SEPTAL AREA LESIONS IMPAIR SPATIAL WORKING MEMORY IN HOMING
PIGEONS (*COLUMBA LIVIA*)

Ryan M. Peterson

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Committee:

Verner P. Bingman, Advisor

Richard Anderson

Patricia Sharp

ABSTRACT

Verner P. Bingman, Advisor

The septo-hippocampal system in birds resembles that of mammals, motivating research into the function of the avian hippocampus while surprisingly little attention has been given to the septum. To investigate a possible role of the avian septum in memory, the effects of septal area lesions on a spatial working memory (SpWM) task was tested in homing pigeons. After pre-operative training on an analogue eight-arm (feeders) radial maze, now sham-operated control and septal-lesioned pigeons were then trained again on the same task of locating the four feeders on the test phase of a trial that were not baited during the sample phase of a trial when the four other feeder were baited. During the test phase of a working memory trial, septal-lesioned pigeons, compared to both their own preoperative performance and the performance of the controls, required significantly more choices to select baited feeders not baited during the sample phase of a trial, and they made significantly fewer correct responses to the now baited feeders on their first four choices. The results demonstrate that, like its mammalian counterpart, the avian septal area plays an important role in SpWM, suggesting that at least some functional properties of the septum are evolutionarily conserved in birds and mammals.

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1. INTRODUCTION

Working memory (WM) is the ability to temporarily store, manipulate, and process information used to guide behavior as well as contribute to the storage of information from previously learned episodes (Baddeley, 2003). The ability to employ WM using space as a discriminative stimulus, termed spatial working memory (SpWM), is an adaptive cognitive capacity that supports, among other things, efficient foraging behavior in a range of animal species (Olton, 1985). Robust SpWM ability in many species may explain why they exhibit a propensity to solve foraging challenges on the basis of their spatial characteristics (Balda & Kamil, 1988; Brodbeck, 1994; Brown, Rish, VonCulin & Edberg, 1993; Hurly, Franz & Healy, 2009).

Numerous studies have demonstrated the involvement of the mammalian prefrontal cortex (PFC) and its functional equivalent in the avian brain, the nidopallium caudolaterale (NCL), in the control of executive functions such as WM. This is based, in part, on research demonstrating that ablation of the PFC (Goldman & Rosvold, 1970; Mishkin, 1957) or NCL (Diekamp, Gagliardo & Grüntürkün, 2002; Mogensen & Divac, 1993) yields WM deficits. Additionally, both structures contain populations of neurons that exhibit sustained activity during WM tasks. These *delay neurons* are characterized by tonic, sustained activation during the delay period in a delay-response task when the subject has to maintain previously perceived information to subsequently respond appropriately (mammals: Funahashi, 2006; Fuster, 1973; birds: Diekamp, Kalt & Grüntürkün, 2002; Rose & Colombo, 2005). But in addition to PFC, lesion studies in mammals have found SpWM deficits after damage to the septal area (Numan & Quaranta, 1990; Numan & Klis, 1992; Rashidy-Pour, Motamedi & Motahed-Larijani, 1996). The origin of this effect is likely due, in part, to the strong connections between the septum and

hippocampal formation (HF; Swanson & Cowan, 1979), which is also important for SpWM (Olton, Becker & Handelmann, 1979).

The avian HF has been extensively studied in the context of spatial cognition such as homing (e.g., Bingman, & Mench, 1990), landmark-based navigation (e.g., Gagliardo, Ioalè, Savini, Dell'Omo & Bingman, 2009), and spatial learning (e.g., Kahn & Bingman, 2004) and memory (e.g., Sherry & Vaccarino, 1989; Smulders, Sasson & DeVoogd, 1995; see also Colombo & Broadbent, 2000). Important in the context of the current study, Good and Macphail (1994) reported SpWM deficits in homing pigeons following aspiration of HF. In contrast to HF, the role of the septum in avian cognition, and spatial cognition in particular, has remained unstudied despite the anatomical connections between the avian septal area and HF (Krayniak & Siegel, 1978a, b; Montagnese, Zachar, Balint & Csillag, 2008; Montagnese, Szekely, Adam & Csillag, 2004). The sparse functional research on the avian septal area has predominantly focused on its involvement in heart rate conditioning (Cohen & Goff, 1978) and regulation of social behavior (Goodson, Eibach, Sakata & Adkins-Regan, 1999), understood based on its efferent projections to the hypothalamus. The dearth of research on the possible relationship between the avian septal area and spatial cognition is perhaps even more surprising given that the mammalian septum is homologous to the avian septum. For example, the neurochemically defined subdivisional organization of the avian septum resembles the mammalian septum (Goodson, Evans & Lindberg, 2004). Comparative examination of developmental gene expression also reveals similarities between mouse and chick along both the dorsal (pallial) and ventral (subpallial) portions of the septum (Puelles, Kuwana, Puelles & Rubenstein, 1999; Puelles et al., 2000).

We began this study by hypothesizing that, like its mammalian counterpart, the avian septum participates in the control of SpWM. To test this hypothesis, control and septal lesioned homing pigeons were trained in an analogue radial-arm maze (Spetch & Honig, 1988) to select among eight radially distributed feeders for food reinforcement. The study is designed such that in order to efficiently deplete the available food at the feeders, a pigeon must remember those feeders it had already visited, increasing the WM load after each choice. We predicted that septal lesioned pigeons would display impairment in SpWM by making more choices to feeders that had already been depleted during a trial.

2. MATERIALS AND METHODS

2.1. Subjects

Fourteen unsexed homing pigeons (*Columba livia*) obtained from the pigeon colony at Bowling Green State University were used in this study. Following preoperative testing (see below), 9 birds underwent septal lesion surgery; the remaining 5 underwent a sham-surgical procedure. Because the current study was the first to examine the effect(s) of septal lesions in birds on spatial cognition, we preferred a larger number of experimental pigeons to eventually explore any relationship between the size and location of the lesions and the size of any behavioral deficits. All birds were housed individually in wire mesh cages (26.7 x 29.8 x 28.6 cm) in a temperature and humidity controlled room on a 12-12 hour light/dark cycle (lights on at 07:00). All birds were food deprived to no less than 85% of their baseline weight and allowed ad lib access to grit and water. Each bird's weight and feeding records were monitored daily. All procedures were carried out in accordance with National Institute of Health guidelines and

approved by Bowling Green State University's Institutional Animal Care and Use Committee (ICAUC).

2.2. Surgery

2.2.1. Septal lesions.

After completion of preoperative testing, 9 birds underwent electrolytic lesion of the septum. The pigeons were anesthetized with Isoflourane gas and placed in a stereotaxic apparatus. A portion of the skull was removed to expose the brain and an electrode (stainless steel insect pin insulated with epoxylite (Epoxylite Corp., Westerville, OH) and 1.3mm exposed tip) was inserted into the brain to produce the lesions. Three, bilateral lesion-target coordinates were used to produce the septal lesions: A 6.8, L + 0.8, V 8.5; A 7.4, L + 0.8, V 8.5; A 8.0, L + 0.8, V 8.0. All lesion coordinates were determined using the stereotaxic atlas of the pigeon brain (Karten & Hodos, 1967). For each lesion burn, 3.0 μ A of current was applied for 15 seconds at the most anterior coordinate and 20 seconds at the 2 most posterior coordinates. Following the lesions, the electrode was removed and the skin over the skull was closed with wound clips and swabbed with Betadine to prevent infection. The pigeon was then returned to its home cage and placed on ad lib food and water for 7 days, at which point wound clips were removed and the subject returned to food deprivation.

2.2.2. Sham Surgery.

Control birds underwent a sham surgical procedure once preoperative testing had finished. The procedure was identical to the septal lesion procedure with the exception that the electrode was not inserted into the brain.

2.3. Testing Environment

2.3.1. Testing Room

The testing room and food sites used in this study were developed to closely match those of Spetch and Honig (1988). All training and testing occurred in an open-field, testing room approximately 2.74 x 6.4 x 7.77 m with uniform white walls and gray floor (See Fig 1A). Various 2-dimensional wall posters and three dimensional objects scattered in the room were used to create a spatially heterogeneous environment. All behavior was observed through a small window in a plywood partition separating the testing room and the observer.

2.3.2. Food Sites

Food sites (see Fig 1B) were replicas of those used by Spetch and Honig (1988) and consisted of a platform with a ramp and a feeder. Ramps/platforms were comprised of a wooden ramp (20° incline, 18.5cm L x 6.8 cm H at the top), which led at the top to a 12 x 29 x 4 cm platform made from a piece of hard foam insulation. The ramp rested on the edge of the platform, which led to the feeder. Feeders, attached to the platforms, were constructed from 2-liter milk cartons. The apex of each carton was cut off and a semicircular hole half way down one wall of the remaining carton was made to form an entrance, which led to a food well that a bird could access. Thin poster board was attached to the cartons on the three sides that did not have the entrance to increase the height of the walls to 27 cm. Additionally, 4 cm flaps were also added to the walls on each side of the entrance; see Figure 1B. The cartons were filled with grit to 10 cm below the lowest point of the semicircular entrance. Throughout the study, the feeders were baited by placing approximately 2.5 gm of food (Purina Pigeon Chow, Ralston-Purina, St. Louis, MO) inside the feeder, against the front wall of the carton. Only by standing on top of the platform could a pigeon determine if food was present or not. The purpose for the seemingly complex feeder arrangement was to decrease the probability of a pigeon cycling through the feeders (see Spetch & Honig, 1988).

2.4. Behavioral Training and Testing

2.4.1. *Training*

The pigeons were trained to eat from feeders in three phases as described in Spetch and Honig (1988). In phase 1 training, a single food site was placed in the room (site 1; Fig. 1A). Food was placed inside the feeder and along the ramp leading up to it. A bird was placed in the center of the room with the lights turned off. The lights were turned on and the bird had 30 min to ascend the ramp and eat from the feeder. Phase 1 was repeated until the bird consumed all the food from the feeder. Once a bird had eaten all the food it was advanced to phase 2 training. In phase 2 training, four feeders were introduced to the room (sites 2, 8, 4, and 5; Fig. 1A). Food was placed in each feeder and at the top of the ramp. After each bird had eaten from all four locations within a 30 min session it was advanced to phase 3 training. In phase 3 training, feeders were placed at all eight food sites. Food was placed inside the feeders only. The bird was advanced to SpWM testing after it ate from all eight locations in a single 30 minute session.

