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

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Abstract

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 (DLS) 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 the causal relationship between diminished dopamine transmission and excessive drug use. Thus, together these data provide mechanistic and therapeutic insight into the excessive drug intake that emerges following protracted use.

INTRODUCTION

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 ventromedial striatum (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 in order to maintain an elevated level of 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

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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.

Author information

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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 paradigms that are deemed to best model the transition from moderate drug use to addiction employ protracted access to the drug[19,20], such as extending access from one (short access, ShA) to six hours (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 drug seeking[22], 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

Male Wistar rats with indwelling intravenous catheters were trained to self-administer cocaine during daily ShA sessions and following acquisition 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/kg/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 (i.e., those that elicited a cocaine infusion) are referred to “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: $F_{(1, 23)} = 383.226$, $P < 0.001$; 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: $F_{(3, 69)} = 25.504$, $P < 0.001$; 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 in extracellular dopamine concentration in VMS following active responses ($P < 0.001$; Fig. 2a). This pattern of activation declined during

LgA where 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: $F_{(2,72)} = 10.230$, $P < 0.001$; 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 similar 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].

To test the relationship between the loss of dopamine signaling and escalation of drug consumption, we took advantage of individual differences in susceptibility to escalate drug self-administration during the LgA regimen by separating animals into two groups depending on whether they exhibited significant escalation based upon linear regression of drug consumption over LgA sessions or not (Fig. 3a, b). Validation of this separation of animals demonstrated that non-escalated animals showed no significant increase in active nose pokes over the course of three 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: $F_{(2,26)} = 14.826$, $P < 0.001$; 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: $F_{(2,51)} = 15.507$, $P < 0.001$; 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 compared to 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 six hours of self-administration ($t_{(43)} = 2.599$, $P = 0.013$). Importantly, 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 three weeks of limited cocaine access (ShA) of only one hour per daily session[13]. Therefore, we carried out additional 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 three 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 (i.e., did not exhibit significant escalation) was not significantly different from that of non-escalated animals in the third week of LgA (P = 0.741; Supplementary Fig. 5b). ShA animals that escalated their drug intake, 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 non-significant trend for decreased VMS dopamine compared to 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 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 was predictive of escalation of drug self-administrationacross LgA and ShA drug-access regimens. These data highlight that the germane aspect related to 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/ShA regimen is not, as assessed in a progressive ratio schedule ($P = 0.340$, Supplementary Fig. 6b).

In contrast to 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, a similar correlation was not observed in the DLS, a brain region that has been widely associated with extended drug self-administration[1,14,16,18].

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, 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-DOPA prior to 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. Importantly, the 30 mg/kg dose of L-DOPA was sufficient to completely restore phasic dopamine signaling in the VMS (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 six hours 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, escalation, 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 prior to 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 prior to 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 prior to each session for two 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 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 three weeks. These animals were then treated with L-DOPA or saline prior to self-administration sessions in the third week (Fig. 5b). LgA-trained animals showed a significant increase in cocaine use during the first two weeks compared to ShA-trained animals (main effect of regimen: $F_{(1,51)} = 15.706$, $P < 0.001$; 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: $F_{(1,51)} = 11.884$, $P = 0.001$; Fig. 5b), decreasing cocaine intake in LgA animals ($P = 0.048$), but not ShA animals ($P = 0.210$; Fig. 5b) without effecting inactive responding (LgA, $P = 0.641$; ShA, $P = 0.664$). Importantly, the differential effect of L-DOPA on active nose pokes was more robust when animals were grouped into escalated and non-escalated, 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: $F_{(1,51)} = 4.091$, $P = 0.048$). Importantly, these differences between escalated and non-escalated sub-populations as well as the de-escalating effects of acute and chronically administered L-DOPA are also observed across all six hours of self-administration (Supplementary Fig. 10). Together these findings demonstrate that phasic dopamine release decreases in animals that escalate their cocaine intake and 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

