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NUCLEUS ACCUMBENS DOPAMINE RELEASE IS NECESSARY AND SUFFICIENT TO PROMOTE THE BEHAVIORAL RESPONSE TO REWARD-PREDICTIVE CUES

S. M. NICOLA,* S. A. TAHA, S. W. KIM AND H. L. FIELDS

Ernest Gallo Clinic & Research Center, University of California, San Francisco, 5858 Horton Street, Suite 200, Emeryville, CA 94608, USA

Abstract—The nucleus accumbens is part of the neural circuit that controls reward-seeking in response to reward-predictive cues. Dopamine release in the accumbens is essential for the normal functioning of this circuit. Previous studies have shown that injection of dopamine receptor antagonists into the accumbens severely impairs an animal's ability to perform operant behaviors specified by predictive cues. Furthermore, excitations and inhibitions of accumbens neurons evoked by such cues are abolished by inactivation of the ventral tegmental area, the major dopaminergic input to the accumbens. These results indicate that dopamine is necessary to elicit neural activity in the accumbens that drives the behavioral response to cues. Here we show that accumbens dopamine release is causal to the rats' reward-seeking behavioral response by demonstrating that dopamine in this structure is both necessary and sufficient to promote the appropriate behavioral response to reward-predictive cues.

Key words: discriminative stimulus, motivation, operant behavior, incentive salience, GBR12909, dopamine transporter.

Anatomical and behavioral observations have led to the idea that neurons in the nucleus accumbens (NAc) serve as a limbic-motor interface (Mogenson et al., 1980, 1993). While the importance of the NAc for many goal-directed behaviors is clearly established, it is not clear how its constituent neurons contribute. Dopamine release within the NAc is particularly critical for animals to respond to discrete cues predictive of salient outcomes contingent on specific behaviors. Such cues are known as discriminative stimuli (DSs). DSs are represented by the firing of neurons in limbic system structures that project to the NAc (Sanghera et al., 1979; Nishijo et al., 1988; Watanabe, 1996; Schoenbaum et al., 1998, 1999; Tremblay and Schultz, 2000; Shidara and Richmond, 2002; Matsumoto et al., 2003). Recently, we demonstrated that subpopulations of NAc neurons are excited or inhibited by a discriminative stimulus (DS) that directs rats to perform an operant response (nosepoke) in order to obtain a sucrose reward (Nicola et al., 2004b). These neural responses are abolished by inactivation of the ventral tegmental area (VTA), a midbrain structure that contains the dopamine neurons that project to the NAc, at the same time that the behavioral response to the cues is severely impaired (Yun et al., 2004b). Coupled with findings that dopamine D1 and D2 receptor antagonists injected directly into the NAc reduce responding to predictive cues (Yun et al., 2004a,b), these results support the hypothesis that NAc dopamine increases the cue-evoked NAc neuronal activity that drives or promotes the behavioral response to cues.

This hypothesis states that dopamine release in the NAc is causal to cue responding. If this is the case, then NAc dopamine release should not only be necessary for the animal to emit the appropriate response to a reward-predictive cue; imposing an increase in dopamine should also be sufficient to promote such a response. Evidence in favor of this hypothesis has been obtained from the study of conditioned reinforcement, in which animals perform an operant response to receive a conditioned stimulus (CS) that had previously been paired with a reward. Injection of amphetamine into the NAc robustly potentiates responding reinforced by response-contingent CS presentation, and this effect is dependent on the increase in NAc dopamine caused by amphetamine (Taylor and Robbins, 1984, 1986; Kelley and Delfs, 1991; Wolterink et al., 1993). Further evidence in favor of the sufficiency of elevated NAc dopamine to increase cue responding comes from the study of Pavlovian-instrumental transfer (PIT). In these experiments, animals are trained separately to associate reward with a CS and to lever-press for the same reward in the absence of the CS. In extinction tests, non-contingent presentation of the CS is observed to increase lever-pressing, an effect that is greatly potentiated by NAc amphetamine injection (Wyvell and Berridge, 2000, 2001). Systemic dopamine antagonists reduce the baseline PIT effect (Dickinson et al., 2000), but it is not known whether this reduction is due to an action on NAc dopamine receptors as opposed to dopamine receptors in other brain regions.

Despite the evidence from conditioned reinforcement and PIT studies implicating NAc dopamine in cue responding, the question of whether NAc dopamine release is sufficient to increase responding to DSs has not been studied explicitly. DSs are stimuli that specify an action required to obtain reward or avoid punishment, whereas CSs are simply associated with delivery of the reward or punishment. Several findings suggest that the neural mechanisms that underlie behavioral responding maintained by response-contingent presentation of reward-

*Corresponding author. Tel: +1-510-985-3972; fax: +1-510-985-3101.
E-mail address: nicola@phy.ucsf.edu (S. M. Nicola).
Abbreviations: CS, conditioned stimulus; DS, discriminative stimulus; NAc, nucleus accumbens; NS, non-rewarded stimulus; PIT, Pavlovian-instrumental transfer; PS, probabilistically predictive stimulus; VTA, ventral tegmental area.

