Hippocampal Volume Is Related to Complexity of Nesting Habitat in Leach’s Storm-Petrel, a Nocturnal Procellariiform Seabird

Marsha L. Abbott\textsuperscript{a} Carolyn J. Walsh\textsuperscript{b} Anne E. Storey\textsuperscript{a} Ian J. Stenhouse\textsuperscript{b} Carolyn W. Harley\textsuperscript{a}

\textsuperscript{a}Department of Psychology and \textsuperscript{b}Biopsychology Program, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada

Key Words
Hippocampus \textbullet} Hippocampal complex \textbullet} Procellariiformes \textbullet} Northern fulmar \textbullet} Fulmaris glacialis \textbullet} Oceanodroma leucorhoa \textbullet} Leach’s storm-petrel \textbullet} Birds \textbullet} Spatial memory

Abstract
The hippocampal and telencephalon volumes of the nocturnal Leach’s storm-petrel (\textit{Oceanodroma leucorhoa}, \(n = 15\)) were compared with published data for food-storing and non-storing Passerines. The hippocampus to telencephalon ratio of Leach’s storm-petrels is intermediate between food-storing and non-storing birds. Leach’s storm-petrels taken from nesting burrows in wooded habitat had a larger relative hippocampal volume than those taken from burrows in an open meadow. Relative olfactory volume did not differ between woods and open-nesting storm-petrels. The larger relative hippocampal volume of storm-petrels may be associated with increased spatial demands of returning to their nests at night in the darker, more navigationally complex woods. It is not known whether the larger hippocampus in storm-petrels from the woods is due to selection on different subpopulations or whether experience in a more complex environment results in greater hippocampal volume. Hippocampal volume from the brain of one diurnal Procellariiforme, the northern fulmar (\textit{Fulmaris glacialis}), fell within the range of non-storing species, which supports the view that hippocampal enlargement in the storm-petrel is related to the spatial demand of returning to the nest at night.

Introduction
The hippocampus has been implicated in spatial memory in birds and mammals [Sherry et al., 1992]. Large relative hippocampal volumes have been related to the complexity of spatial tasks, both between and within avian species. For example, bird species that cache food for subsequent retrieval have a relatively larger hippocampal volume than non-storing species [Sherry et al., 1989; Krebs et al., 1989; Healy et al., 1994; Hampton et al., 1995]. Within species artificial selection and sex differences in spatial tasks have been related to hippocampal volume. Pigeons (\textit{Columbia livia}) bred for homing ability have larger hippocampi than other domestic conspecifics [Rehkamper et al., 1988]. Females in brood-parasitic cowbird species, which must track available nests over large territories, have larger hippocampal volumes than do males during egg-laying [Sherry et al., 1993; Reboreda et al., 1996]. This sex difference is absent in a related, nonparasitic species of cowbird [Reboreda et al., 1996].

Relative hippocampal size has not been reported for any non-Passerine except for measurements of the hippocampus proper in pigeons [Rehkamper et al., 1988]. Comparisons
with the Procellariiform seabirds would provide information about whether different spatial tasks have produced hippocampal volume increases in other avian lineages. Leach’s storm-petrels (Oceanodroma leucorhoa) breed in dense colonies in nesting burrows dug into the ground. They are nocturnal at the breeding colony and are more active on dark nights than on bright ones [i.e. with full moon or little cloud cover; Watanuki, 1986; Bretagnolle, 1990; Bryant, 1993]. This predator avoidance strategy [Watanuki, 1986] means that storm-petrels return to their nests on nights with reduced visual cues in the immediate vicinity of their burrows. Storm-petrels may use olfactory cues to return to the burrow area [Grubb, 1974, 1979; Johnson, 1978]. It is unclear, however, whether olfaction helps an individual find its own burrow. In another nocturnal Procellariiform, the Manx shearwater (Puffinus puffinus), masking olfactory cues at burrow entrances did not impede burrow relocation, whereas birds had trouble finding their burrows when nearby visual cues had been altered [James, 1986]. In Leach’s storm-petrels, both sexes share incubation and chick feeding [Huntington et al., 1996]. As both sexes must return to the nest site often, we predict no sex differences in spatial abilities and relative hippocampal volumes.