2.4.2. *Spatial Working Memory Testing*

Immediately after training, the birds were given 20 trials of SpWM testing in the room-analogue radial arm maze. One SpWM trial took place per day with the first 10 trials conducted prior to surgery followed by 10 postoperative trials. A SpWM trial consisted of two parts, a sampling and test phase. In the sampling phase of a trial, four randomly selected sites were baited and made available. The feeders from the other four food sites were removed from the maze, leaving only the ramp and platform. Each pigeon was placed in the testing room with the lights turned off, the lights were then turned on and a pigeon was allowed to eat from all four, baited food sites. After a bird had visited all four locations, it was removed from the testing room for a 2 minute delay period, during which the missing feeders were installed and baited.

The test phase of a trial began once a bird was returned to the testing room (again with the lights turned off before trial onset). At this point all eight food sites were available, however, only the feeders which were not previously present contained food. The task of the pigeon was to find the food from the now four baited food sites while not returning to the four food sites depleted during the sample phase of the trial as well as the food sites depleted during the test phase of a trial. The bird was allowed to choose from the food sites until it had visited all four test-phase baited locations. The feeders, with their ramps and platforms, were randomly moved between the sample and test phase of a trial, as well as between trials, to ensure that the birds could not rely on the visual characteristics of individual feeders to guide their choices. A visit was scored when the animal stepped onto a ramp with both feet. The order of visits to the food sites was recorded during the test phase of each trial, from which all behavioral dependent measures were calculated. Calculated were 1) total number of food sites visited until a bird had eaten from all four newly (test phase) baited sites (TOT) and 2) the number of test-phase and still baited sites (correct choices) a bird visited on its first four (1ST4) test-phase choices. Additionally, two different types of errors were tabulated for each trial. Retroactive errors (RE) were test-phase visits to a food site that was baited and depleted during the sample phase of a trial. Proactive errors (PE) were test-phase revisits to any location already visited during the testing phase of a trial. PE encompassed both re-visits to a now depleted food site that was baited during the test phase of a trial as well as re-visits to a location where a RE had already been scored, thus birds could make a maximum of 4 RE and an unlimited number of PE during the test phase of any single trial

2.5 Statistical Analyses

Choice data from each bird was gathered and pooled across blocks of 2 trials forming 10 two-trial blocks (5 preoperative, 5 postoperative) for each behavioral dependent measure in order to reduce the inter trial variance. Two 2 (group: lesion, control) x 5(trial block) mixed model analysis of variances (ANOVAs) were conducted on each measure. The first ANOVA evaluated preoperative performance and the second focused on postoperative performance. Post –hoc T-tests were computed via the Sidak-Bonferroni procedure to assess differences across trial blocks. For both within group and between group differences, significance was set at $p < 0.05$.

In addition, to directly contrast the postoperative change in performance between the septal lesion and sham control groups, the pigeons' scores for each dependent measure (TOT, 1ST4, PE, RE) were averaged across all 10 postoperative trials and then subtracted from the average scores recorded during the two trials of last preoperative block. This yielded a separate preoperative-postoperative difference score for each measure for each bird and allowed for a formal assessment of whether any differences were due to an impairment or improvement in performance by one or both groups. Of note, the average of all 10 postoperative trials was used in order to encapsulate the entire postoperative performance of each bird while only the last preoperative trial block was used because the birds were learning the task during the initial preoperative trials and the difference score was designed to assess any change in performance within each subject.

2.6. Histology and Lesion reconstruction

After the animals completed testing, the septal lesioned birds were sacrificed to determine the extent of lesion damage. The birds received a lethal injection of a pentobarbital based euthanasia solution (Vortech Pharmaceuticals, Dearborn, MI; 100mg/kg i.p) and perfused

intracardially with approximately 250 mL of 0.9% saline followed by 4% paraformaldehyde. The brains were then harvested and placed into 4% paraformaldehyde for 24 hours after which they were transferred to 30% sucrose solution for 48-96 hours for cryoprotection. Brains were sectioned at 40 μ m on a freezing microtome, with every fourth section mounted on a gel coated slide. Tissue was differentiated with cresyl violet stain.

Lesions were reconstructed and quantitatively analyzed using Stereo Investigator image analysis software (MicroBrightField, Colchester, VT). Accordingly, for each septal lesioned pigeon, the amount of undamaged septal tissue present in each hemisphere was digitally traced at 0.5 mm intervals between A 9.5 - 7.5. Intact septum volume was calculated by multiplying the area of remaining tissue in each section by the interval distance between sections and then summing the products. Additionally, septal volumes were calculated from 2 intact pigeons, and their mean volume served as an undamaged baseline (U). The difference between the volume of remaining tissue in each lesioned bird and U was divided by U and then multiplied by 100 to produce a percent septal damage in each bird relative to the undamaged baseline. We recognize that this method is subject to some error because septal volume would not be the same in all birds. However, the error produced would be small because the birds were all close to the same weight and would have had similar brain/septal volumes. Separate calculations were made for each hemisphere and for total septum, medial septum (SM) and lateral septum (SL) damage.

3. RESULTS

3.1. Lesion Damage

Figure 2 summarizes the damage to the septal area and surrounding regions in the lesioned birds. Although some variability in lesion damage was found among subjects, most

birds sustained substantial damage to the septal area. In some pigeons, some modest damage extended into the ventral hippocampus, as well as the most medial portions of the mesopallium, nidopallium, striatum and bed nucleus of the stria terminalis. Visual inspection of septal damage in a few birds suggested differences between the hemispheres. Accordingly, a paired samples t-test was calculated to examine whether both hemispheres were damaged equally across all pigeons. There was no significant difference between left and right septal damage ($t_8 = 0.53$, NS).

3.2. Behavior

3.2.1. Preoperative performance

During pre-operative testing, no difference was found between the lesion and control groups with respect to the number of total choices to reach all four baited feeders (TOT; see Fig.3A, $F_{1,12} = 0.128$, NS) or the number of correct choices within the first 4 responses (1ST4; see Fig.4A, $F_{1,12} = 0.208$, NS). There was a significant difference across preoperative trial blocks for both TOT ($F_{4,48} = 8.05$, $p < 0.001$) and 1ST4 ($F_{4,48} = 13.08$, $p < 0.001$), but there was no trial x group interaction for either TOT ($F_{4,48} = 0.356$, $p > 0.05$) or 1ST4 ($F_{4,48} = 0.843$, $p > 0.05$). Paired T-tests revealed that birds made significantly fewer total choices to reach all baited feeders during the test (working memory) phase of a trial ($t_{13} = 3.9$, $p < 0.002$) and more correct choices on their first 4 responses ($t_{13} = 7.78$, $p < 0.001$) during the last preoperative block (TOT: mean = 6.21, S.E.M.=0.36; 1ST4: mean = 2.92, S.E.M. = 0.09) compared to the first block (TOT: mean = 9.67, S.E.M.= 0.88; 1ST4: mean = 1.92, S.E.M.= 0.112) as all birds improved significantly with repeated training on the task.

Additionally, the lesion and control groups did not significantly differ in the number of proactive errors (PE, see Fig. 5A, $F_{1,12} = 1.26$, NS) or retroactive errors (RE, see Fig. 6A, $F_{4,48} =$

0.427, NS). There was as a significant difference across all preoperative trial blocks for both PE ($F_{4,48} = 6.38, p < 0.001$) and RE ($F_{4,48} = 6.03, p < 0.001$). However, a significant interaction was not detected for either PE ($F_{4,48} = 0.347, NS$) or RE ($F_{4,48} = 1.19, NS$). Paired T-tests revealed significantly fewer PE ($t_{13} = 2.72, p < 0.02$) and RE ($t_{13} = 6.00, p < 0.001$) during the last preoperative trial block (PE: mean = 0.5, S.E.M.= 0.29; RE: mean = 1.71, S.E.M.=0.16) compared to the first block (PE: mean = 2.67, S.E.M.=0.75; RE: mean = 3.0, S.E.M.=0.21).

In summary, both groups of pigeons performed equally well on the task, showing the same pattern of improvement during the course of training. Both groups were performing well above chance on the SpWM task by the end of training.

3.2.2. Postoperative performance

In contrast to their preoperative performance, postoperatively the septal lesion and sham control groups differed with respect to both TOT (see Fig. 3A, $F_{1,12} = 156.4, p < 0.001$) and 1ST4 (see Fig. 4A, $F_{1,12} = 73.13, p < 0.001$). The origin of this difference was clearly the larger TOT score of the septal lesioned birds compared to controls for both the first (Figure 3B, $t_{12} = 2.97, p < 0.01$; control: mean = 6.10, S.E.M. = 0.43; lesion: mean = 11.33, S.E.M. = 1.26) and last (Figure 3B, $t_{12} = 3.43, p < 0.005$; control: mean = 5.90, S.E.M. = 0.36; lesion: mean = 8.94, S.E.M. = 0.61) postoperative trial block, as well as their fewer correct choices on 1ST4 again during the first (Figure 4B; $t_{12} = 6.04, p < 0.001$; control: mean = 2.88, S.E.M. = 0.1; lesion: mean = 1.8, S.E.M. = 0.11) and last (Figure 4B, $t_{12} = 3.24, p < 0.01$; control: mean = 2.80, S.E.M. = 0.2; lesion: mean = 2.00, S.E.M. = 0.11) postoperative trial block. The two groups did not, however, exhibit a significant change across postoperative trial blocks in TOT ($F_{4,48} = 1.11, NS$) or 1ST4 ($F_{4,48} = 1.56, NS$), nor was a trial x group interaction detected for TOT ($F_{4,48} = 0.742, NS$) or 1ST4 ($F_{4,48} = 1.38, NS$).

The types of errors committed by the septal lesioned and control pigeons significantly differed with respect to both PE (see Fig. 5A, $F_{1,12} = 5.28, p < 0.05$) and RE (see Fig. 6A, $F_{1,12} = 8.67, p < 0.05$). The origin of this difference was clearly the greater number of PE committed by the septal lesioned birds compared to controls for both the first (Fig. 5B, $t_{12} = 2.67, p < 0.05$; control: mean = 0.3, S.E.M. = 0.2; lesion: mean = 4.27, S.E.M. = 1.88) and last postoperative (Fig. 5B, $t_{12} = 2.57, p < 0.05$; control: mean = 0.4, S.E.M. = 0.18; lesion: mean = 2.38, S.E.M. = 0.55) trial block, as well as the greater number of RE again during the first (Fig. 6B; $t_{12} = 2.84, p < 0.05$; control: mean = 1.8, S.E.M. = 0.33; lesion: mean = 3.05, S.E.M. = 0.28) and last (Fig. 6B, $t_{12} = 2.35, p < 0.05$; control: mean = 1.5, S.E.M. = 0.44; lesion: mean = 2.55, S.E.M. = 0.34) postoperative trial block. However, neither measure was found to significantly vary across postoperative trial blocks (PE: $F_{4,48} = 0.942$, NS; RE: $F_{4,48} = 0.604$, NS), nor was a trial x group interaction for PE ($F_{4,48} = 0.949$, NS) or RE ($F_{4,48} = 0.238$, NS) detected.