In the present study, 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 comparable changes were not observed in tonic dopamine in the current study, in previous work using the same regimen in rats[27] or related self-administration paradigms in non-human primates[32,33]. There have been a number of reports of reduced phasic dopamine function during drug withdrawal (tested between 18 hours and seven days 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 is 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 seconds 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 which predicted 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 processe where 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 occurs 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 do not escalate their drug intake, attenuation of phasic dopamine release does not take place even though these animals exhibit 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]. Conceptualizations 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 towards 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 (e.g., 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 following 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 of 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 which mediates the shift from recreational to uncontrolled drug use.

Methods

Animals

Adult male Wistar rats from Charles River (Hollister, CA, USA) weighing between 300g and 400g 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 with 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 cannula were secured with cranioplastic cement anchored to the skull by screws. Following surgery, rats were administered with 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 one week later.

Implantation of intravenous catheters

Rats were anesthetized with isoflurane, administered with 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 polyvinylpyrolidone (PVP) and heparin (1000 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 five days.

Cocaine self-administration

Self-administration sessions were conducted between 0900 and 1700 hr. 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, 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 (3–4 months old) were trained to obtain cocaine following an operant response on 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 ten seconds) paired with a 20-second presentation of an audiovisual stimulus (illumination of the light inside the nose poke port and tone; conditioned stimulus, CS). During CS presentation, a 20-second 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 pre-training 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 one hour per day (short access; ShA) for one week and then six hours per day (long access; LgA) for three weeks (five days 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 to 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 $1.4^{(T-1)}$ lever presses, starting at 1 lever press (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 total number of infusions earned prior to a thirty-minute period during which no infusions were obtained.

