EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, Vol. 38, pp. 2572–2588, 2013 doi:10.1111/ejn.12253

BEHAVIORAL NEUROSCIENCE

Development of behavioral preferences for the optimal choice following unexpected reward omission is mediated by a reduction of D2-like receptor tone in the nucleus accumbens

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Keywords: behavioral flexibility, dopamine, motivated behavior, rat, reward

Abstract

To survive in a dynamic environment, animals must identify changes in resource availability and rapidly apply adaptive strategies to obtain resources that promote survival. We have utilised a behavioral paradigm to assess differences in foraging strategy when resource (reward) availability unexpectedly changes. When reward magnitude was reduced by 50% (receive one reward pellet instead of two), male and female rats developed a preference for the optimal choice by the second session. However, when an expected reward was omitted (receive no reward pellets instead of one), subjects displayed a robust preference for the optimal choice during the very first session. Previous research shows that, when an expected reward is omitted, dopamine neurons phasically decrease their firing rate, which is hypothesised to decrease dopamine release preferentially affecting D2-like receptors. As robust changes in behavioral preference were specific to reward omission, we tested this hypothesis and the functional role of D1- and D2-like receptors in the nucleus accumbens in mediating the rapid development of a behavioral preference for the rewarded option during reward omission in male rats. Blockade of both receptor types had no effect on this behavior; however, holding D2-like, but not D1-like, receptor tone via infusion of dopamine receptor agonists prevented the development of the preference for the rewarded option during reward omission. These results demonstrate that avoiding an outcome that has been tagged with aversive motivational properties is facilitated through decreased dopamine transmission and subsequent functional disruption of D2-like, but not D1-like, receptor tone in the nucleus accumbens.

Introduction

Motivated behavior, such as foraging, is necessary for survival and reproductive goals (Kelley & Berridge, 2002; Aragona & Wang, 2009; Becker, 2009) and is paramount for fitness (Pyke, 1984; Stephens & Krebs, 1986). As food availability is highly dynamic, flexibility in reward-seeking behavior is critical for survival. When resource availability depletes, animals must be able to recognise this alteration and rapidly adjust their behavior accordingly. This can be studied in the laboratory by modeling foraging conditions and manipulating reward availability, magnitude, or quality (Papini & Dudley, 1997).

Neurobiologically, mesolimbic dopamine (DA) has been strongly implicated in motivated behavior (Nicola, 2007; Berridge, 2012). Although DA has long been known to be involved in appetitive, reward-seeking behaviors (Schultz, 1998; Brown & Peters, 2004; Phillips *et al.*, 2007; Dalley & Everitt, 2009), there is growing evidence that mesolimbic DA is also involved in aversive motivation (Young, 2004; Anstrom *et al.*, 2009; Badrinarayan *et al.*, 2012; Salamone & Correa, 2012). Previous studies have demonstrated that the reduction or omission of an expected reward is a salient and aversive event that can significantly alter behavior (Tinklepaugh, 1928; Miller & Stevenson, 1936; Amsel, 1958; Daly, 1974; Kerfoot *et al.*, 2008), and aversive responses to reward omission are phylogenetically ancient (Vindas *et al.*, 2012). Although the nucleus accumbens (NAc) core has been shown to mediate behavioral flexibility (Cardinal *et al.*, 2001; Corbit *et al.*, 2001; Floresco *et al.*, 2006; Haluk & Floresco, 2009), little is known about the role of DA in this system following decreased responding when reward is omitted (Annett *et al.*, 1989; Reading & Dunnett, 1991).

Electrophysiological recordings demonstrate phasic reductions in firing rate by conventional putative DA neurons [projecting to the NAc core (Ikemoto, 2007; Lammel *et al.*, 2008)] when an expected reward is omitted (Schultz *et al.*, 1997; Roesch *et al.*, 2007), and this is believed to cause a phasic decrease in DA concentration ([DA]) in terminal regions. Modeling data demonstrate that these phasic decreases reduce D1- and D2-like receptor occupancy to 0% (Dreyer *et al.*, 2010). The impact of reducing DA receptor tone has

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Received 9 April 2013, revised 28 April 2013, accepted 12 April 2013

been understudied, and it remains unknown which DA receptor subtype impacts behavior following unexpected reward omission. However, phasic decreases in [DA] are hypothesised to preferentially alter D2-like receptor occupancy, as these receptors have greater affinity for DA (Richfield *et al.*, 1989), and therefore a higher baseline occupancy (Dreyer *et al.*, 2010; Marcellino *et al.*, 2012). It has therefore been suggested that behavioral alterations resulting from phasic decreases in [DA] are mediated by D2, but not D1, receptors (Frank, 2005; Bromberg-Martin *et al.*, 2010).

Here, we utilised an operant behavioral task that allowed subjects to 'forage' for reward in two different locations (two spatially distinct levers). After demonstrating that rats rapidly develop a robust preference for the rewarded option, we tested the aforementioned hypothesis by administering D1- and D2-like receptor agonists and antagonists into the NAc core prior to the first sessions of the reward omission task.

Materials and methods

Subjects

A total of 158 Sprague–Dawley rats between 57 and 64 days of age (males, 251–275 g; females, 176–200 g) were used in these experiments. Rats were obtained from Charles River Laboratories (Winington, MA, USA) and were pair-housed with a same-sex cagemate in transparent plastic cages with metal tops. Animals were kept on a 12/12 h reverse light/dark cycle. Experiments were run daily between 9:00 and 17:00 h during the dark phase.

Mild food restriction was employed to train rats to lever-press for the food reward. As rats naturally continue growing, daily feeding accounted for natural growth over time, which was important to maintain consistent motivation levels throughout the experiment. Subjects were food restricted to approximately 90% of their free-feeding weight accounting for natural growth (Baker *et al.*, 2012). Natural growth curves for free-feeding male and female rats were obtained from Charles River Laboratories. After the operant session each day, rats were weighed and fed based on their weight between 15:30 and 16:30 h each day during the dark cycle. Rats had free access to water in their home cages. Subjects experiencing reward reduction (described below) were fed less than subjects experiencing reward omission to equate the motivational states of the two groups, as reward reduction rats earned larger rewards than reward omission subjects.

Behavioral paradigm

Behavioral training was conducted in chambers (Med Associates, Georgia, VT, USA) that were modified locally by Marc Bradshaw at the University of Michigan. Each chamber was equipped with two cue lights, a pellet dispenser, a reward port, a white noise generator, and two levers (Coulbourn, Whitehall, PA, USA). The reward port was centrally located, equidistant between the two levers (see Fig. 1A). The food reward used throughout the experiments was 45 mg BioServ chocolate-flavored dustless precision reward pellets (Bio Serv, Frenchtown, NJ, USA).

Initially, subjects received two magazine training sessions, in which 25 reward pellets were delivered throughout the session with the inter-trial interval varying from 40 to 80 s. Rats then learned to press two spatially distinct levers (see Fig. 1) to earn up to 50 reward pellets on each lever in 1 h. Once subjects earned 100 reward pellets (50 on each lever) in < 60 min for two consecutive sessions (mean number of sessions, 5.2 ± 0.7), the next phase of

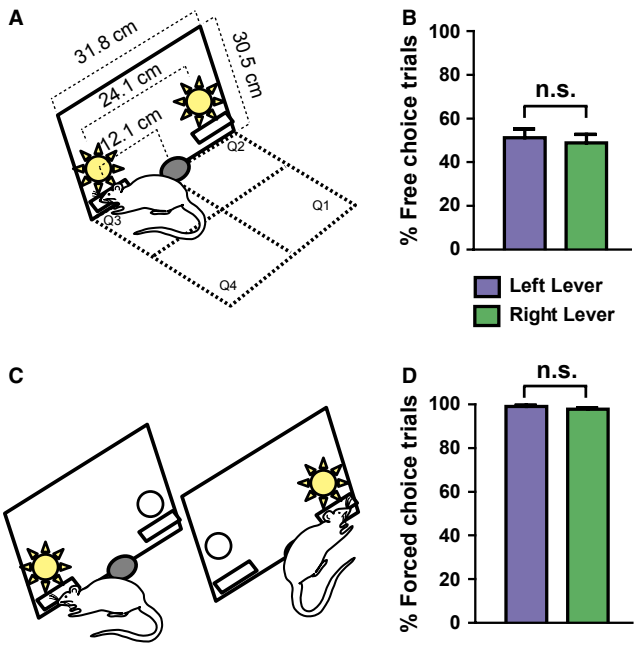

FIG. 1. Appetitive operant behavioral paradigm for examining foraging preference in rats. (A) Free-choice trials facilitate the assessment of an animal's preference for one lever over the other. During training, choosing either lever resulted in equal amount of food reward. (B) Once trained on the task, rats accurately completed free-choice trials, showing no reliable side bias. (C) During forced-choice trials, although both levers were extended, subjects only received a food reward for pressing the lever under the illuminated cue light. (D) Subjects learned to complete forced-choice trials with near perfect accuracy. n.s., not statistically significant. Error bars indicate mean + SEM.

training began in which subjects learned to discriminate between the cue lights. During these trials, one of the two cue lights would illuminate, and 5 s later both levers would extend into the chamber for 15 s or until one lever was pressed. If the lever under the illuminated cue light was chosen, a reward was delivered into the food receptacle 2 s later. Choosing the non-illuminated lever was defined as an error; the cue light would turn off, levers would be retracted, and no food pellet would be delivered. These sessions contained 100 trials (50 with each cue light). Once subjects completed two consecutive sessions with at least 90% accuracy (mean number of sessions, 5.7 ± 0.6), they progressed to the final behavioral task described below.

Consistent with previous studies (Day *et al.*, 2010, 2011; Gan *et al.*, 2010; Sugam *et al.*, 2012), the operant paradigm contained two trial types, termed 'free-choice' and 'forced-choice' trials (Fig. 1A and C). A cue light above the levers signaled which lever, if chosen, would yield reward. At 5 s after one or both of the cue lights illuminated, both levers extended into the behavioral chamber. During free-choice trials, both cue lights illuminated and a response on either lever yielded reward (Fig. 1A), whereas on forced-choice trials, subjects would receive the reward only if they chose the lever below the illuminated cue light (Fig. 1C). Pressing the non-illuminated lever counted as an error and no reward was delivered.

