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Nucleus Accumbens Dopamine Modulates Response Rate but not Response Timing in an Interval Timing Task

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Abstract

While previous work has demonstrated that systemic dopamine manipulations can modulate temporal perception by altering the speed of internal clock processes, the neural site of this modulation remains unclear. Based on recent research suggesting that changes in incentive salience can alter the perception of time, as well as work showing that nucleus accumbens (NAc) shell dopamine (DA) levels modulate the incentive salience of discriminative stimuli that predict instrumental outcomes, we assessed whether microinjections of DA agents into the NAc shell would impact temporal perception. Rats were trained on either a 10-second or a 30-second temporal production procedure and received intra-NAc shell microinfusions of sulpiride, amphetamine and saline. Results showed that NAc DA modulations had no effect on response timing, but intra-NAc shell sulpiride microinfusions significantly decreased response rates, relative to saline and amphetamine. Our findings therefore suggest that neither NAc shell DA levels, nor the resultant changes in incentive salience signaled by this structure, impact temporal control.

Keywords

Interval Timing; Nucleus Accumbens; Peak-Interval Procedure; Rats

Interval timing (i.e., timing in the seconds to minutes range) is a flexible and adaptive process that allows organisms to initiate time-keeping processes at arbitrary moments, time durations of different lengths, and keep records for the durations between critical events (Gibbon & Church, 1990). These time-keeping processes allow for efficient behavior in response to the temporal regularities of the environment, and it has been proposed that timing may underlie both foraging behavior (Brunner, Kacelnik, & Gibbon, 1992) and associative learning (Gallistel & Gibbon, 2000). Several conceptual models of interval timing have been developed that account for a substantial degree of temporally controlled behavior (e.g., Scalar Expectancy Theory (SET), (Gibbon, 1977); A Behavioral Theory of Timing (BET), (Killeen & Fetterman, 1988), and despite important differences in these models, it has been argued that each model is understandable within an information-processing framework composed of clock, memory, and decision stages (Church, 1997).

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In SET, the clock stage is composed of a pacemaker that releases pulses, which are integrated by an accumulator, such that the clock values grow in linear relation to the passage of time. Different durations are encoded by different clock values (Gibbon, Church, & Meck, 1984), and a decision stage determines the relative similarity between current clock values and previously reinforced clock values stored as temporal memories. In contrast, in BET, the “clock values” are the animal's own behavioral states, and pacemaker pulses generate transitions between these states (Bizo & White, 1994; Killeen & Fetterman, 1988). As such, different durations are timed by changes in the speed of the pacemaker. Specifically, pacemaker speed is proposed to be proportional to reinforcement density, which is inversely proportional to the duration being timed. In other words, shorter durations are timed using a faster pacemaker speed, and longer durations are timed using a slower pacemaker speed. Thus, pacemaker speed can be thought of as the “temporal memory”.

Research on the neural basis of timing has suggested that dopamine (DA) modulates the speed of internal clock processes (Cheng, MacDonald, & Meck, 2006; Gooch, Wiener, Portugal, & Matell, 2007; Macdonald & Meck, 2005; Maricq, Roberts, & Church, 1981; Matell, Bateson, & Meck, 2006; Matell, King, & Meck, 2004; Meck, 1983, 1996). Specifically, increased brain DA levels resulting from the administration of systemic DA agonists (antagonists) have been demonstrated to shift response times to the left (right), in a manner consistent with an increase (decrease) in clock speed. Importantly, the degree to which responding is altered is proportional to the duration being timed, thereby suggesting that DA modulates clock speed, rather than impacting impulsivity or the latency to start timing.

Though the effect of systemic DA injections on clock speed has been replicated multiple times, the neural locus for the effect remains elusive. Despite this, several recently developed models of timing have been formulated with respect to the computational mechanisms used by the brain areas thought to subsume timing (Almeida & Ledberg, 2010; Buonomano & Merzenich, 1995). One such biologically inspired timing model is the Striatal Beat Frequency (SBF) model (Matell & Meck, 2000, 2004), in which temporal control is instantiated by cortico-striatal-thalamic circuitry. In SBF, elapsed time is encoded by the coincident activity of cortical neurons, as detected by striatal neurons and relayed through the basal ganglia output structures through the thalamus to the cortex for behavioral expression. The clock speed effects of DA were proposed to occur either within the cortex or the striatum. Consistent with SBF, dorsal striatal involvement in timing has been supported by a number of studies (Chiba, Oshio, & Inase, 2008; Coull, 2004; Drew, et al., 2007; Harrington, et al., 2004; Hinton & Meck, 2004; Matell, Meck, & Nicolelis, 2003; Meck, 2006; Nenadic, et al., 2003; Rao, Mayer, & Harrington, 2001; Tregellas, Davalos, & Rojas, 2006; Wiener, Turkeltaub, & Coslett, 2010).

