BRIEF COMMUNICATION

Inhibition, the Final Frontier: The Impact of Hippocampal Lesions on Behavioral Inhibition and Spatial Processing in Pigeons

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Two commonly accepted functions of the mammalian hippocampus are the processing of spatial information and behavioral inhibition. Although there is clear evidence that the avian hippocampus is also critical for the processing of spatial information, there is a dearth of evidence that it plays a role in behavioral inhibition. In Experiment 1, the effect of hippocampal lesions on behavioral inhibition in pigeons was assessed. Control and hippocampal lesioned pigeons were trained to peck stimuli that were associated with one of three probabilities of reinforcement (40%, 70%, or 100%). The number of pecks that control pigeons distributed to each stimulus was directly related to its respective probability of reinforcement. In contrast, hippocampal lesioned pigeons distributed their pecks evenly across the three stimuli. In Experiment 2, we tested whether these pigeons also displayed the well-accepted spatial deficit. Consistent with previous work, control pigeons acquired the radial arm maze in significantly fewer trials than the hippocampal lesioned pigeons. Together, Experiments 1 and 2 demonstrate that, much like the mammalian hippocampus, the avian hippocampus is critical to behavioral inhibition and spatial processing.

Keywords: hippocampus, behavioral inhibition, spatial processing, lesion, comparative neuroscience

Early investigations of the hippocampus largely ascribed it a unitary spatial function. For example, O’Keefe and Nadel (1978) proposed that the hippocampus functioned solely as a cognitive map, allowing an animal to encode the spatial properties of its environment and use them for navigation. However, two findings suggested that a unitary view of hippocampal function was not adequate. First, in addition to spatial deficits, hippocampal lesions were also shown to produce deficits in behavioral inhibition (e.g., Cheung & Cardinal, 2005; Jarrard, 1968; Rawlins, Feldon, & Butt, 1985). Second, the dorsal hippocampus alone can account for the spatial deficits observed after hippocampal lesions (e.g., Bannerman et al., 1999; Hock & Bunsey, 1998; Moser, Moser, Forrest, Andersen, & Morris, 1995). That is, rats with dorsal hippocampal lesions display comparable spatial deficits to rats with complete hippocampal lesions (Bannerman et al., 2003). In contrast, rats with ventral hippocampal lesions perform at a comparable level to sham-operated controls (Bannerman et al., 2003). This latter finding raises the intriguing question of what function the ventral hippocampus serves.

A long-held view of hippocampal function suggests that it is part of the brain system or systems responsible for anxiety and behavioral inhibition (Gray & McNaughton, 2000). Building on this view, a growing body of work suggests that the ventral hippocampus may play a role in this process (Bannerman et al., 2002, 2003; Richmond et al., 1999). For example, Bannerman et al. (2002) compared rats with complete hippocampal lesions, or lesions restricted to the dorsal or ventral hippocampus, on a range of spatial and behavioral inhibition tasks. Consistent with earlier work, rats with ventral hippocampal lesions performed at a comparable level to sham-operated controls on two standard measures of spatial processing (the t-maze and water-maze tasks) whereas rats with complete or dorsal hippocampal lesions performed at around chance. In contrast, on two tasks of behavioral inhibition (the time in the open arm vs. closed arms of a plus maze and the consumption of novel foods tasks), the opposite result was obtained, with rats with ventral hippocampal lesions behaving like those with complete hippocampal lesions whereas rats with dorsal hippocampal lesions behaved similar to the sham-operated controls.

The hippocampus in monkeys also displays functional differentiation, in this case along its anterior-posterior axis (Colombo, Fernandez, Nakamura, & Gross, 1998). Specifically, consistent with data from rats, the posterior hippocampus of monkeys (the equivalent of the dorsal hippocampus of rats) plays a preferential role in the processing of spatial information (Colombo et al., 1998). It is important to note that there is also evidence that hippocampal lesions reduce behavioral inhibition in monkeys. For
example, when monkeys are required to reach over a fear-provoking stimulus (e.g., a rubber snake or spider) to obtain food, monkeys with hippocampal lesions show shorter food retrieval latencies and fewer emotional responses to the stimulus (Chudasama, Izquierdo, & Murray, 2009; Chudasama, Wright, & Murray, 2008). Although the mapping between location (i.e., anterior and posterior) and function (i.e., behavioral inhibition and spatial processing) is not yet as clear for monkeys as it is for rats, the data collected thus far are intriguing.