One-third of each session's trials (30 trials) were free-choice trials, and an equal number of forced-choice trials for the left and right levers (30 of each) were given in each session. Trial types were interspersed throughout each session, and the inter-trial interval varied from 10 to 30 s. Each subject received a 1 h training session per day containing 90 trials. Consistent with previous work (Day *et al.*, 2010), the schedule of reinforcement progressed from fixed

ratio schedule or reinforcement (FR)1 to FR2 to FR4 across sessions. To advance to each FR schedule, subjects must have completed all trial types with at least 90% accuracy for two consecutive sessions. The average number of sessions to progress from FR1 to FR2 was 3.1 ± 0.4 sessions, and the mean number of session to advance from FR2 to FR4 was 2.5 ± 0.3 sessions.

Throughout training, some subjects (which later experienced reward omission) always received one reward pellet following a correct response. Others (which later experienced reward reduction) always received two pellets after a correct operant response. Rewards were given in this way so that both conditions would be a reduction of one reward pellet. The fact that some subjects received more food reward during the operant sessions was accounted for in daily feeding to maintain equivalent motivational states among all subjects (see 'Subjects' section above). Regardless of whether subjects earned one or two reward pellets per trial, all subjects readily consumed their rewards throughout the session, showing no evidence of satiety.

Once stable responding, defined as a minimum of three consecutive days with at least 90% accuracy on each trial type, occurred on the FR4 schedule of reinforcement, subjects experienced one of two negative contingency switches. During the negative contingency switch sessions, the reward normally resulting from a correct operant response on one lever was either reduced by 50% or completely omitted. Conversely, the other lever remained reinforced on the same schedule (i.e. its contingency was unchanged). In both reduction and omission manipulations, reward was reduced by one reward pellet. In other words, for subjects that received two reward pellets for a correct operant response during training, a response on one lever was reduced to one reward pellet during reward reduction (n = 5 males and 12 females). For subjects that received one reward pellet for each correct operant response during training, during reward omission a response on one lever yielded no reward pellets (n = 6 males and 13 females). More female rats were tested than male rats to ensure we tested females across all stages of the estrous cycle (see Data S1).

Whether the lever that ceased to be reinforced was the right or left lever was counterbalanced across subjects, and this had no consequence on the results (data not shown). Although no statistically significant lever bias was observed, if an individual rat tended to have a lever bias, the 'biased lever' was chosen to be the one in which responding led to altered response contingencies (i.e. reward reduction or omission). This ensured that any changes in behavioral preference were due to the contingency switch manipulation and not a potential underlying individual lever bias. Behavior during the contingency switches did not differ between rats with no prior lever bias and those with a trending bias before the switch (data not shown).

After the three sessions of reward reduction or reward omission, male subjects received six post-switch sessions. These sessions contained free-choice trials and forced-choice trials, and both levers were once again equally reinforced, identically to the sessions prior to the contingency switches. These extra sessions allowed us to test the longer-term effects of reward reduction and omission on behavioral preference. Specifically, if the behavioral preference for the optimal choice was due simply to learning which lever yielded greater reward, when both levers were once again equally reinforced, subjects would be expected to choose them equally (just like they did prior to the contingency switch). However, if the lever yielding reduced or no reward was tagged with lasting aversive properties, the worse-choice lever would be expected to be avoided even when both levers were equally reinforced again.

Analysis of behavior

Performance on the free-choice and forced-choice trials was automated by MED Associates software. To compare choice behavior between the reward omission and reward reduction groups, preference scores were calculated for each rat (percentage of free-choice trials choosing the optimal choice minus the percentage of free-choice trials choosing the worse choice, i.e. the smaller reward lever in reward reduction and the omitted reward lever in reward omission).

Behavior during a baseline session of training, as well as the first sessions of reward reduction and reward omission, was recorded onto DVDs. Videos were scored using BEHAVIOR TRACKER software to determine the locations of subjects throughout the sessions. For scoring purposes, the behavioral chambers were divided into four equal-size quadrants plus another portion of the chamber above the reward port into which rats sometimes climbed (see Fig. 1A for partitioning of quadrants). One quadrant was directly in front of each lever and corresponding cue lights. The percentage of time rats spent in each quadrant was analysed.

The individuals scoring the videos were blind to the experimental manipulations and hypotheses. To maintain a high level of inter-rater reliability, certain videos were scored by multiple raters. Behavioral scoring was very consistent (above 96%), with any measure varying by no more than 4% across raters.

Surgery

All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Michigan Committee on the Use and Care of Animals. Subjects in the behavioral pharmacology experiment underwent surgery after initial behavioral training. They were returned to free-feeding the day prior to surgery. On the day of surgery, rats were anesthetised with an intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg) and implanted with 22 gauge bilateral stainless-steel guide cannulas (Plastics One, Roanoke, VA, USA) above the NAc core (AP, +1.4 mm; ML, +1.3 mm relative to bregma; DV, −3.0 mm from the surface of the skull). Guide cannulas were permanently fixed in place with two stainless-steel surgical screws and dental acrylic. Stainless-steel obturators flush with the end of the guide cannulas were inserted until the experimental day. After surgery, all subjects were given ketoprofen (5 mg/kg) for pain relief and *ad-libitum* access to food and water until fully recovered.

Once fully recovered from surgery (as determined by the return of normal weight gain), food restriction resumed, and subjects were retrained on the FR4 operant task for a minimum of 4 days or until stable performance (defined as a minimum of three consecutive days with at least 90% accuracy on each trial type) on the task was observed. Obturators were removed, cleaned, and reinserted daily to keep the cannulas unclogged.

Drugs and microinfusion procedure

As the behavioral manipulations in this study have previously been shown to phasically alter putative DA neurons (Schultz *et al.*, 1997) projecting to the NAc core (Ikemoto, 2007), we extensively examined the effects of DA transmission in the NAc core on changes in behavior resulting from reward omission. We tested two doses of a variety of dopaminergic agents. The D1-like receptor agonist SKF-38393 (0.1 and 1.0 μg), D1-like receptor antagonist SCH-23390

(0.1 and 1.0 μg), D2-like receptor agonist quinpirole (0.1 and 1.0 μg), and D2-like receptor antagonist eticlopride (0.1 and 1.0 μg) were chosen, and doses were selected based on previous studies showing these compounds to be behaviorally relevant when infused into this brain region, especially at the higher dose (Wolterink *et al.*, 1993; Ranaldi & Beninger, 1994; Swanson *et al.*, 1997; Pezze *et al.*, 2007; Haluk & Floresco, 2009; Moreno *et al.*, 2013; Stopper *et al.*, 2013). These drugs were obtained from Sigma Aldrich (St Louis, MO, USA) and dissolved into sterile saline (Haluk & Floresco, 2009). Drugs were mixed fresh on each day of behavioral testing (i.e. the experimental day).

On the day of behavioral testing, stainless-steel injectors (28 gauge) attached to PE-20 polyethylene tubing (Plastics One) were inserted into the secured guide cannulas and extended approximately 3.5 mm below the tip of the guide cannulas (i.e. into fresh tissue), resulting in accurate targeting of the NAc core (see Fig. 3). Each subject received a bilateral infusion into the NAc core (saline control, $n = 6$; 0.1 μg SCH-23390, $n = 6$; 1.0 μg SCH-23390, $n = 6$; 0.1 μg SKF-38393, $n = 7$; 1.0 μg SKF-38393, $n = 6$; 0.1 μg eticlopride, $n = 7$; 1.0 μg eticlopride, $n = 9$; 0.1 μg quinpirole, $n = 8$; 1.0 μg quinpirole, $n = 5$). The infusion volume of 0.5 μL per side was delivered over 60 s via a 10 μL Hamilton syringe (Reno, NV, USA) and pump (Harvard Apparatus, Holliston, MA, USA). Injectors remained in place for an additional 60 s following the end of the infusion to allow the drug to diffuse; injectors were then removed, obturators were reinserted, and behavioral testing began 10 min later (Haluk & Floresco, 2009; Hanlon *et al.*, 2010). To ensure that observed drug effects were not attributed to drug spreading outside the NAc core, a subset of quinpirole (i.e. the drug that produced a robust behavioral effect) subjects ($n = 7$) received the effective dose of quinpirole, except in a smaller volume (1.0 μg/0.3 μL per side), infused into the core. Additionally, to determine if the quinpirole effect was unique to the NAc core subregion or more broadly to the NAc, additional rats ($n = 6$) received the effective dose of quinpirole (1.0 μg quinpirole/0.3 μL) into the medial shell. The volume of 0.3 μL was chosen as previous work has successfully utilised this volume to study NAc core vs. shell differences in the rat (Pulvirenti *et al.*, 1994; Pierce & Kalivas, 1995; Floresco *et al.*, 2006, 2008). This is important, as many studies have revealed differences in core vs. shell regulation in motivated behavior (Di Chiara, 2002; Meredith *et al.*, 2008; Reynolds & Berridge, 2008; Aragona *et al.*, 2009).

In total, 67 subjects with accurate bilateral injector placements in the NAc core were included in the analyses (saline controls, $n = 6$; 0.1 μg SKF-38393, $n = 7$; 1.0 μg SKF-38393, $n = 6$; 0.1 μg quinpirole, $n = 8$; 1.0 μg quinpirole, $n = 12$; 0.1 μg SCH-23390, $n = 6$; 1.0 μg SCH-23390, $n = 6$; 0.1 μg eticlopride, $n = 7$; 1.0 μg eticlopride, $n = 9$). Six subjects receiving 1.0 μg quinpirole had placements in the NAc medial shell and were included in the analyses.

Locomotor testing

As drugs acting on DA receptors in the NAc can alter general locomotor activity, which could affect behavioral performance and therefore impact the results, we tested the locomotor effects of the higher dose (1.0 μg) of each of the chosen D1-like and D2-like receptor agonists and antagonists. A separate drug-naive group of rats was used so that drug infusions were made into fresh, undamaged tissue, as previous work has shown decreased spread of drug effect from repeated microinfusions (Mahler *et al.*, 2007; Richard & Berridge, 2011). These subjects (total $n = 48$) were implanted with guide cannulas as described above. A between-subjects design, whereby each subject only received one drug, was utilised to exclude the possibility of sensitisation effects (Henry *et al.*, 1998; Vezina, 2004). Once fully recovered from surgery, subjects were maintained at approximately 90% of their free-feeding weight so they would be in the same motivational state as those tested in the behavioral pharmacology experiments.

Consistent with previous work (Badiani *et al.*, 1995; Crombag *et al.*, 1999), locomotor testing was conducted in plastic rectangular cages (45 × 24 × 18 cm) with a block in the center so rats could only explore the perimeter of the cage. These cages were equipped with photobeams to quantify two measures of locomotor activity: total number of photobeam breaks and number of crossovers, defined as moving from one end of the cage to the other. Crossovers captured locomotion across the cage and not the repetitive disruption of a single photobeam (Robinson & Camp, 1987; Paulson *et al.*, 1991). Subjects were run in waves of six to eight rats with saline control animals in every wave to account for any potential variation across days of testing sessions (which is why more saline control rats were tested than drug treatments). Forty-nine rats received infusions, which were conducted as described above and locomotor activity was monitored for 1 h, the same length of time as the operant reward-seeking sessions. One outlier was excluded, so 48 subjects were included in the analysis (saline controls, $n = 20$; 1.0 μg SKF-38393, $n = 7$; 1.0 μg quinpirole, $n = 9$; 1.0 μg SCH-23390, $n = 7$; 1.0 μg eticlopride, $n = 5$).