L-DOPA/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 minutes prior to 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 these L-DOPA prior to each self-administration session for a period of up to two 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 ten minutes prior to session start. After the infusion, the cannulas were left in place for two minutes before removal to allow for 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 upon electrically stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated based upon the average post-implantation sensitivity of electrodes[25]. Prior to analysis of average concentration, all data were smoothed with a 5-point within trial running average. The concentration of dopamine was averaged over seven seconds (approximate duration of the observed phasic signal) following the operant response (post-response) or non-contingent presentation of the CS and was compared to the average concentration over the two seconds prior to the operant response (baseline). The CS was presented non-contingently during every recording sessions conducted in the second and third weeks (twice per session for 20 seconds 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) prior to transcardial perfusion with saline followed by 4%-paraformaldehyde. Brains were removed and post-fixed in paraformaldehyde for twenty-four hours 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 in visualization of anatomical structures and the electrode-induced lesion or infusion sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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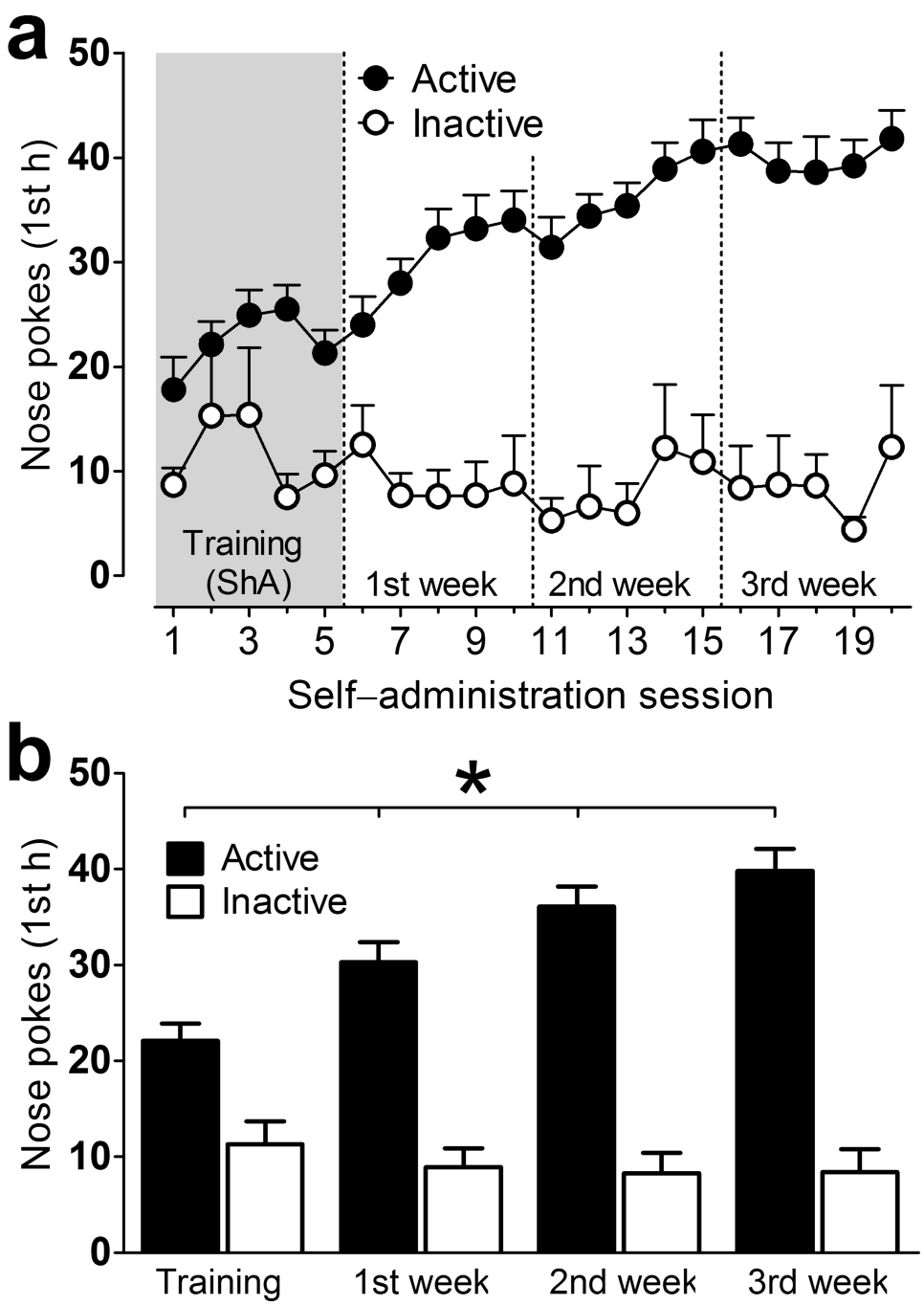

Figure 1. Escalation of drug taking over the course of weeks

a, Nose pokes into the active (closed circles) and inactive (open circles) ports (excluding pokes outside the time-out period) over 5 days of ShA training (gray background) and the first hour of 15 days of LgA (white background) cocaine self-administration (n = 24 rats). **b**, The number of active nose pokes (closed bars) increased significantly across weeks, whereas the number of inactive responses (open bars) remained stable. Data are mean+SEM. *P<0.05, one-way ANOVA.