In contrast to monkeys and rats, evidence for deficits in behavioral inhibition in birds with hippocampal lesions has been difficult to obtain. Indeed, although there is unequivocal evidence that the avian hippocampus plays a critical role in the processing and retention of spatial information (Broadbent & Colombo, 2000; Colombo & Broadbent, 2000; Colombo, Broadbent, Taylor, & Frost, 2001; Colombo, Cawley, & Broadbent, 1997; Watanabe & Bischof, 2004), there is a dearth of evidence that the avian hippocampus plays a role in behavioral inhibition. The only evidence to date that the avian hippocampus may play a role in behavioral inhibition was a brief observation by Colombo and Broadbent (1999) that during the initial phases of training on a t-maze, pigeons were less likely to “freeze” when an experimenter was present in the room during training. This finding is similar to data collected with rats (Bannerman et al., 2002) and monkeys (Chudasama et al., 2009, 2008) using tasks that induce anxiety or fear.

There is evidence that hippocampal lesions in rats produce general deficits in behavioral inhibition rather than deficits specific to tasks or contexts likely to induce anxiety or fear (e.g., Douglas & Isaacs, 1964; Jarrard, 1968; Whishaw & Mittleman, 1991). An open question is whether pigeons also display deficits in behavioral inhibition in nonanxiogenic-inducing contexts. Several studies have demonstrated that rats with hippocampal lesions display less efficient behavior, eliciting more responses per reinforcer compared with control subjects, when trained to press a lever on a differential reinforcement of low rates (DRL) schedule (e.g., Bannerman et al., 1999; Sindén, Rawlins, Gray, & Jarrard, 1986). Uncovering this finding prompted us to reassess unpublished data we originally collected to investigate the effect of hippocampal lesions on reward processing (Pow, 2008). The paradigm, similar to the DRL schedule, provides a measure of behavioral inhibition by investigating whether subjects modulate or scale their behavior in relation to the probability of reinforcement. Consistent with the DRL studies conducted with rats, pigeons with hippocampal lesions failed to scale their behavior to the probability of reinforcement. This finding adds to the evidence that the avian hippocampus serves similar functions as the mammalian hippocampus (see Colombo & Broadbent, 2000; Mayer, Watanabe, & Bischof, 2013, for reviews).

Experiment 1

Method

Subjects. Sixteen naïve pigeons, eight with bilateral hippocampal and area parahippocampalis lesions and eight sham-operated controls, served as subjects. Subjects were individually housed in a colony room. The room was maintained at 20 °C, and overhead fluorescent lights were turned on daily at 7:00 a.m. and turned off 12 h later. Grit and water were provided ad lib. Subjects were maintained at 80–85% of their free-feeding weight for the duration of the experiment. All experimental procedures were approved by the University of Otago Animal Ethics Committee and conducted in accordance with the University of Otago’s Code of Ethical Conduct for the Manipulation of Animals.

Surgery. The animals were anesthetized with a 100-mg/mL concentration of ketamine hydrochloride. A Revzin stereotaxic adapter (Karten & Hodos, 1967) was used to immobilize the head, and a topical anesthetic Xylocaine (10%) was applied to the scalp. A midline incision was made, the scalp was retracted, and the skull covering the hippocampus and area parahippocampalis was removed. The hippocampus and area parahippocampalis were removed bilaterally by aspiration according to the location given by Karten and Hodos (1967). After closing the incision, additional topical anesthetic was applied to the site, and subjects recovered in a padded recovery cage. The control animals underwent the same procedure but no tissue was aspirated.

On completion of the experiments, the pigeons were deeply anesthetized with halothane anesthesia and perfused intracardially with physiological saline, followed by 10% formalin-saline. The brain was removed from the skull and fixed in sucrose-formalin (30%), embedded in paraffin, and sectioned at 25 µm, with every fourth slice stained with cresyl violet.

