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Regional Variation in Phasic Dopamine Release during Alcohol and Sucrose Self-Administration in Rats

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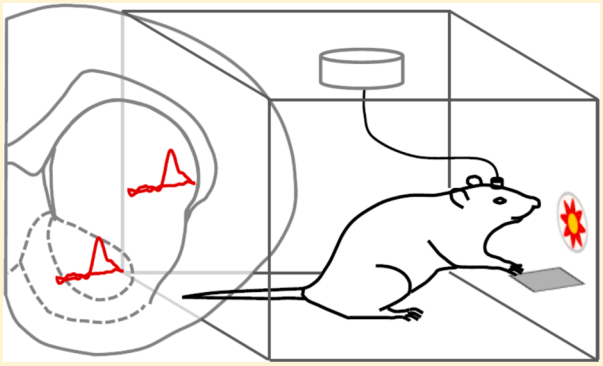
Supporting Information

ABSTRACT: While dopamine input to the dorsal striatum is well-known to be critical for action selection, including alcohol-motivated behaviors, it is unknown whether changes in phasic dopamine accompany these behaviors. Long-term alcohol abuse is believed to promote alterations in the neurocircuitry of reward learning in both ventral and dorsal striatum, potentially through increasing dopamine release. Using fast-scan cyclic voltammetry, we measured phasic dopamine release in the dorsal and ventral striatum during alcoholic and nonalcoholic reward-seeking behavior and reward-related cues in rats trained on a variable-interval schedule of reinforcement. We observed robust phasic dopamine release in the dorsolateral striatum after reinforced lever presses and inconsistent dopamine release in the dorsomedial striatum. Contrary to our expectations, alcohol did not enhance dopamine release in rats drinking alcoholic rewards. Cue-induced dopamine release was also observed in the nucleus accumbens core of rats drinking the reward solutions. These data demonstrate that alcoholic and nonalcoholic reward self-administration on a variable-interval schedule of reinforcement in rats is accompanied by phasic dopamine release time-locked to reinforcement in the dorsolateral striatum and the nucleus accumbens, but not the dorsomedial striatum.



KEYWORDS: *Dorsal striatum, accumbens, voltammetry, alcohol, dopamine, operant conditioning*

Alcohol drinking can be compulsive in subjects with alcohol use disorder.[1] While mechanisms of compulsive alcohol intake are unclear, it has been postulated that, with progression of alcohol drinking, control over this behavior shifts from the prefrontal cortex and the ventral region of the striatum to the dorsal striatum, particularly to the dorsolateral striatum.[2,3] Based on anatomical connectivity with the cortex and functional differences in activity during behavioral control, the striatum can be subdivided into three broad areas: ventral striatum or nucleus accumbens (NAcc), dorsomedial striatum (DMS) or caudate, and dorsolateral striatum (DLS) or putamen. The NAcc plays a major role in forming associations between specific stimuli and alcohol reward,[4] the DMS regulates goal-directed alcohol seeking behavior, and the DLS is thought to control habitual or compulsive alcohol seeking driven by the environmental stimuli previously conditioned with the alcoholic reward.[5−7]

Previously in our laboratory, we demonstrated that self-administration of alcohol in rats on a variable-interval (VI) schedule of reinforcement engages phasic firing activity of presumed medium spiny neurons in the DMS and DLS.[7] The medium spiny neurons in the striatum are GABAergic cells projecting to downstream structures such as globus pallidus and substantia nigra and are major participants in reward learning and action selection. Firing activity of the neurons is modulated by dopamine arising from the ventral tegmental area (VTA) and substantia nigra (SN). Dopamine modulates cortical and thalamic glutamatergic inputs to the striatum by acting on D1- and D2-type receptors, increasing or decreasing excitability of the medium spiny neurons, respectively.[8,9] Depletion of DLS dopamine disrupts the formation of habitual behavior in rats.[10] Phasic dopamine release has been measured in the DLS of rats during cocaine self-administration with fast-scan cyclic voltammetry and is not initially apparent, but emerges after extended training.[11] Together, these findings support the hypothesis that dopamine signaling in the DLS contributes to the expression of reward-seeking behavior, including alcohol seeking, after extended training.

Dopamine neurons fire in two general patterns in the brain: tonic, lower frequencies and phasic bursts of firing at higher frequencies.[12−15] Phasic firing of dopaminergic neurons produces transient, high concentrations of dopamine that would be sufficient to activate low-affinity D1 receptors and modulate firing of striatal neurons.[16] In vivo evidence of dopaminergic modulation of striatal neuronal activity includes

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a report from Owesson-White and colleagues, in which phasic dopamine release was found to precede neuronal activation in the NAcc of rats self-administering cocaine.[17] Similarly, local application of dopamine receptor antagonists inhibited phasic neuronal activity in the NAcc evoked by reward-predictive cues.[18,19] The critical role of NAcc phasic dopamine signaling in reward-conditioning, learning, and self-administration behavior has been intensively investigated for the past decade. However, much less is known about how phasic dopamine transients in the dorsal striatum contribute to a behavior driven by reward or reward-associated stimuli. Moreover, to our knowledge, no previous studies have demonstrated phasic dopamine release in the dorsal striatum during alcohol self-administration.