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associated cues (i.e. conditioned reinforcement) are different from the neural mechanisms that underlie responding elicited by non-contingent presentation of reward-predictive cues such as DSs. For instance, DSs are capable of causing reinstatement of drug-seeking behavior when presented either contingently or non-contingently (McFarland and Ettenberg, 1997; Weiss et al., 2000, 2001; Alleweireldt et al., 2001; Di Ciano and Everitt, 2003; Yun and Fields, 2003; Ciccocioppo et al., 2004), whereas CSs increase drug-seeking only when presented contingently upon the animal's drug-seeking behavior (i.e. as conditioned reinforcers) (Kruzich et al., 2001; Deroche-Gamonet et al., 2002; Di Ciano and Everitt, 2003). Non-contingent presentation of CSs increases NAc dopamine levels, but contingent presentation does not (Neisewander et al., 1996; Di Ciano et al., 1998; Ito et al., 2000). Furthermore, even though disruption of NAc dopamine function reduces the potentiating effects of NAc amphetamine on conditioned reinforcement, such disruption has no effect on the baseline rate of responding maintained by response-contingent CS presentation (Taylor and Robbins, 1986; Wolterink et al., 1993). These results indicate that although NAc dopamine is sufficient to increase responding maintained by contingent cue presentation, it is not necessary for contingent cues to elevate responding. On the other hand, NAc dopamine may be both necessary and sufficient to increase responding to non-contingently presented cues.

NAc dopamine is clearly necessary for animals to respond to non-contingently presented DSs at least under some conditions (Di Ciano et al., 2001; Wakabayashi et al., 2004; Yun et al., 2004a,b). Furthermore, DS presentation excites putative dopamine neurons in the midbrain (Ljungberg et al., 1992) and causes the release of dopamine in the NAc (Bassareo and Di Chiara, 1999; Weiss et al., 2000; Roitman et al., 2004). However, to our knowledge there is no evidence regarding whether NAc dopamine release is sufficient to promote responding to DSs. Determining whether dopamine is sufficient to promote responding to reward-predictive cues is complicated by the fact that well-trained animals respond to nearly all presentations of a cue that is 100% predictive of reward. This imposes a ceiling effect on performance, such that cue-evoked responding cannot be further increased by manipulations that increase NAc dopamine release. Therefore, we designed a task in which animals respond to about half of all cue presentations, by using a cue that predicts reward probabilistically: instead of predicting reward upon 100% of correct responses, only a fraction of cue responses resulted in reward delivery. Animals responded to only 50% of such cue presentations, allowing ample room to observe an increase in their responding as a result of increasing NAc dopamine release by microinjection of the selective dopamine transporter blocker GBR12909.

EXPERIMENTAL PROCEDURES

Animals

Male Long-Evans rats (275 g) were obtained from Harlan (Indianapolis, IN, UA) and maintained on a 12-h light/dark cycle; experiments were performed during the light phase. Upon receipt, animals were allowed at least 1 week of *ad libitum* food and water before being placed on a restricted diet. Except where indicated, this consisted of 13 g of BioServ pellets (1 g each) and 20 ml of water each day. Training began after 1 week of restriction. All procedures were approved by the Ernest Gallo Clinic & Research Center Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines. Every attempt was made to minimize the number of animals required and to minimize their suffering.

Apparatus

Standard Med-Associates (St. Albans, VT, USA) operant chambers were used; each was enclosed within a sound- and light-isolating plastic outer chamber. Two nosepokes were situated on one wall of the operant chamber, with a reward receptacle between them. Photobeams detected nosepoke behavior. The reward receptacle contained a small well into which a liquid 10% sucrose reward was pumped using a syringe pump. Two white houselights were on throughout experiments, and white noise (65 dB) was present at all times. An additional speaker was used to present auditory stimuli (85 dB). The stimuli were (A) an intermittent 4 kHz tone that was on for 40 ms and off for 50 ms (a 90 ms cycle period), and (B) a siren in which the frequency was ramped from 4 kHz to 8 kHz and back with a 400 ms cycle period.

Tasks

Animals were trained on one of two tasks: the probabilistically predictive stimulus (PS)-DS task or the NS-DS task. In the PS-DS task, two tone cues (up to 10 s long) were presented: a DS, which predicted that reward would be delivered after 100% of correct operant responses emitted during DS presentation, and a PS, which predicted that only a fraction of correct responses would be rewarded. For half the animals, the DS was the intermittent tone and the PS was the siren tone; the opposite relation held for the other half. The left nosepoke was designated the active nosepoke hole for half the animals, and the right nosepoke was designated active for the other half. Responses in the other (inactive) nosepoke hole had no programmed consequence at any time. A response into the active nosepoke hole during DS presentation always terminated the DS and resulted in delivery of 60 μl of 10% sucrose into the reward receptacle. Each PS had a 15% chance of resulting in the same reward, contingent on an active nosepoke response during PS presentation. An active nosepoke response during PS presentation caused the cue to be terminated whether or not reward was delivered. The next cue was presented 30 s after cue termination if no reward was delivered or 40 s after cue termination if reward was delivered. The cue presented on each trial (DS or PS) was chosen randomly by the computer.