Apparatus. The birds were trained and tested in two standard operant chambers. Three of the chamber’s interior panels were black, and the front panel housed a 17-inch LCD monitor. Responses to stimuli were recorded using an infrared touch frame positioned between the front panel and computer monitor. A ventilation fan situated in the rear of the operant chamber masked any extraneous noise. A houselight located on the top center of the back wall was on at all times during training and testing. Each chamber was controlled by a Pentium PC computer. The stimuli were three forms: a plus shape, the command icon shape found on Mac computers, and a triangle. Stimuli were approximately 27 × 27 mm and appeared as white forms on a black background.

Procedure. Subjects were first magazine trained and then autoshaped to peck a white disk (Brown & Jenkins, 1968). After autoshaping, subjects were transferred to the operant task. On each trial, after a variable intertrial interval (ITI) of 10–50 s, one of the three stimuli was presented for 5 s followed by a 2-s reward. The probabilities of reinforcement differed across the three stimuli and were as follows: the plus stimulus was rewarded on 100% of trials, the command stimulus was rewarded on 70% of trials, and the triangle stimulus was rewarded on 40% of trials. For ease of explanation these will be referred to as the CS100, CS70, and CS40 stimuli, respectively. A session consisted of 30 trials with each stimulus appearing 10 times within a session. Training continued for 14 days.

Although a failure to scale the level responding to the probability of reward may reflect a deficit in behavioral inhibition, it could also simply reflect an inability to distinguish between the three stimuli or their respective probabilities. To test this possibility, immediately after the training phase, subjects were given a 30-trial session in which all three of the stimuli were presented simultaneously for 5 s and the responses to each recorded. This preference test was conducted in extinction. The left-to-right order in which the stimuli appeared on the screen was counterbalanced such that they appeared an equal number of times in each position.
Results and Discussion

Reconstructions of the brains of the eight hippocampal lesioned pigeons are shown in Figure 1. For all birds except B13 the lesions were complete and included the hippocampus and area parahippocampalis, encroached onto the caudal part of the hyperpallium apicale, and in several animals extended laterally into the area corticoidea dorsolateralis (e.g., see section +6 of B7 and B9). Bird B13 differed from the rest in that there was some sparing of the anterior portions of the hippocampus. In the case of B15, there was some minimal damage sustained to the neostriatum caudale (see section +5).

The number of responses emitted by the control and hippocampal animals to the CS100, CS70, and CS40 stimuli was summed separately for the first 7 days (Block 1) and last 7 days (Block 2) of training. For each subject, the proportion of responses made to the CS100, CS70, and CS40 stimuli was then calculated separately for Blocks 1 and 2 (see Figure 2). All data were arcsine transformed before statistical analysis. The data for each block were subjected to separate two-way analyses of variance (ANOVAs) with conditional stimulus (CS; 3: 100, 70, and 40) as a repeated measure and Group (2: hippocampal and control) as a between-subjects factor.

For Block 1, there was a significant effect of CS, $F(2, 28) = 7.52, p < .05$, and no effect of Group, $F(1, 14) = 3.77, p = .07$, qualified by a significant CS × Group interaction, $F(2, 28) = 3.37, p < .05$. To investigate the significant CS × Group interaction, separate one-way ANOVAs with CS (3: 100, 70, and 40) as the repeated measure were performed for the control and hippocampal animals. For the control animals, there was a significant effect of CS, $F(2, 14) = 8.32, p < .05$, and, consistent with the view that control animals scaled their responding to the probability of reinforcement, a significant linear trend, $F(1, 7) = 13.87, p < .05$, with the linear component accounting for 100% of the variance (see Figure 2, upper

