Reversible Inactivation of the Hippocampal Formation in Food-storing Black-capped Chickadees (*Poecile atricapillus*)

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bird was rechecking an already visited feeder or merely perching at the feeder. If a bird visited the baited feeder before any other feeders on the first entry, then the trial was discarded, since it is
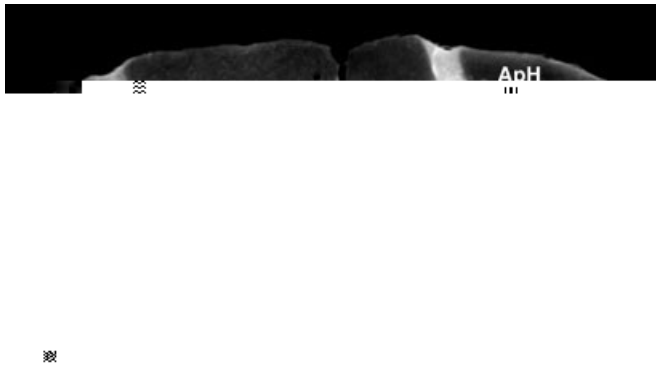
FIGURE 3. Distribution of ͺuorescein-labeled dextran infusions for one subject. Fluorescence appears in the right hippocampal formation (HF) and lateral to the HF in left hemisphere. With a few exceptions, ͺuorescence from the labeled dextran amines was conﬁned to the hippocampal formation (which consists of the hippocampus proper and the area parahippocampalus). ApH, area parahippocampus; Hp, hippocampus; S, septum. Scale bar = 1 mm.

location and spread of fluids from the cannula infusions. Alternate sections were stained with cresyl violet and were coverslipped and examined with a light microscope.

Statistics

To compare a subject's performance for different test series within a treatment condition as well as across treatment conditions, we used a repeated-measures general linear model analysis of variance (ANOVA) and paired *t*-tests. The dependent variable for all experiments was the number of feeders the bird visited. When examining performance on the spatial and visual task, the fixed factors in the GLM equation were entries (1–5) and Drug (lidocaine or saline). When examining the retrieval of spatial memory, paired t-tests were performed for the different delay conditions comparing performance on the probe after lidocaine or saline infusions.

RESULTS

Histology

Fluorescence from the labeled dextran amines could be detected within the HF in every animal (Fig. 3). The positions of cannulae were identified in cresyl violet-stained sections; they revealed small amounts of tissue damage (Fig. 4). In two birds, fluorescence and corresponding cannulae positions were found lateral to the left HF.

Effects of Hippocampal Inactivation on Short-term Spatial and Visual Memory

A total of five birds were tested in the spatial task. In this task, feeders were the same color such that birds had to rely on spatial cues to remember the location of the baited feeder. There was an overall tendency for birds to improve their performance across

entries ($F_{4,16}$ 8.74; P 0.001), however no main effect of drug ($F_{1,4}$ 2.21; P 0.21). There was a significant entry   drug interaction ($F_{4,16}$ 9.56; P 0.001) (Fig. 5A), indicating that improvement on the spatial task occurred after saline infusions, but not after lidocaine infusions.

Five birds were tested in the visual-spatial task, in which each feeder was painted a unique pattern of colors. For birds in the visual-spatial task, there was an overall tendency to improve performance ($F_{4,16}$ 11.46, P 0.001), but no main effect of drug was found ($F_{1,4}$ 0.83, P 0.41). Unlike the spatial task, there was no entry   drug interaction ($F_{1,4}$ 2.54, P 0.08). (Fig. 5B) Thus inactivation of the HF in food-storing birds did not impair the acquisition of a task that can be solved with visual patterns as well as spatial memory.

Effects of Hippocampal Inactivation During the Retrieval Process

Five birds received a single spatial test series of five consecutive entries, followed by a delay of either 15 min or 3 h. For subjects in both the 15-min delay condition and the 3-h delay condition,
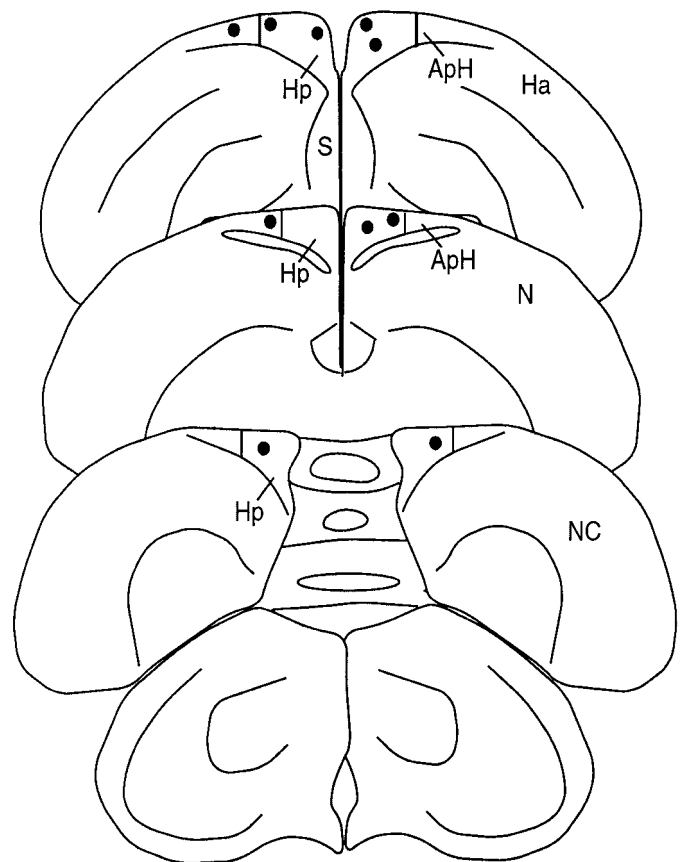