In summary, the postoperative, SpWM performance of the septal lesioned pigeons was characterized by an easily detected impairment compared to the sham control birds. Furthermore, the unchanging performance across the postoperative trial blocks indicates that lesioned subjects failed to recover from their postoperative impairment. As such, the septal lesions resulted in an enduring deficit in SpWM.

3.2.3. Preoperative-postoperative contrasts

Examination of Figures 3A and 4A suggests that the between group difference in postoperative performance between the two groups was a consequence of the septal lesioned pigeons performing poorly relative to their pre-operative performance and not to any change in the performance of the sham-operated pigeons. To formally examine this impression, the scores for each dependent measure (TOT, 1ST4, PE, RE) were averaged across all 10 postoperative trials

and then subtracted from the average scores recorded during the two trials of the last preoperative block. This yielded a separate difference score for each dependent measure for each bird and allowed for a between group comparison of the change in performance after surgery. A one-way ANOVA revealed a significant difference between the lesion and control groups for TOT (see Fig. 7A, $F_{1,12} = 7.32, p < 0.01$), 1ST4 (see Fig. 7B, $F_{1,12} = 13.9, p < 0.01$) and PE (see Fig. 7C, $F_{1,12} = 5.67, p < 0.04$). The contrast for RE did not quite reach significance (see Fig. 7C, $F_{1,12} = 3.58, p = 0.08$). The data in Figure 7, together with the data in Figures 3B and 4B, demonstrate that the group differences were based on the change in (poorer) performance of the septal lesioned pigeons compared to no change in the sham-control birds after surgery. Septal lesions led to more TOT and fewer correct choices during 1ST4, generally reflecting compromised SpWM.

3.2.4. Can the amount of septal damage explain the variability in individual performance?

As summarized above, the extent of septal lesion damage across the experimental pigeons was characterized by some inter-individual variability. It was of interest, therefore, to examine if size of the WM deficit displayed by different individuals could be explained, at least in part, by the amount of septal lesion damage. Rather than carry out an exhaustive series of regressions, we chose to examine the possible relationship between lesion damage and performance by looking at 1ST4 (number of correct choices among the first four choices) during the first postoperative block. We chose this measure because it displayed the largest amount of inter-individual variability, and consequently, was most likely to reveal a correlation. Figure 8 shows the regression, which was not significant ($R^2 = 0.26, F_{1,8} = 2.52, NS$). However, we present this analysis because visual inspection of Figure 8 suggests one outlier among the

pigeons, bird 183, which displayed poorer performance than might have been expected based on its relatively small lesion. By excluding this apparent outlier, the regression among the remaining 8 birds was significant ($R^2 = 0.55$, $F_{1,7} = 7.301$ $p = 0.03$). Although perhaps not compelling, the significant regression supports the idea that larger septal damage leads to a larger spatial working memory deficit.

4. DISCUSSION

Overall, the results of the present study demonstrate that the avian septal area plays an important role in spatial working memory, highlighting its functional similarity with the mammalian septum and possible cooperation with the avian hippocampal formation in supporting at least some aspects of spatial cognition. The essential findings were that septal lesioned homing pigeons made fewer correct choices within the first four responses and more choices to reach all the baited feeders on SpWM trials.

4.1. Spatial Cognition and the Avian Septum

The avian and mammalian telencephalic septal area is evolutionarily conserved. Both the avian and mammalian septal areas contain similar medial and lateral subdivisions based on chemoarchitectonic organization (Goodson et al., 2004; Risold & Swanson, 1997) and developmental gene expression (Puelles et al., 2000). Additionally, both have similar afferent and efferent connections; the septal area serving as an important node between the HF and hypothalamus (Swanson & Cowan, 1979; Montagnese, et al., 2004; 2008). The results of the present study are the first to demonstrate that this homology translates into a similar functional role in spatial cognition. As is the case in mammals, the well known role of the avian HF in spatial cognition (Macphail, 2002), and the reciprocal anatomical connections between the septal

area and HF, suggest that the septal area and HF cooperate in the control of SpWM. Damage to either the mammalian septal area, HF or fimbria-fornix yields impaired spatial processing or SpWM (Numan, 2000; Olton et al., 1979). In birds, HF damage leads to impaired spatial processing and SpWM, but non-spatial WM remains largely intact (e.g., Good & Macphail, 1994; Hampton & Shettleworth, 1996). In fact, when presented with a spatial vs. landmark conflict task HF lesioned birds can successfully solve the task based on non-spatial cues to a greater extent than control birds (Kahn & Bingman, 2009), leading to the conclusion that the avian HF is primarily involved in spatial processing (see Colombo & Broadbent, 2000).

Closer inspection of the results also provides some indication of the nature of the deficit that accompanies septal damage. Postoperatively, the performance of septal lesioned birds on the test phase of working memory trials dropped to chance levels when examining their first four choices but not total choices. This suggests that the lesioned birds may have retained some information about the food sites already visited within the test phase of each trial. Although purely speculative, the septal lesioned birds better than chance performance on one measure of the SpWM task, although still substantially poorer than controls, may have reflected spared WM for non-spatial features that did not change within the testing phase of a trial (e.g., the appearance of a ramp, or the food sites already visited during the test phase of a trial). An alternative explanation is that following septal lesions the birds adopted a stereotypical response strategy (such as sequentially visiting each adjacent food site), which was not dependent on the processing and retention of spatial information. Such a strategy has been observed in birds following HF damage (Colombo, Broadbent, Taylor & Frost, 2001) as well as in rats after septal damage (Janis, Bishop & Dunbar, 1994). A stereotypical response strategy would keep the

number of total choices required to reach all the food sites above chance (no proactive errors) while the number of correct first four choices would remain at chance levels.

Results of the current study indicate that the avian septum is important for spatial cognition. Consistent with this conclusion, Shiflett, Gould, Smulders, and DeVogd (2002) found that the volume of the septal area was greater in a species of food-storing bird, black-capped chickadees (*Poecile atricapillus*), with their greater reliance on spatial memory, than in two non-food-storing birds, blue tits (*Parus caeruleus*) and great tits (*Parus major*). Also in black-capped chickadees, septal area volume increased in the fall, consistent with the onset of food-storing behavior. The pattern of a larger and seasonally varying septal volume in black-capped chickadees parallels their larger and seasonally varying HF volume, which also increases during parts of the year when a bird is more dependent on spatial memory.

4.2. Theta Rhythm

In mammals, the septal area is believed to support memory processes by modulating hippocampal slow rhythmic activity, commonly referred to as hippocampal theta rhythm. Mammalian hippocampal theta is driven by afferent neurons from the medial septum (SM) and diagonal band of Broca (DBB), which fire rhythmically and synapse on HF pyramidal cells that regulate synchronous neuronal depolarizations (Vinogradova, Kitchigina, & Zenchenko, 1998). Damage to the SM/DBB significantly reduces HF theta (Andersen, Bland, Myher, & Schwartzkroin, 1979). In pigeons, the existence of hippocampal theta rhythm (Siegel, Nitz, & Bingman, 2000), although at a lower frequency than in rats, and cholinergic input into HF from the septal area (Krebs, Erichsen, & Bingman, 1991) suggests that the avian septum modulates HF mnemonic processes in a manner similar to mammals.

4.3. Interpretive Caveats

We have made the case that septal lesions in homing pigeons lead to a profound and persistent deficit in SpWM. However, there are some aspects of our study that need to be considered before endorsing this claim. Our analysis did not specifically distinguish between reference memory and working memory components of the task. Reference memory is the stable retention of salient information, which elicits a particular behavior in the presence of specific discriminative stimuli. It is remotely possible that the septal lesions disrupted the task rules stored in reference memory rather than the spatial working memory of the food sites. However, we can exclude such a possibility because when the septal lesioned birds were placed in the experimental space, they moved among the food sites as they did pre-operatively. The birds clearly had intact memory for the procedures of the task; they were impaired only in recalling the location of depleted food sites.

Another concern is the possible damage to fibers entering or leaving the HF as a consequence of the septal lesions. It is possible that part of the observed deficit in some pigeons was a result of some disruption to HF. However, the suggestive correlation between septal damage and size of the SpWM deficit, and the observation that some of the pigeons did not experience septal damage medial enough to damage passing HF fibers, indicate that the surgical damage to the septum was principally if not exclusively the source of the SpWM deficit. However, we acknowledge that future research will need to untangle how the septum and hippocampus cooperate in regulating aspects of spatial cognition like SpWM.

4.4. Conclusions

The results of the current study provide direct evidence that damage to the avian septal area impairs SpWM. As such, the results offer evidence that the avian septum shares a functional

role with the mammalian septum in some aspects of spatial cognition. However, what the study does not do is identify if the septal lesions interfered with spatial processes, working memory processes or some interaction between the two. Important now would be to test septal lesioned homing pigeons on tasks of spatial cognition that do not require WM as well as non-spatial working memory tasks. Given the close relationship between the septum and HF, we would expect general involvement of the avian septum in spatial cognitive processes, an idea that remains to be tested.

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FIGURE LEGENDS

Figure 1. A: Schematic representation of the experimental arena. Radial arrangement of foodsites (1-8), three-dimensional ground (a, broom; b, industrial sink, c, ruler; d, glass jar; e, triangular partitions) and two-dimensional wall (w, large X made with duct; x, small portrait; y, partition made of brown plywood and pink foam insulation; z, multicolored poster) landmarks are shown. Objects (a-e, x-z) were placed throughout the room to better enable the pigeons to discriminate the experimental space. During the sampling phase of a trial, 4 randomly selected feeders were removed, leaving 4 baited food sites and only the ramps at the other 4 locations (see below). During the test phase of a trial, all feeders were present, but only those not present during the sample portion of a trial were baited. **B:** Diagram of a foodsite composed of a feeder (F) and ramp (R). Feeders were constructed from a milk carton and placed on the ramp. In order to locate and obtain the food reinforcement located in the feeder, the bird was required to walk to the top of the ramp (see also Spetch & Honig, 1988).