Figure 1. Coronal sections illustrating the extent of the hippocampal lesions. For each brain the sections are arranged in 1-mm increments from 8 mm (top) to 4 mm (bottom) anterior of earbar zero. A = archistriatum; AV = archistriatum pars ventralis; Cb = cerebellum; CDL = area corticoidea dorsolateralis; DA = tractus archistriatalis dorsalis; E = ectostriatum; HV = hyperstriatum ventrale; N = neostriatum; NC = neostriatum caudale; V = ventricle.
For the hippocampal animals, there was no effect of CS, $F(2, 28) = 101.83, p < .001$, but no significant effect of group, $F(1, 14) = 0.13, p = .72$, and no Group $\times$ CS interaction, $F(2, 28) = .68, p = .42$. The absence of a significant main effect for group, and the absence of a Group $\times$ CS interaction, reflects the fact that, just like the control subjects, subjects with hippocampal lesions displayed preferences that are consistent with each stimuli respective reward value.

The findings of Experiment 1 suggest that the avian hippocampus plays a role in behavioral inhibition, adding to its established role in spatial processing (Colombo & Broadbent, 2000). For thoroughness, and to show behavioral inhibition and spatial deficit in a single group of pigeons, in Experiment 2 we trained the birds from Experiment 1 on an open-field version of the radial arm maze, a task of which the essence is directing movements through space and that has repeatedly shown to be sensitive to hippocampal lesions in birds (Colombo et al., 2001, 1997).

**Experiment 2**

**Method**

Subjects, apparatus, and stimuli. The subjects were the same birds used in Experiment 1. Details of the apparatus have been described elsewhere (Colombo et al., 2001, 1997). In brief, eight identical blue cups (7.3 cm high $\times$ 7.5 cm in diameter) were attached to wooden blocks (4.2 cm high $\times$ 9 cm long) and mounted onto a white table (1.2 m $\times$ 2.4 cm) that was elevated 76 cm from the floor. A circle, 30 cm in diameter, was drawn around each cup. If a pigeon placed its foot on or inside of the circle this was considered a visit. The circle was drawn so that the tallest bird was unable to see whether the cup contained a reward if it stood outside of the circle.

The room that the maze was in contained several contextual cues such as cabinets, computers, and bookcases. An externally controlled light hung above the maze and was switched on at the beginning of each trial and off once the trial was completed.

Procedure. The birds completed three shaping phases to become accustomed to the maze. In Phase 1, two peas were placed on each of the eight circles and the blocks, rims, and inside of the cups. Birds were required to eat from at least five of the eight circles within 10 min. In Phase 2, two peas were placed on the blocks, rims, and inside of the cups. Birds were required to eat from at least five of the eight blocks within 5 min. If a bird failed to complete Phase 2 for 5 consecutive days, they were retrained on Phase 1. This process was repeated until the animal was consistently eating from the blocks, rims, or inside of the cups. In the final phase, two peas were placed on the rims and inside of the cups. Birds were required to eat at least one pea from inside of the cups for at least five of the eight cups within 5 min.

Acquisition. Acquisition began after the successful completion of Phase 3. In the dark, each animal was placed in the middle of the table until the experimenter left the room. The light was then switched on and the pigeon was given 5 min to explore the maze and locate the peas. There were two peas in each cup. If the pigeon completed this task within 5 min, then the time was recorded and...
the session was terminated. Cup visitation was recorded, which was
defined as the bird placing its foot on or inside of the circle. The
number of commission errors (i.e., visiting a previously vis-
it cup) and omission errors (i.e., failing to visit a cup) was also
recorded. Subjects were trained until they satisfied a criterion of
two sessions with no more than three errors, and no more than two
errors on any one of the two sessions.

Results and Discussion

Hippocampal animals required a greater number of trials to
reach the criterion ($M = 21$ sessions) compared with control
animals ($M = 13$ sessions), $t(14) = 2.23$, $p < .05$. Rather than
reflecting a spatial deficit, it is possible that the hippocampal
animals’ increased acquisition was the product of poor inhibition.
That is, similar to Experiment 1, it is possible that hippocampal animals had greater difficulty inhibiting their approach to a previ-
ously visited cup. To address this possibility, we calculated the proportion of commission errors that control and hippocampal animals made across the first five sessions of training. Consistent
with the view that hippocampal animals’ increased acquisition reflects a spatial, rather than inhibitory, deficit, there was no difference in the proportion of commission errors made by control
and hippocampal animals, $t(14) = .08$, $p = .94$.

The findings of Experiment 2 are consistent with previous work
with pigeons (Colombo et al., 2001, 1997; Pearce, George, Has-
grove, Erichsen, & Good, 2005) and rats (e.g., Olton, 1977; Olton,
Becker, & Handelmann, 1979; Olton & Papas, 1979; Olton & Samuelsen, 1976). In addition, it confirms that this group of
pigeons displays a deficit in behavioral inhibition and spatial
processing.

General Discussion

The findings of the experiments presented here suggest that,
much like the mammalian hippocampus, the avian hippocampus
plays a role in response inhibition (Experiment 1) and spatial
processing (Experiment 2). Although the effect of hippocampal
lesions on spatial processing in pigeons is not new (Colombo et al.,
2001, 1997; Pearce et al., 2005), the finding that the lesions also
impair response inhibition is somewhat novel and extends Co-
ombo and Broadbent’s (1999) early observation of reduced freez-
ing behavior by demonstrating a deficit in response inhibition in a
nonanxiety-inducing context. This finding is consistent with pre-
vious work demonstrating deficits in response inhibition in mam-
als after hippocampal lesions (Bannerman et al., 2002, 2003,
1999; Cheung & Cardinal, 2005; Chudasama et al., 2009, 2008;
Richmond et al., 1999).

One issue that the findings presented here obviously cannot
speak to is whether behavioral inhibition and spatial processing are
subservied by different areas of the avian hippocampus. As noted in
the introduction, there is evidence that the dorsal and ventral
hippocampi of rats play a preferential role in spatial processing
and response inhibition, respectively (Bannerman et al., 2002,
2003, 1999). In addition, there is also evidence for functional
differentiation along the anterior-posterior axis of the hippocam-
pus in monkeys (Colombo et al., 1998). One issue that complicates extending this work into avian species is the lack of consensus
over the location and number of subdivisions contained within the
avian hippocampus and, consequently, whether there is correspond-
ence with regions of the mammalian hippocampus. Initially, the
avian hippocampus was divided into the hippocampus and area
parahippocampalis (Karten & Hodos, 1967). Since this initial
division, the avian hippocampus has been said to contain between
five and seven subdivisions (Erichsen, Bingman, & Krebs, 1991;
Krebs, Erichsen, & Bingman, 1991; Siegel, Nitz, & Bingman,
2000). Most recently, using a combination of Nissl staining and
tract-tracing, Atoji and Wild (2004, 2006) identified the follow-
ning seven subdivisions: dorsolateral, dorsomedial, triangu-
lar, V-shaped layer, magnocellular, parvocellular, and cell-poor
regions. With respect to correspondence, the V-shaped layer is
thought to be comparable to the mammalian dentate gyrus, and the
dorsomedial subdivision has components of Ammon’s horn and
the subiculum (Atoji & Wild, 2004, 2006), although further work
is needed to fully elucidate the correspondence between specific
subdivisions (Shanahan, Bingman, Shimizu, Wild, & Güntürkün,
2013) and the relationship of these areas to the rodents’ dorsal/
ventral subdivisions or the primates’ anterior/posterior subdivi-
sions. Given that most evidence suggests that the avian hippocam-
pus is an analogue of the mammalian hippocampus, and that the
inhibition aspect of mammalian hippocampal function has now
been shown in birds, we would predict that within due course there
will be evidence of functional differentiation within the avian
hippocampus.

Conclusion

Bullock (1984) noted that “Comparative neuroscience is likely
to reach insights so novel as to constitute revolutions in under-
standing the structure, functions, ontogeny, and evolution of the
nervous system.” We concur with Bullock (1984) that cross-
species comparisons will be critical to developing a full under-
standing of human brain function, and the study presented here
represents a modest contribution to this endeavor. To use Beach’s
(1950) Lewis Carroll analogy, neuroscience as a field must resist
the temptation to focus almost exclusively on the rat or else they
may find that the Snark (i.e., an understanding of human brain
function) they seek is really a Boojum (i.e., an understanding of a
single species brain function).

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