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Research Report
Pharmacological manipulations of food protection behavior in rats: Evidence for dopaminergic contributions to time perception during a natural behavior
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ABSTRACT

Operant procedures combined with pharmacological manipulations have implicated a role for the dopaminergic system in the perception and production of temporal intervals. Because studies have suggested that animals use temporal information to organize food protection behavior, the current study investigates whether dopaminergic systems are involved in timing during this natural behavior. The experiment examined the influence of a dopaminergic agonist (amphetamine) and an antagonist (haloperidol) on food protection behavior initiated to avoid theft by a conspecific. Amphetamine increased the time spent dodging and decreased the time spent bracing during the consumption of a hazelnut. On the other hand, haloperidol decreased the time spent dodging while showing no systematic changes in bracing. Topographic and kinematic analyses of rat movement conflicted with motivational, motoric, and social accounts of drug-induced changes in food protection behavior organization. These observations provide evidence that rats use temporal information to organize movements in the natural behavior of protecting food from theft by a conspecific, and this organization is influenced by both a dopaminergic agonist and an antagonist.

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1. Introduction

Animals use multiple sources of information to organize their behavior. Among these, information involved in time perception is particularly advantageous. Knowledge about the duration of behaviors or events in the environment helps an animal structure behavior in order to compete for and maximize food availability (Gallistel, 1990; Bateson, 2003). Previous work has demonstrated that rats display consistent movement organization when competing for food with a conspecific (Whishaw and Tomie, 1987, 1988). If a rat is approached by a conspecific during the consumption of a food item, the animal will make

rapid movements away from the conspecific to protect the food item from theft. In a past study, the organization of these food protection behaviors was suggested to depend on the rat's perception of time (Whishaw and Gorny, 1994). Time remaining to consume a food item was the best predictor for the magnitude of food protection behavior associated with food items of varying size (20 mg, 45 mg, 94 mg, 190 mg, 500 mg, 750 mg, 1000 mg) and density (pearl barley, Mung bean, spring wheat, Azuki bean). In general, longer consumption times were associated with movements that displaced the rat farther away from the conspecific. Therefore, the organization of food protection behaviors appears to depend on an animal's ability

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to generate an accurate estimate of time in the seconds-to-minutes range (i.e., interval timing).

Previous research investigating interval timing has supported a role for three stages of information processing in estimating temporal intervals (Gibbon et al., 1984; Matell and Meck, 2000). The clock stage accumulates pulses from a pacemaker as the temporal interval increases. The memory stage stores values from the accumulator into long-term memory. The decision stage involves comparing the current contents of the accumulator to values stored in memory. This view of interval timing has been critical in understanding the influence of behavioral or pharmacological manipulations on interval timing (Meck, 1996; Meck et al., 1987; Wearden, 1999, 2005).

Several lines of evidence have implicated the nigrostriatal dopaminergic system in the clock stage during interval timing. First, dopaminergic agonists and antagonists have been shown to selectively influence the perception and production of temporal intervals (Meck, 1983; Maricq and Church, 1983; Buhusi and Meck, 2002). These observations have led researchers to suggest that dopaminergic agonists increase the rate at which subjective time passes, while dopaminergic antagonists decrease this rate. Second, brain pathology or lesions that compromise the function of the nigrostriatal dopaminergic system have been shown to disrupt interval timing (for a review, see Matell and Meck, 2004). Third, the firing properties of dorsal central striatal neurons have been shown to be tuned to temporal durations associated with the delivery of reinforcement (Matell et al., 2003). These characteristics of interval timing support a role for the nigrostriatal dopaminergic system in controlling the rate of a pacemaker mechanism (Meck, 1996; Gibbon et al., 1997; MacDonald and Meck, 2004; Matell and Meck, 2004; Buhusi and Meck, 2005).

Recent evidence has also supported a role for the dopaminergic system in the temporal organization of natural behaviors. For example, pharmacological manipulation of the dopaminergic system has been shown to systematically alter the timing of grooming behavior in rats (Matell et al., 2006). Furthermore, damage specific to the nigrostriatal dopaminergic system has been shown to disrupt the organization of food protection behaviors (Whishaw and Tomie, 1988). Based on these findings, it is predicted that pharmacological manipulations of the dopaminergic system will produce systematic influences on food protection behavior. For example, the estimated time to consume a food item is based on the perceived rate of food consumption. Under normal conditions, the rate of food item consumption may be perceived as 1000 mg/60 s (16.6 mg/s). Although pharmacological manipulations may influence subjective time, the quantity of the food does not change. Manipulations that increase or decrease the speed of the clock will result in lower (1000 mg/80 s or 12.5 mg/s) or higher (1000 mg/40 s or 25 mg/s) perceived rates of consumption, respectively. Slower perceived rates of food item consumption will be associated with food protection behaviors that maximize the distance from an approaching conspecific. As the perceived rate of food item consumption increases, the need to engage in food protection behaviors that maximize distance should decrease. Therefore, pharmacological manipulations that influence the speed of the clock should also influence the organization of food protection behavior.