We addressed this gap in the literature by investigating the dynamics of phasic dopamine release in the dorsal striatum during sweetened alcohol- and sucrose-seeking behavior and reward-related cues in rats trained on a VI reinforcement schedule. Interval-based reinforcement schedules have a low perceived action-outcome contingency and have been demonstrated to engage both DMS and DLS neurons.[7] We hypothesized that phasic dopamine release would occur in the DLS to reward-related cues, as those stimuli would trigger the response to consume reward, and this would manifest as a predominance of cue-evoked dopamine transients in the DLS compared to the DMS. Moreover, as alcohol activates dopamine release,[20−22] we predicted that dopamine transients would be amplified in rats drinking sweetened alcohol compared to those drinking only sucrose.

RESULTS AND DISCUSSION

To test our hypothesis, we used fast-scan cyclic voltammetry (FSCV) to measure real-time, phasic dopamine activity in the DLS, DMS, and NAcc of rats to determine how phasic dopamine release corresponds to reward-associated cues during alcohol and sucrose self-administration on a variable interval schedule of reinforcement. Note that alcohol was sweetened with sucrose in this study to maintain robust self-administration during voltammetric recording, similar to other studies investigating mechanisms of alcohol self-administration (for example, refs 20 and 23). To determine the contribution of alcohol to dopamine release, we compared dopamine transients in rats drinking sweetened alcohol to those drinking only sucrose.

Acquisition and Performance of Self-Administration on a VI-30 Schedule of Reinforcement. An experimental timeline is shown in Figure 1A. In this study, rats were trained for 8 weeks to self-administer either 10% sucrose (10S) or 10% sucrose plus 10% ethanol (10S10E). Figure 2A shows the number of lever presses performed by rats during the first week of training (averaged across rats for each day) and during remaining weeks (averaged across rats for each week). Day 0 (pretraining) consisted of noncontingent reward deliveries on a random-time schedule, and starting on day 1 rats were trained to press a lever for 0.1 mL of reward. Reinforcement started on a fixed-ratio schedule (1 press earns 1 reward) and gradually shifted to the VI-30 schedule (after a variable interval of time elapsed that averaged 30 s, then 1 press earned 1 reward; Figure 2A, left). Over the first 4 days of operant behavior, the number of presses significantly increased from 120 ± 17 to 216 ± 51 in the 10S group and 131 ± 17 to 218 ± 29 in the 10S10E group. The repeated measures (RM) two-way ANOVA analysis revealed a main effect of schedule ($F_{3,48} = 6.9$, $p < 0.001$) with no significant main effect of group ($F_{1,48} = 0.8$, $p = 0.4$) or group by schedule interaction ($F_{3,48} = 0.6$, $p = 0.6$). Importantly, rats from both 10S and 10S10E groups showed no difference in acquisition of operant self-administration on the VI-30 schedule of reinforcement, and both groups maintained the behavior across the

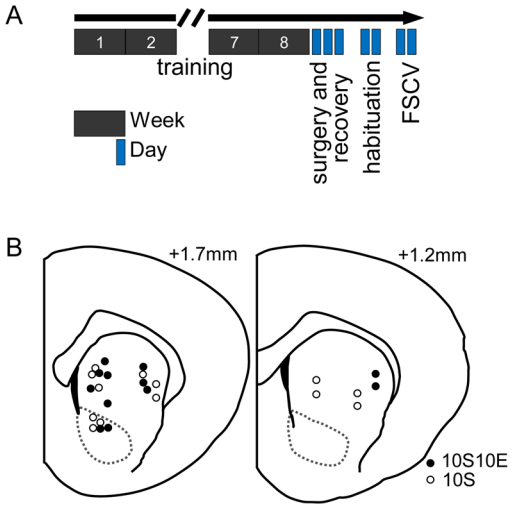

Figure 1. (A) Timeline of the experiment and (B) representation of carbon-fiber electrode placements within the dorsolateral striatum, dorsomedial striatum, and nucleus accumbens core.

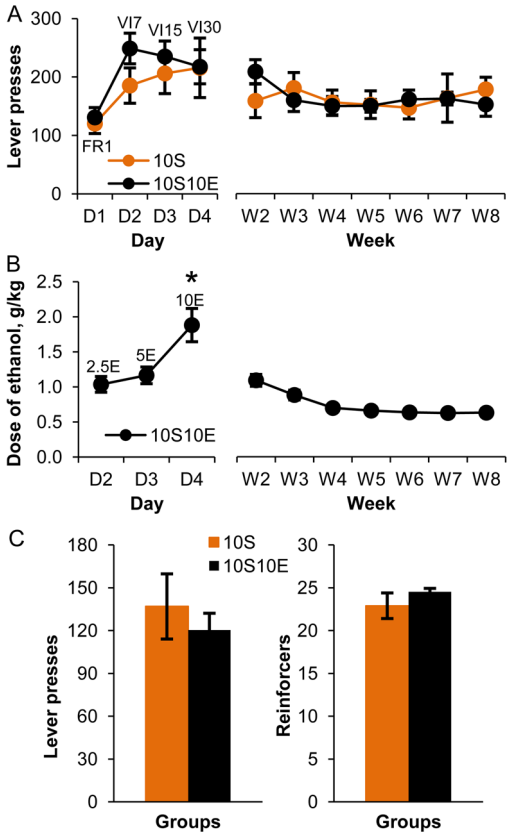

Figure 2. Operant behavior during acquisition and performance of self-administration with 10S and 10S10E solutions. (A) Number of lever presses during acquisition (left, by day) and maintenance (right, by week) of self-administration on the VI-30 schedule of reinforcement. (B) Doses of ethanol obtained by rats in the 10S10E group during acquisition (left, by day) and maintenance (right, by week) of self-administration. (C) Number of lever presses performed (left) and reinforcers obtained (right) by rats during voltammetric measurements. Data are mean ± SEM; n = 8−10 rats/group.