Histology

Upon completion of operant and locomotor testing, all subjects were euthanised with an overdose of ketamine (200 mg/kg) delivered intraperitoneally, and brains were extracted for histological verification. After soaking in formalin solution, brains were rapidly frozen and sliced on a cryostat in 50 μm sections. Brain sections were stained with cresyl violet and viewed under 10 × magnification. Placements were identified by where the end of the tract from the injector tip was located and compared with the brain atlas of Paxinos & Watson (1998).

Statistics

Statistical analyses were performed using SPSS Statistics 19 (IBM, Armonk, NY, USA), and data were graphed using GRAPHPAD PRISM version 5.0 (San Diego, CA, USA). Statistical significance for all statistical tests was defined with an α level of 0.05. Bonferroni corrections were applied to *post-hoc* tests to reduce the risk of Type I errors (Sarter & Fritschy, 2008).

Consistent with previous behavioral and pharmacological studies (Haluk & Floresco, 2009; Day *et al.*, 2010), two-way (multivariate) ANOVAS were used to examine behavioral data during baseline sessions, the contingency switch sessions, and post-switch sessions as well as to screen for sex differences. Metestrus and diestrus data showed no statistically significant differences and therefore were combined for analysis (Lynch *et al.*, 2000). The estrous cycle stage was included as a covariate in analyses (Girard & Garland, 2002; Pawluski *et al.*, 2006) to determine if it modulated the development of behavioral preferences.

In the behavioral pharmacology experiments, two-way (multivariate) ANOVAS and *post-hoc* tests with Bonferroni corrections were used to examine the effects of drugs and doses on choice preference during free-choice and forced-choice trials. As the 0.3 and 0.5 μL volumes of 1.0 μg quinpirole in the NAc core did not statistically differ from each other, they were combined to increase power to

confidently interpret the null result. Because of the robust effect of reward omission and the increased sample size of quinpirole subjects from combining both infusion volumes in the core, the quinpirole-induced blockade of the development of a behavioral preference is interpretable, and the likelihood of it being a Type 2 error is very low. Specifically, the effect of reward omission on behavioral preference is very robust, causing a statistically significant choice preference in groups with as few as five subjects, and throughout the study the robust behavioral effects of reward omission were replicated in behavior-only subjects as well as in many of the drug treatment groups.

Additionally, one-way ANOVAS were used to analyse performance on specific trial types (free-choice trials choosing non-rewarded lever and rewarded forced-choice trials) among drug and control conditions. Dunnett's *post-hoc* tests were used to compare drug groups with controls. Locomotor data were analysed using a one-way ANOVA with planned contrasts (Gonzalez, 2009).

Results

Establishing behavioral preference for the optimal choice during reward reduction and omission

Rats were initially trained to press two levers that yielded equal reward. During one-third of the trials termed 'free-choice trials,' cue lights above both levers illuminated and subjects could earn a reward pellet by pressing either lever (Fig. 1A). During these trials, subjects earned rewards from both levers, and showed no reliable preference for one lever over the other during free-choice trials (Fig. 1B; $t_{10} = 1.489$, $P = 0.167$), which was expected as both levers were equally rewarded. Conversely, during two-thirds of the trials (30 trials for each lever) termed 'forced-choice trials', cue lights above the levers signaled which lever, if chosen, would result in a food reward (Fig. 1C). Rats learned to distinguish between the two cue lights with near-perfect accuracy revealing no side bias during forced-choice trials (Fig. 1D; $t_{10} = 0.305$, $P = 0.767$).

Once stable responding on this task was observed, subjects experienced one of two negative contingency switches. In one contingency switch, the reward following a correct operant response on one lever was reduced by 50% (from two pellets to one pellet); in the other contingency switch, reward was completely omitted (from one pellet to no pellets). In both cases, the reward was decreased by one pellet, and the unchanged lever remained reinforced as normal.

Reducing reward by 50% on one lever did not induce a behavioral preference during the first session; however, a preference for the optimal choice emerged over subsequent reward reduction sessions (Fig. 2A; main effect of reinforcement, $F_{1,48} = 86.270$, $P < 0.001$; interaction of reinforcement by session, $F_{2,48} = 18.082$, $P < 0.001$). Specifically, during the first session of reward reduction, subjects did not exhibit a behavioral preference for one lever over

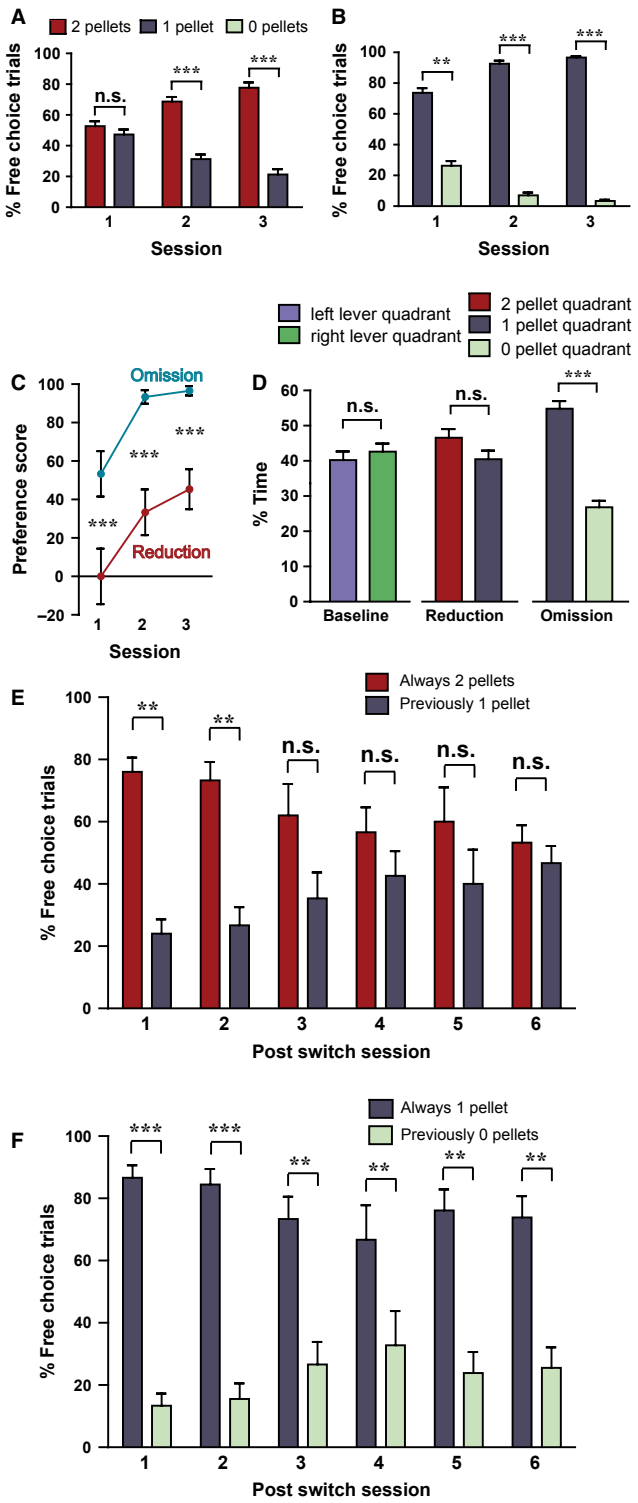

FIG. 2. Effects of unexpected reward reduction and reward omission on choice preference in male and female rats. (A) During the first session of reward reduction when the reward resulting from a correct operant response on one lever was decreased by 50%, subjects displayed no preference for the lever yielding twice as much reward. By the second and third sessions of reward reduction, rats exhibited a preference for the lever yielding greater reward during free-choice trials. (B) When the reward resulting from a correct operant response on one lever was unexpectedly decreased by 100%, a robust preference for the rewarded lever was observed that continued during all three sessions of reward omission. (C) Preference for the better option during free-choice trials was significantly stronger for subjects experiencing reward omission than for those experience reward reduction. (D) Percentage of time spent in the quadrants containing the levers did not differ during baseline sessions or the first session of reward reduction; however, during the first session of reward omission, rats spent significantly more time in the quadrant containing the rewarded lever than the quadrant containing the non-reinforced lever. When the levers were once again equally reinforced, subjects that had experienced reward reduction (E) lost the behavioral preference by the third session, whereas subjects that had experienced reward omission (F) maintained a preference for the lever that continually had been reinforced. n.s., not statistically significant, $^{**}P < 0.01$, $^{***}P < 0.001$. Error bars indicate mean + SEM.

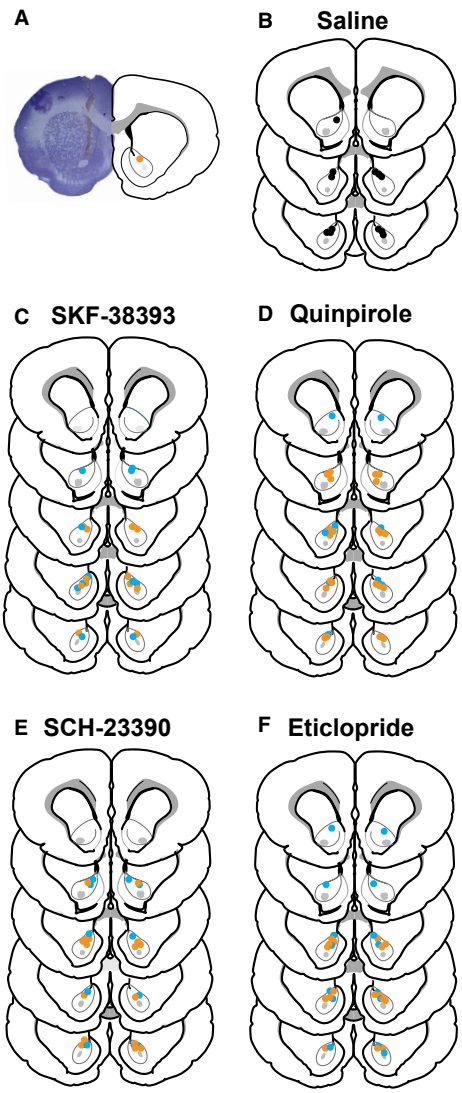

FIG. 3. Histological verification of injectors targeting the NAc core. (A) Representative image of injector placement and corresponding cartoon image. (B–F) Placements of injector tips in the NAc core where drug was infused prior to the first session of reward omission. Black circles represent control saline infusions (B). Color circles indicate where the D1-like agonist (C), D2-like agonist (D), D1-like antagonist (E), and D2-like antagonist (F) were infused into the NAc core. Orange circles indicate 1.0 μg of drug, and cyan circles represent 0.1 μg of drug.

the other during free-choice trials ($P = 0.545$). By the second ($P < 0.001$) and third ($P < 0.001$) sessions of reward reduction, subjects showed a significant preference for the lever yielding twice as much reward. These data demonstrate that the rats learned this contingency switch; however, a 50% reduction in reward was not a sufficiently salient reduction to prompt an immediate alteration in behavior.