The NS-DS task was exactly the same as the PS-DS task, except a non-reward predictive stimulus (NS) was used instead of a PS. The NS had 0% chance of resulting in reward delivery, but it was terminated if the animal made an active nosepoke.

Training on the PS-DS task

(1) Training proceeded in steps, as follows. From training step 3 and onwards, including during all experimental manipulations, all sessions were 2 h long. All animals were initially given 1 session to familiarize them with the reward receptacle. Entry into the reward receptacle caused 60 μl of 10% sucrose reward to be delivered, followed by a 20 s timeout. Animals earned 100 rewards in this session.

(2) The next day, animals had to nosepoke in either of the two nosepokes to obtain reward, followed by an 8 s timeout. Sessions lasted 1 h.

(3) The next day, one nosepoke was chosen to be the active nosepoke, and animals received reward only for responding on

the active nosepoke. Animals were trained on this paradigm for a maximum of two days.

(4) One of the two stimuli described in "Apparatus," above, was chosen to be the DS. This stimulus was presented for up to 60 s. An active nosepoke terminated the DS and caused reward to be delivered. Reward delivery or termination of the DS after 60 s resulted in a 60 s timeout until the next DS presentation. Nosepokes in the absence of the DS had no consequence. These sessions continued until animals earned >60 rewards in a 2 h session.

(5) The stimulus presentation time was reduced to a maximum of 10 s, with 30 s (or, if the animal earned a reward, 40 s) between termination of the stimulus and onset of the next stimulus. Each stimulus was either the DS or the PS (randomly chosen); however, at this stage, responding to the PS was not rewarded. Animals typically responded to about half of PS presentations at this stage and to almost all DS presentations. Training on this step continued for 2–3 days, until PS responding fell to <20% of all PS presentations (i.e. a <20% PS response probability).

(6) The protocol was changed such that the PS had a 30% chance of resulting in reward delivery, provided the animal responded with an active nosepoke (i.e. the PS reward probability was 30%). Over the next several days, the animals' probability of responding to the PS gradually increased.

(7) When the PS response probability reached 60–70%, the PS reward probability was gradually reduced, over 2–3 weeks, to 15%. When animals responded stably to 30–70% of PS presentations, they were judged ready for surgery.

Throughout training and all experiments, the DS reward probability remained at 100%. By the end of training, the DS response probability was near 100%, the PS reward probability was 15%, and the PS response probability ranged from 30 to 70%.

Training on the NS-DS task

Training proceeded as above through step 5. In step 5, responding to the PS is not rewarded, and the task is identical to the NS-DS task. Instead of continuing with steps 6 and 7, rats in the NS-DS group were simply maintained on the NS-DS task, in which the NS is never rewarded, for several weeks, until the NS response probability averaged ~10% and the DS response probability averaged >90%.

Surgery

Animals were initially anesthetized with ketamine (30–60 mg/kg) and xylazine (10 mg/kg), placed in a stereotax, and anesthesia was maintained with isoflurane (0.5–2%). Bilateral 27 gauge stainless steel guide cannulas (Plastics One, Roanoke, VA, USA) were implanted such that the 30 ga injector cannulas would extend 2 mm below the end of the guides and reach the NAc at the border between the core and the shell. Target coordinates of the injectors relative to bregma and the top of the skull were (in mm) AP 1.6, ML ±1.1, DV 7.5 (Paxinos and Watson, 1998). Guide cannulas were secured to the skull with bone screws and dental acrylic, and wire obturators were inserted into the guide cannulas; the ends of the obturators were flush with the ends of the guide cannulas.

PS-DS experiments

After recovery from surgery, animals were retrained on the PS-DS task, with the PS reward probability fixed at 15% for all subsequent sessions. We noticed that the PS response probability of some animals drifted upwards over time, sometimes reaching 80 or 90%. Since we wanted the baseline response probability to remain at about 50%, we increased the amount of food given to these animals each evening in 1 g increments, until the PS response probability stabilized at 30–50%. The DS response probability was unaffected by the additional food and remained near 100%. The animals were maintained on the increased daily ration throughout all experimental sessions; the ration was not changed once injection experiments began. A total of 12 of 26 rats in the PS-DS group were given an increased ration. For these rats, the total daily amount of food ranged from 14 to 22 g, and averaged 17.8 g. The remaining 14 rats received 13 g daily.

All 26 PS-DS animals received both vehicle and two doses of GBR12909 (one rat died before the highest dose was administered), and a subset of 12 rats received SCH23390. The order of injections was randomized. In a subset of 17 PS-DS animals, the effects of extinction of the PS-reward relationship were examined at the end of injection experiments. The PS reward probability was set to 0% for five consecutive sessions, while the DS reward probability remained at 100%.