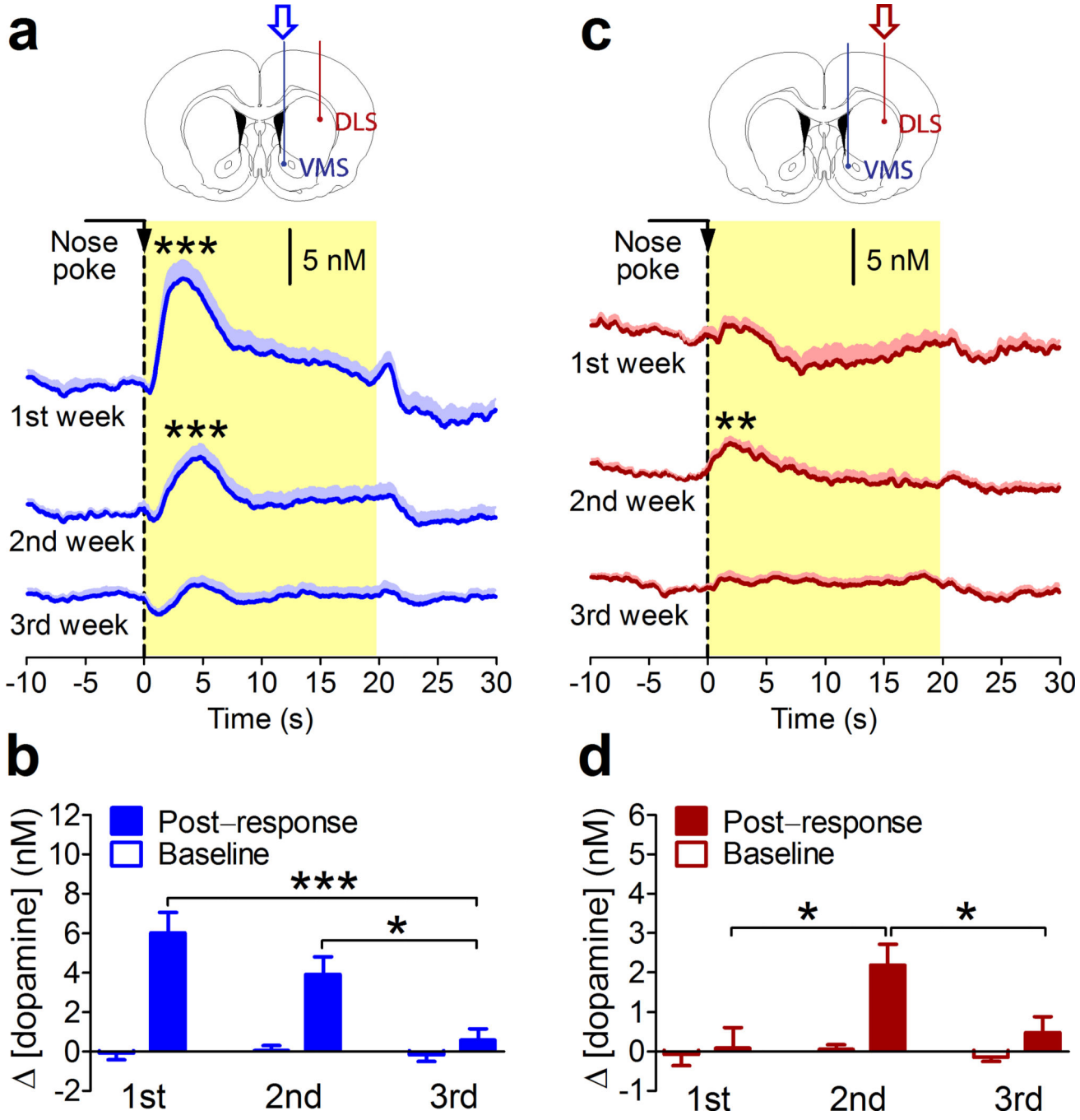

Figure 2. Dopamine signaling in VMS and DLS over the course of weeks

A nose poke (dashed line) into the active port elicited an infusion of cocaine (0.5 mg/kg/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. **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 release in the second week were greater in amplitude than those in the first and third weeks. Data are mean+SEM. *P<0.05, **P<0.01, ***P<0.001, post-hoc two-sided t-tests following two-way ANOVA.