Figure 2. Schematic coronal sections of the septal area (A) and lesion reconstructions (B) at 0.5 mm intervals from anterior (A 9.5) to posterior (A 7.5) according to the atlas of Karten and Hodos (1967), labeled according to the revised nomenclature (Reiner et al., 2004). Black areas identify damage in at least 7 of 9 pigeons, gray areas identify damage in at least 5 pigeons, and hashed areas identify damage in at least 2 of 9 pigeons. Abbreviations: AC, anterior commissure; CcS, caudocentral septum (from Goodson et al., 2004); DBB, diagonal band of Broca; Hp, hippocampus; SL, lateral septum; SM, medial septum; M, mesopallium; N, nidopallium; SH, septohippocampal pathway.

Figure 3. Mean number of total choices required to reach all 4 baited food sites for each group during the test phase of the SpWM task. **A:** Mean total choices for both groups for all 20 trials pooled across blocks of 2 trials. Note: vertical line separates pre-operative trials (1-10) from post-operative trials (11-20). Chance performance was calculated at 14.8, denoted by the dotted line. Standard error bars are shown. **B:** Total mean choices for trial blocks 9-10 (pre-op), 11-12 (first post-op), and 19-20 (last post-op), highlighting a sustained increase in the number of choices taken by the septal-lesioned group following surgery compared to their pre-operative performance and the performance of the control pigeons; $**p < 0.01$.

Figure 4. Mean number of correct choices among the first 4 responses for each group during the test phase of the SpWM task. **A:** Mean total correct choices for both groups for all 20 trials pooled across blocks of 2 trials. Note: vertical line separates preoperative trials (1-10) from post-operative trials (11-20). Chance performance was calculated at 1.8, denoted by the dotted line. Standard error bars are shown. **B:** Mean total correct choices for trial blocks 9-10 (pre-op), 11-12 (first post-op), and 19-20 (last post-op) highlighting sustained fewer correct choices in the septal-lesion group following surgery compared to their preoperative performance and the performance of the control pigeons; ** $p < 0.01$; *** $p < 0.001$.

Figure 5. A: Mean number of proactive errors (PE, visits to a food site that was visited and depleted during the *test* phase of a trial) made during SpWM testing by both groups for all 20 trials pooled across blocks of 2 trials. Note: vertical line separates preoperative trials (1-10) from post-operative trials (11-20). Standard error bars are shown. **B:** PE for trial blocks 9-10 (pre-op), 11-12 (first post-op), and 19-20 (last post-op) highlighting the sustained increase in errors in the septal lesion group postoperatively; ** $p < 0.01$; * $p < 0.05$.

Figure 6. A: Mean number of retroactive errors (RE, visits to a food site that was baited and depleted during the sample phase of a trial) made during SpWM testing by both groups for all 20 trials pooled across blocks of 2 trials. Note: vertical line separates preoperative trials (1-10) from post-operative trials (11-20). Standard error bars are shown. **B:** RE for trial blocks 9-10 (pre-op), 11-12 (first post-op), and 19-20 (last post-op) highlighting the sustained increase in errors in the septal lesion group postoperatively; ** $p < 0.01$; * $p < 0.05$.

Figure 7. Pre-/postoperative difference scores (see text for detailed explanation) for the control (black) and septal-lesioned (white) groups with respect to (A) total choices (TOT), (B) number of correct choices within the first 4 responses (1ST4), as well as (C) proactive (PE) and retroactive (RE) errors. Only the septal-lesioned birds displayed significantly impaired postoperative performance; control birds did not exhibit a significant change in any measure after surgery; ** $p < 0.01$; * $p < 0.05$.

Figure 8. Number correct choices within the first 4 responses for each septal lesion bird in the immediate postoperative block (trials 11-12) plotted against the percent of septal damage. A

regression carried out on all subjects revealed a non significant correlation ($R^2 = 0.26$, $p = 0.15$). However, examination of the figure suggests that one bird (183, circled) behaved as an outlier compared to the others. Taking 183 out of the analysis, a significant negative correlation emerged indicative that a larger volume of septal damage predicted poorer performance; $p < 0.05$.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Table 1. Difference score means (*standard errors*) for each measure.

Measure	Group	
	control	septal lesion
RE	-0.14 (0.31)	-1.0444 (0.3)
PE	0.5 (0.75)	-2.8222 * (0.94)
TOT	0.36 (0.92)	-3.8667** (1.03)
1st4	-0.18 (0.2)	-0.9222** (0.08)

Note: positive values indicate an improvement in performance, while negative values indicate impaired performance postoperatively compared to preoperative performance, ** $p < 0.01$; * $p < 0.05$..

APPENDIX I

MODELS OF MEMORY

The concept of the vertebrate brain containing multiple memory systems has been generally accepted (Squire, 2004; Tulving, & Schacter, 1990; Eichenbaum, 1997). Research in humans (Rempel-Clower, Zola, Squire, & Amaral, 1996; Milner, Corkin, & Teuber, 1968) and animals (Packard, Hirsh, & White, 1989; Hampson, Jarrard, & Deadwyler, 1999; Kahn, & Bingman, 2004; Sherry & Schacter, 1987) supports the existence of multiple types of memory prompting the development of several theoretical models. Atkinson and Shiffrin (1968) illustrate the relationship between short and long term storage in their Information Processing Model, Squire (1986) distinguishes between different types of long term memory, while Baddeley and Hitch (1974) discriminate between the faculties comprising WM. Each model offers a new perspective on the storage of information. Moreover, they provide the theoretical framework for the neurobiological study of memory.

I.1. A Temporal Model

Atkinson and Shiffrin (1968) developed the Information Processing Model, which remains among the most influential memory processing models (see Fig. I-a). This model was developed around three temporal categories of information storage in humans: immediate or sensory stores, short-term store (STS), and long term memory (LTM). Sensory systems are activated when stimulated, storing information only a fraction of a second before immediately degrading, unless the information is consciously attended to. By attending to sensory stores, the information is placed into STS which can last from seconds to minutes. Maintenance of a memory in short term store is dependent upon conscious rehearsal and attention. With rehearsal a memory in STS will be placed in long term store forming a LTM. A LTM can be retrieved throughout one's life by bringing it back into short-term store. Thus, the Information Processing Model proposes that information from the environment flows through a series of temporary sensory registers into a limited capacity STS which feeds information into and out of LTM. This model continues to provide the fundamental framework for temporal memory processing. In

contrast, recent scholars have focused on the classification of memory by categories of stored information.

Figure I-a. Atkinson and Shiffrin's (1968) Information Processing Model. Information from the environment (visual auditory, haptic, etc.) is initially registered by the sensory stores and, if attended to, can flow into a short-term store (STS). Once in the STS, information can be (a) maintained via rehearsal, (b) coded into long term store where it is maintained indefinitely for later retrieval, (c) acted upon (eliciting a behavioral response), or (d) discarded. Information can be forgotten at any point during the memory process resulting in the loss of information.

I.2. A Model of LTM

Squire (1986) proposed a model of LTM that taxonomically differentiates memories as either consciously explicit (declarative) or those which can occur unconsciously (nondeclarative; see Fig.I-b). This dichotomy is based on previous work (i.e. Tulving, Schacter, & Stark, 1982; Graf & Schacter, 1985) highlighting the differences in the type of information carried by each memory and how each system operates. Declarative memory, which includes both episodic and semantic memories, refers to explicit and consciously accessible memories. Episodic memory pertains to specific events, which are bound to the time and place in which the event occurred (Tulving, 2002). In contrast, semantic memories are facts and/or general knowledge lacking a spatiotemporal context.

The second type of memory, nondeclarative memory, is a blanket term used to encompass all forms of memory that can be expressed unconsciously. Priming and classical conditioning, habituation, and sensitization are all examples of nondeclarative memory. The

Information Processing Model and Squire's model offer separate yet noncompeting categories of memory systems. Nonetheless, Squire's model includes nondeclarative memories, which are absent in the Information Processing Model. Thus, the Information Processing Model can only be applied to information that is consciously attended to and Squire's model is lacking in overall scope in that it does not include WM.

Figure I-b. Squire's (1986) taxonomy of long term memory LTM offers a categorical distinction between declarative and non declarative memories. Declarative memories require conscious attention in order to be encoded and recalled. Non-declarative memories can be processed in the absence of conscious thought and encompass classical conditioning, priming, procedural skills, as well as forms of non-associative learning, namely sensitization and habituation.

I.3. A Model of WM

Baddeley and Hitch (1974) essentially coined the term *working memory* (WM) as an alternative to short term store in order to emphasize WM's functional role in the manipulation of information in their Multi-Component Model. According to the model, WM is the cognitive elaboration of an individual's central executive, visuospatial sketchpad and phonological loop. The central executive is the most important component of the Multi-Component Model and includes autonomic and attentional control without differentiating the two. The central

executive temporarily stores information in the episodic buffer. The visuospatial sketchpad and phonological loop have a limited capacity to hold object and verbal memories, respectively, and they may be considered WM extensions of episodic and semantic memories. Additionally, the visuospatial sketchpad is involved in visual imagery, spatial orientation, and is believed to be necessary in forming semantic memories about the appearance of an objects and the object's use. Baddeley (2000) expanded on his previous model of WM to include interactions with LTM and an additional WM component, the episodic buffer (see Fig.I-c). The episodic buffer emphasizes the capacity of WM to manipulate and create new representations rather than simply recalling LTM. In addition to serving as a limited store, the episodic buffer binds information together to form integrated episodes (Baddeley, 2003). Thus, the buffer assumes the activation of a different system than that involved in LTM.

Figure I-c. Baddeley's (2003) multi-component model of WM. Accordingly, the central executive controls attention and draws on the three subsidiary storage systems, the visuospatial sketchpad, episodic buffer, and phonological loop, which serve as active stores for information. The subsystems can interact with each other allowing information to flow in a fluid-like capacity. Additionally the three subsystems can interface with long-term memory which holds crystallized cognitive systems such as vocabulary and episodic memories.

APPENDIX II

MEMORY IN NON-HUMAN ANIMALS.