NS-DS experiments

After recovery from surgery, all 18 rats in this group were retrained on the NS-DS task until performance stabilized at DS response probability >90% and NS response probability ≈10%. At this point, all rats were maintained on 13 g of food daily. GBR12909 (15 nmol) and vehicle were injected in random order. Next, to control for the fact that some animals in the PS-DS group received extra food, we gave a subset of 12 rats 18 g of food daily, the average amount of food given to rats in the PS-DS group that received extra food. After two weeks (during which animals were run daily on the NS-DS task but no injections were made), the GBR12909 and vehicle injections were repeated in this subset of animals, with the dose order randomized. Lastly, the 11 surviving animals were given *ad libitum* food and water for two weeks (again, animals were run daily) and then injected with SCH23390 and vehicle, in random order.

Microinjections

After retraining, animals were injected bilaterally in the NAc prior to every other session with either drug or vehicle. Drugs used were the dopamine reuptake blocker GBR12909 (5 and 15 nmol per side) and the dopamine D1 receptor antagonist SCH23390 (2 μg per side). Injection volume was always 0.5 μl per side. To inject animals, the obturators were removed and the bilateral injectors inserted into the guides. After a 1 min pre-injection period the entire volume was injected over 2 min. After a 1 min post-injection wait, the injectors were removed, the obturators were replaced and the animal was immediately placed into the behavioral chamber and the session began. All sessions were 2 h long. The order of drug doses was randomly chosen for each animal.

Data analysis

In all analyses (except that shown in Fig. 1B), the PS or DS response probability was computed for each animal injected with each drug dose, by dividing the number of PS responses in the session by the number of PS presentations in the session. The response probabilities were then averaged across animals, and the effects of the drugs were determined using paired *t*-tests or repeated measures ANOVA followed by Holm-Sidak comparisons to vehicle. For Fig. 1B, each data point was computed by dividing the total number of PS responses by the total number of PS presentations in all the animals' non-injected sessions in which the DS response probability was >80%. Comparisons were made using χ^2 tests.

Perfusion and histology

At the end of experiments, animals were deeply anesthetized with pentobarbital and perfused with saline and 10% formalin. Sections from the NAc were cut on a microtome and stained with Neutral Red, and cannula positions were determined. In all cases, cannulas were within the NAc.