Another Procellariiform species, the northern fulmar (Fulmarus glacialis), nests at open sites along cliff ledges [Hatch and Nettleship, 1998] with lower nest density than Leach’s storm-petrel. Unlike storm-petrels, breeding fulmars typically come to and leave the nest site during day light [Warham, 1990; Hatch and Nettleship, 1998], and thus the nest site and the partner and/or chick would be easy to locate visually. The two species have similar total incubation and fledging periods, and similar frequency of visits by both parents [Huntington et al., 1996; Hatch and Nettleship, 1998; review in Warham, 1990], and thus no sex differences would be expected in this species as well. Both species show high levels of nest site fidelity across years, so that after the initial prospecting stage, birds usually return to the same nest site each breeding season. Food (fish and marine invertebrates), foraging ranges during breeding and maximum migratory distances are comparable for storm-petrels and fulmars [Huntington et al., 1996; Hatch and Nettleship, 1998]. Both species forage at night and during the day [Warham, 1990; Hatch and Nettleship, 1998], and both have been shown to use olfactory cues to find prey [Grubb, 1972; Hutchison and Wenzel, 1980]. Given the storm-petrels’ more complex spatial task of finding the burrow entrance at night in dense colonies, we predicted that Leach’s storm-petrels would have a relatively larger hippocampus than the northern fulmar if a spatial map (or ‘memory for what is where’) is critical for nest location.

Leach’s storm-petrels on Great Island dig their burrows either in the woods around tree and fern roots or in open meadows [Stenhouse, 1998]. Birds with burrows in the woods have higher reproductive success than birds with burrows in the open habitat [Stenhouse, 1998]. However, burrows in the woods may be harder to relocate because burrow density is higher [Stenhouse, 1998] and nocturnal light levels are lower than in open areas. Sooty shearwaters (Puffinus griseus), another nocturnal Procellariiforme) landed closer to their burrows when nesting in open areas than in woods, and woods-nesting shearwaters tended to drop through the same hole in the forest canopy on successive returns [Warham, 1990]. Efficient burrow location in the woods would reduce the risk of collisions with trees (observed by Grubb, 1974) and would reduce the time that storm-petrels are vulnerable to gull predation after landing in the colony.

In preliminary analyses it was found that a sample of birds from open habitat had a smaller relative hippocampal volume than a sample of birds taken from the woods habitat. If the hippocampus mediates the final stages of burrow site relocation, then returning to the burrow in the darker, more complex 3-dimensional woods environment would make a greater demand on hippocampal function than burrow location in the open meadow habitat. This spatial task would be similar to homing, and it has been shown that pigeons with hippocampal lesions can return to the loft area, but are unable to find the loft [Bingman et al., 1984]. Given the differences between habitats, we predicted that Leach’s storm-petrels nesting in the woods would have a larger hippocampal volume than those nesting in the open.

**Materials and Methods**

Adult Leach’s storm-petrels were collected in July 1996 at Great Island, Witless Bay, Newfoundland. The storm-petrels were hand-removed from their burrows during the breeding season and weighed immediately. Six petrels (4 females, mean weight = 46.9 g; 2 males, mean weight = 52.0 g) were taken from open habitat. Nine petrels (6 females, mean weight = 50.8 g; 3 males, mean weight = 50.7 g) were taken from wooded habitat. They were transported to Memorial University of Newfoundland in dark plastic pipes which were 10 cm in diameter and approximately 30 cm long; the pipes had been stuffed with sod and the ends were taped shut and contained air holes.

Dr. Bernice Wenzel generously provided slides from an adult northern fulmar which had previously been used for drawings in the fulmar atlas [Matochik et al., 1991].