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remaining 7 weeks of training (Figure 2A, right). Maintenance was evaluated by averaging across the five daily sessions of each week; by week 8, rats from 10S and 10S10E groups made similar numbers of lever presses (149 ± 17 and 127 ± 17, respectively), on par with operant behavior in our previous study.[24]

Figure 2B demonstrates doses of alcohol consumed by rats from the 10S10E group across training. During acquisition, alcohol concentration was gradually increased in the drinking solutions which significantly elevated the dose of alcohol consumed by rats from 1.0 g/kg to 1.9 g/kg (RM one-way ANOVA: main effect of day, $F_{2,18} = 7.3$, $p < 0.05$). Post hoc comparison revealed that the alcohol dose on the last day of acquisition, when alcohol content was 10%, was significantly higher than that from the two previous days (Holm-Sidak method, $t > 3$, $p < 0.05$ versus both days). During week 8, 10S10E rats consumed an average of 0.6 ± 0.01 g/kg alcohol per session, equivalent to ∼2 standard drinks in a person.[25] In the current study, we did not analyze blood alcohol content after the self-administration sessions, but a previous study from our lab[24] measured blood alcohol levels of ∼30 mg/dL in rats self-administering 0.5 g/kg alcohol under similar conditions.

When evaluating behavior during the FSCV session (Figure 2C), there was no significant difference between the two groups of rats. The number of lever presses performed by rats during the FSCV sessions was 137 ± 23 and 120 ± 12 in the 10S and 10S10E groups, respectively (unpaired t test, $t_{21} = 0.7$, $p > 0.5$). Similarly, the rats obtained similar amounts of reinforcement (Mann−Whitney rank sum test, T = 115, $p > 0.6$) and 10S10E rats consumed 0.57 ± 0.02 g/kg alcohol. Together, these data demonstrate that the 10S and 10S10E rats were well-matched for operant behavior, suggesting that any differences observed in dopamine release would be due to the reinforcing fluid rather than behavioral output.

Dopamine Release in the Dorsal Striatum of Rats During VI-30 Self-Administration. The DLS, with its connectivity with the sensorimotor cortex, is a critical part of the motor circuit[26] and is important for stimulus-response associations.[27] We hypothesized that phasic dopamine transients in the DLS facilitate reward-seeking in rats performing self-administration on this schedule, and the expression of dopamine transients during lever-press behavior, although correlational, would be critical evidence in support of this hypothesis. Figure 3A illustrates phasic dopamine release observed in the DLS of individual rats at the time of the reinforced and unreinforced lever presses (each averaged across ≥21 trials). Reinforced presses resulted in cue light illumination, signaling reward delivery. Both the color plots and the concentration-versus-time traces demonstrate an increase in dopamine concentration ([DA]) that occurs immediately after the lever presses; in the color plots, dopamine oxidation is indicated by the purple-to-green change in current around time 0. The VI-30 reinforcement schedule produces a sequence of reinforced and unreinforced trials, and an animal cannot predict which trial will be rewarded until the reinforcement cues are presented. Therefore, we evaluated dopamine activity associated with both reinforced and unreinforced trials; dopamine fluctuations prior to the lever press should be similar between the two trial types, but fluctuations after the press should be linked to the appearance or absence of the reward-predictive cues. Figure 3B demonstrates fluctuations in [DA] recorded 5 s before and after the lever press and averaged across the rats within a group (10S, 5 recordings in 4 rats; 10S10E, 7 recordings in 5 rats). Note that [DA] in the initial 2 s period of the window was considered as baseline for the