The present experiment investigates the effects of a dopaminergic agonist (D-amphetamine) and antagonist (haloperidol) on the organization of food protection behaviors. Food protection behaviors are classified as either dodging or bracing. Dodging behavior reflects any attempt to move away from a robber (i.e., a food deprived conspecific that has been consistently exposed to an animal that has received a food item) that involves transferring the food item to the mouth prior to using the fore- and hindlimbs to make a lateral movement away from the approaching rat (see Supplementary dodging videos). A rat that fails to dodge may engage in bracing food protection behaviors. Bracing behaviors reflect attempts to evade the robber in which the food item is not transferred to the mouth and just the hindlimbs are used to move away from the approaching robber (see Supplementary bracing videos). Dodging behaviors displace the rat and food item a farther distance from the robber, relative to bracing behaviors (Whishaw and Tomie, 1987; Whishaw and Tomie, 1988). Early in the consumption of a food item, rats primarily engage in dodging behavior to protect the food item. As the rat continues to consume the food item, dodging behavior subsides and bracing behavior increases. Finally, as the consumption of the food item concludes, rats primarily engage in bracing behavior. Observing that D-amphetamine and haloperidol systematically influence the transition between dodging and bracing behavior would support a role for temporal estimates in organizing food protection behavior (Whishaw and Gorny, 1994). Observing nonsystematic effects of these pharmacological manipulations would suggest that the organization of food protection behavior is independent of temporal estimates. In addition, several measures were developed to evaluate the role of motivational and motoric factors in the effects of the pharmacological manipulations on food protection behaviors (Magour et al., 1974; Wellman, 1990).

2. Results

The ANOVA conducted on hazelnut consumption times under each drug testing session when the robber was present or absent failed to find a significant main effect of drug [$F(2,10)=0.398$, $p=ns$], robber [$F(2,10)=0.098$, $p=ns$], or Drug \times Robber interaction [$F(2,10)=2.22$, $p=ns$]. Dodgers consumed the hazelnut in 98.25 s on average, collapsed across eating conditions and drug conditions. The failure to find significant main effects or an interaction suggests that the drugs did not influence the dodger's motivation to consume the hazelnut.

Time spent dodging during each of the samples under saline, amphetamine, and haloperidol testing sessions is plotted in the top left hand panel of Fig. 1. The ANOVA conducted on subjects' time spent dodging revealed a significant main effect of drug [$F(2,10)=6.724$, $p<0.05$] and sample [$F(4,20)=4.061$, $p<0.05$]; however, the Drug \times Sample [$F(8,40)=1.115$, $p=ns$] interaction was not statistically significant. Subsequent post hoc analysis of the sample main effect revealed a significant linear trend [$F(1,20)=5.068$, $p<0.05$] in time spent dodging. Time spent dodging decreased as a function of hazelnut sample. Tukey LSD post hoc tests on differences between drug conditions revealed that rats spent more time dodging under amphetamine relative to time spent dodging

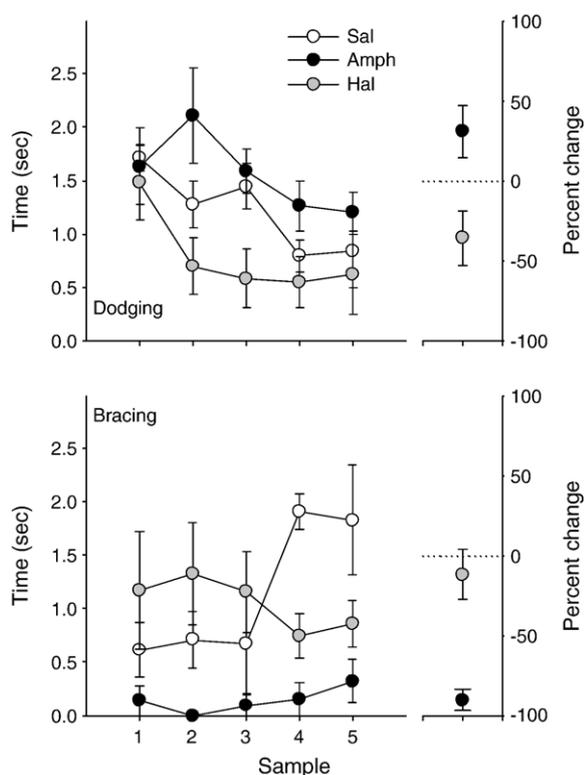


Fig. 1 – Average amount of time dodgers spent engaging in dodging food protection behavior (top left-hand panel) under saline, amphetamine, and haloperidol testing sessions is plotted for each five-second sample. The average percent change in time spent dodging (top right-hand panel) under amphetamine and haloperidol was collapsed across samples. Average amount of time dodgers spent engaging in bracing food protection behavior (bottom left-hand panel) under saline, amphetamine, and haloperidol testing sessions is plotted for each five-second sample. The average percent change in time spent bracing (bottom right-hand panel) under amphetamine and haloperidol was collapsed across samples.