*Nest-Site Characteristics of Leach’s Storm-Petrel and Northern Fulmars*

The storm-petrel colony on Great Island is estimated at approximately 250,000 nesting pairs [Skelepovych and Montecuccchi, 1989]
and there may be as many as 50,000 additional unoccupied burrows on the island. The wooded habitat is a mix of balsam fir (Abies balsamea) and black spruce (Picea mariana) with an average 2.2 burrows/m² (maximum 4.7/m²). The open areas used by storm-petrels are grass-Rubus meadows, and average burrow density is significantly lower (average 1.5 burrows/m²; maximum 2.7 burrows/m²; Stenhouse, 1998). Returning storm-petrels land in the general vicinity of the burrow and then walk to their nest-sites [Grubb, 1974].

In contrast, northern fulmars nest at open sites along the cliff ledge and the minimum average distance between nests is 1.5–2.0 m [Hatch and Nettleship, 1998].

**Histology**

Approximately 4.5 h after capture, storm-petrels were anesthetized with somnotol (15 mg/kg) and ketamine (50 mg/kg). Birds were then perfused transcardially with saline followed by 10% formalin. Brains were stored for one week in 10% formalin at 4°C then weighed and placed in 30% sucrose-formalin (4%) for cryoprotection. They were maintained in this solution at 4°C until sectioned at 40 µm on a cryostat and mounted on slides; brains were encased in ice (made up from a 1.5% ethanol solution to enhance brain integrity) prior to sectioning. Brains from three males and eight females were sectioned coronally and brains from two males and two females were sectioned parasagittally. Alternate sections were stained with cresyl violet. Coronal sections from one brain were sent to Sue Healy (The University, Newcastle upon Tyne) for assistance with localization of the lateral boundary of the hippocampal complex as delineated in previous studies of storing and non-storing birds.

The fulmar brain had been sectioned parasagittally at 50 µm with alternate sections stained with cresyl violet [Matochik et al., 1991].

**Morphometry**

All digitizing was performed blind with respect to habitat. Slides were alphabetically labeled by an independent observer. Every alternate cresyl violet stained section was placed on a microprojector and enlarged 21.5 x. The outer boundaries of the telencephalon, the hippocampal complex and, where available, the olfactory bulbs were traced. Tracings were digitized using a Jandel digitizing tablet, microcomputer and Jandel PC3D software. Area measurements were generated by the Jandel program and volume estimates were calculated by using the formula for a truncated cone [Sherry et al., 1989]. We digitized 54 to 64 sections for each brain sectioned coronally and 33 to 37 sections for each brain that was sectioned parasagittally.

Two storm-petrel brains that had been sectioned parasagittally were used for comparison with the fulmar slides. The slides that contained comparable sections from the storm-petrel and fulmar brains were traced and digitized. We digitized 26 sections for the fulmar brain and 11 to 12 sections digitized for each storm-petrel brain in the comparison. The volume obtained for the available telencephalon and hippocampal complex of the fulmar brain was converted to an estimated volume for an entire brain based on the constants obtained when extrapolating the corresponding regions of the storm-petrel brain to the entire petrel brain. The telencephalon volume was multiplied by 1.48 and the hippocampal volume was multiplied by 2.33.

**Statistical Analysis**

A difference in hippocampal volume between open and wooded habitats was tested by analyzing hippocampal volume as a proportion of telencephalic volume using a t-test for unequal variances (which adjusts degrees of freedom for a more conservative test, Hinkle et al., 1979). Males and females did not differ in body weight (males, 51.2 g; females, 49.3 g); telencephalon volume (males, 390.7 mm³; females, 363.4 mm³) or hippocampal volume (males, 9.5 mm³; females 8.2 mm³). Finally, hippocampal volume as a proportion of telencephalic volume did not differ between males (mean = 0.024; SE = ±0.003) and female (mean = 0.022; SE = ±0.004). Both sexes were grouped together for comparison between habitats.

To compare the two Procellariiforme families to earlier analyses of passerine families, the proportion of hippocampal volume to telencephalon volume was computed for previously published passerine data [Sherry et al., 1989] (table 1). In addition, the residuals of the regression between the logs of the hippocampal complex volume and telencephalon volume were plotted against residuals of the regression between the logs of the hippocampal complex volume and body weight for the 13 families and subfamilies from the earlier analysis [Sherry et al., 1989] (fig. 1), together with the two Procellariiforme families.