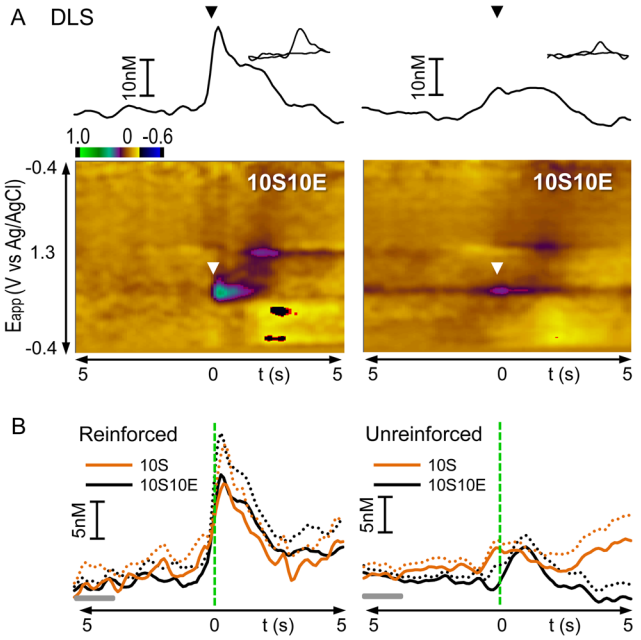

Figure 3. Phasic dopamine activity in the dorsolateral striatum (DLS) 5 s before and 5 s after reinforced (left) and unreinforced (right) lever presses recorded in rats self-administering 10S or 10S10E solutions. (A) Representation of phasic dopamine release recorded in the DLS of individual rats. In the color plot, currents resulting from oxidation/reduction on the carbon-fiber surface are depicted in color across applied potentials (y-axis) and time (x-axis); dopamine oxidation occurs at ∼0.65 V. In the traces above the plot, dopamine concentration, converted by principle component regression from the electrochemical signal, is plotted versus time. The time of lever press is indicated by the triangles (white in the color plot, black in the trace). (B) Fluctuations in dopamine concentration in the DLS measured 5 s before and 5 s after reinforced and unreinforced lever presses and averaged across rats within 10S and 10S10E groups (see text for n values). Solids lines indicate mean, and dashed lines indicate SEM. Gray horizontal bars indicate the 2 s period considered as "baseline". Vertical green dashed lines indicate the time of lever press.

subsequent statistical analysis, similar to prior studies.[28,29] In both 10S and 10S10E rats, [DA] increases after the lever press; however, the increase is dramatically amplified after reinforced as compared to unreinforced lever presses.

The DMS is important for control of behavior based on an action-outcome association when performance of the action is highly dependent on the value of the outcome.[30] We next evaluated [DA] fluctuations in the DMS during operant behavior with low contingency between the rate of an action and its outcome. Figure 4A depicts phasic dopamine release observed in the DMS of two rats: the right plot shows dopamine release at reinforced lever presses in a 10S rat and the left plot shows dopamine release at unreinforced lever presses in a 10S10E rat. Both the color plots and the concentration-versus-time traces demonstrate modest phasic dopamine release in the DMS, but the timing was different in the two rats. We collapsed the data by group across reinforced or unreinforced trials (Figure 4B; 10S, 5 recordings in 4 rats; 10S10E, 6 recordings in 5 rats). The concentration-versus-time traces demonstrate that, in 10S10E rats, [DA] in the DMS slightly increased leading up to either reinforced or unreinforced lever presses, while in the 10S group, [DA] was slightly increased after reinforced lever presses. However, these changes in [DA] were not as robust as in the DLS, and the cyclic voltammograms and color plots indicate that

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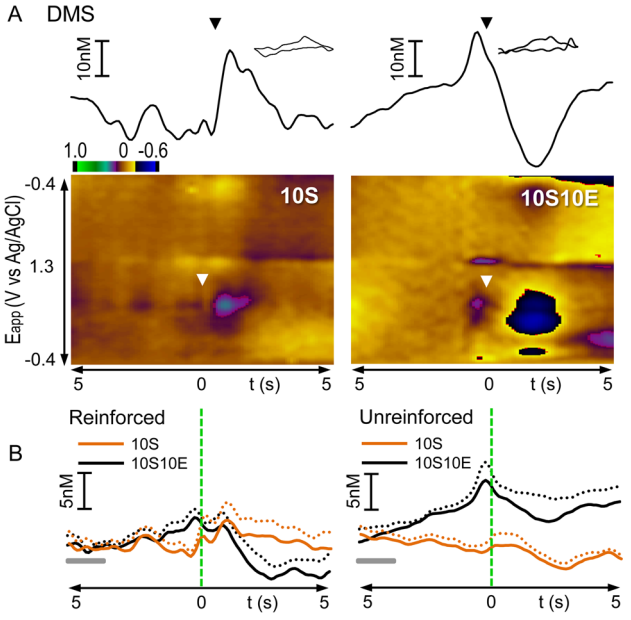

Figure 4. Phasic dopamine activity in the dorsomedial striatum (DMS) 5 s before and 5 s after reinforced (left) and unreinforced (right) lever presses recorded in rats self-administering 10S or 10S10E solutions. (A) Representation of phasic dopamine release recorded in the DMS of individual rats drinking 10S (left) or 10S10E (right). In the color plot, currents resulting from oxidation/reduction reduction on the carbon fiber surface are depicted in color across the changes in applied potential (*y*-axis) and time (*x*-axis); dopamine oxidation occurs at ∼0.65 V. In the trace above the color plot, dopamine concentration, converted by principle component regression from the electrochemical signal, is plotted versus time. The time of lever press is indicated by the triangles (white in the color plot, black in the trace). (B) Fluctuations in dopamine concentration in the DMS measured 5 s before and 5 s after reinforced and unreinforced lever presses and averaged across rats within 10S and 10S10E groups (see text for *n* values). Solids lines indicate mean, and dashed lines indicate SEM. Gray horizontal bars indicate the 2 s period considered as "baseline". Vertical green dashed lines indicate time of the lever press.