under haloperidol ($p < 0.05$). Time spent dodging under saline condition was not found to be significantly different from amphetamine or haloperidol conditions. Previous studies, using similar drug doses and conventional interval timing tasks, express drug effects as a percent change from baseline. The top right hand panel of Fig. 1 plots percent change in time spent dodging under both drug conditions, collapsed across samples. Amphetamine produced a 31.08% (SEM: 16.22) increase in the time spent dodging; whereas haloperidol produced a 35.63% (SEM: 16.81) decrease in the time spent dodging.

The bottom left hand panel of Fig. 1 presents time spent bracing during each sample under saline, amphetamine, and haloperidol testing sessions. The ANOVA conducted on subjects' time spent bracing revealed a significant main effect of drug [$F(2,10) = 23.882$, $p < 0.05$] and a significant Drug \times Sample [$F(8,40) = 2.578$, $p < 0.05$] interaction. Post hoc analysis of time spent bracing involved running separate ANOVAs for each drug condition. The ANOVA conducted on

time spent bracing during saline samples revealed a significant effect of sample [$F(4,20) = 3.751$, $p < 0.05$]. Subsequent trend analysis revealed that bracing increased as a function of sample [$F(1,20) = 11.301$, $p < 0.05$]. The ANOVAs conducted on time spent bracing during amphetamine [$F(4,20) = 1.645$, $p = \text{ns}$] and haloperidol [$F(4,20) = 0.475$, $p = \text{ns}$] did not reveal a significant effect of sample. Tukey LSD post hoc tests on differences between drug conditions revealed that rats spent significantly less time bracing while under the amphetamine condition than observed under saline or haloperidol conditions ($p < 0.05$). The bottom right hand panel of Fig. 1 plots percent change in time spent bracing under both drug conditions, collapsed across samples. Amphetamine (91.42%; SEM: 6.74), but not haloperidol (11.92%; SEM: 15.57), produced a decrease in the time spent bracing during the consumption of the hazelnut.

As indicated in the previous paragraph, dodging and bracing behaviors were unevenly distributed across the samples under different drug conditions. This precluded combined analysis of speed, distance, and distance between heads during the five samples under each drug condition. First, dodging and bracing behaviors observed over the five samples were classified into early and late samples based on a median split for each rat. Second, the unreliable occurrence of dodging behavior under haloperidol prompted a comparison between saline and amphetamine restricted to dodges during early and late samples. Finally, the absence of bracing behavior under amphetamine necessitated a comparison limited to bracing behaviors that occurred during early and late samples under saline and haloperidol conditions.

The average speed associated with dodging behaviors under saline and amphetamine testing conditions were analyzed separately for dodgers and the robber. The ANOVA conducted on dodger speeds while engaged in dodging behavior failed to reveal significant main effects of drug [$F(1,5) = 0.137$, $p = \text{ns}$], sample [$F(1,5) = 0.604$, $p = \text{ns}$], or a Drug \times Sample interaction [$F(1,5) = 0.205$, $p = \text{ns}$]. The ANOVA conducted on robber speeds while the dodger was engaged in dodging behavior failed to reveal significant main effects of drug [$F(1,5) = 1.227$, $p = \text{ns}$], sample [$F(1,5) = 0.916$, $p = \text{ns}$], or a Drug \times Sample interaction [$F(1,5) = 4.143$, $p = \text{ns}$]. The average speed associated with bracing behavior under saline and haloperidol testing conditions was also separately analyzed for dodgers and the robber. The ANOVA conducted on dodger speeds while engaged in bracing behavior failed to reveal significant main effects of drug [$F(1,5) = 0.002$, $p = \text{ns}$], sample [$F(1,5) = 1.679$, $p = \text{ns}$], or a Drug \times Sample interaction [$F(1,5) = 0.470$, $p = \text{ns}$]. The ANOVA conducted on robber speeds while the dodger was engaged in bracing behavior failed to reveal significant main effects of drug [$F(1,5) = 0.031$, $p = \text{ns}$], sample [$F(1,5) = 0.012$, $p = \text{ns}$], or a Drug \times Sample interaction [$F(1,5) = 2.439$, $p = \text{ns}$]. The failure to observe a significant effect of drug condition on the dodger's speed conflicts with amphetamine-induced hyperkinesia and haloperidol-induced bradykinesia accounts of changes in movement organization. Observing that the robber's speed associated with the pursuit of the dodger did not vary across drug conditions discounts robber-induced changes in dodger movement organization.