The nucleus accumbens (NAc) is the ventral extension of the striatum, and has similar neurophysiological characteristics as the dorsal striatum (Izenwasser, Werling, & Cox, 1990; Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Like its dorsal counterpart, the NAc is densely innervated by DA from the ventral tegmental area (VTA), and to a lesser degree, the substantia nigra pars compacta (Nauta, Smith, Faull, & Domesick, 1978; Nicola, Surmeier, & Malenka, 2000; O'Donnell & Grace, 1995; Wise & Rompre, 1989). Based on experimental findings showing that NAc DA release occurs in response to both rewarding as well as aversive stimuli such as foot shock and tail pinch (McCullough, Sokolowski, & Salamone, 1993; Thierry, Tassin, Blanc, & Glowinski, 1976), recent hypotheses have suggested that the NAc functions as an “incentive-property constructor” in both appetitive and aversive contexts by forming incentive representations (Berridge & Robinson, 1998; Horvitz, 2000; Ikemoto & Panksepp, 1999; Schoenbaum & Setlow, 2003; Wise, 2004). However, this broad characterization of NAc function may be an

oversimplification, as sufficient evidence suggests that the NAc core and shell subregions play different roles in reward-based learning (Deutch & Cameron, 1992; Maldonado-Irizarry, Swanson, & Kelley, 1995; Pothuizen, Jongen-Relo, Feldon, & Yee, 2005).

Research that seeks to understand the functional differences in the accumbens subregions is often mixed with regard to the separate roles played by core and shell. However, several studies suggest that the NAc shell responds to discriminative stimuli for reward (Bassareo & Di Chiara, 1997; Corbit, Muir, & Balleine, 2001; Johnson, Goodman, Condon, & Stellar, 1995), whereas the NAc core responds to the reward value of the consequences that follow behavior (Corbit, et al., 2001; Sokolowski & Salamone, 1998). Though some studies report opposing results (Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999), a greater portion of the literature seems to agree that the NAc core is involved in selecting behaviors based on instrumental outcomes, whereas the NAc shell is involved in selecting behaviors based on the incentive value of the discriminative stimuli that predict these outcomes. Because animals in the present experiment were trained on the peak-interval (PI) procedure, in which responses are made in anticipation of reward, all experimental manipulations were carried out in the NAc shell.

As described previously, the pacemaker speed of BET is thought to be directly tied to reinforcement density, and factors contributing to reinforcement density are strongly implicated in the determination of incentive value (Green & Myerson, 2004; Ho, Mobini, Chiang, Bradshaw, & Szabadi, 1999; Mazur, 2001). Consistent with these theoretical links, recent work has indicated that alterations in incentive value achieved via the modulation of motivational factors can modulate the temporal control of behavior in a manner reminiscent of DA manipulations (Galtress & Kirkpatrick, 2009). It should be noted, however, that while the shifts resulting from reinforcer devaluation in this study grew with the increasing durations being timed, they were not strictly proportional as has been seen with DA manipulations. At present, the neural sites responsible for DA's role in modulating clock speed remain unclear, as does the question of whether DA is the intermediary between changes in incentive and alterations in response time.

We hypothesized that DA in the NAc shell, by providing a signal indicative of the incentive salience of a discriminative stimulus for reward, could contribute to DA-mediated changes in clock speed. To this end, we infused DA agents directly into the NAc shell while rats were performing in a temporal production task, in order to assess whether NAc shell DA would modulate response time (clock speed) and/or response rate (incentive value).

Methods

Subjects

Twenty adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), approximately three months of age at the beginning of the experiment, were randomly assigned to one of two duration groups (10 or 30 seconds). All rats were kept on a 12-hour light:dark cycle (lights on at 8:00 AM), and all behavioral testing took place during the light phase. Rats were given ad libitum access to drinking water, but feeding was restricted (Harlan 2019 Rat Diet) such that rats were maintained at 85-90% of their free-feeding body weights, adjusted for growth.

Apparatus

All subjects were trained and tested in standard operant conditioning chambers (30.5 × 25.4 × 30.5 cm – Coulbourn Instruments). Each chamber has an aluminum front wall, back wall, and ceiling, ventilated Plexiglas sides, and a stainless steel floor. Chambers are equipped with a houselight and a seven-tone audio generator. A pellet dispenser located outside of the chamber delivers 45 mg sucrose pellets (Formula F; Noyes Precision, Lancaster, HH) to a

food magazine located on the chamber's front wall. On the back wall of the chamber are three, equidistant nosepoke response holes (2.5 cm opening diameter). The interior of each response hole has yellow and green LED cue lights as well as a photobeam circuit to detect entries and exits. In between each nosepoke hole and extending from the chamber's back wall are aluminum panels (e.g., "hallways") (30.5 cm wide $\times$ 8.5 cm deep) that prevent rapid behavioral switching between the three nosepoke holes. A standard operant-conditioning control program (Graphic State, Coulbourn Instruments) with a temporal resolution of 20 ms was used to achieve stimulus control and data acquisition.