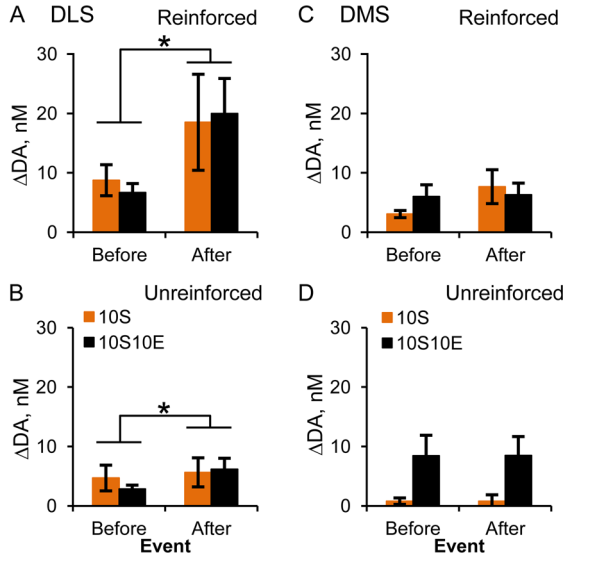

Figure 5. Phasic dopamine activity in the dorsolateral striatum (DLS, left) and dorsomedial striatum (DMS, right) in the 1 s before and 1 s after lever presses recorded in 10S and 10S10E rats. (A, C) Peak change in dopamine concentration in the DLS and DMS before and after reinforced lever presses, expressed as changes from baseline, or Δ[DA], and averaged across rats. (B, D) Peak change in dopamine concentration in the DLS and DMS before and after unreinforced lever presses. Data are mean ± SEM (see text for *n* values). Asterisk (*) indicates $p < 0.05$, main effect of time.

the dopamine signal overlapped with other changes in current. To this point, note that the current associated with dopamine was distinguished from current associated with changes in pH (the major interference) by using principle component regression[31] before further statistical analysis.

To statistically compare dopamine activity between 10S and 10S10E rats, we evaluated the maximal amplitude of [DA] within the 1 s bins before and after lever presses relative to the baseline period (Figure 5), designated as Δ[DA]. In the DLS, the Δ[DA] recorded after reinforced lever presses was 18.5 ± 8 and 20 ± 6 nM in the 10S and 10S10E groups, respectively, and these signals were significantly higher compared to the second before the press (Figure 5A, main effect of time $F_{1,10} = 7.9$, $p < 0.05$, with no effect of group $F_{1,10} = 0.002$, $p = 0.9$, or interaction $F_{1,10} = 0.2$, $p = 0.6$). When lever presses were not reinforced, there was also a significant increase in [DA] after the press in both groups of rats; however, the Δ[DA] was smaller: 5.6 ± 2 nM and 6.2 ± 2 nM in 10S and 10S10E groups, respectively (Figure 5B, two-way ANOVA, main effect of time $F_{1,10} = 6.7$, $p < 0.05$, with no effect of group $F_{1,10} = 0.07$, $p = 0.8$ or interaction $F_{1,10} = 2$, $p = 0.2$). In the DMS, phasic dopamine release was observed in rats from both 10S10E and 10S groups before or after lever presses (Figure 5C, D), although a two-way ANOVA of Δ[DA] yielded no significant effects of group or time around either reinforced lever presses (no main effects of group $F_{1,9} = 0.1$, $p = 0.7$, or time $F_{1,9} = 2.5$, $p = 0.1$, or significant interaction $F_{1,9} = 1.9$, $p = 0.2$) or unreinforced lever presses (no main effects of group $F_{1,9} = 4.4$, $p = 0.07$, or time $F_{1,9} = 0.001$, $p = 0.9$, or significant interaction $F_{1,9} = 0.003$, $p = 0.9$). It was possible that the lack of reliable Δ[DA] in the DMS was due to restricting the analysis window to ±1 s around the lever press. We addressed this by analyzing Δ[DA] in an expanded window of ±5 s around lever presses (Supporting Information Figure 1). However, the results of this analysis replicated those presented in Figure 5. Moreover, plots of Δ[DA] for individual rats (Supporting Information Figure 1) revealed that while [DA] in the DLS tends to increase after the lever press in the majority of rats, that is not the case in the DMS. This analysis revealed that dopamine transients observed in the DMS recordings were inconsistent between animals; as such, Δ[DA] was not apparent after signal averaging across animals. Overall, the lack of transients that were consistently time-locked to the lever press in the DMS suggests that operant performance on schedules of reinforcement with low contingency between the rate of responding and the outcome might require less DMS engagement in the behavior, as demonstrated in previous studies.[7,32] In contrast, the most intense and reliable alterations in [DA] were observed upon reinforcement in the DLS of rats self-administering 10S and 10S10E.

We previously demonstrated that phasic excitation of DLS neurons preceded both reinforced and unreinforced lever pressing in rats trained to self-administer 10% alcohol on a VI-30 reinforcement schedule.[7] As dopamine modulates excitability of medium spiny neurons, we expected to observe dopaminergic activation in the DLS *before* the lever press, consistent with the increased firing activity of DLS neurons. Instead, we observed increased dopamine release *following* the lever press. While this timing contradicts the idea that phasic dopamine release in this case is directly modulating the prepress phasic excitation of DLS

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neurons, it is still consistent with a role for dopamine in plasticity, perhaps modulating the synaptic strength of circuitry underlying stimulus-response associations. Indeed, some DLS neurons (17%) exhibited activation in the second following a reinforced press,[7] and dopamine transients may regulate these neurons. The present findings are consistent with phasic dopamine activation in the DLS that occurred after a nose-poke response in rats self-administering cocaine after prolonged training.[11] Moreover, the present data demonstrate that phasic dopamine activity in the DLS is amplified by the reward-associated cue light and reward delivery accompanying reinforced lever presses, suggesting that DLS dopamine transients are triggered by reward-associated stimuli rather than by motoric aspects of lever pressing. However, Isomura and colleagues demonstrated that motor-associated excitation of DLS neurons in rats was amplified by reward-predicting stimuli preceding movement.[33] It is possible that in the present study the cue and associated dopamine release facilitated approach to the dispensing cup and reward consumption, as opposed to lever pressing. Nevertheless, our results show that phasic dopamine in the DLS accompanies operant responding for reward in rats and is amplified at the onset of reward-associated cues.