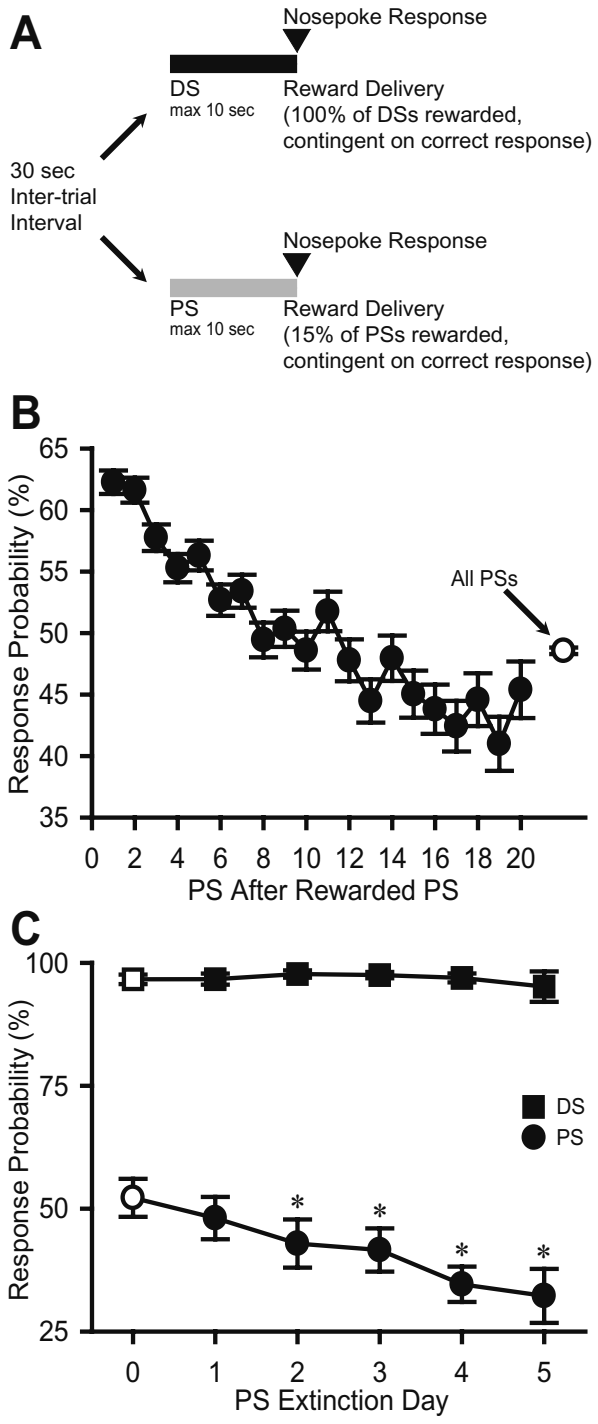

Fig. 1. PS responding is maintained by reinforcement. (A) Diagram of the PS-DS task. Every 30 s, either the DS or the PS was presented (in random order). The cues were on for up to 10 s. An active nosepoke response terminated the cue. Immediately after 100% of correct responses to the DS, sucrose reward was delivered into a receptacle near the nosepoke operandum. The same reward was delivered after only 15% of correct responses to the PS. (B) The PS response probability depends on how recently a reward was delivered as a result of a response to the PS, indicating that rewards earned as a result of responding to the PS reinforce future PS responding. Each data point represents the probability of a response at the *x*th PS after a PS to which the animal responded and earned reward ("rewarded PS"), where *x* is the *x* axis value. Response probabilities in this panel were calculated by dividing the total number of responses to the *x*th PS by the total number of *x*th PS presentations across all non-injected sessions in all PS-DS trained animals; error bars are standard error of the proportion. The 3rd through 20th PS response probabilities were significantly lower than the 1st PS response probability (χ^2 $P<0.05$; *N*'s range from 2565 PS presentations for the 1st PS to 467 for the 20th). (C) Setting the PS reward probability to 0 causes animals to extinguish responding to the PS. On day 0 (open symbols), the DS reward probability was 100% and the PS reward probability was 15%. On days 1 through 5 (closed symbols), the DS reward probability remained at 100%, but the PS reward probability was set to 0. In this panel and in subsequent figures, the response probabilities were calculated by averaging the probabilities across animals, and error bars are S.E.M.. * Significant difference from PS responding on day 0 (Holm-Sidak $P<0.05$; $F_{5,77}=12.9$, $P<0.001$; $n=17$ rats). DS response probability was unchanged across all sessions ($F_{5,77}=0.55$, $P>0.7$).

RESULTS

During experimental sessions, both PS cues (15% of correct responses to the cue were rewarded) and DS cues (100% of correct responses to the cue were rewarded) were presented in random order (Fig. 1A). Whereas animals responded to nearly 100% of DS presentations, they responded to only about 50% of PS presentations. The PS response probability was clearly dependent on the occasional reinforcement of PS responses and was not due to stimulus generalization from the DS to the PS. Three observations directly support this conclusion. First, analysis of responding within behavioral sessions showed that the probability of responding to the PS was highest immediately after earning a reward from a PS response, and became smaller as successive unrewarded PSs were presented (Fig. 1B). Second, reducing the PS reward probability from 15% to 0% (while maintaining the DS reward probability at 100%) over five extinction sessions caused a decrease in PS response probability (Fig. 1C). Finally, in the NS-DS task, the NS response probability averaged ~10% (see below), substantially lower than the PS response probability of 50% in the PS-DS task. Thus, the animals were capable of differentiating the two cues, indicating that PS responding was maintained by reinforcement of PS responses.

To determine whether dopamine release in the NAc is sufficient to promote responding to predictive cues, we microinjected the dopamine transporter blocker GBR12909 directly into the NAc prior to behavioral sessions on the PS-DS task. GBR12909 dose-dependently increased the PS response probability (Fig. 2A; $F_{2,49}=3.9$, $P<0.03$, $N=26$ rats) while the DS response probability remained near 100% (Fig. 2B). To ascertain whether NAc dopamine release is necessary for animals to respond to the PS and DS, we injected a subset of 12 animals with 2 μg of the D1 receptor antagonist SCH23390 (dose based on Yun et al., 2004b). SCH23390 significantly reduced both PS response probability (Fig. 2C; $t_{11}=7.5$, $P<0.001$) and DS response probability (Fig. 2D; $t_{11}=30.1$, $P<0.001$). Thus, dopamine release in the NAc is both necessary and sufficient to promote the operant response directed by reward-predictive stimuli such as the PS.