The majority of theoretical models of memory are designed to explain human memory systems. Indeed, all of the previously described models (Atkinson & Shiffrin, 1968; Squire, 1986; Baddeley, 2000) correspond to different aspects of human memory systems. However, little work has been devoted to develop a universal model of memory between humans and nonhumans (e.g. Eichenbaum, Otto, & Cohen, 1994; Gallagher, 1997). Consequently, in animal research, scholars often broadly use the terms reference memory (RM; interchanged with LTM) and WM when utilizing non-human subjects (Walsh, 2000). RM and WM are emphasized in this paper based on their special relevance to DNMS task. In this context, WM is used to describe retention of information within trial (e.g., locations previously visited in a given trial), while RM is reserved for retention between trials (food is only located at food sites; food sites are only baited once) as the task is trial independent.

II.1. Reference Memory

RM is analogous to parts of LTM as it refers to the retention of salient information, which produces a particular behavior in the presence of specific discriminate stimuli. Thus, a task that remains the same across time such as running through a maze or swimming to a platform in the same position across sessions requires the animal to utilize RM (Honig, 1979). In this sense, RM comprises both ‘semantic’ like memory and procedural or habit memory from Squire’s model of LTM. Episodic memory, however, has been traditionally excluded from RM due to the lack of empirical evidence in animals’ for the need to remember specific episodes. Nevertheless, scientists have recently speculated about the capability of some animals in producing episodic memory (Crystal, 2010; Salwiczeka, Watanabeb & Clayton, 2010).

II.2. Episodic-like Memory

The episodic-like memory system of the brain can recall *what* a particular event was, *where* it happened, and *when* it occurred (Tulving, 2002). This system was once thought to occur only in humans as it is impossible to test whether an animal consciously remembers a

particular episode. However, Clayton and Dickinson (1998) observed food caching in jays which led to the widespread acceptance of episodic-like memory in animals. They found that the jays displayed a memory for the (what) type of food was cached, (where) the location the food was cached, as well as (when) the amount of time between caching and revisiting the food site. Similar results have been replicated in other species including pigeons and rats (Zentall, Singer, & Stagner, 2008; Ergorul & Eichenbaum, 2004). Additionally, the finding of episodic-like memory in nonhuman animals has lead researchers to speculate whether animals are capable of mental time travel and prospective thinking (Zentall, 2005; Raby & Clayton, 2009).

II.3. Working Memory

WM has been observed in a variety of species including dogs (Macpherson & Roberts, 1983), rodents (Zahrt, Taylor, Mathew, & Arnsten, 1997), and birds (Lissek & Grüntürkün, 2004; Spetch & Honig 1988) and is a critical in supporting foraging, communication, problem solving and formation of long-term memories. Human and non-human WM are nearly identical in that they both contribute to the learning and retention of a particular response in the presence of different discriminate stimuli. Becker and Morris (1999) contend the chief distinction between the two is the incorporation of language into human WM. Nonetheless, an important distinction can be made. Cognitive psychologists consider WM as system with a limited capacity to temporary store and manipulate information in complex cognitive tasks such as comprehension, learning, and reasoning, while animal researchers define WM in terms of the ability to store information across multiple trials within the same day, as demanded by tasks such as the radial arm maze, which is likely to be at least partially dependent on LTM for the storage of information (Baddeley, 2000; Olton, Collison, & Werz, 1977) .

Although WM was discovered separately in mammals and birds, it essentially refers to an identical cognitive process in both taxa (Grüntürkün, 2005; Lando et al., 2001). In this context, WM is comparable to short term store in the Information Processing Model and episodic memory in Squire's model. Additionally, while it is possible for WM to be episodic-like, WM does not account for all aspects of episodic-like memory and should be considered functionally different. Griffiths and Clayton (2001) illustrated this through their explanation of a classic delay non-match to sample task (DNMS). Although it is possible that an animal may

episodically recall the events from the first part of a session, Griffiths and Clayton contend that a much simpler explanation exists. Accordingly, it is more likely that an animal learns to simply avoid the most familiar object(s). Consequently it does not need to remember the when and where of locations previously visited in the first part of a trial, eliminating the need for episodic recall. Moreover, unlike episodic memory, WM requires the animal's attention to maintain a memory. In comparison to the enduring applicability of a LTM, the usefulness of any WM is limited to a specific and brief period of time. Thus, information stored specific to a given trial or moment in time is commonly referred to as WM.

II.4. Spatial Working Memory

The elaboration of WM in a situation where different locations in space need to be discriminated is commonly referred to as spatial working memory (SpWM). This subclass of WM has been particularly studied by behaviorists because a basic component of conditioned behavior is the ability of an animal to remember previously visited locations and attend to where it has been (Olton, 1977; 1985). Moreover, numerous species exhibit a propensity to solve tasks primarily on the basis of its spatial characteristics (Balda & Kamil, 1988; Brodbeck, 1994; Brown, Rish, VonCulin, & Edberg, 1993). SpWM can easily be distinguished from nonspatial WM through examination of task specific recall. While both SpWM and nonspatial WM involve the temporary storage of information, only SpWM requires an animal to remember a spatial location(s). In contrast, tasks of nonspatial WM often uses cue (color/shape) based recall, irrelevant of space. Consequently, a substantial proportion of WM tasks designed in the laboratory assess SpWM. This is most apparent when utilizing the radial arm maze paradigm, in which an animal is placed in the center of the maze with between 4 - 17 arms (usually 8) extending from the center in a radial fashion with the end of each arm baited. The animal must travel down each arm to receive reinforcement and return to the center to make subsequent choices. Thus, to optimally solve the maze the animal must remember among all possible choices which arms it has already visited, increasing the WM load after each choice.

APPENDIX III

NEURAL SUBSTRATES OF WORKING MEMORY

Extensive research has illuminated the involvement of certain brain structures in the elaboration of WM. Among the brain structures of principal interest are the mammalian prefrontal cortex (PFC) and avian nidopallium caudolaterale (NCL). Additionally, the mammalian septal area, through its connections with the hippocampal formation (HF), is critically involved in spatial processing, particularly in the context of WM.

III.1. Prefrontal cortex

The mammalian PFC is a pivotally involved in the orchestration of higher order cognitive functions. Notably, WM, among other cognitive endowments such as executive control, distinguish PFC function (Fuster, 1973; Komjima, Hikosaka, Tsitui, Tsukada, & Watanabe, 2007; Postle, Berger, & D'Esposito, 1999). According to Curtis and D'Esposito (2004), prefrontal ablations in experiments that took place over a century ago were pivotal in demonstrating that damage to this area yields impaired performance on WM tasks. Later studies (e.g. Goldman & Rosvold, 1970; Mishkin, 1957) demonstrated that the lateral portion of the PFC is necessary for fully functioning WM capacity. Additionally, the ability to record electrophysiological activity from a single neuron has led to the discovery of pyramidal cells within the PFC that exhibit sustained activity during WM tasks. These “delay neurons” are characterized by tonic, sustained activation during the delay in a delay-response task when the subject has to maintain previously perceived information (Funahashi, 2006; Fuster, 1973). Sustained activity in delay neurons is only observed prior to correct performance; conversely, when subjects make an incorrect choice, sustained activity in these neurons is not observed (Funahashi, Bruce, & Goldman-Rakic, 1989). Furthermore, when the delay period is lengthened or shortened, delay activity is prolonged or shortened accordingly (Kojima & Goldman-Rakic, 1982).

Recent neuroimaging and lesion studies have attempted to dissociate spatial and non-spatial WM neurological pathways within the PFC. Specifically, PFC lesions restricted to the dorsolateral subregion (DLPFC; principal sulcus) lead to impairment in SpWM equivalent to or

greater than complete PFC ablation (Goldman & Rosvold, 1970). However, lesions of the DLPFC do not substantially impair non-spatial WM (Levy & Goldman-Rakic, 1999). For example, monkeys with DLPFC lesions retain the ability to maintain the representation of object stimuli (Petrides, 2000). Likewise, PET and fMRI imaging has found persistent activity in the DLPFC throughout the entire delay period of SpWM tasks (Curtis, Rao, & D'Esposito, 2004; Curtis & D'Esposito, 2003; Owen, Downes, Shallice, Polkey, & Robbins, 1990). Conversely, damage to the ventrolateral PFC (VLPFC) as well as other regions of the cortex leaves SpWM largely intact (Levy & Goldman-Rakic) while causing considerable impairment of non-spatial WM (Passingham, 1975). Thus, it is generally agreed that “the DLPFC is primarily engaged in “online maintenance” of spatial information while the VLPFC supports non-spatial memoranda” (Curtis & D'Esposito, 2004 p. 530).

III.2. Nidopallium caudolaterale

The nidopallium caudolaterale (NCL), a region within the posterior avian forebrain, has been recognized as the functional equivalent of the mammalian PFC (Grüntürkün, 2005). The NCL contains dense dopaminergic immunoreactive neurons as well as numerous afferent and efferent connections similar to the PFC (Divac, Mogensen, & Bjorklund, 1985; Leutgeb, Husband, Ritters, Shimizu, & Bingman, 1996; Konecny, & Grüntürkün, 1999). Aside from the chemoarchitectonic similarities, it is a critical forebrain structure for WM and executive control in birds (Lissek & Grüntürkün, 2004; Rose & Colombo, 2005) and ablation of the NCL yields WM deficits analogous to those observed in humans and apes after damage to the PFC (Gagliardo, Mazzotto, & Divac, 1997; Mogensen & Divac, 1993). Furthermore, the NCL contains single neurons whose activity is modulated during WM comparable to that of PFC delay neurons (Diekamp, Gagliardo, & Grüntürkün, 2002; Diekamp, Kalt, & Grüntürkün, 2002; Rose & Colombo). Based on anatomical, electrophysiological and behavioral evidence, the NCL is the functional equivalent to the mammalian PFC, both supporting WM in a seemingly similar manner.

III.3. Mammalian Septal Area

Occupying a central position within the basal forebrain, the mammalian septum and its constituent nuclei are commonly referred to as the septal area (Swanson, 1977). As an extension of the limbic system, the septal area contains numerous efferent connections to a variety of structures including the amygdala, hypothalamus, HPC, ventral tegmental area (VTA), thalamus and preoptic area (POA; Swanson & Cowan, 1979). Notable areas of afferent projections to the septal area include the hypothalamus, the brainstem monoaminergic cell groups, the HF, the amygdala and the ventral subiculum (Risold & Swanson, 1997; Swanson & Cowan). These connections, as well as strong interconnections from the lateral septum (LS) to the medial septum (MS), allow the septal area to integrate limbic, telencephalic structures associated with higher level cognition and motivation with lower hypothalamic and brainstem areas related to endocrine autonomic functions (Leranth & Vertes, 2000). In essence, the mammalian septum can be viewed as playing a role in initially processing subcortical information about the activating effects or biological significance of episodes or events, and then subsequently modulating the responsiveness of its efferent targets. Therefore, given the mammalian septal area's importance in integrating neural signals across a range of neural systems, it is uniquely suited to support higher order cognitive processes such as WM.