FIGURE 4. Position of cannulae. Black circles represent position of cannulae tips for different subjects. The position of cannulae was mainly conﬁned to the hippocampal formation, which consists of the area parahippocampus (ApH) and hippocampus proper (Hp). Ha, hyperstriatum accessorium; N, neostriatum; NC, neostriatum caudale; S, septum. Sections correspond roughly with Karten and Hodos. 1967. templates A6.75, A5.75, and A4.00, rostral to caudal.

performance improved across the five initial entries preceding the drug infusion (15-min delay: $F_{4,16}$ 8.88; P 0.001; 3-h delay: $F_{4,16}$ 8.44; P

birds is not caused by any disruptive effects of the infusion procedure itself, but by the pharmacological action of the lidocaine. Lidocaine uniformly blocks Na channels; therefore, both local circuit-based and fibers of passage through the HF might also be disrupted with our treatment. We therefore cannot distinguish our results between the effects of lidocaine on altered synaptic integration within the HF and altered communication with other brain regions. Drugs targeting synaptic transmission may provide a more precise means of assessing HF function.

We have shown that inactivation of the HF immediately before memory retrieval impairs retrieval of short-term (15-min) memories. However, HF inactivation does not impair the retrieval of long-term (180-min) memories. These results suggest that short-term retrieval of spatial memories is dependent on the HF and that over a longer duration, they become independent of the HF. That 180 min is a sufficient period for memory consolidation is somewhat surprising, as longer delays are typically necessary for memory consolidation to occur in mammals. The rapid consolidation of memories may be a feature unique to birds. Alternatively, after 180 min memories may still reside in the avian HF but over time become more robust and less susceptible to our technique of partial inactivation of the HF. In either case, some sort of consolidation of the memory trace occurs within the longer delay.

The spared performance of chickadees on the visual-spatial task after HF inactivation eliminates a number of alternative explanations for our results. Specifically, the observed deficit in the spatial task is not due to a loss of response inhibition (Hazeltine et al., 2000), which would be the case if birds could not inhibit the behavior of pulling knots when presented with feeders. Furthermore, the lack of impairment in the visual-spatial memory test argues against HF inactivation causing a generalized deficit in acquiring memory, or in motivation to perform accurately. The addition of visual cues prevented the lidocaine-induced memory impairment observed in the spatial memory task, which suggests that the observed deficit in learning is specific to spatial memory. However, since birds could still be using positional as well as visual information to encode the location of the baited feeder during the visual-spatial memory task, it is possible that the spared performance during the visual-spatial memory task represents spared spatial ability rather than the workings of a hippocampal-independent visual memory system.

Another interpretation of our results is that the HF is involved in processing information about the relationships between landmarks to allow navigation, without involvement in the process of storing or retrieving memories about these relationships (Bolhuis et al., 1994). The memory acquisition task does not rule out a general navigational deficit without a memory component, since the differently colored feeders could act as beacons, making integration of spatial landmarks unnecessary for this task. However, this concern is addressed by our memory retrieval task. The lack of impairment after 180 min rules out the idea that the HF is involved in a purely navigational process.

Our results show that in the spatial task, lidocaine-infused birds

trained to revisit the same location. It is possible that the hoarding situation, which resembles a working-memory paradigm, and the task used here, which resembles a reference memory paradigm, placed different demands on the hippocampus during memory retrieval.

Our results suggest the HF in food-storing black-capped chick-adees functions during short-term learning and retrieval o:l](adees)-retriev[(ade.0Tcy)-378.4(42d)-246.6(42d)-ma8.4(42d)-have.4(42d)-2.4(42d
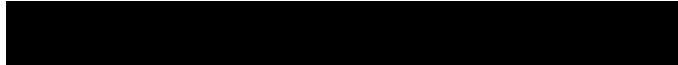
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