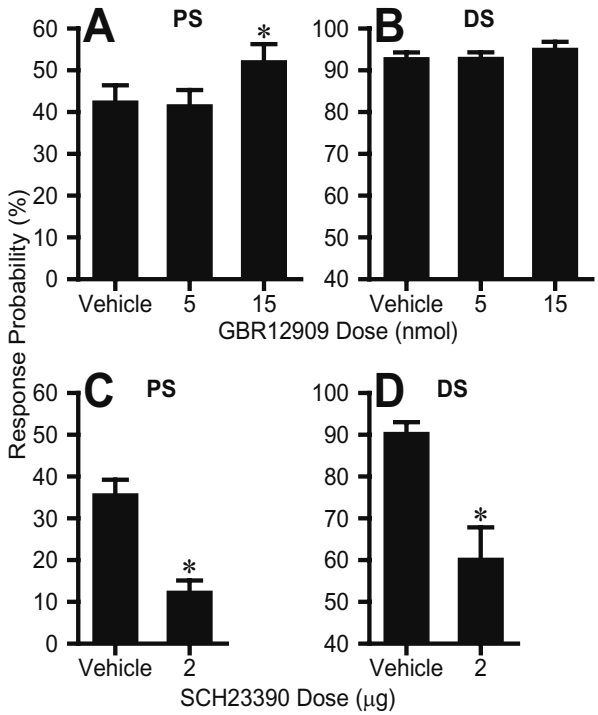

Fig. 2. PS responding is enhanced by injection of a dopamine re-uptake blocker (GBR12909) and reduced by injection of a D1 receptor antagonist (SCH23390) into the NAc. (A) GBR12909 increases PS response probability. (B) DS response probability remains near 100% after GBR12909 injection. (C) SCH23390 reduces PS response probability. (D) SCH23390 reduced DS response probability. * Significant difference from vehicle ($P<0.05$).

The enhanced PS response probability caused by GBR12909 could in principle be due either to a specific enhancement of cue responding or to a general increase in the animals' activity. To exclude the latter possibility, we first determined whether the rate of uncued active nosepoke responding (the rate of nosepokes in the absence of the PS and DS) was enhanced after GBR12909 injection. In vehicle, the rate was 1.00±.16 responses/min; in 5 nmol GBR12909, it was 1.10±.23; and in 15 nmol GBR12909, it was 1.35±.30. There was no significant difference among these values ($F_{2,49}=1.5$, $P>0.2$). This failure to observe an increase in uncued nosepoking could theoretically be due to the fact that animals are less likely to be in the vicinity of the nosepoke during the intercue interval than during the PS, such that a nonspecific increase in motor activity would be less likely to result in an increased nosepoke rate in the absence of cues than during the PS. However, if the GBR12909-induced increase in PS response ratio were due to a nonspecific increase in motor behavior, the latency to respond to the PS after GBR12909 injection should be greater than in vehicle (because non-goal-directed, "accidental" responses during the PS should have longer latencies than goal-directed responses to PS onset). The PS onset-to-nosepoke response latency was not different under the three experimental conditions (vehicle: 2.9±0.1 s, 5 nmol GR12909: 3.1±0.2 s, 15 nmol GBR12909: 3.0±0.2 s; $F_{2,48}=1.2$, $P>0.3$), suggesting that the increased PS response ratio in GBR12909 was due to a specific increase in goal-directed responding to the PS. Next, we trained a separate group of 18 rats on the NS-DS task, in which responding to the NS was never rewarded. We reasoned that if GBR12909 causes a nonspecific increase in operant responding, this should be observed as an increase in NS response probability. However, neither DS nor NS response probability was affected by 15 nmol GBR12909 injected into the NAc (Fig. 3A; $t_{17}=1.6$, $P>0.1$ for NS response probability).

Some rats in the PS-DS group were maintained on extra daily food (see Experimental Procedures). Different levels of food restriction may impact the degree to which

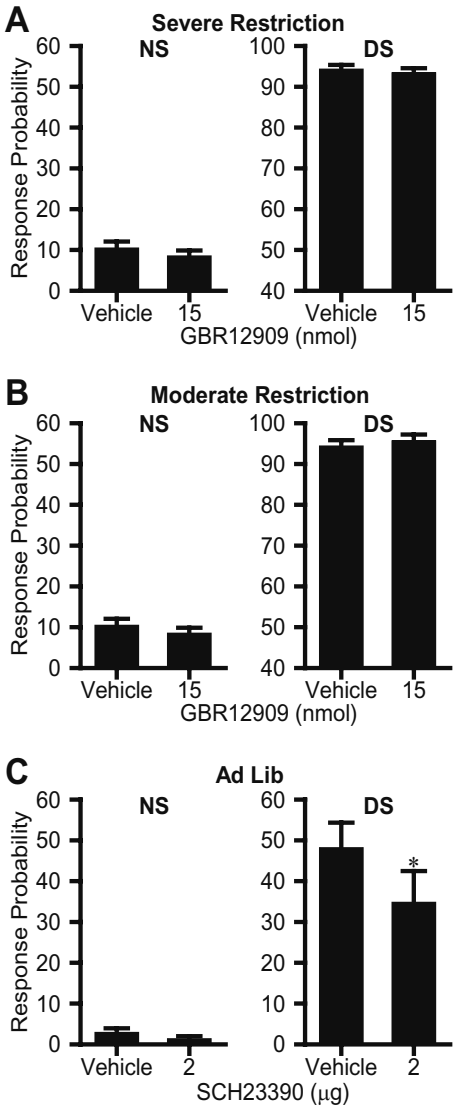

Fig. 3. In the NS-DS task, responding to the NS is not affected by injection of the dopamine re-uptake blocker GBR12909 into the NAc. (A) In animals given 13 g of food per day, GBR12909 does not affect either NS or DS response probability. (B) The same result is obtained in animals given 18 g of food daily. (C) In animals given food and water *ad libitum*, the DS response probability is lower than in restricted animals. Injection of the D1 dopamine receptor antagonist SCH23390 further reduces the DS response probability.