Surgery

Once rats reached a minimum of three months of age, they were implanted bilaterally with stainless steel guide cannulas (26 gauge, Plastics One, Roanoke, VA) into the NAc shell. As was noted previously, the shell subregion was selected based on research showing that it responds to discriminative stimuli for reward (e.g., Bassareo & Di Chiara, 1997; Corbit, et al., 2001; Johnson, et al., 1995), and rats in the present experiment were trained on a discrete trials temporal production procedure, in which they responded to a discriminative stimulus in anticipation of sucrose reinforcement. The stereotaxic coordinates for the shell region relative to bregma were anteroposterior (AP) + 1.6 mm; lateral (L) $\pm$.8 mm; and dorsoventral (DV) -6.8 mm (Corbit, et al., 2001). Prior to surgery, rats were anesthetized with a cocktail of Ketamine (Bioniche Pharma USA Inc.; Bogart, GA; 100mg/kg body weight, i.p.) and Xylazine (Lloyd Laboratories; Shenandoah, IA; 100 mg/kg body weight, i.p.), and secured in a stereotaxic device with an incisor bar set to maintain a level bregma-lambda plane. Cannulas were fixed to the skull with dental acrylic cement and four stainless steel anchoring screws. Rats were given one week to recover before behavioral training began.

Procedure

Two to three days prior to training, subjects received ten 45 mg sucrose pellets (Formula F; Noyes Precision, Lancaster, HH) in their home cage to acclimate them to the reinforcer prior to training. Next, rats progressed through the following training sequence: (1) Nosepoke training, (2) fixed-interval (FI) training, (3) peak-interval (PI) training, (4) microinjections and PI-testing. Rats were run five days per week in two-hour training sessions that took place at the same time daily. During the testing stage, each rat received intra-NAc shell injections of two different doses of d-Amphetamine, sulpiride, and saline in a pseudo-randomly assigned order as described below. Rats were given at least 48 hours between successive microinjections to ensure drug washout. PI performance was compared across drug injection days.

Nosepoke Training (2-6 Sessions)

The nosepoke training protocol delivered sucrose pellets on a continuous reinforcement schedule, such that each entry into the center nosepoke aperture yielded one sucrose pellet. A two second time-out followed the delivery of each pellet in this phase of training to prevent the food magazine from jamming due to the rapid delivery of multiple reinforcers which could occur due to repeatedly triggering the photobeam during a single nosepoke. The procedure terminated when 60 reinforcers were earned or two hours elapsed. Rats were required to earn at least 60 reinforcers on two sequential training sessions in order to progress to the next stage of training.

FI Training (Approximately 5 Sessions)

A discrete-trials FI procedure was used in which the discriminative stimulus (a 4 kHz steady tone) signaled availability of a sucrose pellet after a fixed delay. The duration of the tone

was manipulated between groups, such that one group had a 10 second tone-food availability delay, and the other group had a 30 second tone-food availability delay. After the criterion duration elapsed, the first entry into the center nosepoke aperture yielded one reinforcer, and the tone terminated. No cue was provided to indicate that the criterion delay had elapsed, and there were no consequences for nosepokes made before the delay elapsed. All trials in this phase, as well as in all subsequent phases, were separated by a variable inter-trial interval ranging between 60-90 seconds, uniformly distributed, and sampled with replacement.

PI Training (Approximately 20 Sessions)

The PI training protocol was identical to the FI protocol, with the exception that a random 50% of the trials were non-reinforced probe trials. Probe trials presented the same 4 kHz tone that was used on FI trials, but the tone was presented for three to four times its FI duration and terminated independently of behavior. In order to progress to PI testing with concurrent microinjections, rats were required to meet two criteria. First, rats' peak times were required to fall within 30% of the criterion duration on four consecutive days (i.e., 7-13s for the 10-s group and 21-39s for the 30-s group). Second, rats' individual coefficients of variation (CV) for each of the four days were required to fall within 25% of the average CV computed across those days.

PI Testing and Concurrent Microinjections (6 sessions)

Rats were moved on an individual basis to this phase of the experiment (two rounds of PI testing with concurrent microinjections) when they demonstrated temporal accuracy and precision as determined by the above described criteria. Rats in this phase received microinjections approximately fifteen minutes prior to being run on the PI procedure described above. Microinjections/PI testing were first carried out in all rats at the lower dose of each drug, followed by a second round in which microinjections/PI testing were carried out using a higher dose of each drug.