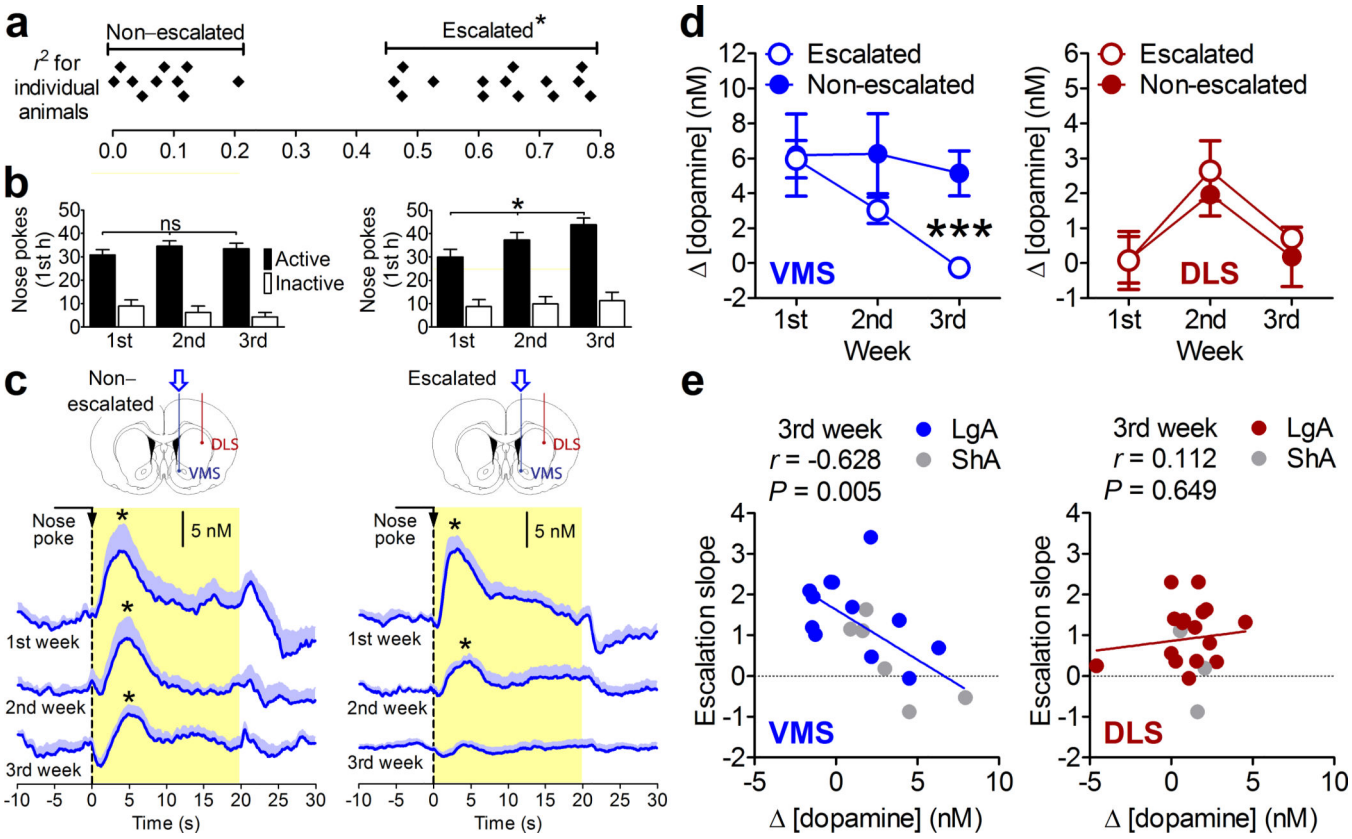

Figure 3. Individual differences in drug-taking behavior and striatal dopamine signaling

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 in cocaine intake over the course of LgA (left), whereas escalated rats increased their intake significantly (right). **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, whereas release in VMS of escalated rats (right) was observed during the first and second, but not third, 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+SEM. *$P<0.05$, ***$P<0.001$, post-hoc two-sided t-tests following ANOVA.

