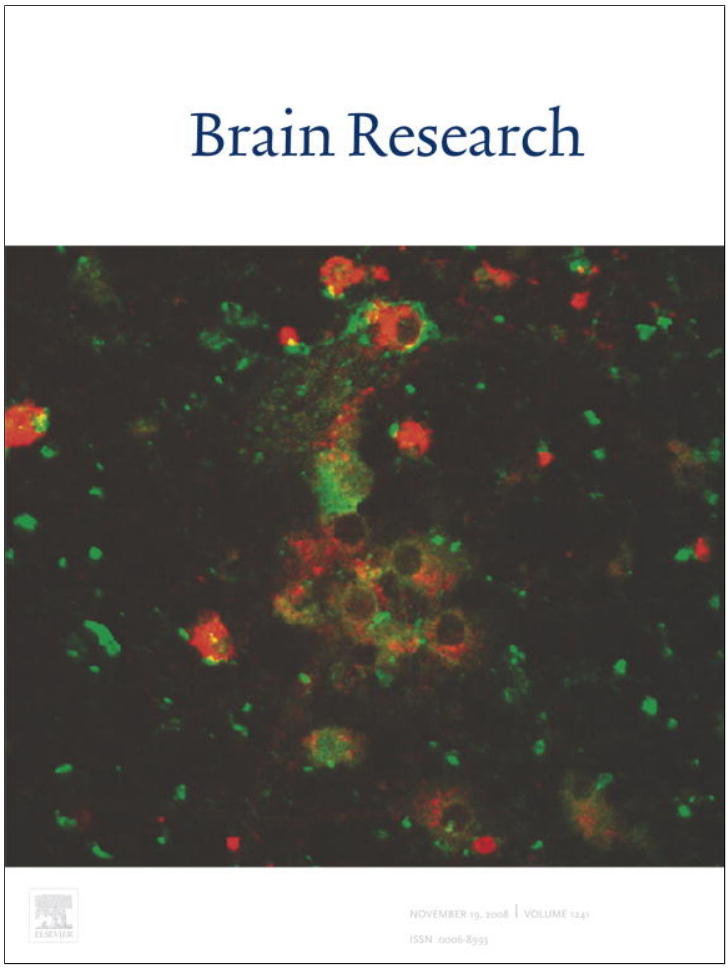

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Research Report

# Organization of food protection behavior is differentially influenced by 192 IgG-saporin lesions of either the medial septum or the nucleus basalis magnocellularis

*Megan M. Martin*[a], *Shawn S. Winter*[a], *Joseph L. Cheatwood*[b,c], *Lynniece A. Carter*[a], *Jeana L. Jones*[a], *Scott L. Weathered*[a], *Steven J. Wagner*[a], *Douglas G. Wallace*[a,*]

[a] *Department of Psychology, Northern Illinois University, De Kalb, IL 60115-2892, USA*
[b] *Department of Neurology, Loyola University, Chicago, IL, USA*
[c] *Research Service, Hines VA Hospital, Hines, IL USA*

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ABSTRACT

Converging lines of evidence have supported a role for the nucleus basalis magnocellularis (NB) in attentional mechanisms; however, debate continues regarding the role of the medial septum in behavior (MS). Recent studies have supported a role for the septohippocampal system in the online processing of internally generated cues. The current study was designed to investigate a possible double dissociation in rat food protection behavior, a natural behavior that has been shown to depend on external and internal sources of information. The study examined the effects of intraparenchymal injections of 192 IgG-saporin into either the MS or NB on the organization of food protection behavior. NB cholinergic lesions reduced the number of successful food protection behaviors while sparing the temporal organization of food protection behavior. In contrast, MS cholinergic lesions disrupted the temporal organization of food protection behavior while sparing the ability to successfully protect food items. These observations are consistent with a double dissociation of NB and MS cholinergic systems' contributions to processing external and internal sources of information and provide further evidence for the septohippocampal system's involvement in processing internally generated cues.



## 1. Introduction

Rats use multiple sources of information to organize their naturally occurring behaviors (Wallace et al., 2008). The organization observed in spontaneously occurring food protection behaviors suggests that rats use external and internally generated cues to organize their movements (Whishaw, Tomie, 1987; Whishaw, 1988; Whishaw, Gorny, 1994). Although the approach of a conspecific during the consumption of a food item will initiate a lateral movement away from that animal, the magnitude of the lateral movement appears to be related to the rat's estimated time to consume the food item (Whishaw, Gorny, 1994). Long consumption times are associated with larger magnitude lateral movements (i.e., dodging behavior) in which the forelimbs are removed from the food item to facilitate locomotion. Short consumption

* *Corresponding author*. Fax: +1 815 753 8088.
E-mail address: dwallace@niu.edu (D.G. Wallace).

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times are associated with smaller magnitude lateral movements (i.e., bracing behavior) in which forelimbs remain in contact with the food item. Recent work has suggested that the transition from dodging behavior to bracing behavior observed during the consumption of a food item is mediated by processes related to interval timing (Wallace et al., 2006). Separate groups of cholinergic neurons within the basal forebrain have been postulated to mediate processing external (e.g., approaching conspecific) and internally generated (e.g., interval timing) sources of information (Dunnett et al., 1991; McGaughy et al., 1996; Martin et al., 2007; Martin, Wallace, 2007).

Basal forebrain cholinergic neurons have been implicated in different cognitive functions (Everitt, Robbins, 1997). The medial septum area (MS) provides the main source of cholinergic inputs to the hippocampus (Amaral and Kurz, 1985) and also sends cholinergic projections to cingulate and entorhinal cortices (Mitchell et al., 1982; Woolf, 1991; Risold, 2004). Initial work demonstrated that nonselective lesion techniques impaired performance on a variety of spatial tasks, thereby supporting a role for the MS in mnemonic processes (Miyamoto et al., 1987; Kelsey, Landry, 1988; M'Harzi and Jarrard, 1992; Hepler et al., 1985; Hagan et al., 1988; Mizumori et al., 1990). Recent studies using the selective immunotoxin 192 IgG-saporin have demonstrated spared performance on spatial tasks despite large reductions in markers of cholinergic function in the hippocampus (Baxter et al., 1995; Baxter, Gallagher 1996; McMahan et al., 1997; Jonasson et al., 2004; Frielingsdorf et al., 2006; Gibbs, Johnson, 2007; however see Berger-Sweeney et al., 1994; Lehmann et al., 2003). Many of the spatial tasks used to examine mnemonic function in these studies provided rats with access to multiple sources of information. Therefore, spared performance may reflect the use of one source of cues to compensate for impaired processing of another set of cues. A recent study has supported this compensatory account of spared performance by examining the effects of selective MS lesions on the use of external and internal cues to guide navigation in the food hoarding paradigm (Martin, Wallace, 2007). Although rats with MS 192 IgG-saporin lesions were accurate in carrying food to a cued or hidden refuge, impairments in returning to the refuge were observed under dark conditions or when the refuge location conflicted with prior experience. These results were consistent with rats' ability to use environmental cues to compensate for impaired processing of internal cue information. The exact contribution of MS cholinergic function to internal cue processing remains to be determined.

In contrast, the nucleus basalis magnocellularis (NB) provides the major source of cholinergic projections to the cortex (Amaral, Kurz, 1985; Woolf, 1991). Similar to early work using nonselective MS lesion techniques, NB lesions have shown impaired performance on a variety of tasks (Santucci, Haroutunian, 1989; Connor et al., 1991). Although these observations suggested a potential role for the NB in mnemonic processes, the combination of more selective lesion techniques and behavior analyses has demonstrated a role for the NB in sustained attention (Dunnett et al., 1991; Ammassari-Teule et al., 1993; McGaughy et al., 1996). For example, both nonselective (Robbins et al., 1989) and selective (McGaughy et al., 2002; Lehmann et al., 2003) NB lesions disrupt performance on the five-choice serial reaction time task. These results have been supported by studies demonstrating an impairment in sustained attention yet spared mnemonic functions following NB lesions in a behavioral vigilance task (McGaughy et al., 1996; Burk, Sarter, 2001; Lehmann et al., 2003). Examining the contribution of the NB cholinergic neurons to food protection behavior organization provides an opportunity to evaluate the extent that the sustained attention account of NB cholinergic function generalizes to a spontaneously occurring natural behavior.