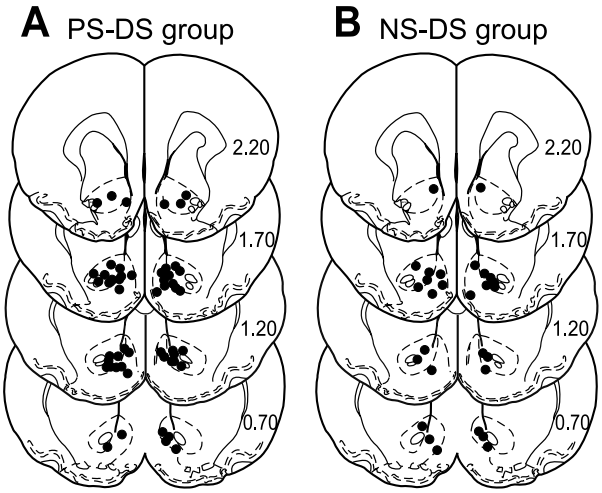

Fig. 4. Cannula placements were in the NAc. Diagrams show coronal sections (Paxinos and Watson, 1998) with verified injector placements for the PS-DS group (A) and for the NS-DS group (B). Numbers on individual sections indicate the distance anterior to bregma (mm).

the dopamine system controls some reward-seeking behaviors (Bechara et al., 1992). To be certain that GBR12909 did not cause a nonspecific effect in the less-restricted PS-DS animals, we repeated the GBR12909 and vehicle injections in a subset of 12 animals in the NS-DS group after giving them a similar amount of extra food for 2 weeks. GBR12909 (15 nmol) did not affect NS response probability under these conditions (Fig. 3B; $t_{11}=.44$, $P>0.6$). Lastly, to verify that NAc dopamine receptor activation is necessary to respond to predictive cues even in sated animals, we determined the effects of NAc SCH23390 injection in NS-DS animals given food and water *ad libitum* for two weeks. Under these conditions, the animals' DS response probability was 48%, substantially less than under food-restricted conditions. SCH23390 (2 μg) injection into the NAc caused a further decrease in DS response probability (Fig. 3C; $t_{10}=2.6$, $P<0.03$) whereas NS response probability was unaffected (although a floor effect may have prevented observation of a decrease).

Histological assessment of cannula placements showed that they were within the NAc (Fig. 4). The intended coordinates were at the border between core and shell, and placements were clustered in this area. Given the relatively large volume of the injections (0.5 μl), it is likely that the drugs diffused to both core and shell in all cases.

DISCUSSION

In the PS-DS task, animals responded to nearly all DS presentations, but responded to only approximately 50% of PS presentations. This behavior is consistent with previous observations that animals "match" their responding to the probability that reward will result from a particular action (Staddon and Cerutti, 2003). In this case, animals overmatched, since the response probability (50%) was greater than the reward probability (15%). The substantial degree of overmatching is likely due to the fact that the animals were food- and water-restricted, since response probability became smaller when animals were given more food. The relatively low energetic cost of making a nosepoke response likely also contributed to overmatching.

Matching behavior must be due to a neural computation that scales the response probability to the observed probability of earning reward. Because the magnitude of the transient burst of dopamine neuron firing triggered by reward-predictive cues is proportional to the probability of reward predicted by the cue (Fiorillo et al., 2003; Satoh et al., 2003), cue-evoked dopamine release in the NAc may increase the probability of a response. This idea is supported by several additional observations. First, presentation of reward-predictive cues causes the transient release of dopamine in the NAc (Robinson et al., 2002; Roitman et al., 2004). Second, subpopulations of NAc neurons fire in response to reward-predictive DSs (Ghitza et al., 2003; Nicola et al., 2004b). Third, inactivation of the VTA, the major dopaminergic input to the NAc, abolishes both the NAc neuronal firing response to a DS as well as the reward-seeking behavioral response (Yun et al., 2004b). Fourth, treatment of the NAc with dopamine receptor antagonists reduces both DS and PS response probability (Yun et al., 2004a,b, present work). Taken together, these findings suggest a mechanism by which dopamine released in the NAc facilitates the appropriate behavioral response to cues: the cue-evoked dopamine transient facilitates the cue-evoked firing of NAc neurons (Nicola et al., 2004a) which increases the probability that the animal will perform the learned reward-seeking response appropriate to the cue.

Our findings that GBR12909 injection increases the PS response probability but does not change the NS response probability support this hypothesis. These results are consistent with the idea that NAc dopamine gates or facilitates a response encoded by neurons upstream of the NAc. When the NS is presented, presumably these inputs to the NAc are relatively silent, and thus increasing NAc dopamine release with GBR12909 has no effect. Furthermore, like DS responding, PS responding was reduced by injection of the dopamine receptor antagonist SCH23390 into the NAc, indicating that PS responding requires dopamine release in this structure. Thus, our results indicate that dopamine is both necessary and sufficient to promote a behavioral response to a reward-predictive cue such as the PS, and therefore that dopamine, by acting on neurons in the NAc, causes animals to respond to the cue.