Drugs and Infusions

Each rat received two rounds of intra-NAc shell injections of each of the following drugs in a randomized order (lower dose in round 1): (1) d-Amphetamine sulfate (Sigma-Aldrich Co.; St. Louis, MO; 10 µg/µL, 20 µg/µL), (2) sulpiride (Sigma-Aldrich Co.; St. Louis, MO; 2 µg/µL, 4 µg/µL) and (3) 0.9% physiological saline. Drugs and doses were chosen based upon their use in other studies with similar methodologies (Cador, Robbins, & Everitt, 1989; Hodge, Samson, & Haraguchi, 1992; Koch, Schmid, & Schnitzler, 2000; Wyvell & Berridge, 2000). In addition, amphetamine has recently been shown to increase clock speed in rats (Taylor, Horvitz, & Balsam, 2007), and is less neurotoxic than methamphetamine (Cheng, Etcheberry, & Meck, 2007), while sulpiride is a D2 antagonist, and this subtype has been linked to clock slowing in rats (Meck, 1986). Amphetamine was dissolved in physiological saline. Sulpiride was dissolved in saline after its pH was lowered with acetic acid and subsequently adjusted to pH 6-7 using sodium hydroxide (NaOH). Due to post-operative complications, two rats (one in the 10-s group and another in the 30-s group) received no microinjections and were removed from the study, and a third rat (30-s group) received only sulpiride and saline injections during the low-dose round of microinjections. PI training sessions without injections were run in between drug injection days. Microinjections through the implanted guide cannulas were made using a 10 µL Hamilton syringe that was driven by a SP2001 syringe pump. All injections were made in a total volume of 0.5µL per side at a rate of 0.1µL/minute. Approximately one minute was given after each injection to allow for drug dispersal, after which dummy cannulas were replaced.

Histology

Once behavioral testing was complete, animals were injected with sodium pentobarbital (Fort Dodge Animal Health; Fort Dodge, IA; 100 mg/kg body weight, i.p.) and perfused intracardially with 0.9% physiological saline followed by formalin (Fisher Scientific Company L.L.C; Kalamazoo, MI). Brains were stored in formalin after removal and moved to a 30% sucrose solution two days prior to being sliced. Brains were fixed to a frozen microtome and sliced into coronal sections (50 μ m). Approximately every other section was saved for histological analysis, and slices were stained using a Nissl stain to verify cannula placement. Cannula locations are displayed in Figure 1. Results of our histological analysis indicated that in five rats (two rats from the 10-s group and three rats from the 30-s group) cannulas were implanted into regions other than the NAc shell subregion. Data from these five rats were removed from our statistical analyses.

Data Analysis

Mean functions—Plots of the average rate of nosepoking as a function of the time elapsed in a trial (a peak function) were constructed using 1 sec bins. These peak functions were fitted with a modified Gaussian curve, and measures of peak time, peak rate, peak spread, and coefficient of variation (CV) were collected from the functions (MATLAB 7, The MathWorks, Inc., Cambridge, MA). The CV was computed by normalizing the peak spread by the peak time. In the 10-s group, the first 30 seconds of all probe trials for each injection day were included in data analyses. In the 30-s group, the first 90 seconds of all probe trials for each injection day were included in data analyses.

Single-trial Analyses—As mean peak functions are derived from all of the PI trials for a given session pooled together, the Gaussian distributions obtained during PI sessions do not characterize behavior on individual trials. The response pattern on individual probe trials typically takes the form of a low-high-low step pattern, in which rats initially respond at a low rate, abruptly switch to a high response rate near the expected time of reinforcement, and abruptly return to a low rate after the target duration elapses (Cheng & Westwood, 1993; Church, Meck, & Gibbon, 1994; Matell, et al., 2006). Single-trial analyses permit an assessment of rats' response rates during high and low states of responding, as well as the times at which the transitions from low- to high- to low states of responding occur. By collecting similar measures of performance from mean functions and single step functions, we can assess whether NAc shell DA manipulations affect performance similarly in a session-wise average as well as at the level of individual trials, or, alternatively, whether a session-wise average obscures the effects of NAc DA modulations on single trials. These analyses were conducted by exhaustively fitting the response pattern on each trial (1-s bins) with a set of three response rate states. From these fits, rats' start times, stop times, and spread of responding, as well as rats' response rates during the initial low state, high state, and final low states of responding were collected. The start time (s_1) is the time at which responding shifts from a low rate to a high rate, the stop time (s_2) is the time at which responding shifts from a high rate to a low rate, and the spread ($s_1 - s_2$) is the duration of the high response rate state.