In contrast to reward reduction, when the reward following a correct operant response on one lever was unexpectedly omitted (i.e. reduced to no pellets), subjects displayed a robust behavioral preference for the rewarded lever during the very first session (Fig. 2B; main effect of reinforcement, $F_{1,54} = 949.129$, $P < 0.001$; interaction of reinforcement by session, $F_{2,54} = 37.372$, $P < 0.001$; session 1, $P < 0.001$). A strong preference for the rewarded lever continued during free-choice trials of the second ($P < 0.001$) and third ($P < 0.001$) sessions of reward omission. In fact, the preference for the rewarded lever was even stronger during the second and third sessions of reward omission ($F_{1,12} = 51.280$, $P < 0.001$); subjects chose the omitted reward lever significantly fewer times during the second ($P < 0.001$) and third ($P < 0.001$) sessions compared with the first session. Choice preference did not significantly increase between the second and third sessions of reward omission ($P = 0.227$) possibly due to a ceiling effect; subjects were almost exclusively choosing the rewarded lever during free-choice trials already by the second session (Fig. 2B).

Although both reward reduction and omission spurred a preference for the more valuable option, reward omission prompted a more rapid, robust preference for the rewarded option during the very first session, whereas the preference for the lever yielding greater reward during reward reduction was more modest, developing over sessions (Fig. 2C; main effect, $F_{1,33} = 56.451$, $P < 0.001$). Indeed, reward omission subjects showed a significantly stronger preference for the better option lever during free-choice trials than reward reduction subjects during all three contingency switch sessions (Fig. 2C; session 1, $P < 0.001$; session 2, $P < 0.001$; session 2, $P < 0.001$). Reward omission was the only contingency switch that produced robust changes in behavioral preference on the first day.

As sex differences exist in a variety of rodent behavioral tasks (Van Haaren *et al.*, 1990; Jonasson, 2005; Becker & Taylor, 2008; Dalla & Shors, 2009; Sutcliffe, 2011), and should be examined in new behavioral models (Becker *et al.*, 2005; Beery & Zucker, 2011), we included both sexes to determine if male and female rats respond differently to reduction and omission of an expected reward. Male and female rats did not differ in baseline performance of the task (Fig. S1), and both male and female subjects displayed a preference for the lever yielding greater reward during the second and third, but not the first, sessions of reward reduction and a preference for the rewarded lever during all three sessions of reward omission (Fig. S2). No statistically significant differences were found in behavioral performance of this task between male and female rats. Furthermore, the lack of sex difference in development of choice preference was not due to estrous cycle effects. Using vaginal lavage (Becker *et al.*, 2005), we monitored the estrous cycle and tested females during each estrous cycle state. No significant differences in choice preference between males and females across the estrous cycle stages were found (Fig. S3).

Reinforcement learning theory (Glimcher, 2011) would predict that, through experiencing reward omission, subjects would learn to associate the cue light above the non-reinforced lever with receiving zero reward and learn that the cue light over the other lever predicts reward availability. Whereas reinforcement learning importantly focuses on learning and the predictability of outcomes, frustration theory addresses the emotional component of reward omission, stating that the omission of an anticipated reward is aversive and 'frustrating' (Amsel, 1958). As the utilised reward omission paradigm prompts a rapid and robust preference for the rewarded lever, we hypothesised that the cues for the reward omission lever would develop aversive qualities. Specifically, we predicted that subjects would avoid the quadrant of the behavioral chamber containing the reward omission lever and reduce responding during forced-choice trials on the reward omission lever. As reward reduction did not cause a choice preference during the first session, we did not expect that the reduced reward lever would become aversive as determined by avoidance, and that responding on forced-choice trials for the smaller reward would be reduced during the first session.

Behavioral videos were analysed to determine where rats were spending time throughout the sessions. During the inter-trial inter-

vals when levers were unavailable (which, in total, accounted for over 50% of the length of the session), subjects could freely explore the chamber, and, as levers did not become available until 5 s after the cue light was illuminated, subjects had time to approach the lever from any place in the chamber.

Video analysis revealed that, during baseline sessions when both levers were equally reinforced, subjects did not spend more time in the quadrant in front of one lever than the other (Fig. 2D; $t_{16} = -0.326$, $P = 0.748$). Similarly, subjects experiencing reward reduction did not display a significant preference for the quadrant containing the lever yielding optimal reward during the first session of reward reduction (Fig. 2D; $t_{14} = 1.007$, $P = 0.331$). Supporting our hypothesis, subjects experiencing reward omission spent significantly less time in the quadrant of the chamber containing the extinguished lever and significantly more time in the quadrant containing the reinforced lever (Fig. 2D; $t_{14} = 7.084$, $P < 0.001$). These results demonstrate the salience of reward omission on goal-directed behavior and support the theory that cues signaling reward omission acquire aversive properties and are therefore avoided.

Furthermore, performance on forced-choice trials during reward reduction did not significantly differ during the first session (Table 1). During sessions two and three, performance on forced-choice trials for the smaller reward (the reward that has been reduced by 50%) modestly, but significantly, decreased (Table 1). Conversely, subjects experiencing reward omission showed a robust decrease in performance on omitted forced-choice trials, whereas performance on rewarded forced-choice trials remained very high over all three sessions (Table 1). Together with the quadrant analyses and free-choice results, these data demonstrate that reward omission causes greater avoidance of the suboptimal choice than reward reduction and are consistent with frustration theory showing that the omission of an expected reward is a salient and aversive event.

After three sessions of reward reduction or omission, responding on both levers was once again equally reinforced for six sessions. Although a response on either lever yielded the same reward, subjects did not initially choose both levers equally during free-choice trials (main effect for performance on free-choice trials: subjects that had experienced reward reduction: Fig. 2E; $F_{1,5} = 19.298$, $P < 0.001$; subjects that had experienced reward omission: Fig. 2F; $F_{1,5} = 85.810$, $P < 0.001$). *Post-hoc* analyses revealed that subjects that previously underwent reward reduction displayed a significant preference during the first two post-switch sessions for the lever that had previously signaled the larger reward (Fig. 2E; session 1, $P = 0.003$; session 2, $P = 0.006$), but did not display a significant preference for one lever over the other during the remainder of the post-switch sessions (session 3, $P = 0.097$; session 4, $P = 0.373$; session 5, $P = 0.207$; session 6, $P = 0.670$).

In contrast, subjects that had undergone reward omission maintained a significant preference for the lever that had consistently been rewarded and chose the lever that had previously resulted in an omitted reward less across all six post-switch sessions (Fig. 2F; session 1, $P < 0.001$; session 2, $P < 0.001$; session 3, $P = 0.003$; session 4, $P = 0.024$; session 5, $P = 0.001$; session 6, $P = 0.002$). The decreased responding on the previously non-rewarded lever was not attributable to a deficit in learning (i.e. not knowing that choosing this lever would result in reward), as performance on forced-choice trials for both levers was nearly perfect by the second session (Table 2; main effect, $F_{1,5} = 9.416$, $P = 0.005$). Subjects that had undergone the reward reduction contingency switch performed equivalently on forced-choice trials for both levers (Table 2; main effect, $F_{1,5} = 0.889$, $P = 0.355$). These data are consistent with frustration theory (Amsel, 1958) and support the hypothesis that, during reward omission, the cues predicting an omitted reward are tagged with aversive motivational properties (Liu *et al.*, 2008).

TABLE 1. Percentage of forced-choice trials accurately completed

	Reward reduction Two pellets	One pellet	Reward omission One pellet	No pellets
Session 1	96.47 ± 1.32 $F_{1,15} = 0.901$, $P = 0.358$	94.29 ± 1.71	97.72 ± 0.88 $F_{1,17} = 24.849$, $P < 0.001$	71.57 ± 4.58
Session 2	99.216 ± 0.45 $F_{1,15} = 8.555$, $P = 0.010$	93.33 ± 1.43	98.42 ± 0.78 $F_{1,17} = 28.085$, $P < 0.001$	59.82 ± 6.16
Session 3	98.24 ± 0.91 $F_{1,15} = 22.594$, $P < 0.001$	85.69 ± 2.26	98.42 ± 0.74 $F_{1,17} = 208.197$, $P < 0.001$	27.19 ± 4.53

TABLE 2. Percentage of post-switch forced-choice trials accurately completed

	Post-reward reduction Always two pellets	Previously one pellet	Post-reward omission Always one pellet	Previously no pellets
Session 1	98.67 ± 0.82 $P = 0.569$	98.00 ± 1.33	99.44 ± 0.56 $P < 0.001$	77.78 ± 8.59
Session 2	99.33 ± 0.67 $P = 0.260$	99.33 ± 0.67	100.00 ± 0.00 $P = 0.635$	98.33 ± 0.75
Session 3	98.00 ± 1.33 $P = 0.569$	98.67 ± 0.82	100.00 ± 0.00 $P = 0.430$	97.22 ± 1.34
Session 4	98.00 ± 1.33 $P = 1.000$	98.00 ± 1.33	99.44 ± 0.56 $P = 0.874$	100.00 ± 0.00
Session 5	99.33 ± 0.67 $P = 0.569$	98.67 ± 1.33	99.44 ± 0.56 $P = 1.000$	99.44 ± 0.56
Session 6	99.33 ± 0.67 $P = 0.569$	98.67 ± 0.82	100.00 ± 0.00 $P = 0.874$	99.44 ± 0.56

During these trials, responses on both levers were once again equally rewarded.