Overall, these data are consistent with our hypothesis that phasic dopamine release occurs in the DLS to lever-press behavior, with a predominance of dopamine transients in the DLS compared to the DMS in the seconds around the lever press. However, the results of this study do not support the hypothesis that dopamine transients are amplified in rats drinking sweetened alcohol compared to those drinking sucrose, even though it is well-known that alcohol activates dopamine release.[20−22] The lack of an effect of alcohol on dopamine release might be due to the low concentration of alcohol accumulating during the self-administration session. While microdialysis studies have reported increases in extracellular dopamine levels during alcohol self-administration that surpassed those in rats self-administering sucrose,[20,34] those recordings were made in the NAcc and they may be due to alcohol-associated cues rather than pharmacological effects of alcohol.[35,36] Another interpretation of the data is that both reinforcers used in this study are equally rewarding for the rats and, therefore, the stimuli associated with them elicited equivalent dopamine release. This explanation is supported by the lack of any difference in self-administration behavior between rats from 10S and 10S10E groups.

Functional Overlap of Phasic Dopamine Release between Dorsal and Ventral Striatum. In the present study, one interpretation of the lack of large dopamine fluctuations time-locked to lever presses in the DMS was that dopamine activity was dampened in those rats. As a positive control, we made additional FSCV recordings in rats from the DMS groups on a subsequent day, lowering a fresh carbon-fiber electrode to the NAcc. The NAcc, as a part of the basal ganglia complex, is highly associated with control over motivated behavior and action selection,[37] and dopamine transients in the NAcc were previously reported to occur upon the delivery of unexpected rewards and reward-associated cues.[38] Thus, we expected to observe dopamine transients upon reinforcement in the NAcc of these rats (two 10S10E rats and three 10S rats). As shown in Figure 6A, the color plot and [DA]-versus-time trace from a single rat illustrate phasic dopamine release occurring immediately after reinforced lever presses (averaged across trials). Composite data are shown in Figure 6B, with a robust increase in [DA] after reinforced, but not unreinforced, lever

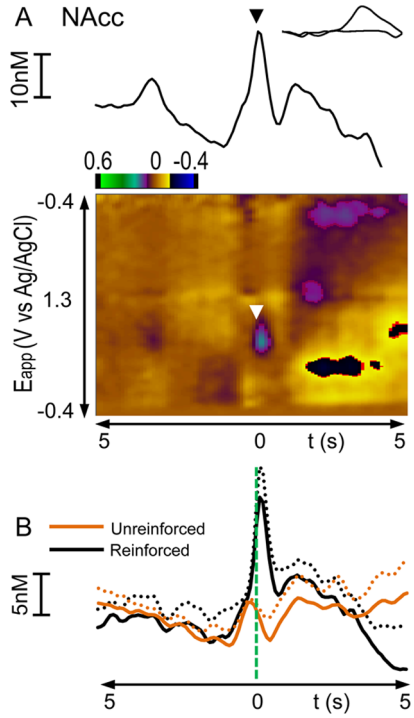

Figure 6. Phasic dopamine activity in the nucleus accumbens (NAcc) 5 s before and 5 s after lever presses. (A) Representation of phasic dopamine release recorded in the NAcc of an individual rat. In the color plot, currents resulting from oxidation/reduction reduction on the carbon fiber surface are depicted in color across the changes in applied potential (*y*-axis) and time (*x*-axis); dopamine oxidation occurs at ∼0.65 V. In the trace above the color plot, dopamine concentration, converted by principle component regression from the electrochemical signal, is plotted versus time. The time of lever press is indicated by the triangles (white in the color plot, black in the trace). (B) Fluctuations in phasic dopamine around reinforced and unreinforced lever presses. Data are collapsed across rats from 10S and 10S10E groups (n = 5). Solids lines indicate mean, and dashed lines indicate SEM. Vertical green dashed line indicates time of the lever press.

presses. These data are consistent with multiple reports of NAcc dopamine transients upon presentation of reward-associated cues.[28,38−40] Therefore, in the present study, NAcc dopamine release was similar to release in the DLS at reinforced lever presses.

In summary, dopamine release was reliably observed in the DLS and NAc within the second after reinforced lever presses, but not in the DMS. [DA] in each region is compared in Figure 7. Under the VI-30 reinforcement schedule, the reward-associated cue following a reinforced lever press was presented to rats for 3.5 s while the reward was delivered. During this time, rats typically oriented toward the cue light before turning to the cup to drink. For Figure 7, we combined measurements from 10S and 10S10E rats to compare fluctuations in [DA] measured in DLS, DMS and NAcc across a 3 s window around reinforced lever presses. After the onset of the cue light (time zero), triggered by the lever press and indicating reward delivery, [DA] increased in both the NAcc and DLS but not in the DMS (as previously shown in Figures 3, 4, and 6). While this evidence shows similarity in the timing of the dopamine transients in the DLS and NAcc, the functions of these striatal regions within the sensorimotor and limbic corticostriatal circuits, respectively, suggest that these dopamine signals may serve different roles during the behavior.[41,42] The importance of brain connectivity within the basal ganglia and the

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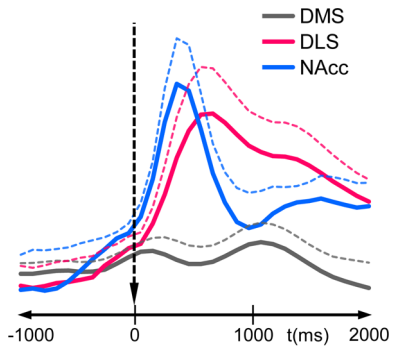

Figure 7. Comparison of composite phasic dopamine activity in the dorsolateral striatum (DLS), dorsomedial striatum (DMS), and nucleus accumbens core (NAcc) during reinforced lever presses. Dopamine concentration-versus-time traces from each region are collapsed across 10S and 10S10E groups and superimposed. Solids lines indicate mean, and dashed lines indicate SEM. Vertical arrow indicates the lever press and onset of the cue light.