Statistics—All statistical analyses were conducted using SPSS (version 16). Separate $2 \times 3 \times 2$ (Dose $\times$ Drug $\times$ Group) mixed factors analyses of variance (ANOVA's) were conducted for the proportion of trials on which rats responded, as well as rats' peak rates (on trials in which at least one response occurred), peak times, and CV's of responding. Additionally, separate Dose $\times$ Drug $\times$ Group mixed factors ANOVA's were conducted for the data obtained from single-trial analyses (i.e., start time, stop time, spread, initial low rate, high rate, and final low rate of responding). In all statistical analyses, group was the between-subjects factor, and drug and dose were within-subjects factors. The Greenhouse-Geiser

correction was used for significance testing for all cases in which the sphericity assumption was violated, and the significance criterion was set at $p < .05$.

Results

Mean peak functions (Figure 2) show that NAc DA manipulations had no effect on timing (i.e., peak times are similar across microinjections) in either the 10-s or the 30-s group, but rats' peak rates of responding in both groups appeared lower after sulpiride microinjections relative to saline and amphetamine microinjections.

The lowered response rate following sulpiride was confirmed by repeated measures ANOVAs for peak rate with Group as a between-subjects factor and Drug and Dose as within-subjects factors. The results demonstrated a significant Drug $\times$ Group interaction, $F(2,20) = 4.57$, $p < .05$, as well as significant main effects of drug, $F(2,20) = 19.62$, $p < .001$; dose, $F(1,10) = 7.72$, $p < .05$; and group, $F(1,10) = 12.47$, $p < .01$ (Figure 3 bottom). Post hoc paired t tests revealed that at the low dose, peak rates of responding in the 10-s group were lower after sulpiride injections ($M = .55$, $SD = .33$) than after saline ($M = 1.46$, $SD = .54$), $t(6) = -4.29$, or amphetamine injections ($M = 1.80$, $SD = .71$), $t(6) = -3.77$, $p < .01$ in both cases, which did not differ from one another. Peak rates of responding as a function of drug did not differ in the 30-s group at this dose. At the high dose, peak rates of responding in the 10-s group were lower after sulpiride injections ($M = 1.22$, $SD = .59$) than after saline ($M = 1.77$, $SD = .74$), $t(6) = -3.48$, or amphetamine injections ($M = 1.82$, $SD = .38$), $t(6) = -3.48$, $p < .01$ in both cases, which did not differ from one another. In the 30-s group, there was a trend for sulpiride to decrease peak rates of responding ($M = .61$, $SD = .28$) relative to saline ($M = 1.07$, $SD = .47$), $t(4) = -2.34$, $p = .079$. Peak rates of responding in this group did not differ between sulpiride and amphetamine ($M = .78$, $SD = .62$), or between saline and amphetamine.

Similarly, an ANOVA was conducted on the proportion of trials on which rats responded. The analysis indicated a significant main effect of drug, $F(2, 20) = 29.61$, $p < .001$, but no other significant main effects or interactions. Post hoc paired t tests with the data collapsed across the group and dose variables revealed that sulpiride significantly decreased the proportion of trials on which rats responded ($M = .56$, $SD = .24$) relative to saline ($M = .87$, $SD = .30$), $t(23) = -4.20$, and amphetamine ($M = .90$, $SD = .15$), $t(23) = -6.29$, $p < .001$ in both cases, which did not differ from one another (Figure 3 top).

As was suggested by the mean functions, intra-NAc DA manipulations had no effect on rats' peak times of responding, but rats' coefficients of variation (CVs) of responding differed as a function of group. The ANOVA comparing peak times of responding normalized by the programmed criterion durations revealed no significant effects of drug, $F(2,20) = 1.27$; dose, $F(1,10) = .65$; or group, $F(1,10) = .15$, $p > .05$ in all cases. However, the ANOVA comparing rats' CVs of responding indicated a significant main effect of group, $F(1,10) = 8.80$, $p < .05$, in that rats' CV's of responding were significantly greater in the 30-s group ($M = 1.13$, $SD = .32$) than in the 10-s group ($M = .91$, $SD = .23$). No effects of drug, $F(2,20) = .04$; or dose, $F(1,10) = 1.57$; nor any interactions, were significant for CV's, $p > .05$ in all cases.

Additional mixed factors ANOVA's were conducted for the data that were obtained from the single-trials analyses. These data include three response rate variables (i.e., initial low rate of responding, high rate, and final low rate of responding), and three measures of temporal control (i.e., start time, stop time, and spread of responding). Intra-NAc sulpiride injections significantly decreased rats' rates of responding during the initial low state of the step function relative to saline and amphetamine. Specifically, there was a significant main effect

of drug, $F(2,20) = 3.92$, $p < .05$, but no other significant main effects or interactions. Post hoc paired t tests with the data collapsed across the group and dose variables revealed that sulpiride significantly decreased rats' initial low rates of responding ($M = .15$, $SD = .09$) relative to saline ($M = .22$, $SD = .14$), $t(23) = -2.67$, $p < .05$, and amphetamine ($M = .22$, $SD = .14$), $t(23) = -2.87$, $p < .01$, which did not differ from one another.

During the high state of the step function, rats' rates of responding differed as a function of group. The ANOVA comparing rats' high response rates indicated a significant Drug $\times$ Group interaction, $F(2,20) = 6.89$, $p < .01$, as well as significant main effects of drug, $F(2,20) = 19.97$, $p < .001$; and group, $F(1,9) = 13.13$, $p < .01$. Post hoc paired t tests with the data collapsed across dose revealed that in the 10-s group, sulpiride significantly decreased rats' high state rates of responding ($M = 1.60$, $SD = .50$) relative to saline ($M = 2.17$, $SD = .47$), $t(12) = -3.64$, $p < .01$, and amphetamine ($M = 2.31$, $SD = .34$), $t(12) = -4.82$, $p < .001$, which did not differ from one another. In the 30-s group, sulpiride significantly decreased rats' high state rates of responding ($M = 1.28$, $SD = .24$) relative to saline ($M = 1.62$, $SD = .32$), $t(9) = -5.10$, $p = .001$. However, rats' high state rates did not differ between sulpiride and amphetamine ($M = 1.46$, $SD = .48$), or between saline and amphetamine, in this group.

NAc DA modulation did not influence rats' rates of responding during the final low state of the step function. The ANOVA comparing rats' final low rates revealed no significant main effects of dose, $F(1,10) = .15$; drug, $F(1,20) = 3.07$; or group $F(1,10) = 1.15$; and no significant interactions.

When the effects of NAc DA modulation on STA measures of timing (i.e., start times, stop times, and spread of responding) were assessed, no significant effects were obtained. Specifically, the ANOVA comparing start times normalized by the programmed criterion durations revealed no significant effects of drug, $F(2,20) = .73$; dose, $F(1,10) = .003$; or group, $F(1,10) = 3.35$, $p > .05$ in all cases. The ANOVA comparing stop times normalized by the programmed criterion durations also revealed no significant effects of drug, $F(2,20) = 1.40$; dose, $F(1,10) = .47$; or group, $F(1,10) = .03$, $p > .05$ in all cases. Finally, the ANOVA comparing spread of responding normalized by the programmed criterion durations also indicated no significant effects of drug, $F(2,20) = 1.72$; dose, $F(1,10) = .88$; or group, $F(1,10) = 2.88$, $p > .05$ in all cases.

We assessed the fit quality of the step functions for both the 10-s and 30-s groups that were yielded by our single trials analyses. Results showed that the step functions provided significantly better fits for the 10-s group data than the 30-s group data. The ANOVA comparing the variance accounted for (i.e., η^2) by the step functions indicated a significant main effect of group, $F(1,10) = 34.37$, $p < .001$, in that the functions accounted for significantly more of the variance in the 10-s group ($M = .85$, $SD = .02$) than in the 30-s group ($M = .64$, $SD = .02$).

We also computed inter-poke intervals to see whether response topographies changed as a function of group and drug. The modal inter-poke intervals on saline (a measure of response speed during a bout) were nearly identical across groups ($M = 0.429$ s in the 10-s group; $M = 0.420$ s in the 30-s group, n.s.). While there was an interaction between dose, drug and group, $F(2,20) = 4.38$, $p < 0.05$, subsequent paired t -tests revealed that the only reliable drug effect was a slight slowing of the poke rate during a bout on the low dose of sulpiride in the 10-s group ($M = 0.50$ s). In contrast, using the per trial average number of inter-poke intervals ranging between 1s and 2s as a measure of pausing, we found highly significant differences as a function of group, $F(2,20) = 42.0$, $p < 0.001$, ($M = 4.0$ pauses per trial in the 10-s group; 15.2 pauses per trial in the 30-s group). There were no significant effects of drug or dose, nor any interactions.

Discussion

The purpose of this work was to determine if modulating nucleus accumbens (NAc) shell dopamine (DA) levels modulates peak time and/or peak rate in a temporal production procedure. Our results showed that sulpiride microinjections significantly decreased both the proportion of trials on which rats responded, as well as rats' peak rates of responding on trials in which they did respond. Analysis of the response patterns on trials in which the rats responded revealed that sulpiride significantly decreased the response rate on both the initial "low response rate" state and the "high response rate" state. Previous work has indicated that independent thresholds are utilized for starting versus stopping high rate responding (Church et al., 1994), and may be differentially impacted by dopaminergic drugs (Matell et al., 2006; Taylor et al., 2007). The current work suggests that the response rates of the three response states are differentially sensitive to DA manipulations as well. Such differential DA sensitivity of the low states may be reflective of differential impulsivity before versus after the criterion duration has elapsed (Matell & Portugal, 2007), as impulsivity has been shown to be modulated by DA drugs (Evenden, 1999). Consistent with these findings, unpublished data collected in our lab has shown that DA agonists in the dorsal striatum selectively enhanced rats' response rates during the initial low state of the single step function (Kurti & Matell, 2009), without impacting the terminal low state or the high state rates, or rate transition times.

As peak rates are viewed as reflecting motivation, the influence of NAc DA modulation on response rates in the present experiment is consistent with Ikemoto and Panksepp's (1999) characterization of the NAc as an "incentive property constructor." Moreover, as our DA manipulations were conducted in the NAc shell subregion, our data also corroborate evidence suggesting that the NAc shell is implicated in responding to discriminative stimuli for reward. In contrast to the effects of NAc shell DA modulation on response rates, these same manipulations had little impact on peak times. Further, while it remains unclear why we found differences in the relative spread of responding between groups (we presume this failure reflects individual differences in decision criteria or error that happened to randomly covary with group assignment), the administration of dopaminergic agents had no bearing on this effect. As such, the current data suggest that neither NAc shell DA levels, nor the resultant changes in incentive signaled by this region, impact the temporal control of behavior. It should be emphasized that the lack of effects on temporal control is not likely to be due to insufficient statistical power, as we obtained consistent significant effects on peak rate and response probability using several indices of performance.

Though intra-NAc shell sulpiride microinjections decreased the proportion of trials on which rats responded, the opposite effect (i.e., increased response likelihood) was not observed after amphetamine microinjections. One possible reason for this asymmetry is that rats were already responding at near maximal response levels. Specifically, the failure to obtain amphetamine effects on the proportion of trials on which responding occurred is likely due to a ceiling effect, as rats were responding on approximately 90% of trials under saline conditions.

A ceiling effect may also explain the lack of change in peak rates and the rates of responding during the "high response rate" state identified by single-trial analyses following amphetamine administration. Rats' peak rates and their response rates during the high state of the step function were greater in the 10-second group than the 30-second group, which at first glance, seems to suggest that rats in the 30-s group had not achieved ceiling high rates under saline conditions. However, one caveat is that the "high response rate" state is often composed of the entire duration of time during which an animal responds in anticipation of reinforcement. Importantly, this state may be composed of bouts of rapid responding

intermixed with periods of pausing, and amphetamine may not eliminate pausing behavior. In other words, it is conceivable that the 30-s group differed from the 10-s group not in terms of response rates during the bouts, but instead, in the particular topography (i.e., pattern) of responding. Indeed, visual inspection of the pattern of responding in the 30-s group suggested that the high state was composed of periods of responding interspersed with short pauses, whereas the high state in the 10-s group was largely composed of a single period of high rate responding without intervening pauses.

While previous work using single trial analysis methods on rats trained to lever press for reinforcement has not reported such differential patterns of behavior across durations (Church et al., 1994), nose-poking occurs at higher rates than lever pressing (Caine, Negus, & Mello, 1999), and the lower rate of lever pressing may have obscured these response bouts within a more prolonged response state. Quantitative support for this interpretation comes from the finding that the step functions yielded by our single trials analyses provided poorer fits for the 30-s group than the 10-s group. Further post-hoc analyses of the inter-poke intervals were also consistent with this interpretation. The modal inter-poke intervals on saline (a measure of response speed during a bout) were nearly identical across durations, with no effect of amphetamine on response speed within a bout. In contrast, using the number of inter-poke intervals ranging between 1s and 2s as a measure of pausing, we found highly significant differences as a function of duration, such that these long pauses were four times more likely in the 30s group than the 10s group, irrespective of drug condition. These data provide clear support for a substantial difference in response topography as a function of duration. Taken together, these data suggest that the failure to see increases in response rates during amphetamine administration likely resulted from a ceiling effect in terms of response rate within a bout.

The differential response rate between the two durations used here is inconsistent with the supposition that response rate and response time are independent (Roberts, 1981, though see Exp 5). However, the durations used in that experiment were relatively more similar (a 1:2 duration ratio versus a 1:3 duration ratio used here), and a higher proportion of reinforced trials were used (i.e., 80% versus 50% used here). While it remains unclear whether these procedural differences are responsible for the inverse relation between peak time and peak rate seen here, other studies using similar discrepancies have reported similar findings (see Fig. 6 & 7 in Cheng & Meck, 2007).

Though our experimental findings showing changes in peak rate, but not peak time, do not support the hypothesis that the NAc shell modulates clock speed based on incentive, our findings are consistent with other work indicating that the NAc may mediate response rate, but not response timing (Meck, 2006; Roberts, 1981). Specifically, rats in Meck were trained on a dual-duration, PI procedure in which reinforcement for lever-pressing was provided on 50% of the trials at both 10-s and 60-s durations. Baseline response patterns indicated that rats' peak times occurred at approximately 10-s and 60-s, with higher peak response rates on 10-s trials. 6-hydroxydopamine lesions of the NAc (both core and shell regions) had no impact on temporal control (i.e., no effect on peak time or peak spread), but led to an elimination of the differential peak rates associated with the different signaled durations. These results were taken to indicate that the NAc is involved in specifying the incentive salience of discriminative stimuli, but is not involved in temporal control.

One important difference between our findings and Meck's (2006) findings that should be noted is that after receiving NAc DA lesions, rats in Meck's study maintained robust peak rates despite no longer discriminating incentive value, whereas decreased NAc DA functionality in the present experiment following sulpiride infusions significantly suppressed response rates relative to saline and amphetamine. While it is unclear why the effect on peak

rates differed between these studies, one possibility is that the extended DA loss following 6-OHDA lesions results in some type of compensatory functionality (e.g., via a dorsal striatal habit-based system), whereas the acute decrease in DA activity following microinfusions do not produce this result. Nevertheless, taken together, the results of both studies show that whether NAc DA manipulations reduce rats' ability to discriminate incentive value (Meck, 2006), or diminish incentive value (present experiment), changes in NAc DA, and consequently expressed incentive, do not modulate clock speed.

In partial contrast to these results, a recent investigation examined the effects of NAc core excitotoxic lesions on rats' sensitivity to changes in both the magnitude and the length of the delay to reward in a peak-interval (PI) procedure (Galtress & Kirkpatrick, 2010). Results indicated that NAc core-lesioned rats timed pre-established reinforcement delays correctly and adjusted anticipatory responding appropriately when the delay to reward was reduced (indicating that the capacity to update temporal control remained intact), but they did not show increases in peak response rates, nor changes in peak time, when the magnitude of reward was increased (indicating that the ability to update incentive representations of discriminative stimuli was disrupted). Conversely, sham-lesioned rats displayed higher peak rates of responding, as well as leftward shifts in the start, middle, and stop times on individual trials, when reward magnitude was increased, as well as when the reward delay was shortened. The finding that NAc lesions left timing, but not incentive motivation, intact further suggests that the NAc is involved in incentive motivational processes but not in generating the temporal control of behavior.

One important difference between our findings and those of Galtress and Kirkpatrick (2010) is that whereas we showed that NAc shell DA manipulations had no impact on temporal control despite altering response rates (thereby suggesting that the NAc shell DA does not regulate clock speed), Galtress and Kirkpatrick showed that the NAc core is necessary for modulating temporal control following incentive manipulations (albeit by eliminating incentive-based changes in both response rate and clock speed). It remains unclear whether the NAc core/shell distinction, and/or the difference in methodology (DA pharmacological manipulations versus excitotoxic lesions), is responsible for these different results. Intriguingly, there are substantial interactions between these structures (Groenewegen, Wright, Beijer, & Voorn, 1999; Wright & Groenewegen, 1995), including both direct bidirectional connections (van Dongen, et al., 2005), indirect connections from shell to core via pallido-thalamo-cortical-pathways (Zahm, 1999; Zahm & Heimer, 1990, 1993), and feedback through DA systems in the ventral tegmental area and substantia nigra pars compacta (Nauta, et al., 1978). As such, the dissociation between NAc core and shell involvement in incentive-induced modulation of timing is a bit surprising. Nevertheless, similar dissociations have been seen in a number of recent behavioral studies (Bassareo, Musio, & Di Chiara, 2010; Murphy, Robinson, Theobald, Dalley, & Robbins, 2008; Nelson, Thur, Marsden, & Cassaday, 2010; Shiflett & Balleine, 2010). Clearly, future work examining incentive modulation of timing following DA manipulations within the NAc core, and excitotoxic lesions of the shell, will be informative in this regard.

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Figure 1.

Locations of cannula tips. Black circles indicate tips in nucleus accumbens shell, and grey circles indicate tips outside of shell (Left panel: 10s group, Right Panel: 30s group). Figures from Paxinos & Watson (1997).

Figure 2.

Average “peak” response functions resulting from microinjections of dopaminergic drugs into the nucleus accumbens shell at low (left) and high (right) doses.

Figure 3.

Top: Proportion of trials on which rats responded as a function of dopaminergic drugs microinjected into the nucleus accumbens shell at low dose (left) and high (right) doses. Top panels: Proportion of trials on which rats responded. Bottom panels: Peak rates of responding on trials in which rats responded.