Although this interpretation is consistent with the evidence to date, alternative explanations cannot yet be excluded. For instance, dopamine neurons fire in response to unexpected rewards, and the dopamine released by this firing has been proposed to contribute to reinforcement (Schultz, 2002). Rewards earned after responding to the PS are relatively unexpected and may trigger dopamine neuron firing. Thus, GBR12909 could increase the magnitude of a reward-associated dopamine transient in the NAc, and, through a dopamine-dependent plasticity mechanism, serve to increase the probability of responding to

subsequent PSs. Notably, facilitation of cue responding by cue-elicited dopamine transients and dopamine-dependent plasticity are not mutually exclusive, and could even occur in tandem. Further experiments are required to explore these hypotheses.

Decreasing the animals' motivation for sucrose by providing them with more food decreased PS response probability, and providing animals with *ad libitum* food and water lowered the DS response probability to about 50%. Because some reward-seeking behaviors are dependent on dopamine in the deprived condition but not in the sated condition (Bechara et al., 1992; Nader et al., 1997), it is possible that dopamine release in the NAc causes DS and PS responding only in deprived animals. However, NAc injection of SCH23390 into animals fed *ad libitum* resulted in a further decrease in DS responding, arguing against this possibility. It is likely that the NAc dopamine release evoked by reward-predictive cues serves as a general mechanism for increasing the likelihood of a reward-seeking response to the stimulus regardless of the animal's satiety state.

Although the reduction in DS and PS response probability caused by injection of NAc SCH23390 is most likely due to reduced dopamine-dependent excitations or inhibitions of NAc neurons by reward predictive cues (Yun et al., 2004b), other explanations are possible. For instance, dopamine could be required for other NAc neurons to facilitate locomotion in general, such that SCH23390 reduces all locomotor activity, including that required for cue responding. This idea is supported by observations that injection of SCH23390 into the NAc at doses similar to that used here reduces locomotion under some conditions (Trevitt et al., 2001; Baldo et al., 2002). However, the fact that in our earlier study only cue-evoked changes in firing were reduced by VTA inactivation argues against this possibility (Yun et al., 2004b), as does our previous finding that SCH23390 does not reduce responding on an uncued fixed-ratio one task (Yun et al., 2004a). These findings lead to the suggestion that the locomotor activity that is suppressed by SCH23390 is driven by the firing of NAc neurons evoked by various cues in the locomotor chamber, some of which may cause approach behavior (Yun et al., 2004a). The specific nature of the GBR12909 effect on PS responding in the present study also argues against a role for NAc dopamine in "general" locomotor behavior. GBR12909 increased PS responding without increasing NS responding or uncued response rate, suggesting that increasing NAc dopamine specifically increases the reward-seeking response to cues. Decreasing the function of NAc dopamine is therefore likely also to cause a specific decrease in cue-evoked reward-seeking, not a general decrease in locomotor activity. Because SCH23390 injection into the NAc and lesion of dopamine fibers in the NAc reduce responding in tasks requiring relatively high behavioral output to obtain reward (Nowend et al., 2001; Salamone and Correa, 2002; Salamone et al., 2005), an intriguing possibility is that the same neurons that dopamine-dependently facilitate the response to reward-predictive cues are also responsible for facilitating the expenditure of effort in high-effort tasks, perhaps because their firing encodes a benefit/cost ratio. Specifically, the dopamine-dependent cue-evoked firing could reflect both the reward predicted by the cues and the level of work, specified by the cues, required to obtain the reward, and the probability of a reward-seeking response could depend on the magnitude of the cue-evoked change in firing.

Although the elevation of responding maintained by presentation of CSs contingent upon the animal's response does not depend on NAc dopamine release (Taylor and Robbins, 1986; Wolterink et al., 1993), non-contingent presentation of CSs increases dopamine release in the NAc core (Ito et al., 2000). Furthermore, lesions of the NAc core impair acquisition of a second-order conditioning task, in which responding is elevated by occasional contingent presentation of a reward-associated cue (Ito et al., 2004). One possibility is that before animals have learned that responding results in CS presentation, the contingently-presented CS serves as a DS that elicits operant responses. Dopamine released onto NAc core neurons could facilitate learning during second-order conditioning by increasing the likelihood of a response to the stimulus. Further investigation is required to confirm this hypothesis.

CONCLUSION

In summary, the dopamine reuptake blocker GBR12909 specifically increased cue response probability in a task where animals respond to about half of cue presentations. The dopamine D1 receptor antagonist SCH23390 reduced responding to such cues. These results support the hypothesis that dopamine release in the NAc is necessary and sufficient for responding to reward-predictive cues, and therefore that NAc dopamine release causes animals to respond to such cues.

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