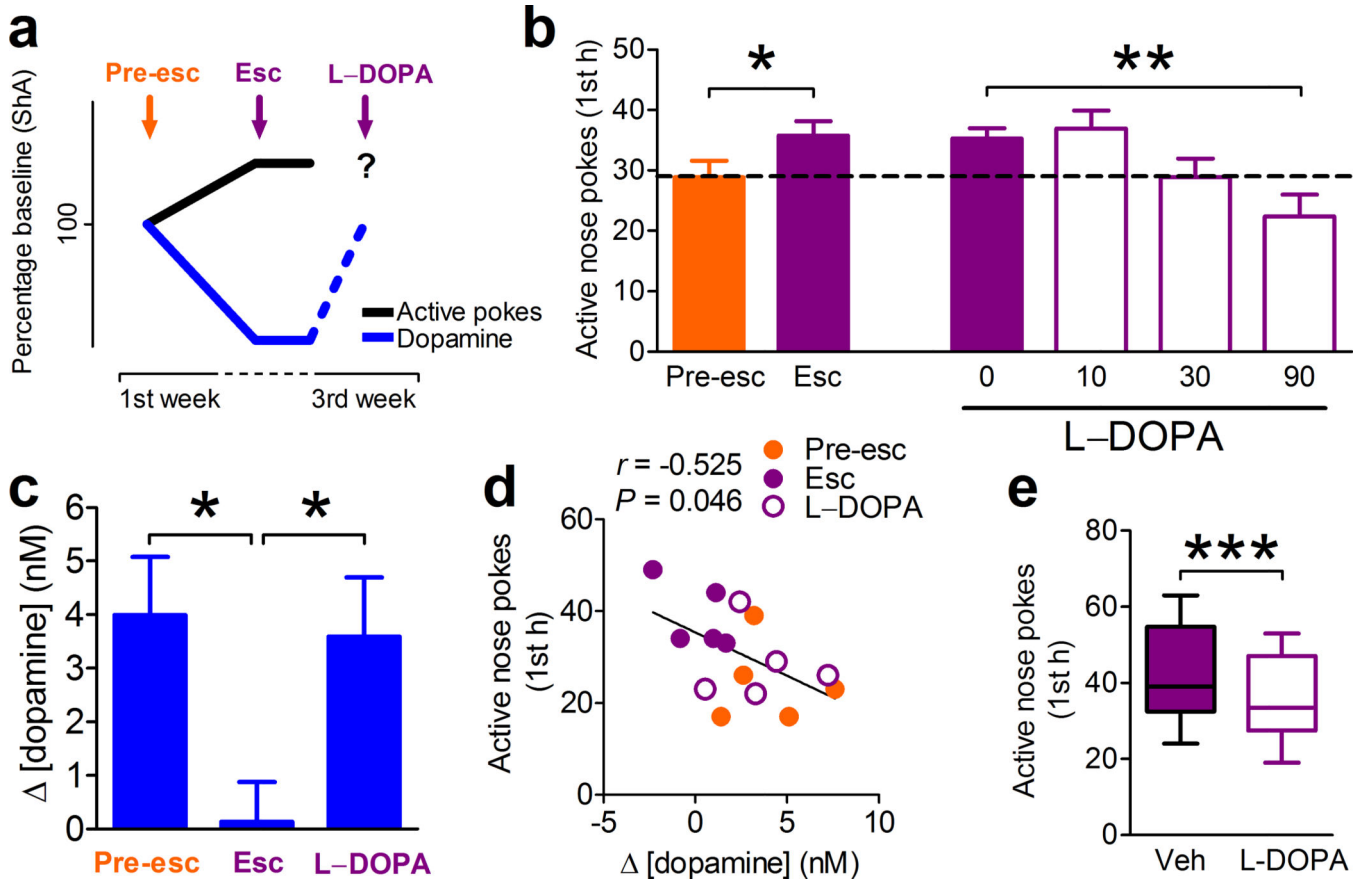

Figure 4. L-DOPA decreases escalated drug intake by replenishing VMS dopamine release

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. **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 minutes prior to a self-administration session during the third week of LgA. **c, d**, Administration of L-DOPA (30 mg/kg) and Benserazide (2 mg/kg) restores escalation-related decremented phasic dopamine release in the VMS following active nose-poke responses. **e**, L-DOPA infused into the VMS of escalated rats is effective at reducing drug consumption. Data are mean+SEM. **P*<0.05, ****P*<0.001, two-sided t-tests. ***P*<0.01, one-way ANOVA.

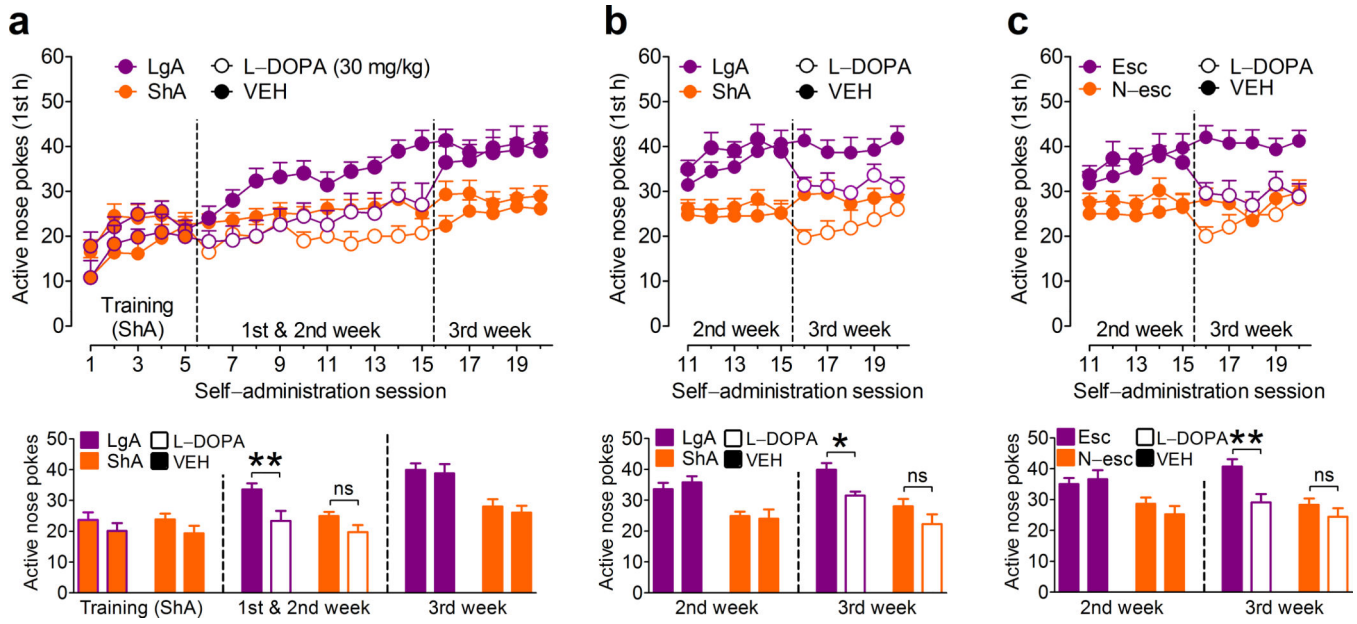

Figure 5. L-DOPA prevents and reverses the escalation of drug intake

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) prior to 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 (closed purple circles). Upon cessation of L-DOPA treatment (third week), no differences were observed 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 (panel b) was more robust when animals were grouped into escalated and non-escalated, instead of ShA and LgA. Data are mean+SEM. ns $P \geq 0.05$, $^{*}P<0.05$, $^{**}P<0.01$, post-hoc two-sided t-tests following two-way ANOVA.