VTA and SN for control of action selection has been discussed in many studies.[37,41,43] Anatomical studies of the reciprocal connectivity between the striatal subregions and the VTA/SN have shown that the dopaminergic projection to the DLS can be modulated by input from the NAcc.[44,45] Thus, it is *possible* that the NAcc core and DLS dopamine signals reported here are functionally linked, but there are two caveats which can contribute to these results. Dopamine activity in the two regions was measured in different animals and individual differences in the performance of the task by rats could contribute to the observed dopamine signals. Another possibility is that measurements in the NAc were necessarily made the day after the DMS experiment, although it is common to make in vivo recordings for several days, albeit at different locations, in rats or primates that have been well-trained on a task.[19,28,46−48] Future studies can address specific functional roles for dopamine release in these regions by blocking or amplifying dopamine transients in the NAcc and measuring the effect on DLS dopamine transients, and by conducting dopamine measurements in both regions within the same animals.

In conclusion, this study provides important evidence of a neurochemical mechanism of reward-seeking behavior in rats under a schedule of reinforcement with low action-outcome contingency. We observed robust phasic dopamine release in the DLS and less in the DMS, supporting the hypothesis that DLS dopamine is more engaged in this type of behavior. Future studies can investigate phasic dopamine activity in the DLS and DMS of rats given short versus extended training, as well as during extinction sessions and contingency degradation, to determine whether DMS dopamine transients are activated under those conditions. DLS dopamine release was amplified by reward-related cues, suggesting that phasic dopamine release in the DLS reflects responses of animals to the reward-associated stimuli or subsequent behavior. Future studies can lengthen the time between the reward-predictive cue and the reward delivery to determine which aspect of reinforcement is associated with DLS dopamine release. No difference was found between animals self-administering sucrose or alcoholic solutions, supporting the interpretation that the dopamine transients reflect aspects of behavioral control rather than a specific reinforcer. Cue-induced dopamine release was also observed in the NAcc of rats, demonstrating similarities between ventral and dorsal striatal dopamine in action selection and control over self-administration in the rat.

METHODS

Subjects. Male Long-Evans rats were purchased from Charles River (Raleigh, NC) at 250−300 g. Rats were individually housed under a 12 h light/12 h dark cycle. Animals were water-restricted for the 5 initial days of behavioral training and thereafter had food and water ad libitum. Experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Variable-Interval Operant Training. A schematic representation of the timeline of the experiment is presented in Figure 1A. Behavioral training was performed in operant chambers (MedAssociates, St. Albans, VT) equipped with a house light, two levers, cue lights located above the levers, and two fluid-dispensing cups.[49] The VI-30 training procedure was implemented as previously described.[7,24] During the first week of training, animals were water-restricted. The variable interval (VI) was gradually lengthened over the first 4−5 sessions. The initial 3 sessions lasted up to 3 h; after day 2, sessions were reduced to 30 min. After session 15, reinforcements were limited to 25 per session to minimize differences in reinforcement history. Rats were trained 5 days a week (Monday−Friday) for at least 8 weeks.

The sucrose-fading procedure was used to facilitate alcohol self-administration in rats.[50] On pretraining (day 0) and day 1 of training, all rats were given 20% sucrose solution. Then rats were randomly assigned to self-administer either 10% sucrose solution (10S group) or 10% ethanol solution sweetened with 10% sucrose (10S10E group). Sucrose concentration was reduced to 10% in both groups on day 2, and alcohol was increased from 2.5 to 10% in the 10S10E group by day 4−5.

Surgery. Rats were surgically prepared for voltammetric measurement of phasic dopamine release in striatum, as previously described.[21,51] Rats were anesthetized with isoflurane (induction at 5%, maintenance at 2%) and placed in the stereotaxic frame on a heated pad. A guide cannula was implanted above the DMS or DLS with coordinates from bregma of 1.0 mm (anterior) and 1.2 or 2.4 mm (lateral), respectively. A subset of rats from the DMS group was used for voltammetric measurement of dopamine activity in the NAcc. Thus, NAcc coordinates for the guide cannulas are the same as those for DMS. A bipolar stimulating electrode was placed in the VTA/SN area at coordinates −5.2 mm (posterior), 1.0−1.2 mm (lateral), and −8.6 mm (ventral) from bregma. Finally, an Ag/AgCl reference electrode was placed in the hemisphere contralateral to the guide cannula. All items were secured with stainless steel screws and dental acrylic. After surgery, rats were given ibuprofen (15 mg/kg daily, p.o.) and monitored closely for 3 days.