The goal of the current study was to examine whether selective cholinergic lesions of the MS or NB differentially influence the organization of food protection behavior. Considering the evidence for a role of MS cholinergic neurons in processing internal cues, it is possible that 192 IgG-saporin lesions of the MS will disrupt the transition from dodging to bracing observed during the consumption of the food item. Provided that NB cholinergic neurons contribute to responding to the behaviorally relevant environmental stimuli, it can be predicted that 192 IgG-saporin of the NB will disrupt the rats' ability to protect the food item from an approaching conspecific. Observing that these disruptions in food protection behavior are unique to each lesion would demonstrate a double dissociation of function associated with MS and NB cholinergic neurons in the basal forebrain.

## 2. Results

One MS lesion rat was removed from analyses due to a lack of reduction in acetylcholinesterase staining in the hippocampus. In addition, one MS lesion, one MS sham, and two NB lesion rats were excluded from analyses because they failed to engage in food protection behaviors (rats exhibited less than 6 behaviors during a session). Therefore, a total of 65 rats were included in the current study: MS lesion ($n=17$), MS sham ($n=16$), NB lesion ($n=17$), NB sham ($n=15$). Four animals from each group did not receive food protection training and were used to determine the extent that lesions spared GABAergic neurons in the MS and NB.

### 2.1. *Histology*

Hippocampal and cortical acetylcholinesterase (AChE) optical densities obtained from posterior coronal sections were initially analyzed with MS sham and NB sham as separate groups. The ANOVA revealed significant main effects of group [$F(3,45)=12.613$, $p<0.001$], region [$F(1,45)=12.487$, $p=0.001$], and Group × Region interaction [$F(3,45)=56.472$, $p<0.001$]. Although post hoc analyses revealed significant differences (Tukey LSD $p<0.05$) between sham and lesion groups, there were no significant differences observed between MS and NB sham groups. Therefore, for all subsequent analyses, MS sham and NB sham groups were collapsed into a common SHAM group. Brain sections are presented for a representative SHAM, MS, and NB rat (see top panel of Fig. 1). Group average optical density scores are listed for each area sampled in the three coronal sections (see Table 1). All ANOVAs conducted on optical densities obtained from each area sampled revealed a significant main effect of group: cingulate cortex [$F(2,46)=34.90$, $p<0.001$], retrosplenial cortex [$F(2,46)=28.54$, $p<0.001$],

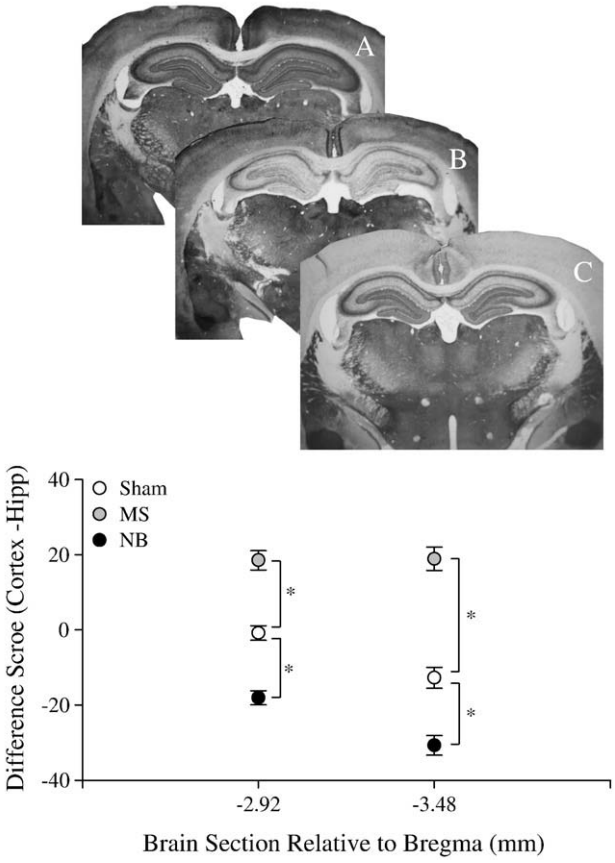


**Fig. 1 – Coronal brain sections are presented for a representative sham (A), MS (B), and NB (C) rat. Each group's average difference in cortical and hippocampal optical density is plotted for anterior and posterior coronal sections (*$p<0.05$).**

anterior dentate gyrus and CA1 [$F(2,46)=49.77$, $p<0.001$], anterior CA2 and CA3 [$F(2,46)=39.51$], posterior dentate gyrus and CA1 [$F(2,46)=42.85$, $p<0.001$], posterior CA2 and CA3 [$F(2,46)=41.37$, $p<0.001$], and medial parietal association cortex [$F(2,46)=15.13$, $p<0.001$]. In general, MS lesions significantly reduced cortical and hippocampal optical densities relative to Sham lesions; whereas NB lesions significantly reduced cortical optical densities relative to Sham lesions (see Table 1 for results of individual post hoc tests). To further characterize the effects of each lesion on cortical and hippocampal cholinergic function, difference scores were computed for cortical and hippocampal (averaged across both areas sampled) AChE optical densities from each animal's two posterior sections (see bottom panel of Fig. 1). The ANOVA conducted on anterior (approximately −2.9 mm relative to bregma) difference scores revealed a significant main effect of group [$F(2,46)=58.24$, $p<0.001$]. The ANOVA conducted on posterior (approximately −3.5 mm relative to bregma) difference scores revealed a significant main effect of group [$F(2,46)=58.60$, $p<0.001$]. At both levels, the MS group had a significantly larger reduction in hippocampal AChE relative to the SHAM group, whereas the NB group had a significant reduction in cortical AChE relative to the SHAM group (Tukey LSD, $p<0.05$). These results are similar to other studies reporting reductions in hippocampal and cortical AChE fiber staining subsequent to immunotoxin 192 IgG-saporin lesions of the MS or NB, respectively (Berger-Sweeney et al., 1994; McGaughy et al., 2002; Lehmann et al., 2003).

Parvalbumin immunohistochemistry demonstrated a minor reduction of parvalbumin-positive GABAergic neurons in the MS (see top panels of Fig. 2) and NB (see bottom panels of Fig. 2) of rats receiving either lesion. These results clearly parallel other studies demonstrating that infusion of the immunotoxin 192 IgG-saporin into the MS or NB has a limited effect on GABAergic neurons in these areas (Berger-Sweeney et al., 1994; McGaughy et al., 2002; Lehmann et al., 2003; Bizon et al., 2003; Dwyer et al., 2007).

### 2.2. *Organization of food protection behavior*

Time to consume the food item in the absence of a conspecific was recorded for each dodger. The ANOVA conducted on time to consume the hazelnut failed to reveal a significant effect of group [$F(2,46)=2.145$, $p=0.129$]. On average, rats spent 122.8 s (SEM: 44.2) consuming the hazelnut.

Total number of food protection behaviors was recorded from the two hazelnut sessions on day six. The ANOVA conducted on total number of food protection behaviors failed to reveal a significant effect of group [$F(2,46)=1.974$, $p=0.151$]. On average, rats exhibited 40.16 (SEM: 18.68) food protection behaviors during the consumption of the last two hazelnut sessions.

