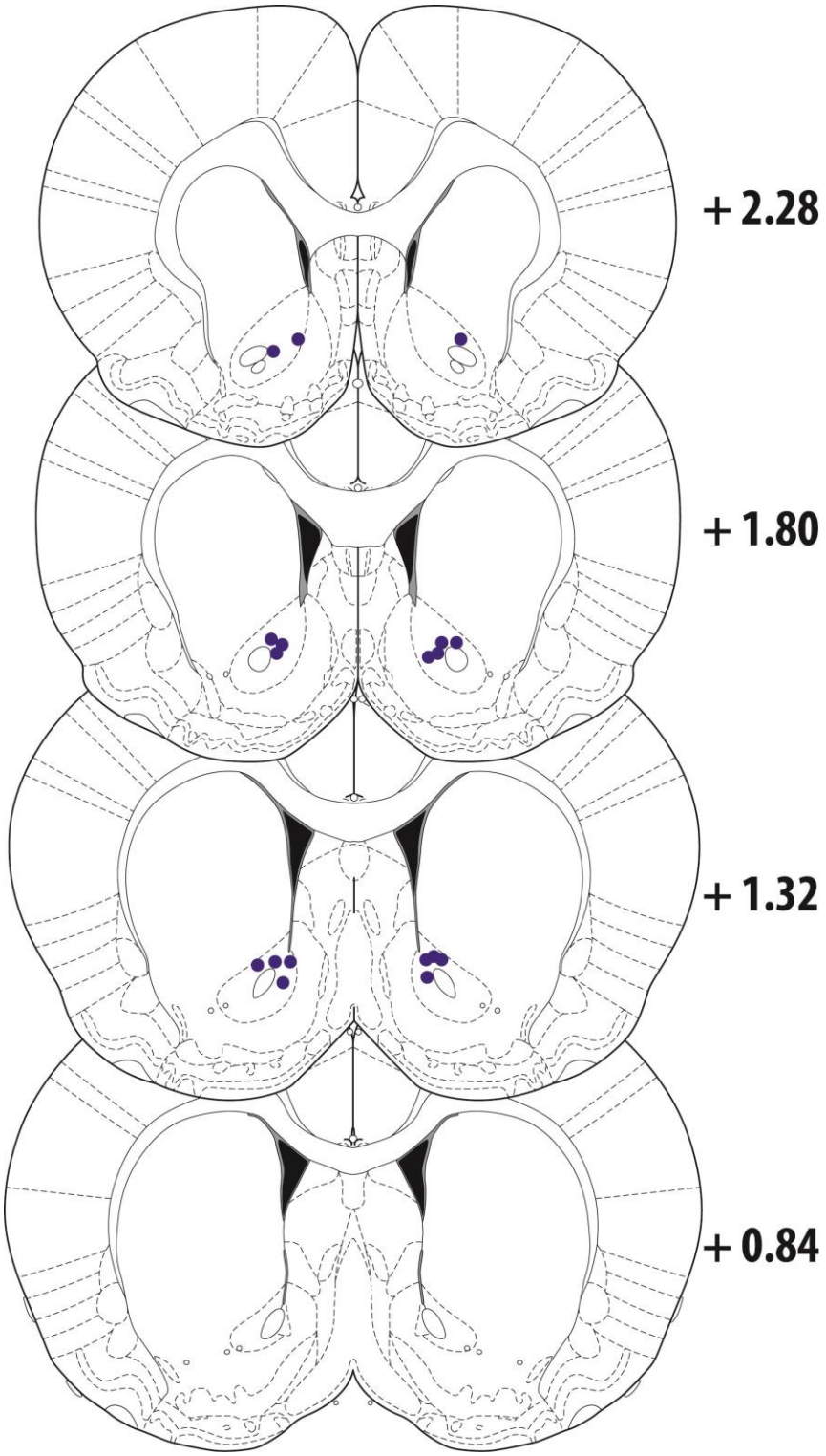
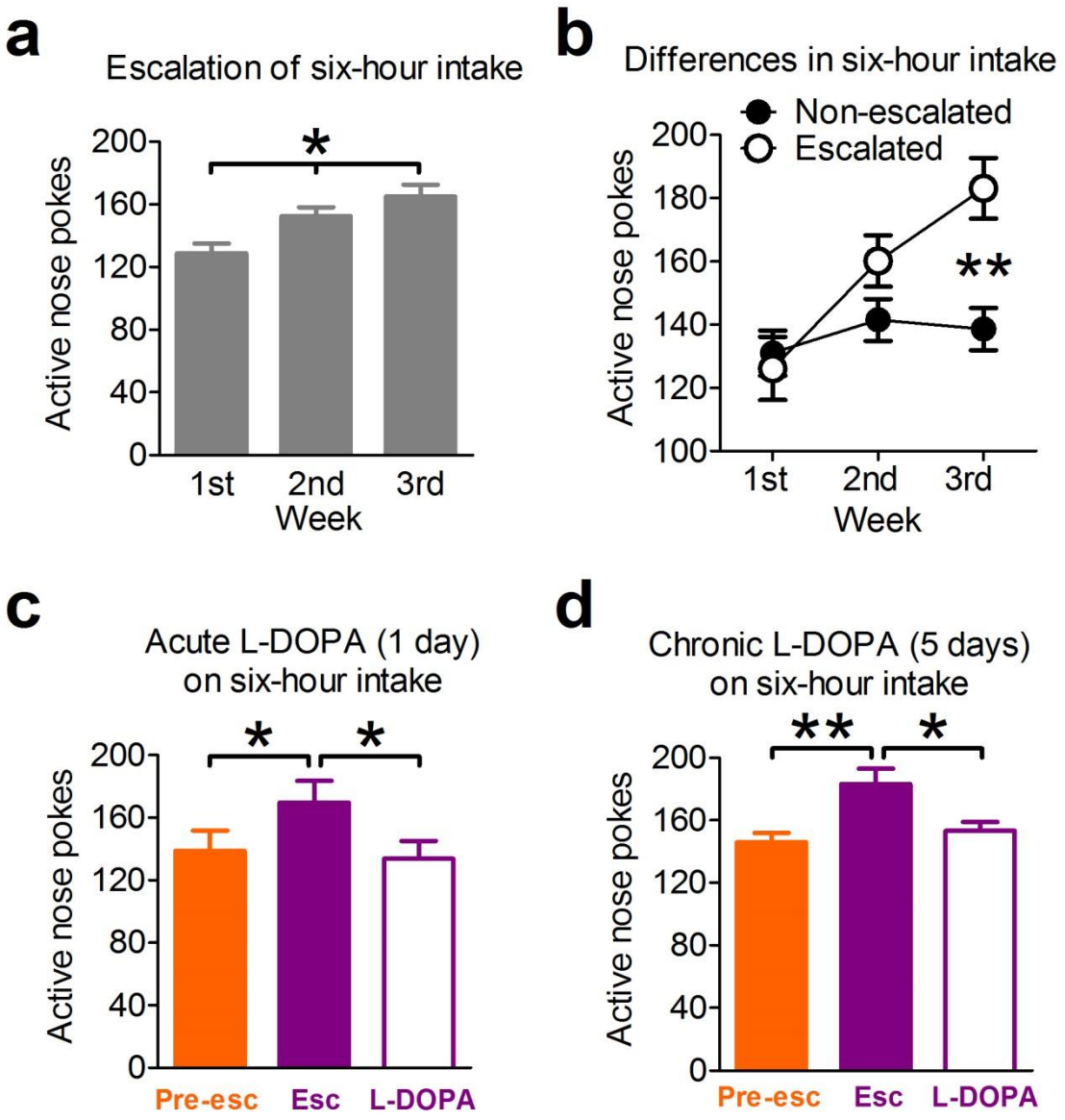


Supplementary Figure 9: **Histological verification of infusion sites in VMS (3rd experiment).** VMS infusion sites (blue circles) were confirmed to be within the nucleus accumbens core. The numbers on each plate indicate distance in millimeters anterior from bregma[48].



Supplementary Figure 10: **Escalation of cocaine intake and effects of L-DOPA during LgA over six hours of cocaine self-administration.** **a**, LgA animals showed a significantly increasing number of active nose pokes during six hours of cocaine self-administration across weeks (n = 24). **b**, Non-escalated animals (n = 10) showed no significant increase in cocaine intake during six hours of self-administration over the course of LgA (closed circles), whereas escalated rats (n = 14) increased their intake significantly (open circles). **c**, A single i.v. injection of L-DOPA (30 mg/kg) and Benserazide (2 mg/kg) prior to session start decreased the escalated number of active nose poke responses (purple bar) to a number (open purple bar) comparable to the pre-escalation stage (orange bar; n = 5). **d**, Repeated i.v. administration of L-DOPA (30 mg/kg) and Benserazide (2 mg/kg) on five consecutive days reliably reduced the escalated number of active nose poke responses (purple bar) and maintain the responses at a rate (open purple bar) comparable to the pre-escalation stage (orange bar; n = 5). Data are mean+SEM. *P<0.05. **P<0.01.