Pharmacologically holding D2-like, but not D1-like, receptor tone in the nucleus accumbens core prevents a behavioral preference for the rewarded option during unexpected reward omission

Reward reduction and omission have been shown to alter the firing of putative DA neurons (Schultz, 1998; Waelti *et al.*, 2001; Matsumoto & Hikosaka, 2009), which affects DA transmission in the NAc core (Ikemoto, 2007). To test whether the effects of altering DA receptor tone are indeed differentially mediated in a receptor-specific manner within the NAc core, multiple doses of D1-like and D2-like receptor agonists and antagonist were microinfused into the NAc core (see Fig. 3A for a representative image and corresponding cartoon representation) 10 min prior to the first session of reward omission (reward omission was chosen as it robustly alters behavior during the first session, unlike reward reduction; Fig. 2). Specifically, subjects received bilateral microinfusions of saline (Fig. 3B), the D1-like agonist SKF-38393 (Fig. 3C), the D2-like agonist quinpirole (Fig. 3D), the D1-like antagonist SCH-23390 (Fig. 3E), or the D2-like antagonist eticlopride (Fig. 3F). Two doses of each drug were tested (see Materials and methods for justifications of the chosen doses).

If a decrease in occupancy of either D1- or D2-like receptors is necessary for establishing a preference for the better option (Dreyer *et al.*, 2010), then both the D1- and D2-like receptor agonists should block the choice preference for the more optimally rewarded option. However, if only D2-like receptors are necessary for suppressing responding to the omitted reward lever [as would be predicted by Frank *et al.* (2004), Bromberg-Martin *et al.* (2010), Hikida *et al.* (2010)], then the D2-like, but not D1-like, receptor agonist would be expected to prevent a choice preference for the rewarded lever. Multiple research groups have suggested that decreases in [DA] preferentially affect D2-like receptors, because D2-like receptors have a higher affinity for DA (Richfield *et al.*, 1989) and higher basal occupancy (Dreyer *et al.*, 2010) than D1-like receptors.

Indeed, drug treatment had a significant effect on preference behavior during free-choice trials (main effect of drug treatment, $F_{8,58} = 3.084$ $P = 0.006$; interaction of choice preference and drug dose received, $F_{8,56} = 2.665$, $P = 0.015$). Similar to behavior-only subjects (Fig. 2D), control rats that received infusions of saline into the NAc core exhibited a robust preference for the rewarded lever on free-choice trials (Fig. 4A; $P < 0.001$). Neither dose of the D1-like receptor agonist prevented subjects from exhibiting a significant preference for the rewarded lever (Fig. 4B; 0.1 μg SKF-38393, $P = 0.003$; 1.0 μg SKF-38393, $P < 0.001$). However, administration of the D2-like receptor agonist dose-dependently prevented a behavioral preference for the rewarded lever during the first session of reward omission, with the higher dose being the effective dose (Fig. 4C). Subjects receiving the lower dose of quinpirole developed a moderate preference for the rewarded lever ($P = 0.048$), but the higher dose of quinpirole attenuated the development of a choice preference during unexpected reward omission ($P = 0.213$). These data support the hypothesis that phasic decreases in DA transmission from unexpected reward omission (Schultz, 1998; Pan *et al.*, 2008) have functional consequences at high-affinity D2-like receptors (Richfield *et al.*, 1989) necessary for altering behavior (Frank, 2005).

In contrast to infusions of DA agonists, which can functionally hold DA tone stable at specific DA receptor subtypes, administration of DA antagonists was largely without effect in this behavioral paradigm. Similarly to controls, subjects receiving either dose of the D1-like receptor antagonist displayed a significant behavioral preference for the rewarded lever (Fig. 4D; 0.1 μg SCH-23390, $P = 0.002$; 1.0 μg SCH-23390, $P < 0.001$). Subjects that received either dose of the D2-like receptor antagonist also developed a preference for the rewarded lever (Fig. 4E; 0.1 μg eticlopride, $P = 0.016$; 1.0 μg eticlopride, $P = 0.041$).

In addition to examining the effects of D1-like and D2-like agonists and antagonists on the development of behavioral preferences for the rewarded lever, we also compared how frequently subjects receiving each drug chose the omitted-reward lever. Rats receiving either dose of the D2-like, but not D1-like, receptor agonist chose the lever yielding no reward significantly more times than controls during free-choice trials (Fig. 4F; main effect of drug treatment, $F_{8,58} = 2.782$, $P = 0.011$; Dunnett's *post-hoc* comparisons: 0.1 μg quinpirole, $P = 0.047$; 1.0 μg quinpirole, $P = 0.004$; 0.1 μg SKF-38393, $P = 0.311$; 1.0 μg SKF-38393, $P = 0.994$). To determine whether the quinpirole effect on choice preference persisted throughout the session, we divided the free-choice trials into two blocks and analysed the percentage of trials in which subjects receiving 1.0 μg quinpirole chose the non-reinforced lever compared with controls. Quinpirole subjects chose the non-rewarded lever significantly more times than controls during both blocks of trials (Fig. 4F, inset; block 1, $t_{16} = 2.879$, $P = 0.011$; block 2, $t_{16} = 2.620$, $P = 0.019$), demonstrating that quinpirole attenuated responding for the optimal choice throughout the session.

Although no significant changes were seen in subjects receiving the D1-like antagonist (0.1 μg SCH-23390, $P = 0.514$; 1.0 μg SCH-23390, $P = 0.926$), subjects receiving the higher ($P = 0.042$) but not lower ($P = 0.162$) dose of the D2-like antagonist chose the lever yielding no reward a modest but significant number of times more than controls. However, this was likely attributable to the significantly altered locomotor activity and/or motivation resulting from eticlopride (see locomotor results below). Indeed, subjects in the high-dose eticlopride group completed fewer rewarded forced-choice trials than controls (Fig. 4G; $P = 0.047$), whereas no statistical differences were seen among any of the other groups ($F_{8,58} = 2.848$, $P = 0.010$; 0.1 μg eticlopride, $P = 1.000$; 0.1 μg SCH-23390, $P = 0.988$; 1.0 μg SCH-23390, $P = 0.702$; 0.1 μg SKF-38393, $P = 1.000$; 1.0 μg SKF-38393, $P = 1.000$; 0.1 μg quinpirole, $P = 0.919$; 1.0 μg quinpirole, $P = 1.000$). This supports the interpretation that differences in performance of eticlopride rats (from D2-like receptor blockage within the NAc core) were at least partially attributable to other effects of the drug. It is important to emphasise that we utilised such high doses, despite their locomotor impairments, to demonstrate that such manipulations have no impact on altering behavioral choice in this paradigm.

Similarly to behavior-only controls (Table 1), saline controls (Fig. 4H, $P = 0.009$) and D1-like receptor agonist groups (Fig. 4I; 0.1 μg SKF-38393, $P = 0.001$; 1.0 μg SKF-38393, $P = 0.015$) completed significantly fewer forced-choice trials for the omitted reward lever than for the rewarded lever. The D2-like receptor agonist, however, dose-dependently altered performance on forced-choice trials (Fig. 4J). Subjects receiving the lower dose of quinpirole completed fewer forced-choice reward-omitted trials (Fig. 4J; $P = 0.019$), whereas the higher dose caused no statistically significant reduction in performance (Fig. 4J; $P = 0.217$) on forced-choice trials even though subjects were not receiving a reward on these trials. Similarly to controls, both D1-like (Fig. 4K, 0.1 μg SCH-23390, $P = 0.015$; 1.0 μg SCH-23390, $P < 0.001$) and D2-like (Fig. 4L; 0.1 μg eticlopride marginally significant, $P = 0.063$; 1.0 μg eticlopride, $P = 0.001$) receptor antagonist groups completed fewer forced-choice trials for the omitted reward lever than for the rewarded lever. These results further support the interpretation that

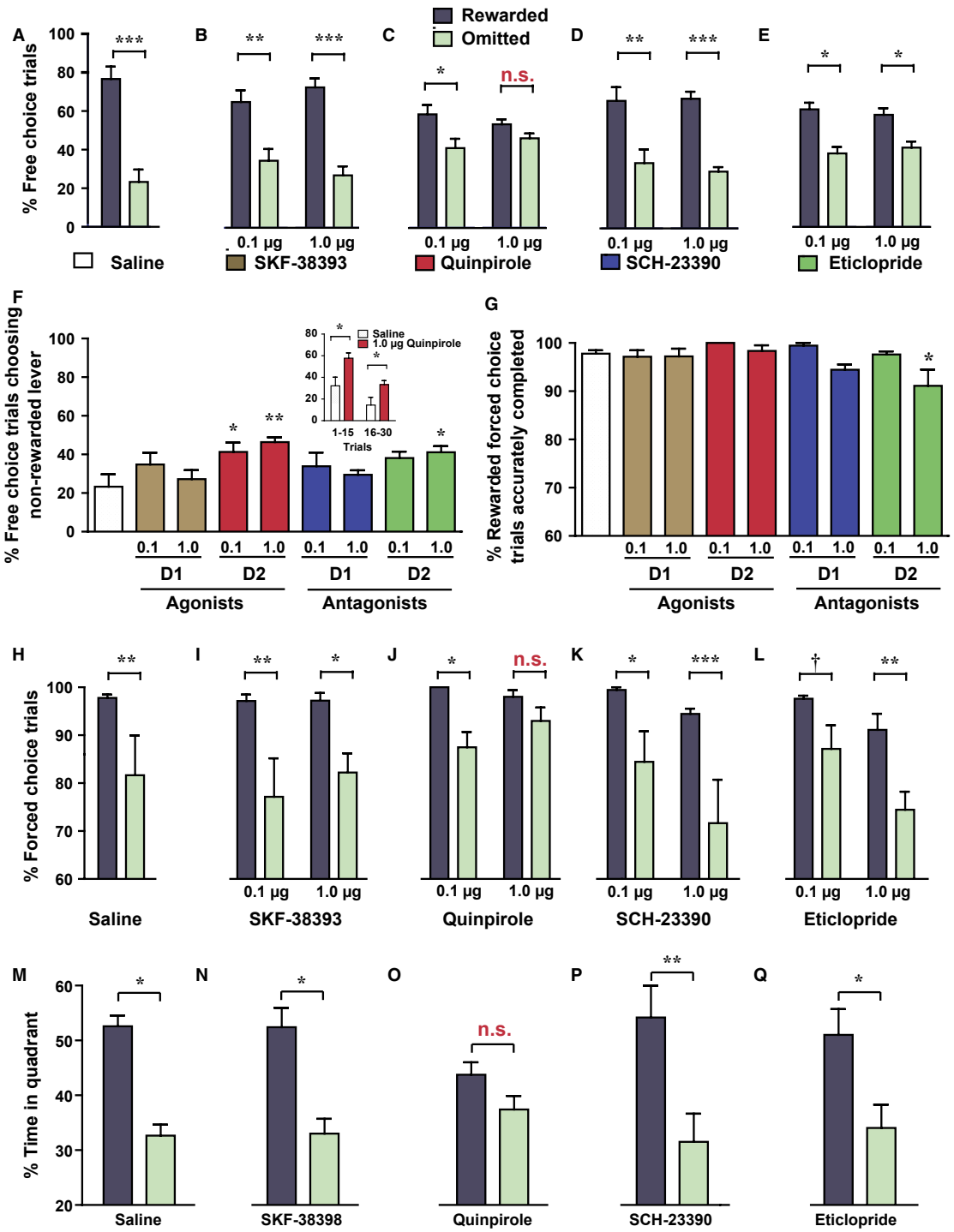

FIG. 4. Disrupting D2-like, but not D1-like, receptor tone in the NAc core prevents the rapid development of behavioral preference for the rewarded option during unexpected reward omission. Similarly to controls (A), subjects receiving either dose of the D1- agonist (B) or the D1- or D2-like antagonists (D and E, respectively) expressed a significant preference for the rewarded lever. However, the D2-like agonist dose-dependently blocked the normally robust behavioral preference for the rewarded lever (C). (F) Interfering with D2-like, but not D1-like, receptor tone resulted in rats choosing the non-reinforced lever more often than controls (white bar) during free-choice trials. Inset: disruption of D2-like receptor tone via quinpirole affects choice preference throughout the session. (G) Only the higher dose of eticlopride reduced the number of correctly completed forced-choice trials for the rewarded lever compared with controls. (H–L) During reward omission, subjects completed fewer forced-choice trials for the non-reinforced lever than for the rewarded lever. Much like controls (H), subjects administered with either dose of the D1-like receptor agonist (I) displayed reduced performance on non-rewarded forced-choice trials. The higher, but not lower, dose of the D2-like agonist prevented the relative decrease in performance on non-reinforced compared with rewarded forced-choice trials (J). Subjects receiving the D1-like (K) or D2-like (L) receptor antagonists completed fewer forced-choice trials for the non-rewarded lever, similarly to controls. (M–Q) During reward omission, rats avoided the quadrant of the chamber containing the omitted-reward lever, spending more time in the quadrant containing the rewarded lever. Similarly to controls (M), subjects receiving the D1 agonist (N) or either the D1-like (P) or D2-like (Q) antagonist spent less time in front of the non-reinforced lever (green bars). However, the D2-like agonist (O) prevented subjects from spending significantly more time in front of the rewarded lever (gray bars). n.s., not statistically significant, $^{\dagger}P = 0.06$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Error bars indicate mean + SEM.

preventing a reduction of D2-like, but not D1-like, receptor tone precludes the alteration in reward-seeking strategy in this task when a 'foraging patch' is depleted.

As reward omission can cause subjects to avoid the quadrant of the chamber containing the omitted-reward lever (Fig. 2D), videos of the sessions of subjects receiving the higher dose of each drug were analysed to determine the effects of DA receptor agonists and antagonists on which quadrants of the chamber rats were occupying throughout the reward omission sessions (see Fig. 1A). Similarly to behavior-only controls (Fig. 2D), saline controls spent significantly more time in the quadrant containing the rewarded lever than the quadrant containing the non-reinforced lever (Fig. 4M; $P = 0.011$). As expected, subjects receiving the D1-like agonist also spent significantly less time in the omitted-reward lever quadrant than the rewarded lever quadrant (Fig. 4N; $P = 0.013$). However, subjects receiving the D2-like agonist spent equivalent time in the quadrants containing the rewarded and omitted levers (Fig. 4O; $P = 0.333$). Also, similarly to controls, subjects infused with the D1-like (Fig. 4P; $P = 0.004$) or D2-like (Fig. 4Q; $P = 0.012$) antagonists spent significantly less time in the quadrant containing the non-reinforced lever compared with time spent in the quadrant containing the rewarded lever.

Together, these results strongly suggest that holding D2-like, but not D1-like, receptor tone stable prevents a choice preference for the reinforced option following a reward omission manipulation, and these data are consistent with previous work revealing that higher (1.0 or 10.0 μg) but not lower (0.1 μg) doses of quinpirole into the NAc core impair strategy set shifting and reversal learning, which are other important assays of behavioral flexibility (Haluk & Floresco, 2009). Although basal, steady-state levels of DA have been estimated to be in the low (6–20) nanomolar range (Kawagoe *et al.*, 1992; Sam & Justice, 1996; Shou *et al.*, 2006; Owesson-White *et al.*, 2012), the higher dose of quinpirole is likely better at maintaining D2-like receptor tone to prevent the putative phasic reduction in D2-like receptor occupancy during reward omission (Hong & Hikosaka, 2011). Our findings support the hypothesis that a phasic reduction in D2-like receptor signaling is necessary for guiding motivated behavior away from suboptimal choices (Frank, 2005; Bromberg-Martin *et al.*, 2010; Dreyer *et al.*, 2010).

It has been proposed that decreases in DA neuron firing from the omission of an expected reward could be signaling motivational disappointment rather than a negative prediction teaching signal (Berridge, 2012). To investigate whether the quinpirole-induced lack of a preference for the rewarded option during reward omission (Fig. 5A) was due to impairment in learning about the omitted reward, rats were tested drug-free in a second reward omission session the following day. If quinpirole caused a learning deficit, quinpirole subjects when tested drug-free would be expected to choose the suboptimal option more times than controls as they would have to learn about the contingency switch during the second session. Conversely, if quinpirole impaired the motivational disappointment of the omitted reward lever without impairing learning, quinpirole subjects when tested drug-free would be expected to immediately perform as well as controls during the second, drug-free session of reward omission, as learning would have occurred even though the expression of the behavioral preference was not observed during the first session (Fig. 5A).

During the second reward omission session when subjects did not receive any microinfusions, a robust behavioral preference was observed for the rewarded lever during free-choice trials (Fig. 5B, main effect, $F_{1,59} = 1032.841$, $P < 0.001$; Bonferroni *post-hoc* results of all drug conditions, $P < 0.001$). Regardless of drug treatment the previous day, subjects chose the omitted reward lever infrequently on free-choice trials (Fig. 5C); no significant differences in the percentage of free-choice trials choosing the omitted reward lever were observed compared with controls ($F_{8,60} = 1.146$, $P = 0.347$). Additionally, subjects did not differ in the number of times they chose the omitted reward option during the first five free-choice trials of the second session compared with controls (Fig. 5D; $F_{8,60} = 1.629$, $P = 0.136$), demonstrating that all subjects, including those that had received quinpirole, performed similarly during the beginning of the second session regardless of previous drug treatment. These results support the interpretation that the D2-like agonist attenuated the behavioral preference for the optimal choice during reward omission without impairing learning about the omitted reward.

Both the nucleus accumbens core and shell subregions facilitate the development of a behavioral preference during unexpected reward omission through reduction of dopamine tone at D2-like receptors

Subregions of the NAc, primarily the NAc core and shell, are anatomically and functionally distinct (Kelley, 1999; Zahm, 1999; Di Chiara, 2002; Aragona *et al.*, 2006, 2008; Ikemoto, 2007; Aragona, 2011). The NAc core and shell subregions can serve different roles in certain types of behavioral flexibility (Floresco *et al.*, 2006). As the NAc shell can facilitate memory of arousing experiences, such as a significant reduction in expected reward (Kerfoot *et al.*, 2008), and it recently has been shown that under certain experimental conditions aversive stimuli phasically decrease [DA] in the shell (McCutcheon *et al.*, 2012), we investigated whether the effect described above, demonstrating that the D2-like receptor agonist prevents the development of a behavioral preference for the rewarded option, is unique to the NAc core or whether it is also true in the NAc shell.

As 1.0 μg quinpirole (0.5 μL volume) in the NAc core blocks the development of a choice preference during reward omission (Fig. 4), we infused this effective dose, but at a smaller volume (0.3 μL), into the NAc core or medial shell prior to the first session of reward omission (Fig. 6A; core, $n = 7$, shell, $n = 6$). Neither the main effect ($F_{1,11} = 2.818$, $P = 0.121$) nor the interaction of choice behavior by subregion ($F_{1,11} = 0.152$, $P = 0.704$) was significant, demonstrating that D2-like agonism in both the NAc core and medial shell attenuates a choice preference for the rewarded option following unexpected reward omission (Fig. 6B). Indeed, whereas control subjects displayed a robust preference for the rewarded lever ($t_7 = 5.347$, $P = 0.001$), subjects receiving quinpirole into the core ($P = 0.363$) or shell ($P = 0.186$) did not significantly prefer the rewarded lever compared with the non-reinforced lever.

General locomotor effects of quinpirole are not responsible for the lack of choice preference following unexpected reward omission

To examine if the quinpirole-induced blockade of the development of the behavioral preference during reward omission was attributable to alterations in locomotor activity caused by the drug, separate groups of drug-naive rats received bilateral microinfusions of saline or the D1-like or D2-like receptor agonists or antagonists (1.0 μg/0.5 μL) into the NAc core. Each subject received only one drug to exclude the possibility of sensitisation effects (Henry *et al.*, 1998; Vezina, 2004). Locomotor behavior was monitored for 1 h – the identical length of time as the operant task. Two measures of locomotor activity were recorded: the total number of photobeam breaks

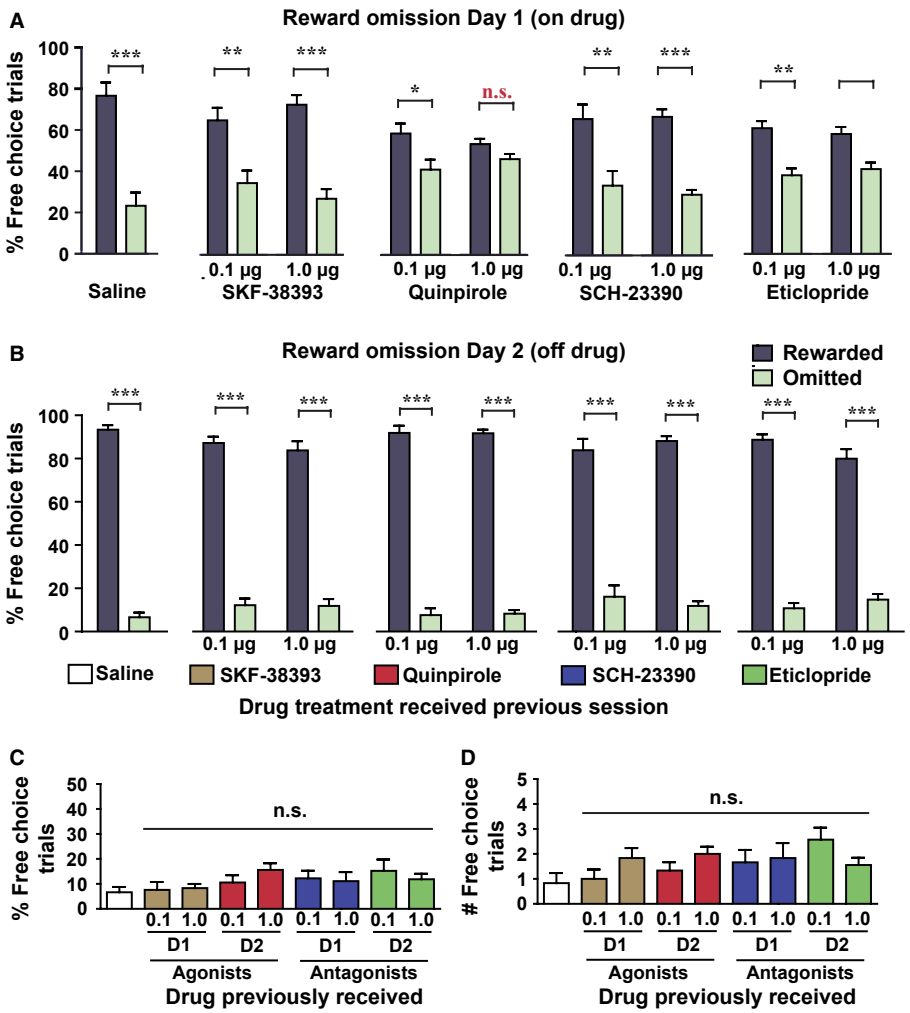

FIG. 5. Behavioral preferences for the optimal option during the second, drug-free session of reward omission reveal that quinpirole did not block learning about the omitted reward. (A) Choice preference from the first session is reshown for comparison. (B) During the second session of reward omission, rats (now drug-free) demonstrated a robust behavioral preference for the rewarded lever during free-choice trials regardless of the drug they received the previous day. Drug received during the previous session did not significantly affect the percentage of free-choice trials in which subjects chose the lever yielding no reward (C) or the number of times rats chose the omitted reward lever during the first five trials of the second switch day when subjects did not receive drug (D). n.s., not statistically significant compared with saline controls, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Error bars indicate mean + SEM.

and the number of 'crossovers' (see Materials and methods for details).

One-way ANOVAS revealed that drug treatment significantly affected locomotor activity assessed by the total number of beam breaks (Fig. 7A; $F_{4,42} = 7.88$, $P < 0.001$) as well as the total number of crossovers (Fig. 7B; $F_{4,42} = 6.866$, $P < 0.001$). Specifically, D1-like activation within the NAc core significantly increased (beam breaks, $P = 0.002$; crossovers, $P = 0.001$), whereas D1-like blockade (beam breaks, $P = 0.007$; crossovers, $P = 0.043$) and D2-like blockade (beam breaks, $P = 0.025$; crossovers, $P = 0.044$) significantly decreased the number of photobeam breaks and crossovers compared with controls (Fig. 7A and B). D2-like activation (via quinpirole), however, did not significantly alter locomotor activity (beam breaks, $P = 0.715$; crossovers, $P = 0.687$).

Both the number of beam breaks and crossovers reveal similar effects of the drugs on locomotor activity. Indeed, there was a significant and robust correlation between these two measures (Fig. 7C; $r_{45} = 0.971$, $P < 0.001$). The similarity between beam break and crossover data within each drug treatment demonstrates that these variables are highly reliable measures of general locomotor activity and are sensitive to alterations from dopaminergic drugs.

These locomotor data demonstrate that all doses of the dopaminergic agents used were sufficient to modulate behavior in a predicted way. Consistent with the literature, both D1-like (McGregor & Roberts, 1993; Baldo *et al.*, 2002; Haluk & Floresco, 2009) and D2-like (Boye *et al.*, 2001; Haluk & Floresco, 2009) antagonists significantly decreased locomotor activity. Conversely, the D1-like agonist significantly increased locomotor activity, consistent with previous work (Dreher & Jackson, 1989; Phillips *et al.*, 1995; David *et al.*, 2004). Quinpirole, as expected (Haluk & Floresco, 2009; Stopper *et al.*, 2013), did not significantly alter locomotion. Although quinpirole did not affect general locomotion here, it is noteworthy that its effects appear to be more variable; some studies have detected that quinpirole site-specifically infused into the NAc modestly increased (Dreher & Jackson, 1989; Phillips *et al.*, 1995) or decreased (David *et al.*, 2004) locomotor activity.

As quinpirole did not significantly alter locomotor activity, the quinpirole-induced attenuation of the behavioral preference during

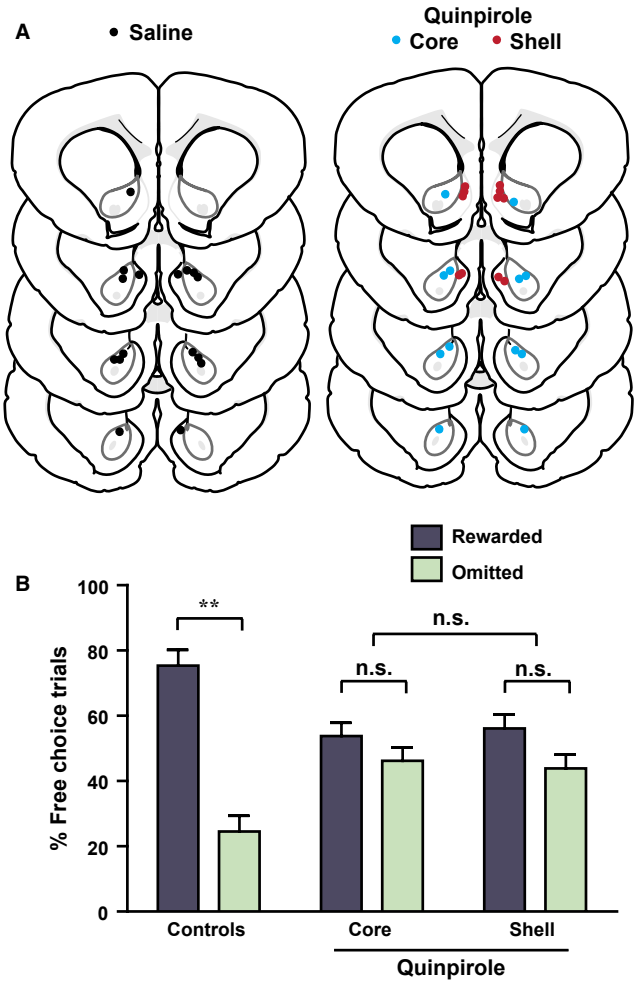

FIG. 6. The D2-like receptor agonist administered into the NAc core or shell prevented a behavioral choice preference during reward omission. (A) Injector placements in the NAc. Black circles represent saline controls. Red circles indicate shell and blue circles indicate core placements. (B) Quinpirole (1 μg) bilaterally infused into the NAc core or shell attenuated the development of a preference for the optimal choice. n.s., not statistically significant. $^{**}P < 0.01$. Error bars indicate mean + SEM.

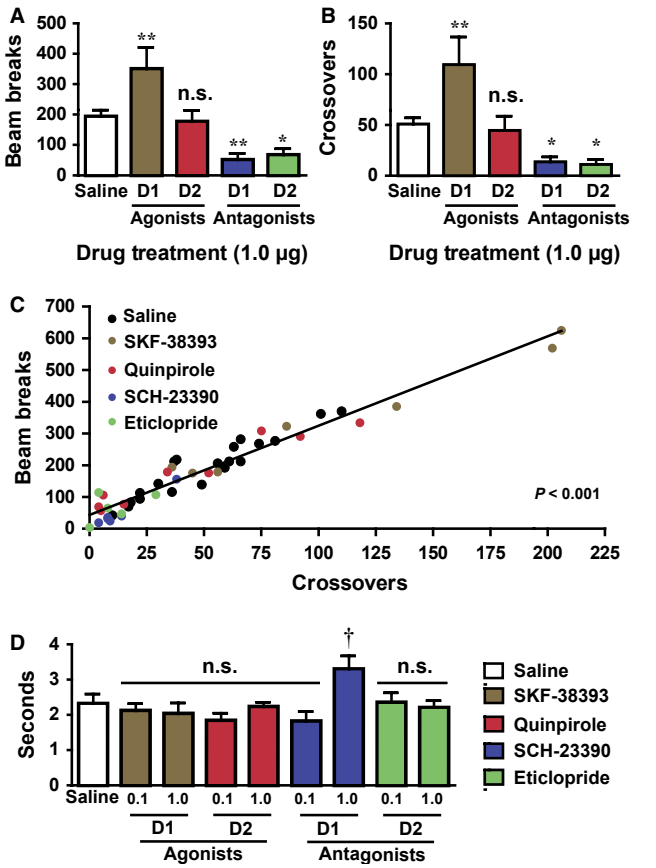

FIG. 7. Locomotor effects of the utilised D1- and D2-like receptor agonists and antagonists. The D1-like receptor agonist increased, whereas the D1- and D2-like receptor antagonists decreased locomotor activity compared with controls in separate, drug-naive groups of rats as measured by total beam breaks (A) and crossovers (B) in a 1 h session. The D2-like agonist did not affect locomotor behavior. (C) The two measures of locomotor activity, beam breaks and crossovers, were highly correlated. (D) Response latencies on free-choice trials during reward omission reveal that only the higher dose of the D1-like antagonist affected performance on the task compared with controls (white bar). n.s., not statistically significant. $^{\dagger}P = 0.06$, $^{*}P < 0.05$, $^{**}P < 0.01$. Error bars indicate mean + SEM.

reward omission cannot be attributed to drug effects on locomotor activity. Importantly, the lack of effects from all other dopaminergic drugs tested in the behavioral choice paradigm was not due to insufficient doses, because the doses utilised were sufficient to alter general locomotor behavior in expected ways.

Although the dopaminergic drugs were given at behaviorally relevant doses (Fig. 7A and B), the response latencies of subjects during free-choice trials in the reward omission experiment revealed that the locomotor effects of these drugs did not affect the ability of subjects to perform the operant response (Fig. 7D). Only the high dose (1.0 μg) of SCH-23390 revealed a trend for increasing response latency ($P = 0.062$). The response latencies of subjects receiving the other drugs and doses did not significantly differ from controls (one-way ANOVA: $F_{8,59} = 3.000$, $P = 0.007$; 0.1 μg SKF-38393, $P = 0.995$; 1.0 μg SKF-38393, $P = 0.970$; 0.1 μg quinpirole, $P = 0.641$; 1.0 μg quinpirole, $P = 0.994$; 0.1 μg SCH-23390, $P = 0.668$; 0.1 μg eticlopride, $P = 1.000$; 1.0 μg eticlopride, $P = 1.000$). Together, these data demonstrate that the dopaminergic manipulations on choice behavior were not attributable to general locomotor effects of the drugs.

Discussion

In the present study we first demonstrated that rats developed a more rapid and robust behavioral preference in a choice assay when reward was omitted compared with an equivalent reduction in reward. We next examined the neurobiological regulation of this behavior in response to reward omission and focused on DA receptor regulation within the NAc core. The only pharmacological manipulation that prevented the development of the first session preference during reward omission was a dose-dependent infusion of the D2-like agonist quinpirole. This finding is consistent with modeling studies, which have predicted that phasic reductions in [DA] reduce DA tone in a functionally significant manner to D2-like, but not D1-like, receptors (Frank, 2005; Dreyer *et al.*, 2010; Hong & Hikosaka, 2011). Thus, we provide evidence that blocking the reduction in D2-like, but not D1-like, tone in the NAc attenuates behavioral alterations in response to reward omission. This finding has many important implications for the nature of DA regulation of adaptive motivated behavior and specifically extends our knowledge that decreased [DA] at D2-like receptors mediates avoiding cues tagged with aversive motivational properties.

Psychological mechanisms underlying the development of behavioral preferences mediated by aversive motivation

It is well established that the absence of an expected reward is a salient event prompting behavioral reactions often described as emotional (Tinklepaugh, 1928; Miller & Stevenson, 1936; Crespi, 1942; Skinner, 1953; Salinas *et al.*, 1997; Salinas & Gold, 2005; Sastre & Reilly, 2006; Young & Williams, 2010; Purgert *et al.*, 2012; Ramot & Akirav, 2012; Veeneman *et al.*, 2012). Based on the many observations that the omission of an anticipated reward is an aversive event that has been described as 'frustrating,' frustration theory emerged (Amsel, 1958; Daly, 1974). In support of this theory, a series of studies have shown that rats will lever press or jump hurdles to escape stimuli that were previously associated with reward but are now associated with the omission of reward (Adelman & Maatsch, 1956; Daly, 1969a,b,c, 1974). Consistent with these studies, our results demonstrate that rats quickly recognise the omission of an expected reward and rapidly develop a choice preference for the optimal choice avoiding the extinguished lever and the quadrant of the behavioral chamber containing that lever.

A 50% reduction of reward (an equivalent decrease in the number of pellets as the omission condition, i.e. one less reward pellet) eventually elicited a similar behavioral preference but not as quickly or robustly as reward omission. Consistent with previous studies (Salinas *et al.*, 1993; Salinas & White, 1998; Sastre & Reilly, 2006; Ramot & Akirav, 2012), reward reduction evoked a significant preference for the more valuable option by the second session (24 h later). Multiple studies that have reduced reward value by 90% have observed behavioral effects during the first session including increased latency to retrieve reward in a maze (Salinas *et al.*, 1996, 1997; Kerfoot *et al.*, 2008) and consuming less of the reward (Salinas & Gold, 2005). Together with our results, these findings indicate that reward reduction can have significant, immediate effects on behavior, but the reduction must be highly salient to the animal, often at levels close to omission.

The lack of sex differences in this paradigm is not surprising as flexibility in foraging strategy is adaptive in both males and females. However, although no sex differences in this behavioral paradigm exist in adult virgin rats, females caring for offspring may develop a preference for the optimal choice more rapidly than males and nulliparous females, as maternal females must forage for their pups in addition to themselves (Kinsley *et al.*, 1999; Love *et al.*, 2005).

Reduction of D2-like receptor tone in the nucleus accumbens mediates behavioral preferences for optimal choices

When an expected reward is omitted, conventional DA neurons [known to project to the NAc core (Ikemoto, 2007; Lammel *et al.*, 2008, 2011; Liss & Roeper, 2008)] phasically decrease their firing rate (Schultz *et al.*, 1997; Schultz, 1998; Pan *et al.*, 2005, 2008; Roesch *et al.*, 2007). This is associated with a decrease in [DA] in the forebrain terminal regions to which they project (Ikemoto, 2007; Dreyer *et al.*, 2010), such as the NAc core (Day *et al.*, 2007). Decreases in striatal [DA] are hypothesised to have greater functional consequences to D2-like receptors because these receptors have greater affinity for DA (Richfield *et al.*, 1989; Marcellino *et al.*, 2012) and therefore a higher baseline occupancy compared with low-affinity D1-like receptors (Frank, 2005; Bromberg-Martin *et al.*, 2010; Hong & Hikosaka, 2011).

A reduction in D2 receptor tone has been hypothesised to promote action suppression and No–Go learning (Frank, 2005; Bromberg-Martin *et al.*, 2010). D2-expressing neurons are predominantly in the indirect pathway (Gerfen & Surmeier, 2011) and have been shown to mediate aversive learning (Hikida *et al.*, 2010). Recently, it has been shown that freezing behavior to an aversive stimulus is strongly associated with phasic decreases in [DA] within the NAc core (Badrinarayan *et al.*, 2012; Oleson *et al.*, 2012), and optogenetic depolarisation of neurons expressing D2 receptors within the dorsomedial striatum [a region that shows similar DA transmission dynamics as the NAc core (Brown *et al.*, 2011)] causes mice to instantaneously freeze (Kravitz *et al.*, 2010). Moreover, mice will avoid a trigger activating neurons that express D2 receptors within this region (Kravitz *et al.*, 2012). These recent studies suggest that phasic reductions in [DA] may activate D2-expressing neurons in the NAc core and may, at least in part, mediate aversive motivated behavior.

Here, using site-specific microinfusions of D1- and D2-like receptor agonists and antagonists, we found that only the D2-like agonist quinpirole dose-dependently prevented the rapid expression of a behavioral preference for the rewarded option. Importantly, the locomotor effects of quinpirole are not responsible for the lack of a behavioral preference. Administration of the D1-like agonist or D1- or D2-like antagonists did not impair the development of a preference for the rewarded lever, which is consistent with previous work demonstrating that DA blockade does not affect the ability of rats to choose a larger reward (Salamone *et al.*, 1994). These findings further support the hypothesis that guiding motivated behavior away from aversive cues is mediated through a phasic reduction in the occupancy of D2-like receptors.

Parkinson's disease, which occurs when DA neurons degenerate, can cause cognitive impairments in addition to the well-known motor impairments (Rana *et al.*, 2013). Our finding that D2-like agonists attenuate the avoidance of a suboptimal choice is consistent with and has implications for research examining reinforcement learning in Parkinson's patients. Specifically, when off their medications in a DA-depleted state, patients with Parkinson's disease have been observed to be better at learning to avoid negative outcomes than positive outcomes; however, when on their medications, primarily D2-like agonists, they did not learn as well from negative feedback (Frank *et al.*, 2004; Cools *et al.*, 2006, 2007; Voon *et al.*, 2010). Importantly, functional magnetic resonance imaging studies reveal that DA agonists that disrupt learning from negative feedback in patients with Parkinson's disease correspond to smaller decreases in ventral striatal activity in response to losses (Voon *et al.*, 2010). In combination with our results, these data strongly support the idea that performance in avoiding suboptimal choices is attenuated by D2-like agonists filling in the phasic dips in DA from reward omission (Frank *et al.*, 2004).

In addition to binding to post-synaptic D2-like receptors, quinpirole also binds to pre-synaptic D2 autoreceptors. Binding of D2-like agonists to autoreceptors can decrease basal levels of DA (Kalivas & Duffy, 1991; Pierce *et al.*, 1995; Koeltzow *et al.*, 2003) and decrease stimulated phasic DA release in the dorsal striatum (Joseph *et al.*, 2002; O'Connor & Lowry, 2012) as well as in the NAc core and shell (Maina & Mathews, 2010). In the present study, even though basal levels of DA may be altered due to quinpirole's effects at autoreceptors, D2-like tone at post-synaptic receptors would be maintained. Indeed, the effective dose of quinpirole in attenuating the preference for the rewarded option is in the range of doses that are presumed to bind to post-synaptic D2-like receptors (Swanson *et al.*, 1997; Boschen *et al.*, 2011). Therefore, regardless of the amount of DA being released, the post-synaptic D2-like receptors would be expected not to functionally experience the reduced binding from decreased levels of DA as the quinpirole would be bound

to these receptors, and the results of this study support this interpretation.

Indeed, basal levels of DA are sufficient to inhibit D2-expressing indirect pathway neurons (Surmeier *et al.*, 2011), and quinpirole inhibits such neurons (Hooper *et al.*, 1997). Together, in combination with the quinpirole attenuation of behavioral response to reward omission, these data are consistent with the hypothesis that decreases in DA, which would preferentially affect high-affinity D2-like receptors (Kreitzer & Berke, 2011), are necessary for altering behavior away from a suboptimal choice.

Conclusion

The present experiments demonstrate that the omission of an expected reward is a salient, aversive event prompting a robust preference for the rewarded option. The expression of this behavioral choice preference is dose-dependently attenuated by a D2-like, but not D1-like, agonist in the NAc. These results support the hypothesis that phasic reductions in [DA], as would occur during the omission of an expected reward, preferentially affect D2-like receptors. Specifically, decreased occupancy of D2-like receptors in the NAc facilitates motivated behavior that drives animals away from a non-rewarded option.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Male and female rats performed the task equally well ***A,*** Neither male nor female rats exhibited a significant preference for one lever or the other. ***B,*** Both males and females completed forced choice trials with near perfect accuracy.

Fig. S2. Male and female rats exhibited the same choice preferences during the two negative contingency switches.

Fig. S3. Estrous cycle did not affect the development of choice preference.

Acknowledgements

This work was supported by PO1DA03165601 to B.J.A. and DA007267 and a Rackham Research Grant to K.A.P.-S. The authors would like to acknowledge Dr Jill Becker for a critical reading of the manuscript and insight into sex difference research; Dr Jennifer Cummings for teaching us to monitor the estrous cycle; Drs Terry Robinson and Ben Saunders for the use of locomotor chambers; Shanna Resendez, Nathan Nartker, Ben Blackmer, and Tom Geesey for technical assistance; and the many undergraduates of the B.J.A. laboratory who helped run rats and score videos.

Abbreviations

DA, dopamine; [DA], dopamine concentration; D1-like, D1-like dopamine receptor; D2-like, D2-like dopamine receptor; FR, fixed ratio schedule or reinforcement; NAc, nucleus accumbens.

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