**Table 1 – Group average acetylcholinesterase optical densities**

| | Hippocampus (AP: −2.9 mm) | | Hippocampus (AP: −3.5 mm) | | Cg (AP: 2.2 mm) | RS (AP: −2.9 mm) | MPtA (AP: −3.5 mm) |
|---|---|---|---|---|---|---|---|
| | DG | CA | DG | CA | | | |
| Sham | 156.07 (3.87) | 159.52 (3.65) | 149.21 (4.38) | 157.69 (3.90) | 154.10 (3.41) | 156.92 (3.53) | 140.66 (3.51) |
| NB | 147.95 (2.98) | 150.78 (2.03) | 145.01 (1.71) | 150.87 (1.48) | 117.81* (2.66) | 131.25* (1.94) | 117.19* (2.45) |
| MS | 95.02*† (6.29) | 106.94*† (6.46) | 91.44*† (6.25) | 106.28*† (5.57) | 114.34* (5.46) | 119.46* (4.81) | 117.74* (4.23) |

Notes: Group Standard Errors are provided in parentheses; * indicates significant difference between sham ($p<0.05$); † indicates significant difference between lesion groups ($p<0.05$). All anterior–posterior (AP) measurements are relative to bregma. Abbreviations: dentate gyrus (DG); Cornue Ammond (CA); cingulate cortex (Cg); retrosplenial cortex (RS); medial parietal association cortex (MPtA).

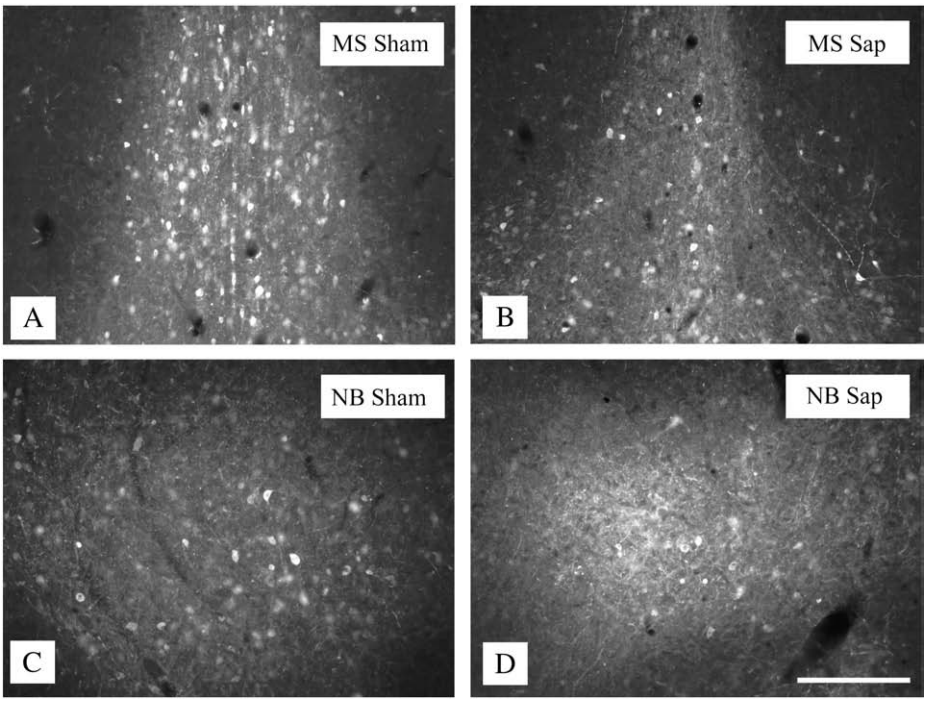


**Fig. 2 – Parvalbumin-positive interneuron cell bodies and processes were visible in the medial septum in both sham (A) and saporin-lesion rats (B). Similarly, parvalbumin-positive neurons were detected in the sham (C) and saporin-lesioned (D) nucleus basalis (scale bar = 100 μm).**

Percent of food protection behaviors in which the dodger successfully protected the food item from theft was calculated from the two hazelnut sessions on day six (see Fig. 3). The ANOVA conducted on percent successful food protection behaviors revealed a significant effect of group [$F(2,46) = 13.178$, $p < 0.001$]. The Sham and MS groups had significantly more successful food protection behaviors relative to the NB group (Tukey LSD, $p < 0.05$). Because theft of the hazelnut occurred just prior to the initiation of a food protection behavior, it could not be determined whether the ability to protect the hazelnut varied between dodging and bracing behaviors. It was observed that the hazelnut was stolen from the NB group during early and late samples; therefore, dodging and bracing behaviors may be equivalent in their ability to protect the food item from theft. All groups engaged in an equivalent number of food protection behaviors; however, the NB group had their hazelnut stolen significantly more often.

The time that dodgers spent engaged in dodging and bracing behaviors was calculated from the hazelnut session with the most food protection behaviors. The session was divided into five equal subsessions. The first 5 s of a subsession that had a food protection behavior (i.e., sample) was captured for analysis. Observing that food protection behaviors did not occur in some subsessions resulted in pooling data into early and late samples (see Fig. 4). The ANOVA conducted on time spent dodging and bracing during early and late samples revealed a significant main effect of behavior [$F(1,46) = 48.107$, $p < 0.001$], Behavior × Sample interaction [$F(1,46) = 8.448$, $p = 0.006$], and Behavior × Sample × Group interaction [$F(2,46) = 7.105$, $p = 0.002$]. Other main effects and interactions were not significant. To further characterize the three-way interaction, separate ANOVAs were conducted for each food protection behavior. The ANOVA conducted on the time each group spent dodging during early and late samples (top left-hand panel of Fig. 4) revealed a significant Sample × Group interaction [$F(2,46) = 4.756$, $p = 0.013$]. Main effects of sample and group were not found to be significant. Although groups spent equivalent amounts of time dodging during early samples, the MS group spent significantly more time dodging relative to either Sham or NB groups during late samples (Tukey LSD, $p < 0.05$). The ANOVA conducted on the time each group spent bracing during early and late samples (top right-hand panel of Fig. 4) revealed a significant main effect of sample [$F(1,46) = 12.420$, $p = 0.001$] and group [$F(2,46) = 4.955$, $p = 0.011$]. The Sample × Group interaction was not found to be significant. All groups displayed a significant

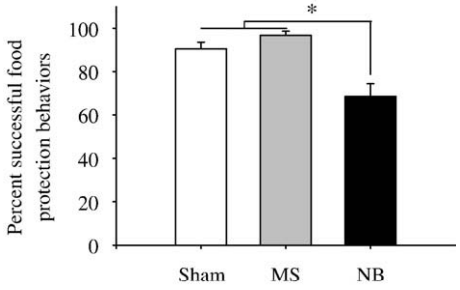


**Fig. 3 – Percent successful food protection behaviors are plotted for each group. Only the NB group displayed a significant reduction in successful food protection behaviors (*$p < 0.05$).**

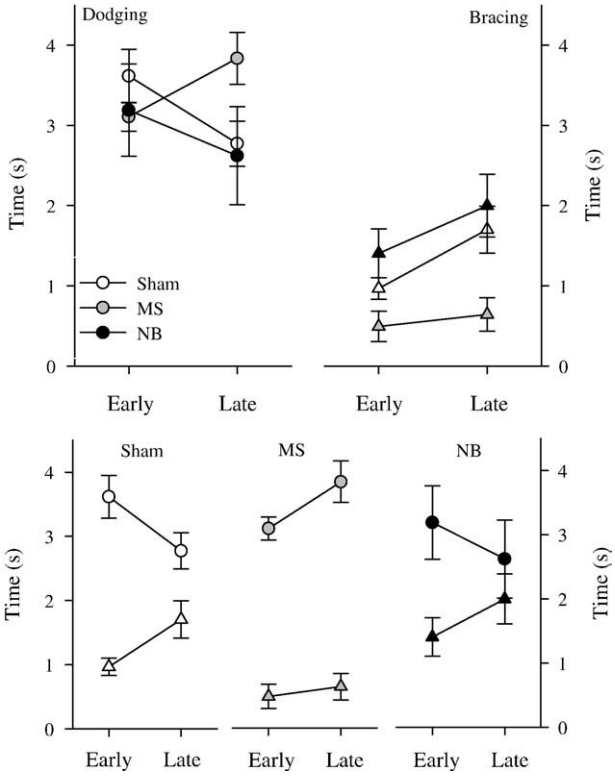


**Fig. 4 – The average time spent dodging during early and late samples is plotted for the Sham, MS, and NB groups (top left-hand panel). The average time spent bracing during early and late samples is plotted to the Sham, MS, and NB groups (right-hand panel). The average time spent dodging (circle) and bracing (triangle) was also plotted for each group (bottom panels). Note: only the MS group exhibited a disruption in the transition from dodging to bracing.**