III.3.1. *Septal Area and Working Memory*

The involvement of the mammalian septal area in WM has primarily been assessed through lesion studies (Numan & Quaranta, 1990; Numan & Klis, 1992; Rashidy-Pour, Motamedi & Motahed-Larijani, 1996). For example, Numan and Quaranta (1990) lesioned the MS in rats and examined performance on five versions of a delayed alternation task. Delay alternation is a version of the delayed response task that requires the subject to choose (between 2 stimuli) the stimulus opposite the one that yielded reinforcement immediately prior to the delay period (Numan, 2000). The first version of Numan and Quaranta's test included trials with no delay period. The second and third versions involved 10 second delays and 20 second delays, respectively, designed to assess SpWM. The fourth version incorporated trials with a 20 second delay followed by a cue light over the left lever to reduce the spatial requirements of the task. Lastly, the fifth version involved trials with a 20 second delay followed by a cue light over the correct lever that eliminated the WM component of the task. They found that the lesioned rats

showed impaired performance on the 10 and 20 second delay and the cued left versions of the task. However, there was no difference between the groups when there was no delay or the correct choice was cued. This indicates a WM-specific impairment following damage to the septal area. While some research has indicated that damage to the septal area results in both RM and WM deficits (i.e. Poucet & Buhot, 1994), other research has concluded that RM errors are essentially the result of WM deficits during training that prevent learning of the RM challenge. Reversible inactivation of the MS with tetrodotoxin or lidocaine immediately prior to training, but not following training, disrupted acquisition of the RM in a water maze (Rashidy-Pour et al., 1996). Taken together, the data suggest that the septal area is involved in the initial acquisition and maintenance of WM, and damage to this region is functionally similar to damage to the frontal cortex (Numan, 2000).

III.3.2. *Septal modulation of the hippocampus*

Although neuronal pathways extend from the mammalian septal area to a number of brain structures, the direct reciprocal connections with the HPC intimately connect the two subregions to such an extent that they are both integral in the expression of SpWM. This may be explained, in part, by septal modulation of HPC slow rhythmic activity.

Neurons in the HPC generate synchronous neuronal depolarizations between 7 and 12 Hz, commonly referred to as theta rhythm. HPC theta activity has been observed during motor activity (Vanderwolf, 1969; de Araujo, Baffa, & Wakai, 2002), emotional situations (Aftanas & Golocheikine, 2001; Pare & Collins 2000), attentional tasks (Dietl, Dirlich, Zvogl, Lechner, & Strian, 1999), and memory tasks (Lisman & Idiart, 1995; Burgess & Gruzelier, 1997; Jensen & Tesche, 2002; Givens & Olton, 1990). Moreover, HPC theta activity facilitates neuronal depolarizations at an optimal frequency to induce long-term potentiation (LTP) within the HPC (Greenstein, Pavlides, & Winson, 1988; Vinogradova, 1995). LTP is a candidate underlying physiological phenomenon that may facilitate the formation of memory (Bliss & Collingridge, 1993). Additionally, HPC theta may reset WM (Givens, 1996; Givens & Olton, 1995).

HPC theta activity is dependent on rhythmic neuronal firing in the septal area. Cholinergic and GABAergic neurons in the MS and diagonal band of Broca (DBB) fire

rhythmically and synapse on HPC pyramidal cells modulating synchronous depolarizations (Macadar, Roig, Monti, & Budelli, 1970; Vinogradova, Kitchigina, & Zenchenko, 1998). Thus, if neurons are firing synchronously at 7 Hz in the MS, one would expect populations of neurons in the HPC to fire at 7 Hz and produce an EEG wave of 7 Hz. Damage to the MS/DBB significantly reduces or abolishes HPC theta (Andersen, Bland, Myher, & Schwartzkroin, 1979). For example, temporary inactivation of MS/DBB neurons with GABAergic agonists and cholinergic antagonists produced a reversible disruption of HPC theta (Givens & McMahon, 1997; Givens & Olton, 1994; 1995).

Notably, only efferent projections from the MS/DBB are primarily involved in HPC theta. Disruption of afferent connections to the MS from the HPC does not abolish or significantly affect theta power or frequency (Rawlins et al., 1979). This suggests that the septal area modulates HPC processes without direct feedback, suggesting that the mammalian septal area initiates or plays a supporting role in behavior controlled by the HPC.

III.4. Avian Septal Area

The mammalian septum is homologous to the avian septum (Montagnese, Szekely, Adam, & Csillag, 2004). Comparative examination of developmental gene expression revealed similarities between mouse and chick along both the dorsal (pallial) and ventral (subpallial) portions of the septum (Puelles, Kuwana, Puelles, & Rubenstein, 1999; Puelles et al., 2000).

III.4.1. *Anatomy of the Avian Septal Area*

Although the characteristics of the avian septum have been grossly defined for over thirty years (Krayniak & Siegel, 1978), it is not until recently has a consensus been reached on its subdivisions. Largely consistent with literature on pigeons (Krayniak & Siegel; Atoji & Wild, 2004; Medina & Reiner, 1994) and chicks (Montagnese et al., 2004), Goodson, Evans and Lindberg (2004) divided the songbird septum into four distinct chemoarchitectonic zones (see Fig. III-a) including the MS, lateral septum (LS), caudocentral septum (CcS) and septohippocampal area (SH).

Figure. III-a. Drawings of the avian septum at rostral (A8.8-A9.0), commissural (A 7.2), and caudal (A7.2) levels depicting the major subdivisions, as determined by Goodson et al. (2004). Left: Rostrally, the septum is comprised of the lateral septum (LS) and medial septum (MS). Middle: The septohippocampal pathway appears at the level of the anterior commissure. Right: at caudal levels, the caudocentral septum arises from the MS, just ventral to the LS. CcS, caudocentral septum; LS, lateral septum; MS, medial septum; SH, septohippocampal pathway.

The MS arises rostrally and expands through commissural levels. It contains an intermediate sized population of cholinergic neurons (choline acetyltransferase (ChAT) immunoreactive), which give rise to HF projections (Medina & Reiner, 1994). Also of note, neuropeptide Y (NPY) reactive fibers are noticeably absent throughout the majority of the MS. Thus, the MS can be easily defined as an area with little immunoreactivity when staining for NPY.

The LS spans the entire length of the septal area, initially developing dorsally and expanding ventral-laterally toward commissural levels, at which point the LS contracts dorsal-laterally with the expansion of the CcS. The subdivisions of the LS are essentially analogous to the subdivisions in the rat LS as proposed by Risold and Swanson (1997). The major afferents to the LS arise from the HF and the LS projects to the MS. Thus, a similar feedback loop between the MS-HF-LS can be observed in both mammals (Swanson & Cowan, 1979) and birds

(Krayniak & Siegel, 1978; Montagnese, Zachar, Balint, & Csillag, 2008). The densest innervations of tyrosine hydroxylase (TH) reactive fibers are located in the LS, especially in the dorsal-most region.

The CcS expands caudally where the MS disappears. This structure may be the avian equivalent to the septofibrial nucleus in mammals (Risold & Swanson, 1997). The CcS has pronounced histochemical diversity and may be considered a posterior extension of the MS.

Structurally similar to the septohippocampal nucleus in the rat (Swanson & Risold, 2000), the SH develops along the midline beginning rostrally and expanding to the level of the CcS. The SH is predominately characterized by an abundance of substance P, NPY, and vasoactive intestinal polypeptide immunoreactive fibers. Additionally, neurons originating in the MS expand through the SH and synapse in the HF.

The avian septal area is a histochemically diverse structure. Nonetheless, only two immunohistochemical markers (TH and NPY) are necessary to delineate the different subregions. The divisional differences between the MS and the LS are largely similar to those seen in mammals. In addition, the pigeon and chick septal areas contain efferent connections to a number of structures, namely the HPC, hypothalamus, dorsomedial thalamus, and midbrain tegmentum, as well as fewer projections to the NCL, archipallium, and nucleus accumbens (Montagnese, et al., 2004; Krayniak & Siegel, 1978). Afferent connections to the septal area include HF, dorsolateral corticoid area, temporo-parieto-occipital area, rostral dorsomedial hyperpallium apicale, POA, hypothalamus, VTA, locus coeruleus and the raphe nuclei (Montagnese et al., 2008; Atoji & Wild, 2004). These connections are, in a large part, similar to those observed in the mammalian septal area. A principal difference between avian and mammalian septal efferent connections is that the avian septum has comparatively fewer projections to the hippocampus. The projections to the HPC in pigeons principally originate from the DBB (Casini et al., 1986; Montagnese, et al., 2004). Research in other bird species (i.e. budgerigars) concluded that the histochemical properties of the MS and LS are essentially reversed. Notably, in budgerigars the major projections to the HPC from the septum arise from the LS (Roberts, Hall, & Brauth, 2002). However, Goodson et al. (2002) argue that Robert's et al. organizational scheme is inconsistent with the established peptidergic features with the LS.

III.4.2. *Extension of limbic system function to birds*

Electrophysiological and neurochemical evidence in the avian brain raises the question of whether the avian septal area modulates HPC processes such as SpWM in a similar fashion as the mammalian septal area. Although HPC theta activity has almost exclusively been studied in mammals, Siegel et al. (2000) observed synchronous rhythmic firing in the pigeon HF, albeit at a slightly lower frequency (4-7 Hz) than theta activity typically observed in rats. Additionally, in the mammalian HPC, theta is driven by afferent cholinergic and GABAergic neurons from the MS/DBB. However, the avian septum contains a modest amount of cholinergic neurons (Goodson et al., 2004; Krebs et al., 1991), which are noticeably different from the mammalian septal area, although prominent numbers of cholinergic neurons (i.e. ChAT immunoreactive) have been observed in the DBB. The largest concentration of cholinergic neurons and fibers within the septum was found at caudal levels of the MS in songbirds. Consequently the avian HF contains a comparatively sparse number of cholinergic afferents from the septal area (Krebs et al., 1991).