**Results**

Storm-petrels had an average body weight of 49.9 g, an average hippocampal volume of 8.63 mm³ and an average telencephalon volume of 372.5 mm³. The average weight of the five fulmars in the Matochik et al. [1991] atlas was 525.0 g. The fulmar brain digitized in the present study had an estimated hippocampal volume of 17.4 mm³ and an estimated telencephalon volume of 1,067.1 mm³.

Table 1 shows that the percentage of telencephalon accounted for by hippocampal volume in the fulmar is similar to that in non-storing passerines, while percentage of hippocampal volume in the storm-petrel is intermediate between storing and non-storing passerines. This difference is seen more clearly in figure 1. Figure 1 shows that storm-petrels have a larger hippocampal complex volume than

<table>
<thead>
<tr>
<th>Families/subfamilies</th>
<th>Percentage of hippocampal volume</th>
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<tbody>
<tr>
<td>Procellariiformes</td>
<td></td>
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<tr>
<td>Hydrobatidae</td>
<td></td>
</tr>
<tr>
<td>Leach’s storm-petrel</td>
<td>2.3%</td>
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<tr>
<td>Procellaridae</td>
<td></td>
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<tr>
<td>Northern fulmar</td>
<td>1.6%</td>
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<tr>
<td>Passeriformes¹</td>
<td></td>
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<tr>
<td>Food storing subfamilies (n = 3)</td>
<td>3.9%</td>
</tr>
<tr>
<td>Non-storing subfamilies (n = 10)</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

¹ Data from Sherry et al. [1989].
predicted by their telencephalon volume; the fulmar, by
contrast, has a smaller hippocampal volume than predicted
either by telecephalon volume or by body weight.

Storm-petrels taken from burrows in the woods had a
larger telencephalon (mean = 408.3 mm$^3$, SE = ±26.8) than
those from burrows in the open (mean = 318.8 mm$^3$, SE = ±17.2; $t$ (13) = 2.8, $p_{2\text{-tailed}} < 0.05$). Hippocampal volume
was also larger in storm-petrels taken from wooded habitat
(mean = 10.0 mm$^3$, SE = ±0.99) than in storm-petrels from
the open (mean = 6.5 mm$^3$, SE = ±0.20; $t$ (13) = 3.4, $p_{2\text{-tailed}} < 0.01$). Body weights between petrels from wooded and
open habitats did not differ (wooded, 48.6 g, SE = ±1.7; open, 50.8 g, SE = ±1.4). Finally, the relative hippo-
campal/telencephalon volume was larger in storm-petrels
from the woods (mean = 0.024, SE = ±0.002) than those
from the open (mean = 0.021, SE = ±0.0006; $t$ (13) = 2.13, $p_{1\text{-tailed}} < 0.05$). Left hemispheres of storm-petrels taken
from the woods (mean = 0.026 mm$^3$, SE = 0.002) were
larger than those of storm-petrels from the open (mean = 0.020 mm$^3$, SE = 0.0007; $t$ (10) = 2.05, $p_{1\text{-tailed}} < 0.05$), whereas right hemispheres did not differ.

Olfactory bulb volume in the eight storm-petrels for
which bulbs were available was 23.0 mm$^3$ (SE = ±2.6), with
no significant differences in olfactory lobes or olfactory
lobes to telencephalon ratios between the two habitats.
The overall olfactory bulb to telencephalon ratio was 0.057
(SE = ±0.003). Olfactory bulb volume correlated signifi-
cantly with telencephalon volume ($r = 0.84$) but not with
hippocampal volume ($r = 0.64$). Olfactory bulbs were com-
pared for four birds from the wooded habitat and three birds
from the open habitat that showed a significant habitat ef-
effect for the hippocampal/telencephalon ratio (mean = 0.023, SE = ±0.0017 woods; mean = 0.019, SE = ±0.0001 open;
$t$ (5) = 2.14, $p < 0.04$ 1-tailed). There were no differences
in the olfactory bulb/telencephalon ratios for birds from the
woods (mean = 0.056, SE = ±0.004) and open (mean = 0.058, SE = 0.006) habitats.

Olfactory bulb volume in the fulmar was estimated to be
42.5 mm$^3$ and the overall olfactory bulb to telencephalon
ratio was 0.040. Using complete brains from more than one
fulmar, Wenzel and Meisami [1987] estimated an olfactory
lobe volume of 60 mm$^3$, which would represent a ratio
of 0.056 with our estimated telencephalon volume, results
nearly identical to ours for the Leach’s storm petrel.

Fig. 1. Residuals of the regression between hippocampal volume and telencephalon volume plotted against residuals
of the regression between hippocampal volume and body weight for Passerines [data from Sherry et al., 1989] and for
two Procellariiforme species, Leach’s storm petrel and the northern fulmar (this study).
Discussion

Leach’s storm-petrels have a hippocampal volume relative to telencephalon that is intermediate between that of food storers and non-storers. Hippocampal volume in the residual analysis was larger than would be expected on the basis of telencephalon volume. This increased size was predicted based on the spatial demands for storm-petrels having to relocate their burrows at night. The inference that increased hippocampal volume is related to nocturnal activity is strengthened by comparison with the diurnal northern fulmar. The fulmar had a smaller hippocampal complex than would be predicted by either body size or telencephalon volume, falling well within the range of non-storing Passerines.

The storm-petrels taken from the wooded habitat had a larger hippocampal/telencephalon ratio than storm-petrels taken from the open. This confirmed our preliminary observations and is not surprising when considering habitat differences. Light levels are lower in the wooded habitat and its 3-dimensional structure and high burrow density makes returning to the burrow more difficult. Despite previous evidence of a role for olfaction in burrow location for petrels [Grubb, 1974, 1979; Johnson, 1978], the present findings for the wooded and open habitats argue that differences in hippocampal volume, not olfactory volume, are significantly related to spatial demands.

Olfactory lobe volumes of diurnal and nocturnal Procellariiformes did not differ in this or other studies [Bang, 1966; Healy and Guilford, 1990], which supports the idea that the hippocampus rather than the olfactory lobes is involved in spatial demands of the nocturnal storm-petrels. There is some evidence that the large olfactory bulbs generally found in Procellariiformes may be important in nocturnal foraging, even in species such as the fulmar that are classified as diurnal based on observations that their visits to the breeding colony occur primarily in daylight [Hutchison and Wenzel, 1980; Warham, 1990; Hatch and Nettelship, 1998].

There are two possibilities that might account for the larger hippocampal size in storm-petrels taken from a wooded area. One possibility is that petrels with smaller hippocampi are unable to navigate the wooded areas and, hence, select burrow sites in the open areas. Given the higher reproductive success in the woods [Stenhouse, 1998], storm-petrels capable of inhabiting the wooded environment should prefer it to the open habitat. Leach’s storm-petrels are thought to return to the area near their natal burrows [Huntington et al., 1996], and thus a larger hippocampal complex might be selected for over generations. If the storm-petrels from the two habitats represent separate subpopulations with little interbreeding, then the differing hippocampal volumes might be genetically determined. Thus, young birds hatched from burrows in the woods might have larger hippocampi that could help them choose and then relocate a burrow site in the woods.

Alternatively, the hippocampus might be larger in those two- to three-year old storm-petrels that start prospecting for nest sites in the woods when they return to the breeding colony. Food-storing species that are deprived of the opportunity to store food have relative hippocampal volumes which are comparable to those of non-storing species [Clayton and Krebs, 1994]. Storing food has been shown to promote hippocampal neurogenesis [Patel et al., 1997]. Leach’s storm-petrels might have a similar mechanism whereby experiencing the more complex woods environment facilitates development of an increased hippocampal volume. At the moment, there is not enough information to know which of these two hypotheses is more likely.

Taken together, these data suggest that the navigational task of finding burrows at night in complex habitat is a further example of increased spatial demands that results in larger relative hippocampal volume. This effect is seen both in the storm-petrel/fulmar comparison and in the comparison between woods and open habitat for Leach’s storm-petrels.

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