Fast-Scan Cyclic Voltammetry. Rats were habituated for 2 days to the tether used to connect the electrochemical instrumentation with the electrodes. The voltammetric measurement of phasic dopamine release in behaving rats was performed as previously described.[21,51] Briefly, a freshly constructed carbon-fiber electrode was lowered into the striatum via the guide cannula as the rat was gently restrained. A triangle waveform potential was applied to the electrode (−0.4 to 1.3 to −0.4 V) at 60 Hz for 20−30 min to condition the electrode, then the frequency of the applied potential was decreased to 10 Hz. Note that there have been few studies using voltammetric measurements of spontaneous dopamine transients within dorsal striatum and especially within DLS. This may be due to detection limitations of dopamine in this brain region due to neurobiological aspects of dopamine neurotransmission. For example, more abundant distribution of the monoamine vesicular transporter and uptake complex was demonstrated in the lateral part of the striatum compared to the medial part[52] and in dorsal striatum compared to the NAcc.[53] This might suggest faster dopamine uptake in the dorsal striatum, and specifically in the DLS, which would limit the amplitude of extrasynaptic dopamine transients in this brain region. Therefore, in this study, the electrode was lowered within the region of interest at ∼75 μm increments and voltammetric measurements were collected every 2−3 min until spontaneously occurring dopamine

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transients and dopamine release evoked by electrical stimulation in the VTA/SN (16−24 biphasic pulses, 2 ms/phase, 40−60 Hz, 125 μA) were detected. These measurements were performed to ensure that the carbon-fiber electrode was positioned near active dopamine terminals. Once dopamine release was detected, the behavioral session began and the voltammetric recording was performed continuously for the entire session. At least one rat in each group underwent two recordings within the DLS or DMS. The second recording was made on a subsequent day with a fresh carbon-fiber electrode, which was placed at least 300 μm lower than the first recording site (ensuring no overlap of recording sites), but still within the targeted region.

Postexperimental calibration of the electrodes was performed as previously described[21] in a flow of TRIS buffer (2.5 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 2.0 mM Na_2SO_4, 1.2 mM NaH_2PO_4, 15 mM TRIS HCl, 126 mM NaCl, pH = 7.4) with known concentrations (up to 1 μM) of dopamine while current was recorded.

Histology. Rats were anesthetized with ≥1.5 g/kg urethane and perfused with 10% formaldehyde. Brains were removed and stored in −80 °C. Brains were sectioned in 50 μm slices and stained with thionin. Guide cannulae damage and estimates of the voltammetric electrode placement were identified under a microscope. Results of histological analysis are schematically presented in Figure 1B.

Data Analysis. *Behavioral Data Analysis.* The effects of group (10S, 10S10E) and time on the number of lever presses during the first week (acquisition) and subsequent weeks (maintenance) of self-administration were analyzed with repeated measures (RM), two-way ANOVA. Doses of alcohol consumed by rats during training were analyzed with RM one-way ANOVA. The number of lever presses and reinforcers during FSCV experiments were compared between groups with unpaired *t* tests or Mann−Whitney Rank sum tests, according to the results of the Shapiro-Wilk test of normality. All statistical data analysis for behavioral and voltammetric data was conducted using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA).

Voltammetric Data Analysis. Dopamine concentrations in a 10 s window around each operant event (unrewarded and rewarded lever presses) were visualized by using color plots.[54] In the color plots, applied potential is indicated on the vertical axis, time is on the horizontal axis, and oxidative and reductive current is expressed in color. The plots corresponding to individual lever presses (trials) were averaged with others across the session to compile average electrochemical plots for all unreinforced and all reinforced lever presses for each rat. Trials with apparent mechanical noise (for example, touching the electrode assembly against the chamber wall) were excluded, and the average number of trials included in the data analysis/rat was 14 ± 6 for reinforced lever presses and 64 ± 44 for unreinforced lever presses. Finally, in these averaged color plots, currents obtained from the oxidative potential of dopamine (~0.65 V versus Ag/AgCl reference) was converted to [DA] by using principal component regression analysis and was plotted over time.[31] All subsequent data analysis was conducted on the average concentration-versus-time traces corresponding to the unreinforced or reinforced lever presses performed by individual rats.

For statistical analysis of changes in phasic dopamine concentrations, the 10-s concentration-versus-time traces were divided into ten 1 s bins.[17] Next, [DA] was averaged within the first 2 bins and set as "baseline". Bins 5 and 6 (e.g., 1 s before and after a lever press) were considered as most associated with the behavioral responses based on evidence from studies that investigated burst-firing activity of dopaminergic neurons during self-administration in animals.[55] Peak amplitudes of [DA] during these periods were obtained and expressed as changes from baseline. RM two-way ANOVA was used to assess effects of group (10S, 10S10E) and time (bins 5 and 6) on phasic dopamine release measured in the DMS and DLS.

■ ASSOCIATED CONTENT

Supporting Information

Supplemental Figure 1. Dopamine release was evaluated in the dorsomedial striatum and dorsolateral striatum in an expanded time window around the lever presses. Data are presented by individual rat as well as by group. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

DLR conceptualized the experiments. TAS collected and analyzed the data. DLR and TAS interpreted the data and prepared the manuscript together.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

[DA], dopamine concentration; DLS, dorsolateral striatum; DMS, dorsomedial striatum; FSCV, fast-scan cyclic voltammetry; NAcc, nucleus accumbens; SN, substantia nigra; VTA, ventral tegmental area

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