Among the most studied areas within the avian (and mammalian) limbic system is the HF. Nevertheless, little research has focused on the HF and WM in birds. In one study, Sherry and Vaccarino (1989) aspirated the HF in black-capped chickadees and observed an inability to reliably recover food caches based on spatial location. However, no effect was found on a similar task that eliminated the spatial component and relied on well learned cues. Moreover, they observed that the HF lesioned group made significantly more revisiting errors in both the spatial task and the cue task. Such an increase in revisiting errors regardless of the type of task is indicative of a WM deficit. Hence, damage to the mammalian HPC and avian HF produces similar behavioral deficits, signifying a functional homology (Colombo & Broadbent, 2000; Colombo, Broadbent, Taylor, & Frost, 2001). While no study has examined the involvement of the avian septal area with respect to any memory system, several studies have hinted at septal involvement in memory. For instance, Shiflett, Gould, Smulders, and DeVoogd, (2002) compared the volume of the septal area in two non-storing species, blue tits and great tits, and one food-storing species, black-capped chickadees. Shiflett et al. reported that the relative septal volume was larger in chickadees than in the non-storing species. They also compared septum and

DBB volume in chickadees over the course of a year and concluded that the relative septal volume, but not DBB volume, varies seasonally. The volume of the septum increased in the fall, consistent with the onset of food storing behavior. This species and seasonal variation parallels the variation in the HF within chickadees and other food sorting birds, with increases during parts of the year when the animal is more dependent on spatial memory. Taken together, the increased septal volume in a food storing species as well as the seasonal variation in septal volume indicate that the septum may be specialized for some aspects of food-storing and spatial memory.

III.4.3. *Conclusion*

The septal area is evolutionarily conserved in mammals and birds. Among the anatomical similarities between the mammalian and avian septal areas is the connection to HPC. The mammalian HPC is critically involved in spatial processing and WM. Lesions to the HPC lead to deficits in SpWM. Comparably, damage to the avian HF has yielded deficits in spatial memory as well as increased WM errors. Moreover, in mammals, lesions to the MS/DBB lead to impaired performance on SpWM tasks, a result likely due to the strong connection between septum and HPC via the fornix. However, no study has attempted to ascertain if avian septal damage yields a similar SpWM deficit. As the integrity of mammalian septohippocampal interconnectivity is essential for SpWM, it is plausible that it may also be the case in birds.

APPENDIX IV

EXAMINATION OF POSSIBLE NON-SPECIFIC EFFECTS OF SEPTAL LESIONS

In addition to examining SpWM, several behavioral assays were performed to assess other possible lesion effects on behavior. Although the results from the behavioral assays do not bare any direct evidence on the SpWM task, they were conducted because the aforementioned experiment is among the first to examine the behavioral effects of septal lesions in birds and the researchers wanted to better understand the role of the septum in behavior. Based on the previous literature indicating that damage to the septal area lead to hyperdipsia (Blass & Hanson, 1970) and septal rage (Gray, 1982), water consumption and neophobia were examined.

IV.1. Hyperdipsia

Previous research in rats has indicated that electrolytic lesions of the septal area, specifically the MS, induce hyperdipsia, or an intense thirst marked by an increase in drinking behavior (Blass & Hanson, 1970). However, such an effect is dependent upon systemic body fluid volume loss, hypovoluma, an effect that can be induced by systemic injection of polyethelene glycol which draws extracellular fluids, reducing blood volume resulting in the relase of angiotensin (Tondat & Almli, 1975). Septal lesions, therefore, damage efferent projections to the vasopressinergic neurons in the supraoptic nucleus (SON) in the hypothalamus, which regulates angiotensin release, blood pressure and drinking. Septal hyperdipsia is not dependent on changes in body fluid osmolality, such as following water deprivation. Of note, septal area lesioned rats drank equivalently to sham controls when water deprived for 24 hrs (Sullivan, et al., 2003). Thus, under normal conditions (e.g. not injected with polyethelene glycol) hyperdipsia is rarely observed. As current experiment is among the first to study the effects of septal lesions in birds, the possibility of hyperdipsia birds was assessed by measuring daily water consumption.

IV.1.1. *Measurement of Daily Water Consumption*

Possible hyperdipsia was investigated during the course of the SpWM study. All birds had ad lib access to water throughout the course of the SpWM study. During SpWM testing, a

stainless steel feeding trough was filled with approximately 300 g¹ of water at the end of each testing day, weighed and made available to the test subject. At the beginning of the next testing day, the trough was weighed again and the difference was subtracted from the weight recorded the previous day, yielding an approximate measure of the amount of water consumed per day. The process was repeated for seven days, both preoperatively and postoperatively.

Figure. IV-a. Mean pre-/postoperative volumes of water consumed each day for the control (black) and septal-lesioned (white) groups. While the statistical analysis revealed significant intra-day variation in water consumption (not shown), the graph clearly highlights the minimal overall change in consumption both between the lesion and control groups as well as between the pre- and postoperative conditions within each group. Standard error bars are shown.

IV.1.2 Results and Discussion

A 2 (lesion/control group) x 2 (pre-/post-operative condition) x 7 (day) mixed-model ANOVA was conducted to assess any difference in water consumption following septal lesions. A significant difference between the septal-lesioned and sham control birds was not detected ($F_{1,10} = 0.78$, NS) nor was a main effect across pre-/post-operative condition detected ($F_{1,10} = 0.008$, NS). However, a significant main effect was found across the seven days ($F_{6,60} = 2.28$, $p < 0.05$) as well as a significant lesion/control group by day interaction ($F_{6,60} = 3.57$, $p < 0.005$) and a

¹ 300 g = 300 mL. Grams were used because water was weighed. Volume of water consumed could not be directly measured accurately due to the design of the water basin.

significant pre-/post-operative condition by day interaction ($F_{6, 60} = 3.21, p < 0.01$). These differences across recoding days may likely be attributed to the daily variation of the temperature and air pressure in the room where the birds were housed as opposed to variation in drinking induced through septal damage. Such environmental variation can affect the amount of water that evaporates each day and subsequently is counted as consumed each day. Further evidence that the significant effects across recording days can be attributed to environmental noise and not an effect of septal damage is a non-significant treatment condition by group interaction ($F_{1, 10} = 0.008$ NS) nor was a 3-way (group x treatment condition x day) interaction detected ($F_{6, 60} = 0.74$, NS). Overall, these data indicate that, although there is substantial amount of environmental noise within these data (indicative by the daily variation in water levels), lesions of the septal area do not result in substantially altered drinking behavior in birds.

It is noteworthy that such findings in birds do not contradict the mammalian literature. Studies in rats (Tondat & Almlı, 1975; Sullivan, et al., 2003) that have observed hyperdipsia following septal damage also induced hypovolemia, typically through injections of a hyperoncotic colloid (e.g. polyethylene glycol). As the drinking assay only measured daily water consumption and did not induce hypovolemia there was little reason to predict an alteration in drinking behavior. Thus, the results provide initial insight indicating a functional similarity between the avian and mammalian septal area but a comprehensive examination of the effects of septal damage on drinking in birds (such as following hypovolemia and alterations in body fluid osmolality) is necessary before generalizing the function of the septal area across taxa.

IV.2. Septal modulation of fear behavior

The septal area has been implicated in the expression of fear-like behavior in mammals (Gray, 1982) as well as aggressive behavior in birds (Goodson, Eibach, Sakata, & Adkins-Regan, 1999). However, conflicting research on the mammalian septum has led to ambiguity in interpreting a possible role of the septum in regulating emotional behavior. Initial investigation on the effects of septal lesions on emotionality in rodents observed a more robust startle response and a higher propensity to attack experimenters and fight with conspecifics in lesioned animals (i.e. King, 1958). This led to the diffusion of the idea that septal lesions producing increased anxiety or ‘septal rage’. Harvey, Lints, Jacobson, and Hunt (1965) supported this viewpoint after

observing an increase in freezing, an innate defensive behavior, in septal-lesioned rats indicating increased anxiety. More recent research has yielded seemingly contradictory findings. Notably, septal lesions lead to a decrease in defensive burying, suggesting a reduced expression of anxiety (Treit, 1991). Moreover, Pesold, and Treit (1992) demonstrated that septal-lesioned animals avoided a shock probe to the same extent as control subjects, indicating no change in fear behavior after damage to the septum.

Sparks and Ledoux (2000) reviewed the literature regarding the seemingly contradictory lines of research and concluded, partly based on the strong septal connections with the HPC and involvement in memory, that the septum is involved in the inhibition of the expression of defensive behavior when the information based on spatial-temporal relationships suggests that a threat may not be present. Thus, damage to the septum yields a lack of inhibition of defensive behavior.

One study that examined the relationship between anxiety and the septal area in pigeons offered a slightly different conclusion. Cohen and Goff (1978) lesioned the septal area in pigeons and subjected each bird to multiple trials of paired light-shock conditioning. Despite the cardioactive function of the septal area via modulation of hypothalamic vasotocin release, they found that lesions did not alter heart rate conditioning nor did the lesions affect the development of the conditioned response. Based on their observations, Cohen and Goff concluded that the avian septal area is not critical in defensive conditioning.

While there is no evidence that avian septal damage affects defensive conditioning, given the paucity of research, the possibility the septum may modulate defensive conditioning and/or levels of anxiety cannot be neglected. Thus, in order to exclude septal modulation of fear as a contributing factor affecting choice behavior on the SpWM task, a behavioral assay of neophobia was conducted on eleven of the fourteen test subject (3 lesioned birds were not included in the anxiety/neophobia testing). Neophobia was assessed by exposing each bird to several novel objects. The time to approach a foodsite next to the object was compared between septal-lesioned and sham control birds in addition to contrasting the latencies between pre- and post-operative conditions.

IV.2.1. *Response to novelty*

Levels of response to novelty were scored by exposing each pigeon, individually, to several novel stimuli including statues, plants, stage props, lamps, and a natural predator (a stuffed broad-winged hawk). Novelty testing took place just prior to pre-operative SpWM testing and immediately after post-operative SpWM testing utilizing the same testing room and one of the eight foodsites (see Fig. 1B). A foodsite was placed at location 1 (see Fig 1A) and was baited with pigeon chow. A bird was then placed in the center of the dark testing room, the lights were turned on and the animal was be free to eat from the feeder. Turning on the lights coincided with starting a stopwatch to measure latency to approach and eat from the feeder. Once the bird placed both feet to the ramp of the foodsite, the stopwatch was stopped, and latency to approach was recorded while the bird was allowed to consume the chow. Once the pigeon stepped off the ramp the trial ended. The lights were turned off and the animal was removed from the room for a 1 min inter-trial interval during which time the feeder was re-baited. The bird was then reintroduced to the room, whereby the next trial commenced. If the bird did not eat from the feeder in 15 min the trial was scored as “fail”, the latency to approach the foodsite was recorded as 15 min, and the trial ended. Of note, nonapproach-trials rarely occurred throughout testing and were only observed during novelty trials (see below). The process was repeated until the bird had completed 5 trials in one day.