increase in time spent bracing across samples (Tukey LSD, $p<0.05$). The MS group spent significantly less time bracing across both early and late samples relative to the Sham and NB groups. To further characterize each group's transition between dodging and bracing food protection behaviors, separate ANOVAs were conducted for each group. The ANOVA conducted on the time the SHAM group (bottom left-hand panel of Fig. 4) spent engaged in either type of food protection behavior during early and late samples revealed a significant main effect of behavior [$F(1,22)=26.183$, $p<0.001$] and Behavior×Sample interaction [$F(1,22)=22.121$, $p<0.001$]; however, the main effect of sample was not significant. The ANOVA conducted on the same measures for the MS group (bottom middle panel of Fig. 4) revealed significant main effects of behavior [$F(1,12)=110.361$, $p<0.001$] and sample [$F(1,12)=4.982$, $p=0.045$]; however, the Behavior×Sample interaction was not significant. The ANOVA conducted on the same measures for the NB group (bottom right-hand panel of Fig. 4) only revealed a significant Behavior×Sample interaction [$F(1,12)=5.460$, $p=0.038$]. Together these results are consistent with both Sham and NB groups exhibiting a similar temporal organization of food protection behavior. Specifically, both groups exhibited dodging behaviors early in the consumption of the hazelnut and bracing behaviors late in the consumption of the hazelnut. In contrast, the MS group did not exhibit a similar transition between dodging and bracing food protection behaviors during the consumption of the hazelnut.

### 2.3. *Organization of dodging behavior*

As indicated in the foregoing section, groups spent varying amounts of time engaged in each food protection behavior. To characterize whether food protection behaviors changed across samples, kinematic analysis of dodging behavior was restricted to animals that exhibited dodges in both early and late samples. Given this criteria, three animals in the NB group ($n=10$) were excluded from the analysis.

Several measures were used to characterize dodging behavior during early and late samples. First, Fig. 5 plots average distance traveled (top left-hand panel) during dodging behaviors. The ANOVA conducted on distance traveled failed to reveal a significant effect of group, sample, or Group×Sample interaction. These results demonstrate that groups were equivalent in the distance traveled during early and late samples.

Second, Fig. 5 plots the average speed of the dodger (top right-hand panel) and robber (bottom left-hand panel). The ANOVA conducted on dodger average speed revealed significant main effects of group [$F(2,43)=3.438$, $p=0.041$] and sample [$F(1,43)=11.460$, $p=0.002$]; however, the Sample×Group interaction was not significant. Although all groups displayed a significant decrease in the dodger average speed from the early to late samples, the MS group's average dodger speed

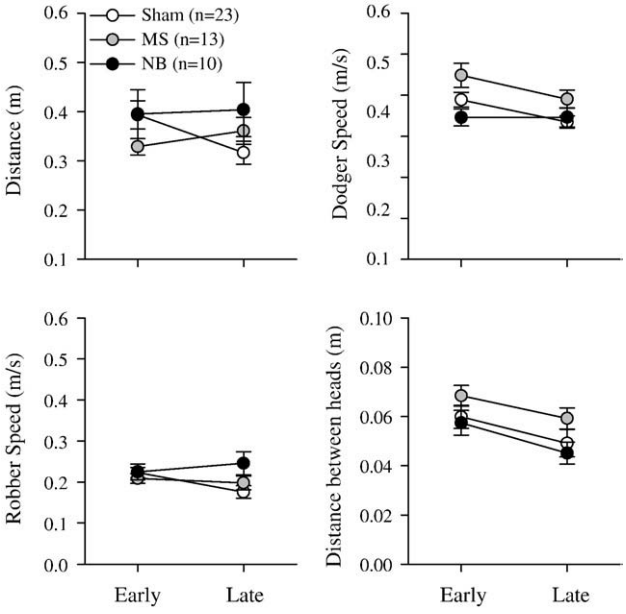


**Fig. 5 – Several measures were used to characterized each group's dodging behavior observed during early and late samples: average distance traveled by the dodger (top left panel), average speed of the dodger (top right panel), average speed of the robber (bottom left panel), and average distance between heads at the start of a dodge (bottom right panel). Note: the number of subjects reflects the rats that exhibited dodging behavior in both samples.**

was significantly faster relative to the other groups (Tukey LSD, $p<0.05$). The ANOVA conducted on the robbers' average speed revealed a significant Sample×Group interaction [$F(2,43)=3.273$, $p=0.048$]. To further characterize the significant interaction, simple effects analyses were conducted for early and late samples. The ANOVA conducted on robber speeds during early samples failed to reveal a significant effect of group, whereas the ANOVA conducted on late samples revealed a marginally significant effect of group [$F(2,43)=3.158$, $p=0.053$]. This latter effect appears to depend on the tendency of the robbers, when paired with NB rats, to travel at slightly faster speeds.

Finally, Fig. 5 plots the average distance between dodger and robber heads (bottom right-hand panel) at the initiation of the food protection behavior during early and late samples. The ANOVA conducted on the distance between heads revealed a significant main effect of sample [$F(1,43)=6.093$, $p=0.018$]; however, the main effect of group and the Group×Sample interaction were not significant. The distance between heads at the initiation of a dodge decreased from early to late samples.

### 2.4. *Organization of bracing behavior*

Similar to the previous section, kinematic analysis of bracing behavior was restricted to rats that exhibited this behavior in both early and late samples. This criteria resulted in four rats excluded from the SHAM group ($n=19$), seven rats excluded from the MS group ($n=6$), and two rats excluded from the NB group ($n=11$).

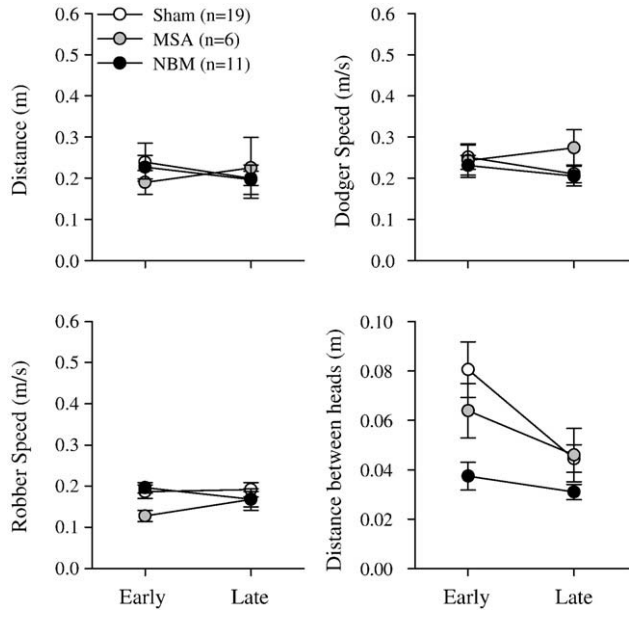


**Fig. 6 – Several measures were used to characterized each group's bracing behavior observed during early and late samples: average distance traveled by the dodger (top left panel), average speed of the dodger (top right panel), average speed of the robber (bottom left panel), and average distance between heads at the start of a dodge (bottom right panel). Note: the number of subjects reflects the rats that exhibited bracing behavior in both samples.**

Average dodger distance traveled during bracing behavior is plotted in the top left-hand panel of Fig. 6. The ANOVA conducted on distance traveled failed to reveal significant effects of group, sample, or Group×Sample interaction. Groups traveled equivalent distances during bracing behaviors independent of sample.

Average speed of the dodger during bracing behavior is plotted in the top right-hand panel of Fig. 6. The ANOVA conducted on the dodgers' average speeds failed to reveal significant effects of group, sample, or Group×Sample interaction. Average speeds of the dodger during bracing behavior did not vary as a function of group or sample. The average speed of the robber during bracing behavior is plotted in the bottom left-hand panel of Fig. 6. The ANOVA conducted on the robbers' average speeds failed to reveal significant effects of group, sample, or Group×Sample interaction. Average speeds of the robber during bracing behavior did not vary as a function of group or sample.

Average distance between the heads of the dodger and robber at the initiation of bracing behavior is plotted in the bottom right-hand panel of Fig. 6. The ANOVA conducted on the average distance between heads revealed significant main effects of group [$F(2,33)=3.960$, $p=0.029$] and sample [$F(1,33)=10.368$, $p=0.003$]; however, the Group×Sample interaction was not significant. The distance between the heads at the initiation of a bracing behavior decreased from early to late samples, with the NB group exhibiting a shorter distance between heads relative to the sham group (Tukey LSD, $p<0.05$).

## 3. Discussion

The current study examined the effects of injecting the immunotoxin 192 IgG-saporin into NB or MS on the organization of food protection behavior. NB lesions impaired the rats' ability to protect the food item from theft, while sparing the transition between food protection behaviors. These observations are consistent with other studies demonstrating a role for NB cholinergic neurons in the ability to sustain or shift attention (McGaughy et al., 1996; McGaughy et al., 2002; Lehmann et al., 2003). In contrast, MS lesions spared the rats' ability to protect the hazelnut from theft; however, rats failed to exhibit the transition between food protection behaviors during the consumption of the hazelnut. This failure to transition between food protection behaviors may reflect impaired processing of temporal information during the consumption of the hazelnut. The following sections consider the evidence that supports and conflicts with these interpretations of the double dissociation observed in the organization of food protection behavior associated with cholinergic neurons originating from the NB and MS.

### 3.1. *Interval timing and food protection behavior*

Interval timing, or the ability to estimate time in the seconds-to-minutes range, is a major source of information that animals use to organize their behaviors (Buhusi, Meck, 2005). For example, in the peak procedure (Roberts, 1981) cues are paired with different fixed-interval (FI) schedules. A tone may

signal a FI-20 schedule (first response after 20 s is reinforced); whereas, a light may signal a FI-40 schedule (first response after 40 s is reinforced). Occasionally probe trials are given in which a cue is presented for a longer duration without delivering the reinforcement. Response rate systematically varies across the probe trial: response rates symmetrically increase and decreases with the peak occurring at the time reinforcement would be delivered (i.e., 20 s or 40 s). The ability to systematically vary response rate during a probe trial has been suggested to depend on the animals' ability to estimate time. Three distinct stages of information processing have been posited to mediate interval timing (Gibbon et al., 1984; Matell, Meck, 2000). First, the clock stage involves the accumulation of pulses from a pacemaker that is initiated by the onset of a stimulus. Next, the memory stage involves encoding the number of pulses associated with the duration of the stimulus. Finally, the decision stage reflects the online comparison of the number of pulses accumulated from the clock stage to the number of pulses represented in the memory stage. Provided that the two values are similar enough, a response is elicited. Although this theory of interval timing has been based on results obtained from studies using operant conditioning procedures, recent work has suggested it may have more general implications for how rats organize their naturally occurring behaviors (Matell et al., 2006; Wallace et al., 2006). Administration of a dopaminergic agonist has been shown to produce a leftward shift in the peak response time without changing the maximum response rate (Buhusi, Meck, 2002). This selective change in performance has been attributed to an increase in the rate at which subjective time passes, or a faster accumulation of pulses in the clock stage. Administration of a dopaminergic agonist has also been shown to increase the time spent dodging during consumption of a hazelnut (Wallace et al., 2006). These authors posit that the dodger continuously monitors the rate at which the hazelnut is being consumed. The rate of hazelnut consumption reflects amount of food consumed per temporal interval (e.g., 1000 mg/60 s=16.67 mg/s). Pharmacological manipulations that increase the accumulation of pulses from the pacemaker would result in a slower perceived rate of consumption (1000 mg/80 s=12.5 mg/s). A slower perceived rate of consumption would translate into longer estimated times to consume the hazelnut and an increased likelihood of engaging in dodging food protection behavior. This interpretation is further supported by observing that dopaminergic antagonists slow the passage of subjective time in the peak-interval procedure and reduce the time spent dodging during the consumption of a hazelnut (Meck, 1983; Maricq, Church, 1983; Buhusi, Meck, 2002; Wallace et al., 2006). These results are consistent with a role for interval timing in the organization of food protection behavior that may be mediated by the dopaminergic system.

Results of the current study provide further evidence that interval timing processes contribute to the organization of food protection behavior. Previous research has demonstrated that rats can stop and start interval timing processes. Specifically, the peak-interval procedure can be modified such that the signal is temporally removed during select trials. On these GAP trials, peak times are shifted to the right by the length of time the stimulus was absent, consistent with stop and start timing processes during the GAP. NB lesions have been shown to spare performance on these GAP trials; however, MS lesions have been shown to produce rightward shifts in peak times equivalent to the interval of time when the signal was present prior to removal and the interval of time when the signal was absent (Meck et al., 1987). The performance associated with MS lesions was consistent with resetting rather than stopping interval timing processes when the signal was removed. This ability to stop and start interval timing processes may contribute to the transition between food protection behaviors. During the consumption of the hazelnut, dodgers are periodically interrupted by an approaching robber. The type of food protection behavior elicited depends on the rat's estimated time to consume the hazelnut. Deriving the estimated time to eat the hazelnut depends on a perceived rate of eating, a representation of time spent eating the current hazelnut, and a memory of the amount of time required to eat a typical hazelnut. It is posited that under normal conditions the dodger is continuously updating the time spent eating until the robber approaches. At the initiation of a food protection behavior, the dodger stops updating the time spent eating and evaluates the time to finish eating the hazelnut. Deriving the time to finish eating depends on comparing the current time spent eating to the memory of the time required to eat a typical hazelnut. As the time spent eating the current hazelnut approaches the time associated with consuming an entire hazelnut, bracing behavior becomes more frequent. Subsequent to the food protection behavior, the dodger returns to updating the time spent eating the current hazelnut. Provided that each food protection behavior is similar to a GAP in the peak-interval trial, the failure of the MS group to exhibit the transition between dodging and bracing may reflect a failure to resume updating the time spent eating the current hazelnut after a food protection behavior has been elicited. Resetting the representation of time spent eating the hazelnut after each food protection behavior would necessitate generating a new estimate of time to consume the hazelnut based entirely on the current consumption rate. Considering that perceived consumption rate should be relatively consistent, estimated times to consume the hazelnut would likely be longer than usual. The observation that the MS group spent significantly more time engaged in dodging behavior, rather than bracing behavior, also supports this interpretation. The possibility that disruptions in food protection behavior are mediated by impairments associated with other interval timing processes will be considered next.

Another modification of the peak-interval procedure has demonstrated that rats can simultaneously time two intervals (Olton et al., 1988). In this procedure, two signals became associated with different fixed-interval schedules (i.e., FI-10 s or FI-20 s). On probe trials, the duration of each signal was extended and was presented either alone or in combination with the other signal. During combined trials, rats displayed two peak response rates associated with the peak time of each signal. MS lesions have been shown to spare performance on single and compound trials; however, NB lesions disrupt performance only on compound trials. The disruption in performance during compound trials was attributed to impaired attentional mechanisms. Studies using the more

selective immunotoxin 192 IgG-saporin technique have also provided evidence to support a role for the NB cholinergic neurons in mediating attentional mechanisms (McGaughy et al., 1996; McGaughy et al., 2002; Lehmann et al., 2003). A disruption in the rat's ability to detect an approaching robber would make the dodger more susceptible to theft of the hazelnut. Interestingly, the NB group also displayed a small yet significant decrease in the distance between heads at the initiation of bracing food protection behaviors. This significant decrease may have afforded the robber a better position to steal the hazelnut. These impairments in protecting the hazelnut from theft were not observed in rats with MS lesions, consistent with sparing of attentional mechanisms. These observations provide further evidence for the dissociation of the roles of the MS and NB in interval timing processes that mediate the organization of food protection behavior.

Studies have consistently shown that NB and MS lesions differentially influence the location of the peak time in the peak-interval procedure. NB lesions produce a rightward shift in peak times; whereas, MS lesions produce a leftward shift in peak times (Meck et al., 1987; Olton et al., 1988). This pattern of responding was attributed to a modification of the remembered time of reinforcement. NB lesions gradually increase the remembered time of reinforcement; whereas, MS lesions gradually decrease the remembered time of reinforcement. Modifications in the remembered duration to consume a hazelnut would be expected to influence the rats' ability to estimate the time remaining to eat the hazelnut; however, symmetrically opposite shifts in time spent engaged in food protection behaviors were not observed. There are several possible explanations for the failure of the present study to observe this dissociation between NB and MS lesions. First, characterization of food protection behavior organization did not extend over multiple days. The gradual shifts in peak speeds associated with NB and MS lesions occurred over the course of multiple training sessions (Meck et al., 1987). Future work examining the effects of NB and MS lesions on the organization of food protection behavior over multiple sessions may provide further insight to the role of these systems for mnemonic processes related to interval timing. Next, the current study employed lesion techniques shown to be more selective for cholinergic neurons than previously used in the interval timing literature. It is possible that sparing GABAergic neurons may have been sufficient for accurate encoding of the remembered duration to consume a hazelnut. Further research examining the effects of lesion techniques selective for GABAergic neurons on the organization of food protection behavior may further dissociate the functions of the NB and MS. The current study provides additional evidence that the organization of food protection behavior depends on processes related to interval timing and demonstrates a role for basal forebrain cholinergic neurons in these processes.

### 3.2. *Neurobiology of food protection behavior*

Previous work has demonstrated a role for cortical and hippocampal function in the success of food protection behavior. For example, hemidecortication has been shown to significantly decrease successful food protection behavior restricted to the side contralateral to the damage (Whishaw, Tomie, 1988). In addition, both transection of the fimbria–fornix (Oddie et al., 2002) or infusion of a cholinergic antagonist into the MS (Oddie et al., 1997) significantly increased the number of food items stolen from the rat. The results of the current study demonstrate that qualitatively different impairments were observed with selective cholinergic lesions of the NB and MS. The impaired ability to protect food items associated with NB lesions is consistent with previous research demonstrating a role for the cortex in protecting food items from theft. It is likely that the selectivity of the 192 IgG-saporin lesion technique was a critical factor in the spared ability to protect food items observed in rats with MS lesions. To be specific, relative to fimbria–fornix lesions, the immunotoxin 192 IgG-saporin spares GABAergic neurons in the MS. A subpopulation of these GABAergic neurons synapses on hippocampal inhibitory interneurons (Tóth et al., 1997). It is possible that the disinhibition of hippocampal pyramidal neurons associated with MS GABAergic neuronal activity is necessary for successful food protection behavior, yet it is not sufficient to mediate the transition between dodging to bracing behaviors. It is also possible that spared GABAergic neurons in the MS may have been sufficient for the development of compensatory mechanisms. For example, the MS group exhibited significantly faster average dodger speeds during dodging food protection behavior relative to the other groups. This change in movement organization may have facilitated the protection of the food item. Either of these interpretations appears to conflict with the previous work demonstrating that cholinergic antagonists (e.g., atropine) infused directly into the MS significantly disrupt a rat's ability to protect food items (Oddie et al., 1997). However, recent work has demonstrated that cholinergic agonists and antagonists modulate the activity of the septohippocampal GABAergic pathway rather than the cholinergic pathway (Wu et al., 2000; Alreja et al., 2000; Wu et al., 2003). Therefore, impairments in protecting food items associated with fimbria–fornix transection or infusion of cholinergic antagonists into the MS may be related to disruption of the septohippocampal GABAergic system. Although this accounts for the spared ability to protect food items from theft observed in rats with 192 IgG-saporin lesions of the MS, further work is needed to determine whether GABAergic MS and NB projections differentially contribute to the organization of food protection behavior.

### 3.3. *Contribution of motivational, motoric, and social factors to food protection behavior*

Although processing external and internal sources of information contributes to the organization of food protection behavior, this organization also depends on the functioning of other systems. Several measures were developed to evaluate the extent to which group differences may have been mediated by a more general disruption in food protection behavior. First, the dodger has to be motivated to consume the hazelnut. Groups did not differ in the amount of time required to consume the hazelnut in the absence of the robber. Although this observation is consistent with groups being equivalent in the motivation to consume the hazelnut, this measure may not be able to detect subtle changes in

motivation. For example, the impaired ability to protect the hazelnut associated with NB lesions may reflect a subtle modification of motivational factors; however, other work has demonstrated that impaired performance on the five-choice serial reaction time task associated with NB lesions was reversed by varying attentional demands of the task (Lehmann et al., 2003). These observations are consistent with NB lesions producing an attentional deficit rather than a motivational deficit. Future work examining the effects of varying attentional and motivational factors on the organization of food protection behavior may provide a better understanding of the deficits associated with MS and NB lesions.

Second, impaired motor coordination (e.g., handling the food item) may have contributed to the reduction in successful food protection behaviors observed in the NB group. Several lines of evidence discount this as a mediating factor. First, equivalent dodging and bracing behavior kinematics (i.e., distance traveled) were observed across groups. Next, success of protecting the hazelnut from theft was independent of observing the transition between dodging and bracing food protection behaviors. Finally, 192 IgG-saporin lesions of the MS or NB have been shown to spare performance on a variety of tasks that require some level of motor coordination (Wrenn, Lappi, Wiley, 1999; Lehmann et al., 2003). MS and NB lesions appear to spare motor coordination; however, an increase in dodging speed was observed in rats receiving MS lesions. This increase in speed during dodging behavior may have contributed to the MS rats' spared ability to protect the food item from theft. Considering that cortical and subcortical motor systems have been implicated in the organization of food protection behavior (Whishaw, Tomie, 1988), additional work should examine whether these systems mediate the compensation associated with MS lesions.

Finally, food protection behavior depends on social interaction; the robber has to approach the dodger to elicit this behavior. A similar number of food protection behaviors were observed across groups. Although this observation is consistent with social interactions not varying across groups, subtle differences were observed. Robbers were closer to the heads of dodgers with NB lesions at the initiation of bracing behaviors. Considering the extensive literature reporting impaired attentional function with these NB lesions, this difference in distance between heads was attributed to impaired attentional function. It is possible that the robber could have learned that NB rats were poor at protecting the hazelnut and modified their approach to gain better access to the hazelnut. In addition, robbers were marginally faster during late dodging samples when paired with an NB rat. Future work is needed to determine the extent that a robber can adapt its approach based on the nature of a conspecific's impairment.

### 3.4. *Role of basal forebrain cholinergic systems in spatial orientation*

The observed disruption in the MS group's ability to transition from dodging to bracing behaviors may be related to spatial disorientation associated with lesions of the septohippocampal cholinergic system. Rats use environmental (i.e., visual, auditory, olfactory) and internally generated or self-movement (i.e., vestibular, proprioceptive, efferent copies of motor commands) cues to maintain spatial orientation. Immunotoxic lesions of the medial septum have been shown to spare performance on tasks in which rats can use environmental cues to guide navigation (Baxter, Gallagher 1996; McMahan et al., 1997; Cahill and Baxter, 2001; Frielingsdorf et al., 2006); whereas, impaired performance has been observed on tasks in which rats were restricted to using trial unique self-movement cues to guide navigation (Martin, Wallace, 2007). These results are consistent with a growing literature positing a role for the hippocampal formation in the online processing of self-movement cues to maintain spatial orientation (Maaswinkel et al., 1999; Wallace, Whishaw, 2003; Martin et al., 2007; for a review see Wallace et al., 2008). In addition, the MS also sends cholinergic projections to the cingulate and entorhinal cortices (Mitchell et al., 1982; Woolf, 1991; Risold, 2004). Damage to either of these areas has also been shown to impair performance on tasks developed to assess trial unique self-movement cues to guide navigation (Whishaw et al., 2001; Parron, Save, 2004). Online processing of self-movement cues depends on evaluating information in the appropriate temporal context (Barlow, 1964; Gallistel, 1990). For example, deriving an estimate of distance traveled from self-movement cues depends at least on detection of linear acceleration and some representation of the temporal context the movement occurred in. Disruptions in the ability to continuously update this temporal context (i.e., resetting rather than stopping and starting interval timing processes) may result in errors in estimating travel distance and result in spatial disorientation under conditions when animals are restricted to using self-movement cues to guide navigation.

### 3.5. *Selectivity of the 192 IgG-saporin lesion technique*

The current study used the immunotoxin 192 IgG-saporin to selectively damage cholinergic neurons in the MS and NB. Several aspects of the histological data are relevant for interpreting the behavioral dissociation observed between MS and NB lesions. MS lesions produced significant reductions in AChE fiber staining in both hippocampus and cortical areas; whereas, NB lesions reduced AChE fiber staining restricted to cortical areas (see Table 1). Observing that both lesions reduced cortical AChE fiber staining suggests that the origin of cholinergic projections and not total cortical cholinergic tone appears to be critical for protection of the hazelnut from theft. This interpretation depends on selectivity of the lesion technique. Although 192 IgG-saporin lesions were shown to spare GABAergic neurons in the MS and NB, the observed reductions in parvalbumin-positive GABAergic neurons parallel reports from other studies using similar lesion techniques (Berger-Sweeney et al., 1994; McGaughy et al., 2002; Lehmann et al., 2003; Bizon et al., 2003; Dwyer et al., 2007). It is possible that the observed reductions in GABAergic neurons may have also contributed to the disruption in the organization of food protection behavior associated with MS and NB lesions. For example, recent work using lesion techniques that target GABAergic neurons (while producing minimal damage to cholinergic neurons) in the MS disrupt performance on spatial tasks (Smith Pang, 2005; Dwyer et al., 2007). The extent that performance in these spatial tasks depends on processing internally generated cues remains to be determined. Future

work examining the effects of selective GABAergic lesions on the organization of food protection behavior may better characterize the role of the MS and NB in processing different sources of information.

### 3.6. Conclusion

The current study demonstrates that neurons in the NB and MS differentially contribute to the organization of food protection behavior. NB neurons appear to mediate attentional processes related to detection of the approaching robber. MS neurons, on the other hand, appear to be involved in making the transition from dodging behavior to bracing behavior during the consumption of a food item. This disruption in food protection behavior organization may reflect an impaired ability to maintain an online representation of the time elapsed since initiation of food item consumption. Therefore, performance on other tasks that depend on online processing of temporal information should also be sensitive to cholinergic MS lesions. For example, dead reckoning based navigation depends on processing self-movement cues in the appropriate temporal context to estimate the direction and distance to the point where movement was initiated (Barlow, 1964; Mittelstaedt, Mittelstaedt, 1980; Etienne, 1980; Gallistel, 1990; Etienne and Jeffery, 2004; Wallace et al., 2008). Interestingly, a growing number of studies have demonstrated a role for the septohippocampal system in dead reckoning based navigation (Maaswinkel et al., 1999; Wallace, Whishaw, 2003; Martin et al., 2007; Martin, Wallace, 2007).

The current study also demonstrates that kinematic analysis of naturally occurring behaviors is a robust paradigm for investigating the neurobiology of cognition. First, multiple processes or mechanisms can be evaluated with food protection behavior. This provides the opportunity to examine whether information processing systems interact with each other or operate in parallel. For example, the current study demonstrated that the processes related to external and internal information appear to operate in parallel. Second, food protection behaviors spontaneously occur with limited exposure to the testing apparatus. This minimizes the dependence on intact implicit learning systems. Finally, the multivariate nature of food protection behavior is relevant for the development and evaluation of novel therapies for neurodegenerative disorders. Improved performance associated with drug treatments may reflect restitution of function or compensatory mechanisms. The molecular description of food protection behavior outlined in the current study has the potential to dissociate these mechanism(s) in which a cognitive enhancer may be influencing performance.

## 4. Experimental procedures

### 4.1. Subjects

Seventy-eight female (90-day old) Long–Evans hooded rats (*Rattus norvegicus*) bred at Northern Illinois University from stock purchased at Harlan Sprague–Dawley were used in the current study. Sixty-two rats were used to examine the effects of MS or NB lesions on food protection behavior. Of these, eight rats did not receive any surgical treatment and served as conspecifics (i.e., robbers) for the other 54 rats (dodgers) that attempted to protect their food item from theft. An additional 16 that did not receive any behavioral testing were included to examine the selectivity of the lesion technique on GABAergic neurons in MS and NB. All rats were housed in groups of two in plastic cages in a colony room maintained at 20–21 °C with a 12/12 h light/dark cycle. Throughout testing, animals' food availability was restricted to maintain them at 80% of their initial body weights. Water was provided ad libitum. All experimental procedures in this study were approved by the local Institutional Animal Care and Use Committee (IACUC), which follows the standards set by the Office of Laboratory Animal Welfare.

### 4.2. Surgery

Rats were deeply anesthetized with a mixture of isoflurane and oxygen throughout surgery. Cholinergic lesions of the basal forebrain were produced by injecting 192 IgG-saporin (0.50 μg/μL; Lot#: 41-105; Advanced Targeting Systems, San Diego, CA) at a rate of 0.20 μL per minute. MS lesions ($n=19$) were produced by injecting 192 IgG-saporin at the following sites relative to bregma and the surface of the dura: AP: +0.30, ML: ±0.20, DV1: −7.5 [0.40 μL each site], DV2: −6.5 [0.30 μL each site]. NB lesions ($n=19$) were produced by injecting 192 IgG-saporin at the following sites relative to bregma and the surface of the dura: AP: −0.75, ML1: ±3.3, DV1: −8.1 [0.40 μL each site], ML2: ±2.3, DV2: −7.8 [0.40 μL each site]. After each injection, the cannula was left in place for 3 min to prevent diffusion up the needle tract. These parameters were selected in light of pilot work and previous studies demonstrating a sparing of GABAergic cells (Berger-Sweeney et al., 1994; McGaughy et al., 2002; Lehmann et al., 2003). Animals receiving MS sham ($n=17$) and NB sham ($n=15$) surgeries were treated identically except that Dulbecco's phosphate buffered saline was injected instead of 192 IgG-saporin.

Four rats from each group did not experience the food protection training. These rats were used to examine the selectivity of the lesion techniques for GABAergic neurons in the MS and NB.

### 4.3. Apparatus

Rats were tested in a transparent Plexiglas cylinder measuring 45 cm high and 44 cm in diameter. The cylinder was located on a table with a transparent top. A mirror was positioned at an angle under the top of the table such that the rats could be filmed from below (Pinel et al., 1992).

### 4.4. Procedure

Rats were habituated to consuming the hazelnut in the testing apparatus and in the presence of a conspecific over 5 days. On day one, dodgers were individually placed in the cylinder followed by the introduction of a conspecific (i.e., robber). At the end of the 15-minute session, both rats were removed. On day two, only dodgers were placed in the cylinder, and tweezers were used to administer the hazelnuts. On days

three through five, the dodger was given two hazelnut sessions per day. A hazelnut session involved consumption of the hazelnut in the cylinder with the robber present. If the robber was successful in obtaining the hazelnut from the dodger, then the robber was removed from the cylinder, the food item was removed from the robber's mouth, the food item was given to the dodger, and the robber was placed back in the cylinder. On day six, dodgers were videotaped during two hazelnut sessions and videotaped while eating a hazelnut in the absence of the robber.

### 4.5. *Analysis of food protection behaviors*

#### 4.5.1. *Organization of food protection behavior*

Several measures were used to evaluate whether groups differed in the general characteristics of food protection behavior. First, time to consume the hazelnut was recorded in the absence of the robber. Second, the total number of elicited food protection behaviors was recorded during the two hazelnut sessions on day six. Finally, the percent of successful food protection behaviors was calculated based on the number of food protection behaviors that resulted in the dodger retaining the hazelnut relative to the total number of attempted robberies. The pattern of group differences across these measures was used to determine the extent that modified hedonic, motoric, social, or attentional factors mediated disruptions in food protection behaviors.

#### 4.5.2. *Organization of dodging and bracing behaviors*

The hazelnut session on day six with the most food protection behaviors was selected for further analyses. This increased the likelihood that food protection behaviors would be observed throughout the consumption of the hazelnut. To ensure quasi random sampling of food protection behaviors during the consumption of the hazelnut, each hazelnut session was divided into five equal subsessions, and the first 5 s of each subsession (i.e., sample) was captured for topographic and kinematic analysis. If no food protection behaviors occurred during this time, then a later segment of the sample was used for analysis. Occasionally, food protection behaviors did not occur during an entire subsession. Rather than eliminate a dodger's data from the analyses, data were pooled into early and late samples. Samples that occurred in the first half of the hazelnut session were pooled and categorized as the early sample, whereas samples occurring in the second half were pooled and categorized as the late sample. The analogue video associated with each sample was converted to a digital file by the Peak Performance (Peak Performance Technologies Inc., Englewood, CO 80112, USA) system at a 30 Hz sampling rate. The orientation of the dodger's body and robber's body during

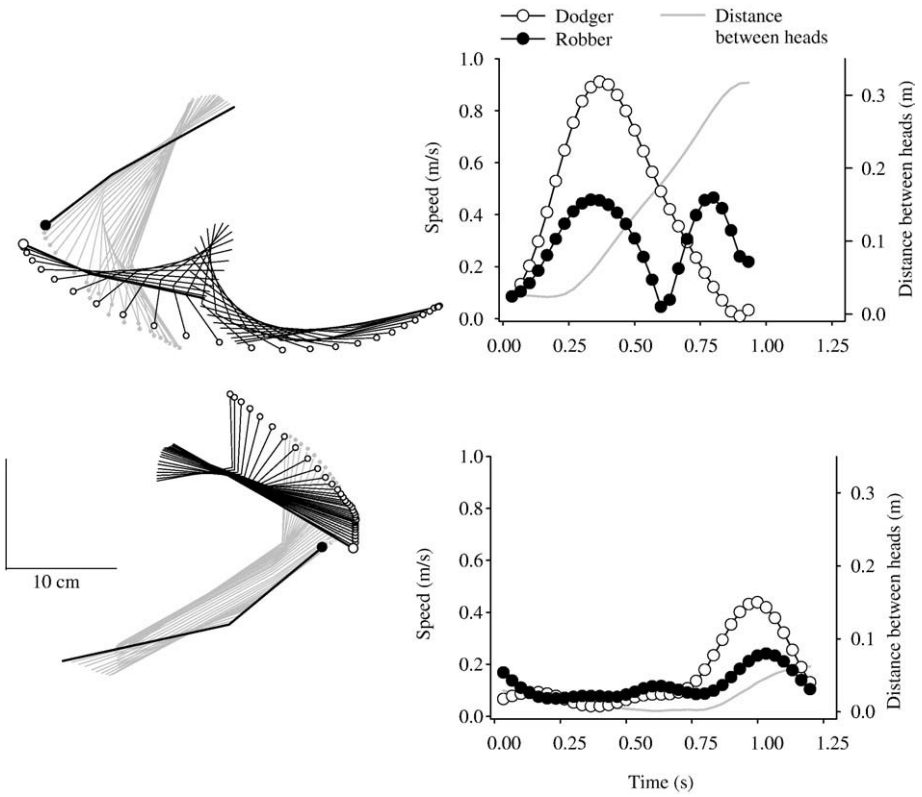


**Fig. 7 – The topography of dodging (top panels) and bracing (bottom panels) food protection behaviors are plotted for a representative dodger (white circles) and robber (black/grey circles). Each rat's initial position of the nose and body is indicated by a large circle and bold line, respectively. Subsequent positions are represented by smaller circles and lighter weight lines. The moment-to-moment speeds and distance between heads are plotted for each rat's head during a dodge and brace. Dodging is characterized as a dramatic increase in speed and distance between heads. Bracing is slower behavior resulting in a smaller distance between heads.**

each frame of the sample was recorded by digitizing the location of the nose, midpoint between the forelimbs, and the base of the tail for both rats. Food protection behaviors were classified as a dodge, a brace, or other (eating, walking, rearing, etc.). Dodging behavior was defined as any attempt to escape the robber that involved transferring the food item to the mouth while using both fore- and hind limbs to move away from the approaching robber (see top panels of Fig. 7). Bracing behavior was also defined as any behavior that involved attempting to escape the robber; however, forelimbs were not removed from the food item during bracing (see bottom panels of Fig. 7). The Peak Performance system used the digitized raw data to calculate instantaneous speeds and scaled *x*–*y* coordinates for each point. Additional measures were used to further characterize successful dodging and bracing behaviors that occurred in early and late samples. First, the time spent engaged in dodging or bracing behaviors was calculated for early and late samples. Second, average distance traveled during dodging or bracing behaviors was calculated for early and late samples. Third, average speeds of the dodger and robber were calculated for both dodging and bracing behaviors during early and late samples. Finally, the average distance between the heads of the dodger and robber prior to the initiation of dodges and braces was calculated for early and late samples.

### 4.6. *Histology*

#### 4.6.1. *Acetylcholinesterase stain*

Subsequent to behavioral testing, rats were deeply sedated and perfused with phosphate buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffered picric acid. Brains were removed and stored in the same solution for two days at 4 °C. Brains were cut into 40 μm sections using a vibrotome, and every third section was mounted on chromalum subbed slides. Slides were stained for acetylcholinesterase (Karnovsky, Roots, 1964).

Three coronal brain sections (approximately 2.2 mm, −2.9 mm, and −3.5 mm, relative to bregma) from each rat were photographed in gray scale using a digital camera (Penguin 600 CL, Pixera Corporation, USA) attached to a microscope (Olympus BH2-RFCA, Olympus America Inc., USA). Digital photographic files were opened with Scion Image for Windows (Scion Corporation, USA; freely available on the Internet at http://www.scioncorp.com). Optical density values were obtained from rectangular areas (52 pixels = 1 mm) in the cingulate cortex (45×70 pixels), retrosplenial cortex (45×70 pixels), hippocampal dentate gyrus and CA1 in both posterior sections (52×52 pixels), hippocampal CA2 and CA3 in both posterior sections (52× pixels), and medial parietal association cortex (52×52 pixels). Optical density values were expressed as a function of the gray scale value (white: 0.0; black: 255).

#### 4.6.2. *Parvalbumin immunohistochemistry*

Subsequent to surgery, rats ($n$=16) remained in their home cage for a duration equal to that experienced by rats receiving food protection training (i.e., approximately 4 weeks). After the delay, the animals were deeply sedated and perfused with phosphate buffered saline followed by phosphate buffered 4% paraformaldehyde. Brains were removed and stored in the same solution for two days at 4 °C. Brains were cut at 50 μm and placed in PBS solution. Sections were then placed in a blocking solution containing 10% donkey serum (DS), TBS, and 0.1% TX for 1 h at 23 °C. Blocking solution was then removed and sections were incubated overnight in a primary antibody solution containing 5% DS, TBS, and 0.1% TX, as well as a mouse monoclonal anti-parvalbumin antibody (1:1000; P3088; Sigma) at 4 °C, as described previously (Dwyer et al., 2007; Smith, Pang, 2005). After incubation, sections were washed in TBS and then incubated for 1 h at room temperature in a secondary antibody solution containing an AF546-labeled donkey anti-mouse antibody (1:1000; Invitrogen), 5% DS, TBS, and 0.1%TX, followed by 3 washes in TBS. Tissue sections were then mounted on gelatin coated slides and coverslipped with aqueous media (Fluoromount; Sigma). Immunofluorescence was assessed and photomicrographed on a Leica fluorescent microscope. Secondary only controls were treated as above, with the omission of the primary antibody solution.

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