Analysis of the average distance traveled by the dodger during dodging and bracing behaviors provided further

assessment of the drug-induced hyperactivity (amphetamine) or bradykinesia (haloperidol) account of changes in movement organization. The ANOVA conducted on distance traveled during dodging behavior failed to reveal significant main effects of drug [$F(1,5)=3.266$, $p=ns$], sample [$F(1,5)=0.831$, $p=ns$], or a Drug×Sample interaction [$F(1,5)=1.565$, $p=ns$]. The ANOVA conducted on distance traveled during bracing failed to reveal significant main effects of drug [$F(1,5)=0.422$, $p=ns$], sample [$F(1,5)=1.467$, $p=ns$] or a Drug×Sample interaction [$F(1,5)=0.164$, $p=ns$]. Observing that the distance traveled during dodging and bracing behaviors did not vary as a function of drug condition provides further evidence against hyperactivity or bradykinesia explanations of changes in movement organization.

Fig. 2 plots the average distances between the heads of the dodger and robber at the start and the end of the dodge phase during early and late samples under saline and amphetamine testing conditions. The ANOVA conducted on distance between heads revealed significant main effects of drug [$F(1,5)=62.101$, $p<0.05$] and dodge phase [$F(1,5)=105.728$, $p<0.05$]. The main effects of sample [$F(1,5)=2.603$, $p=ns$], Drug×Dodge Phase interaction [$F(1,5)=1.444$, $p=ns$], Drug×Sample interaction [$F(1,5)=0.314$, $p=ns$], Dodge Phase×Sample interaction [$F(1,5)=2.377$, $p=ns$], and Drug×Dodge Phase×Sample interaction [$F(1,5)=0.258$, $p=ns$] were not found to be significant. The distance between heads during dodging behaviors was significantly longer under the amphetamine condition relative to the saline condition, independent of dodge phase or sample. The distance between heads was significantly longer at the end of the dodge phase relative to the start, independent of drug condition or sample.

Fig. 3 plots the average distances between the heads of the dodger and robber at the start and the end of the brace phase during early and late samples under saline and haloperidol testing conditions. The ANOVA conducted on distance between heads revealed significant main effects of drug [$F(1,5)=8.435$, $p<0.05$] and sample [$F(1,5)=8.370$, $p<0.05$]. The

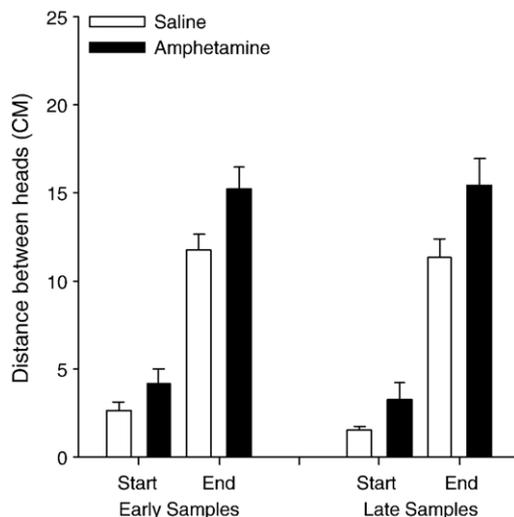


Fig. 2 – Average distance between the heads of the dodger and robber at the start and end of the dodging behavior during saline and amphetamine testing conditions is plotted for early and late samples.

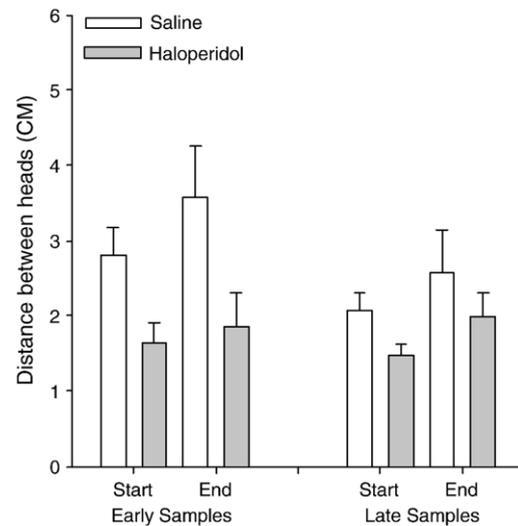


Fig. 3 – Average distance between the heads of the dodger and robber at the start and end of the bracing behavior during saline and haloperidol testing conditions is plotted for early and late samples.

main effects of brace phase [$F(1,5)=2.560$, $p=ns$], Drug×Brace Phase interaction [$F(1,5)=0.219$, $p=ns$], Drug×Sample interaction [$F(1,5)=5.3$, $p=ns$], Brace Phase×Sample interaction [$F(1,5)=0.003$, $p=ns$], and Drug×Brace Phase×Sample interaction [$F(1,5)=1.241$, $p=ns$] were not found to be significant. The distance between heads was significantly smaller under the haloperidol condition relative to the saline condition, independent of brace phase or sample. Distance between heads was significantly smaller on late samples relative to early samples, independent of drug condition or brace phase.

3. Discussion

The goal of this study was to investigate the effects of pharmacological manipulation of the dopaminergic system on the organization of naturally occurring food protection behavior. During the consumption of the hazelnut under saline conditions, dodging behaviors decreased while bracing behaviors increased. Administration of amphetamine increased the time spent dodging during the consumption of the hazelnut, while suppressing bracing behavior. In addition, amphetamine administration significantly increased the distance between heads of the dodger and robber at the initiation and termination of dodging behavior. Administration of haloperidol decreased the time spent dodging throughout consumption of the hazelnut. Although haloperidol initially produced an increase in the time spent bracing during early samples, bracing behavior did not vary as a function of the sample. Haloperidol also produced a significant decrease in the distance between the heads of the dodger and robber at both phases of bracing behavior. These results demonstrate that pharmacological manipulations of the dopaminergic system influence the organization of food protection behaviors and that dodging and bracing behaviors are differentially affected by these manipulations. The following sections examine the potential role for

mechanisms related to interval timing and motivation in mediating the changes in the organization of food protection behaviors observed under amphetamine and haloperidol conditions.

Previous studies have supported a role for estimates of time left to consume the food item as a critical factor in organizing food protection behavior (Whishaw and Gorny, 1994). These claims are based on observations of food protection behavior across food items of varied size and density. The best predictor of dodge probability and magnitude was the estimated time to consume the food item. Longer estimated durations were associated with a higher probability of dodging behavior and dodges of larger amplitude. Assuming that estimates of the time to consume the food item are based on the perceived rate of food consumption, changes in processes related to interval timing should produce predictable changes in food protection behavior. For example, under saline conditions, the rate of food item consumption may be perceived as 1000 mg/60 s (16.6 mg/s); however, under conditions with elevated dopaminergic function (i.e., administration of amphetamine), the quantity of the food does not change, but time estimates increase. Therefore, the rat perceives a slower rate of consumption (e.g., 1000 mg/80 s = 12.5 mg/s) and engages in food protection behaviors that increase the distance from the conspecific (i.e., engaging in dodging behavior and initiating dodges with the robber at a further distance). Conversely, under conditions that suppress dopaminergic function (i.e., administration of haloperidol), time estimates decrease, resulting in the perception of higher rates of food item consumption (e.g., 1000 mg/40 s = 25 mg/s). This would result in selecting food protection behaviors that are less effective in increasing the distance between the conspecific and the rat (i.e., engaging in bracing behavior and initiating braces with the robber at a closer distance). Although the effects of amphetamine and haloperidol on time spent dodging parallel results obtained using conventional interval timing procedures (Meck, 1983, 1996), systematically larger percent changes were observed in the current study. However, these percent changes are consistent with effects observed in the modification of grooming sequences associated with the administration of a selective D1 agonist (Matell et al., 2006). In general, the effects of amphetamine and haloperidol on dodging behavior support a role for the speed of an internal pacemaker or clock in organizing food protection behavior.

The effects of amphetamine and haloperidol on food protection behavior indicate that modifications of clock speed may not be the only factor contributing to the organization of these behaviors. Several studies have suggested that dopaminergic drugs may function to modulate the allocation of attentional resources related to stimulus salience (Santi et al., 1995; Stanford and Santi, 1998). The use of a variant of the peak-interval procedure in which the to-be-timed stimulus is interrupted by a short temporal interval (i.e., gap procedure) has been critical in understanding the effects of dopaminergic drugs on the speed of the clock and attentional processes (Buhusi and Meck, 2002). Specifically, methamphetamine has been shown to influence both the speed of the clock and attentional processes, whereas haloperidol predominately influenced attentional processes alone. Two aspects of the current study provide additional support for the differential effects of dopaminergic drugs.

First, amphetamine increased the time spent dodging while suppressing bracing behavior, consistent with amphetamine's influence on the speed of the clock. In contrast, haloperidol suppressed dodging behavior while having no systematic effect on the time spent bracing, consistent with a minor influence on the speed of the clock. Second, amphetamine increased, and haloperidol decreased, the distance between the animals' heads during food protection behaviors, consistent with both drugs' influence on attentional processes. These results support a role for the modification of clock speed and attentional processes in determining the effects of dopaminergic drugs on the organization of food protection behavior.

Although the present study supports previous work suggesting a role for temporal processes in organizing food protection behaviors, other factors not related to temporal or attentional processing may also contribute to this organization. First, it is possible that the hedonic value of the hazelnut can vary, and this will result in differences in the level of motivation to evade the robber. For example, it has been shown that the magnitude of the unconditioned stimulus in appetitive classical conditioning and the reinforcer magnitude in instrumental conditioning will influence the response topography in these paradigms (Holland, 1979; Ratliff and Ratliff, 1971). In the current study, the hedonic qualities associated with the hazelnut may vary as a function of food item size. Initially, the hazelnut is associated with maximum hedonic value and results in behaviors that maximize food protection (i.e., dodging). During consumption of the hazelnut, the hedonic value of the food item decreases resulting in a reduction in the magnitude of food protection behaviors (i.e., bracing). The results of the present study are congruent with these predictions. In addition, dopaminergic agonists and antagonists have been suggested to influence the hedonic value of reinforcers used during instrumental conditioning (Heyman, 1983; Heyman and Seiden, 1985; Frank et al., 1988; Willner et al., 1990; Frank et al., 1995). Therefore, it is possible that the observed effects of amphetamine and haloperidol on the organization of food protection behavior may be related to a modification of the hedonic value associated with the hazelnut. For example, amphetamine may have increased the hedonic value associated with the hazelnut, thereby resulting in an increased motivation to protect the hazelnut. Based on this account of food protection organization, one would also predict that the overall rate of hazelnut consumption should vary as a function of drug condition. Observing that neither drug condition nor consumption condition (i.e., alone or with the robber) significantly influenced the time required for hazelnut consumption conflicts with this motivational account. In a similar line of reasoning, the appetite suppressant effects of amphetamine (Magour et al., 1974) can be eliminated as a potential factor influencing food protection behavior organization. Finally, food items associated with equivalent consumption times (Azuki bean: 38.29 ± 1.09 s; 750-mg food pellet: 41.97 ± 0.89 s), yet varied size (Azuki bean: 135 ± 0.25 mg; 750 mg food pellet: 750 mg), produced equivalent dodge probability and dodge magnitude in a past study (Whishaw and Gorny, 1994). These observations demonstrate that rats were not simply using size to evaluate the hedonic value of the food item. Although the

hedonic value of a food item may be important in the generation of food protection behaviors, the role of a food item's hedonic value in organizing when dodging and bracing behaviors occur remains to be determined.

Second, pharmacological manipulation of the dopaminergic system has also been shown to influence the ability to move (Magour et al., 1974; Heyman, 1983). Amphetamine may have induced a hyperactive state in the dodger, whereas bradykinesia may have resulted from the administration of haloperidol. Therefore, the changes in the organization of food protection behavior may reflect motoric impairment rather than a modification of interval timing. Drug doses were selected that matched those in previous studies that used traditional interval timing procedures and reported no effects on maximal response rates. In addition, the dodger's average speed during dodging and bracing behaviors did not vary as a function of drug condition. Finally, the distance traveled by the dodger during dodging and bracing behaviors did not vary as a function of drug condition. These observations are inconsistent with a movement account of drug effects on the organization of food protection behavior.

Finally, food protection behaviors depend, in part, on the social interaction between the dodger and the robber. Food protection behaviors are elicited when the dodger is approached by the robber. Drug conditions may have produced subtle changes in the dodger's behavior that signaled the conspecific to approach in a different way, thereby influencing the organization of food protection behavior. Observing that the robber's average speed did not vary as a function of drug condition is inconsistent with the robber mediating the organization of food protection behavior or the drug-induced changes.

Several procedural aspects of the current study limit the ability to generalize these results. First, a majority of the previous research on interval timing have used male rats to avoid potential drug interactions with cycling levels of estrogen in female rats. Studies have observed similar effects of dopaminergic drugs on interval timing in female rats (Cevik, 2003). Although sex-typical movement patterns have been observed during dodging behavior (Field et al., 1996), both males and females exhibit similar organization of dodging and bracing food protection behaviors (Whishaw and Tomie, 1987). Therefore, future work is required to determine the extent that the results of the current study would also be observed in male rats. Second, the order of drug administration was not counter-balanced. Although it is unlikely that the organization of food protection behavior fluctuates across days in a pattern consistent with drug effects, future studies employing a between-subjects design investigating chronic administration may provide additional insight to factors influencing these behaviors. Finally, D-amphetamine is relatively nonspecific in its activation of the dopaminergic system. The development of agonists and antagonists specific to D1 and D2 receptors would provide tools that may more clearly fractionate different components of food protection behavior.

The current study examined the effects of amphetamine and haloperidol on the organization of food protection behavior. This study extends previous work reporting the role of time estimation in the organization of food protection behaviors (Whishaw and Gorny, 1994) by demonstrating that

drugs shown to influence interval timing in classic operant procedures (Meck, 1983, 1996) also influence the organization of a natural behavior, such as food protection. The organization of food protection behavior provides a paradigm that is sensitive to the effects of amphetamine and haloperidol on several factors relevant for interval timing. First, the perceived rate of food item consumption depends on the pace of an internal clock. The perceived eating rate is used to estimate the time remaining to consume the food item. Longer estimates of time remaining to consume the food item are associated with dodging, whereas shorter estimates are associated with bracing. Second, attentional processes are related to evaluating the salience of the approaching conspecific or robber. Although amphetamine appeared to influence both clock rate and attentional processes, haloperidol primarily influenced attentional processes. Third, the hedonic value associated with the food item provides motivation for food protection behaviors. Previous studies using various food items and the absence of drug effects on time to consume the food item in the current study do not support a role for the hedonic value of the food item in organizing food protection behavior. Finally, the topographic and kinematic analysis of food protection behaviors used in the current study assessed the role of motor impairments in mediating drug effects. The equivalent speeds and travel distances observed across testing conditions are inconsistent with hyperkinetic or bradykinetic accounts of drugs effects on the organization of food protection behavior. Observations of naturally occurring behaviors during food competition provide a robust paradigm for investigating the neurobiology of processes related to interval timing.

4. Experimental procedures

4.1. Subjects

Seven adult female Long-Evans rats (University of Lethbridge animal vivarium) were used (6 dodgers and 1 robber) in the current study. They were housed in groups in a colony room maintained at 20–21 °C with a normal 12:12 h light/dark cycle. Throughout testing, animals were food deprived to 75–80% of their initial body weights and given supplemental feeding in their home cages as required. Water was provided ad libitum. All experimental procedures in this study were approved by the University of Lethbridge Animal Care Committee, which follows the standards set by the Canadian Council on Animal Care.

4.2. Apparatus

Testing was conducted in a cylindrical container constructed of thin, transparent Plexiglas measuring 45 cm high and 40 cm in diameter. The cylinder was located on a table with a glass top, under which an inclined mirror was mounted. This arrangement allowed filming (Sony DV camcorder) the rats from below (Pinel et al., 1992).

4.3. Drug administration

Fifteen minutes prior to a testing session, each dodger rat was injected intraperitoneally with either saline, D-amphetamine

(1.5 mg/kg), or haloperidol (0.12 mg/kg). Each dodger received all drug conditions across different sessions.

4.4. Procedure

4.4.1. Habituation and training

Prior to testing with drug administration, dodger rats were habituated to the testing apparatus once per day for 2 days. These training sessions lasted 15 min and allowed the rats to explore the cylinder. For days 3 and 4, the rats were provided with several food items (hazelnut, soy bean, 1 g food pellet, and almond) one at a time and were allowed to remain in the cylinder until each food item was eaten. During the following 7 days, rats were habituated to eating individual food items in the cylinder with a conspecific (i.e., robber). These training days were identical to the testing days except that the dodger did not receive injections prior to placement in the cylinder.

4.4.2. Testing

Animals were injected with saline, D-amphetamine, or haloperidol 15 min prior to the testing session. At the start of a testing session, each dodger rat was placed in the cylinder with the robber rat. The dodger was handed a food item with tweezers. Both animals' behaviors were videotaped until the food item was completely eaten. If the robber was successful

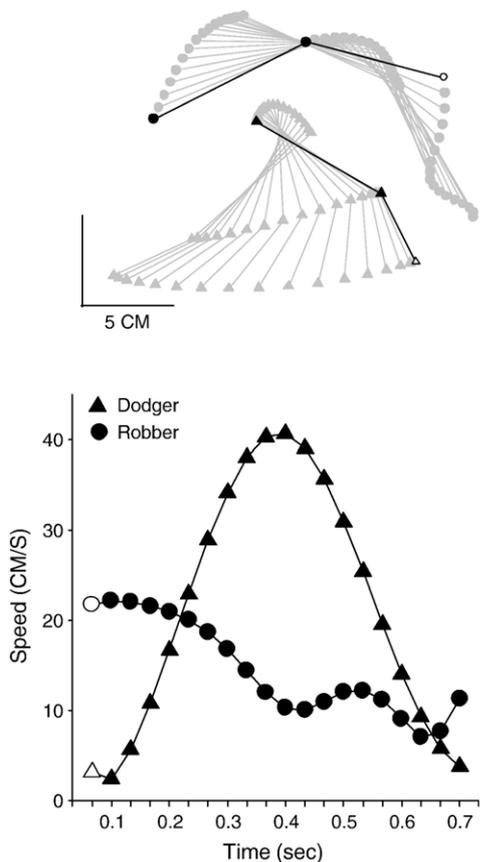


Fig. 4 – Topographic (top) and kinematic (bottom) representations of a single instance of dodging behavior under saline testing conditions are plotted for a dodger (triangles) and robber (circles).

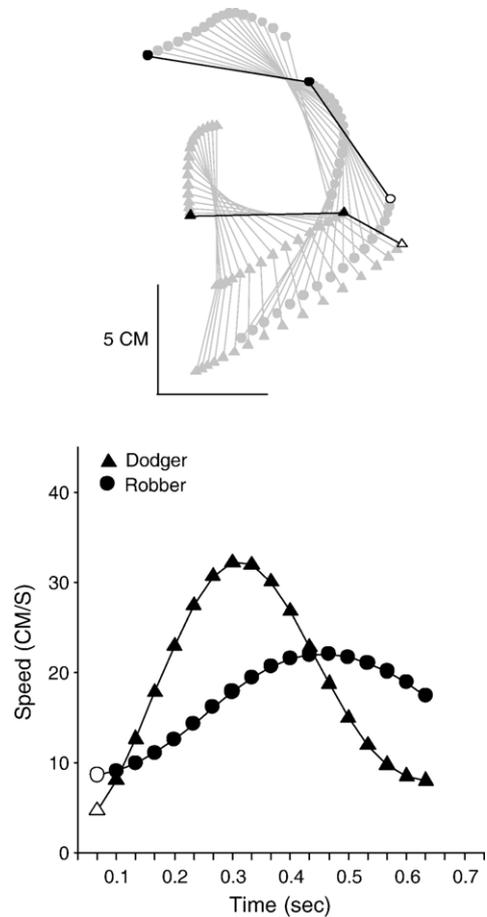


Fig. 5 – Topographic (top) and kinematic (bottom) representations of a single instance of bracing behavior under saline testing conditions are plotted for a dodger (triangles) and robber (circles).

in obtaining the food, the robber was removed from the test apparatus, the dodger was given the food, and the robber was reintroduced.

The order of testing sessions was the same for all rats. On day 1 of testing, the rats were injected with saline. On day 2, amphetamine was injected prior to the testing. Day 3 followed with a saline injection to provide a washout day. On day 4, rats were injected with haloperidol. Subsequent to daily testing, dodging rats were videotaped in the cylinder while consuming each food item in the absence of the robber.

4.5. Analysis of food protection behavior

Observations during testing with the food items revealed that only the hazelnut consistently induced lengthy enough eat times to allow for dodging and bracing behaviors. Short consumption times and dropped food items associated with soy beans, 1 g food pellets, and almonds precluded their inclusion in the current analysis; therefore, only the hazelnut eating sessions were included in the analyses. Each hazelnut session was divided into five sub-sessions, and roughly the first 5 s of each of these sub-sessions was used as samples in

the analysis, although it should be noted that samples with no dodging or bracing behaviors were avoided (i.e., a later portion of the sub-session would be used as the sample). Samples were obtained throughout each hazelnut session such that at least 1 s separated the end of one sample and the beginning of the second sample. The analogue video recordings associated with each sample were converted to a digital file using the Peak Performance motion capturing system (Peak Performance Technologies Inc., Englewood, CO 80112, USA). The system captured individual frames at a sampling rate of 30 Hz. Location and orientation of the dodger's body and the robber's body during each frame of a sample were indicated by tracking three points (tip of the snout, midpoint between the forelimbs, and base of the tail) on each rat. Moment-to-moment speeds and scaled *x*-*y* coordinates were computed from the raw data.

Behaviors occurring during a sample were classified as dodging, bracing, or others. Dodging behavior was defined as any attempts to move away from the robber that involved transferring the food item to the mouth and using the fore- and hindlimbs to move away from the approaching robber (Whishaw, 1988). Topographic and kinematic characteristics associated with a typical dodge are plotted in Fig. 4. Bracing behaviors also involved attempts to evade a robber; however, the dodger did not remove the forelimbs from the food item and only used the hindlimbs to move away from the approaching robber. Fig. 5 presents the topographic and kinematic characteristics associated with bracing behavior. Note that both dodging and bracing occur in similar time frames (<1.0 s). Behaviors not classified as dodging or bracing during the sample were classified as others (e.g., eating or standing).

Several measures were developed to characterize dodging and bracing behaviors that occurred in each sample under saline, amphetamine, and haloperidol testing sessions. First, time required to consume the hazelnut was measured under each testing session while in the presence of the robber. Subsequent to the testing session, time to consume the hazelnut was also measured in the absence of the robber. Second, time spent dodging and bracing was calculated for each sample under saline, amphetamine, and haloperidol testing sessions. Third, average speeds of dodgers and the robber were calculated for each dodge and brace for all samples under the three testing sessions. Fourth, distance traveled by dodgers during each dodge and brace was calculated for each sample under saline, amphetamine, and haloperidol testing sessions. Finally, the distance between the dodger's and robber's heads was calculated at the initiation and termination of each dodging and bracing behavior during samples occurring under each of the testing sessions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.brainres.2006.07.015](https://doi.org/10.1016/j.brainres.2006.07.015).

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