This task utilized 5 trials per session across 4 days (20 trials total). Novelty testing occurred on trials 9, 13 and 18 (the 4th trial of the second day, and the 3rd trial of the third and fourth days). Novelty trials were different from the other (usual) trials in that a novel object was introduced into the room during the inter-trial interval of each novelty trial and was placed either next to or behind the foodsite, depending on the size of the object. The pigeon was then placed in the dark testing room, the trial commenced, and the subject was allowed eat from the feeder. After the completion of the novelty trial, regardless of whether the bird approached the foodsite, the novel object was removed from the room during the inter-trial interval. Postoperatively, a pigeon was exposed to the same procedure using new novel objects. All 4 novel objects were counterbalanced across test subjects. Additionally, each animal was exposed to the hawk twice, once preoperatively to and once postoperatively, both occurring on trial 18.

A latency difference score, the behavioral dependent measure, was calculated for each bird each day as the difference between the latency to approach the food site in the novelty trial and the preceding trial. Additionally, the difference between trials 3 and 4 on day 1 was calculated as a non-exposure control. The difference scores were then analyzed utilizing a 2 (group: lesion, control) x 2 (treatment condition: pre-/post-operative) x 4 (day) mixed model ANOVA.

IV.2.2. *Results and Discussion*

During novelty testing, the latency difference scores did not significantly differ between the septal-lesioned and sham control groups ($F_{1,10} = 0.07$, NS) nor did the differences scores differ across the pre-/post-operative conditions ($F_{1,10} = 3.03$, NS). However, a significant main effect for the difference scores across the four testing sessions was found (see Fig. IV-b, $F_{3,30} = 10.00$, $p < 0.001$). Post-hoc comparisons using the Sidak-Bonferroni procedure determined the latency to approach the foodsite was significantly longer when exposed to the hawk (in session 4) compared to, both, control trials on in the first session, when no item was present ($t_{11} = 4.42$, $p < 0.005$), and when compared to other novelty trials (session 2: $t_{11} = 2.40$, $p < 0.05$; session 3: $t_{11} = 3.39$, $p < 0.01$). Interestingly, a significant group x pre-/post-operative condition interaction was found (see Fig. IV-c, $F_{1,10} = 14.32$, $p < 0.005$). However, a group x session interaction was not detected ($F_{3,30} = 0.23$, NS) nor was a pre-/post-operative x session interaction detected ($F_{3,30} = 1.56$, NS). Additionally, a 3-way (group x treatment condition x session) interaction was not found to be significant ($F_{3,30} = 1.56$, NS).

The birds' latency to approach the feeder did not significantly differ between septal lesion and sham control groups nor did the latencies significantly differ before and after surgery. Additionally, based on our analysis the parameter that produced the greatest variability in approach time was the testing session, indicative that the *type* of addition to the testing environment (hawk vs. novel object or vs. non-exposure control), regardless of whether or not the birds had septal damage, accounted for the variability in approach time. Birds took longer to approach the foodsite when the hawk was present compared to when it was not present (in all other novelty trials). Additionally, the birds took longer to approach the foodsite in novelty trials when a novel object was present in the testing room compared to control trials where no object

was present. Taken together, these results suggest that septal lesions did not affect a bird's behavior when exposed to a novel object compared to both their own preoperative data as well as non lesion sham control birds.

Figure. IV-b. Latency difference scores across all 4 testing sessions for the sham control (black) and septal-lesioned (gray) groups differentiating between the pre- (solid line) and post-operative (hashed) conditions. Difference scores were calculated by subtracting the latency to approach the foodsite on the preceding trial from the novelty trial. Thus, a score greater than 0 indicates a longer latency to approach the foodsite in the novelty trial compared to the preceding trial while a score less than 0 indicates a shorter latency to approach the foodsite on the novelty trial compared to the preceding trial. Session 1 (calculated as trial 4-3) served as a non-exposure control, while a novel object was presented on session 2 (trial 9-8) and session 3 (trial 13-12), and the hawk was presented in session 4 (trial 18-17). Standard error bars are shown.

Figure. IV-c. Mean pre-/post-operative latency difference scores for the sham control (black) and septal-lesioned (white) groups highlighting a significant treatment (pre-/post-operative) condition by group interaction ($p < 0.005$). Standard error bars are shown.

IV.3. General Discussion

As the principal experiment of this thesis is among the first studies to lesion the avian septal area it offered a unique opportunity to observe any possible behavioral changes following septal damage. Moreover, a substantial literature exists implicating the mammalian septal area in drinking behavior as well as in neophobia/anxiety behaviors, providing a starting point for behaviors that can be observed in birds that might have a perceivable change following septal lesions. The assessment of drinking behavior did not reveal a change in water consumption following damage to the septal area. Additionally, the neophobia behavioral assay did not clearly reveal a significant difference in approach time to a novel object following septal damage. Although not exhaustive, the two behavioral assays, they do provide some insight onto the behavioral effects of septal lesions. Nonetheless, a more comprehensive examination of septal damage on drinking or neophobia is necessary before any conclusions can be drawn.

APPENDIX V

EXAMINATION OF SITE SPECIFIC LESION EFFECTS

During our initial histological analysis, it was observed that the extent of septal lesion damage across the experimental pigeons was characterized by some inter-individual variability. Therefore, it was of interest to examine if size of the WM deficit displayed by different individuals could be explained, at least in part, by the amount of total septal damage or amount of damage to a particular subdivision.

In order to assess whether the variance in the amount of total septal damage (S_D) accounts for the variance in performance on the SpWM task, four separate regression analyses were conducted on the four difference scores (one regression per dependent measure: total choices required to reach all 4 baited food sites, TOT; correct choices among the first 4 responses, 1ST4; proactive errors, PE; retroactive errors, RE). Each regression analysis for all four dependent measures did not reveal a significant effect for total septal damage (TOT ($F_{1,8} = 0.615$, NS), 1ST4 ($F_{1,8} = 0.002$, NS), PE ($F_{1,8} = 1.42$, NS), RE ($F_{1,8} = 0.704$, NS)). As such, S_D did not predict behavioral performance in the SpWM task.

Additionally, as amount of total septal damage did not significantly account for the variance in performance, a subdivisional analysis was warranted. First, the lesion damage was quantitatively analyzed for each bird and percent of structural damage was calculated separately for the left and right hemispheres of the medial septum (MS_D), lateral septum (LS_D), and caudal central septum (CcS_D ; see table V-a). The damage to the two hemispheres of each subdivision was then combined to obtain a total percent damage to each subdivision.

Following the determination of the percent septal subdivision damage for each bird, I carried out several multiple regressions using these values to determine if damage to any particular septal subdivision predicted performance in the SpWM task. Accordingly, two backward stepwise multiple regressions were carried out using S_D , MS_D , LS_D , and CcS_D as predictor variables for 1ST4 and TOT difference scores. The regressions failed to reveal any significant differences. Thus, no predictor variable used in the regression analyses predicted performance in the SpWM task.

In a last investigative attempt, a regression was run on the first and last trial block on both 1ST4 and TOT using S_D as the predictor variable. No regression was found to be significant for either the first (TOT ($F_{1,8} = 1.15$, NS), 1ST4 ($F_{1,8} = 2.52$, NS)) or last (TOT ($F_{1,8} = 0.81$, NS), 1ST4 ($F_{1,8} = 1.9$, NS)) trial block. As noted in the text, visual inspection of the scatter plot with the 1ST4 from the first trial block regressions revealed an apparent outlier and following exclusion of that bird, the regression (first trial block of 1ST4) among the remaining 8 birds was found to be significant ($F_{1,7} = 7.301$ $p = 0.03$).

Table V-a. Observed percent volume damage to the right, left, and total (right + left) for subdivisions of the septal area

Subject	total septum			LS			MS			CcS		
	R%	L %	Total %	R%	L %	Total %	R%	L %	Total %	R%	L %	Total %
456	53.15	47.82	50.42	47.91	53.33	50.60	63.62	46.88	54.81	32.81	23.71	28.18
458	48.99	44.63	46.76	64.87	66.65	65.75	44.97	33.02	38.68	0.00	0.00	0.00
408	77.91	58.33	67.89	99.68	83.99	95.13	41.79	22.14	31.45	17.74	23.31	20.57
274	20.81	15.39	18.04	17.69	14.72	16.22	28.84	15.63	21.89	9.59	12.46	11.05
227	70.23	43.42	56.51	75.59	47.20	61.50	56.88	49.97	53.24	54.04	12.46	32.90
217	16.96	47.15	32.40	21.30	45.14	33.13	17.46	43.42	31.12	0.00	45.34	23.05
185	43.43	31.52	37.34	32.29	32.51	32.40	65.89	42.68	53.68	37.67	7.99	22.58
183	60.87	70.36	65.73	54.61	71.29	62.89	68.85	70.09	69.50	44.26	49.92	47.14
165	62.20	69.01	65.68	53.19	74.64	63.84	91.19	77.10	83.78	20.33	24.42	22.41

APPENDIX VI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL LETTER

Office of Research Compliance
308A University Hall
Bowling Green, OH 43403-0183
Phone: (419) 372-7716
Fax: (419) 372-6916
E-mail: hsrb@bgsu.edu

August 28, 2009

Dr. Vern Bingman
Psychology
Bowling Green State University

Re: IACUC Protocol 09-006

Title:

The role of the hippocampus and septal area in homing pigeon working memory

Dear Dr. Bingman:

On **August 26, 2009** the above referenced protocol received **final approval** after review of the requested clarifications by the IACUC. The clarifications have been incorporated into the official copy of your protocol (see attached).

This approval expires on August 25, 2010, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol. Please also remember to keep a copy of the approved protocol in the animal facility room(s) in which your animals are housed and in any associated procedure rooms (contact the UAF staff for assistance in this regard).

Please consult with the staff of the Animal Facility about your requirements to get started on this project. Good luck with your project.

Sincerely,

Hillary Harms
IACUC Administrator

Comments: