Comparative neuroanatomy of various tactile foraging ducks

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Introduction

Many animals are tactile-foragers, including the duck whose bill has specialized touch-sensitive regions. With their bill, they can dabble, or feel around, on land or in water. All somatosensory information (touch, temperature and pain) from the bill is conveyed to the brain through primary sensory neurons located in the trigeminal ganglia (TG). TG neurons that sense force (mechanoreceptors) interact with homologs of human Pacinian and Meissner corpuscles, called Herbst and Grandry corpuscles, respectively. These mechanoreceptors are tuned to detect deep or superficial vibration at different frequencies. Herbst corpuscles are composed of onion-like layers of lamellar cells that ensheath the afferent neuron terminals (Fig 2). Grandry corpuscles are composed of stacks of ~2-3 Grandry cells with afferent neuron terminals 'sandwiched' between them (Fig 3).

Different duck species have different means of locating food. Some species, like Pekin (domestic duck), dabbles for resources. This is a process of rapidly moving the beak on the area to discriminate edible from non-edible. Other ducks use diving, where they capture prey using tactile (Ruddy, Scaup) or visual methods (Merganser). These species also have dramatically different bill morphology, as well as differences in the relative size of the brainstem nucleus that receives input from TG. The domestic duck has different densities of corpuscle at different locations on the bill with the highest density of both Herbst and Grandry corpuscles in the rostro-lateral region of the dorsal bill and the bill tip organ(s) [1]. Here, we measure differences in sizes and densities of Herbst and Grandry corpuscles in two regions of the bill, to determine whether these parameters map onto different foraging abilities.

Objective

Quantification of Herbst and Grandry corpuscles in embryonic bills of various species and various locations to correlate density to foraging methods.

Methods

Bill skin from various embryonic ducks were drop fixed in 4% PFA and stored at -20˚C in cryoprotectant for >3years. 2.413 mm punches from the dorsal bill skin were made as shown in Fig 4. A&C and sectioned to 8 micrometers using a cryostat. Every 9th-10th sample was used for corpuscle quantification, resulting in 9-20 sections per sample. Sections were stained for Tuj1 (R&D systems, 1:500), which binds to beta-tubulin 3 in both neurons and corpuscles using DAB immunohistochemistry according to standard protocols [2]. Finally, a toluidine blue counterstain was applied, and sections were dehydrated in successive ethanol baths. From there, brightfield images were collected on an Olympus BX63 at 10x magnification. Each corpuscle was counted in each section. Each section was also measured along the epidermis to get a side length. The side length was converted to a surface area by multiplying the section thickness. Corpuscle density was calculated using the formula: Density of corpuscles = (#corpuscles*corpuscle size correction factor/surface area) and averaged across all sections. In order to account for error in estimates of corpuscles size, the ferret diameter was measured in 23-147 of each corpuscle per section from each species. Mean± SD can be seen in Table 1. Error of propagation was done by $\frac{\sigma_x}{X} = \sqrt{\left(\frac{\sigma_a}{a}\right)^2 + \left(\frac{\sigma_b}{b}\right)^2}$. Data was analyzed using Microsoft Excel and Igor Pro (Wavemetrics).

Table 1: Mean ferret diameter of all sections with standard deviation.

Species and Sample location	mean herbst size	mean grandry size	stdev herbst	stdev grandry
ABDU	43.63	20.31	10.25	7.205
HADU	40.65	21.185	11.25	7.035
LESC	34.56	20.345	10.25	6.56
MERG	42.17	19.815	13.25	9.175
RUDDY	44.43	18.965	15.23	7.185
WODU	39.35	17.3	12.56	6.755
PEKIN	42.05	15.505	10.97	5.805

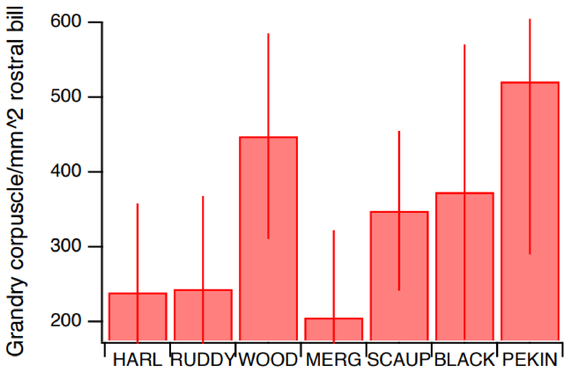

Figure 7: Average density of Grandry corpuscles of various species at the rostral end.

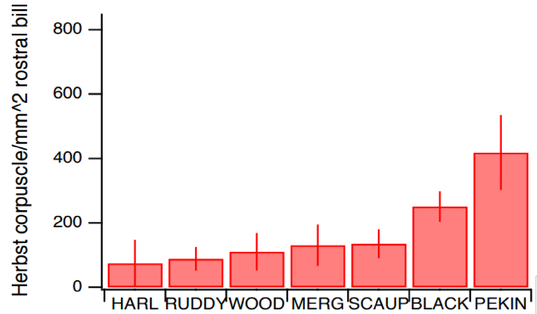

Figure 8:Average density of Herbst corpuscles of various species at the rostral end.

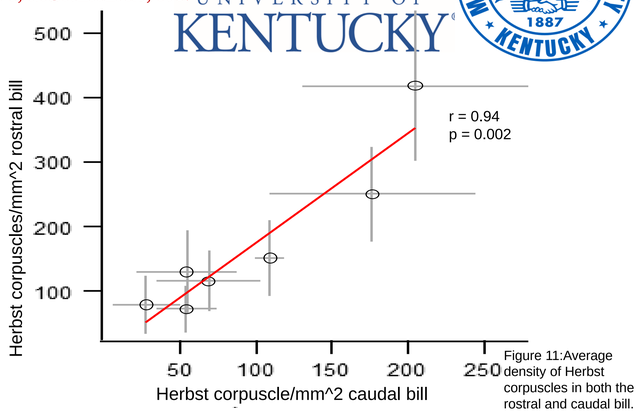

Figure 11:Average density of Herbst corpuscles in both the rostral and caudal bill.

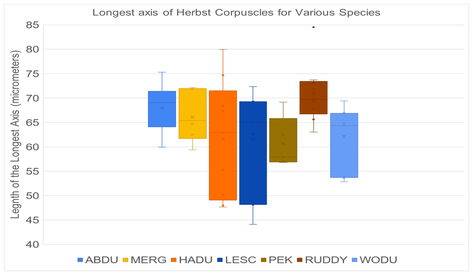

Figure 12: Box and Whisker plot of the axis of the largest Herbst corpuscles across species.

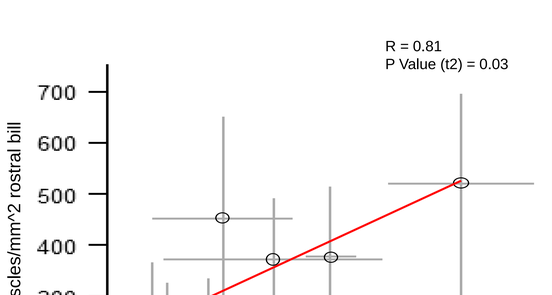

Figure 9: Average density of Grandry corpuscles in both the rostral and caudal bill.

Discussion and Implications

In probe foraging shorebirds the number of bony pits, thought to contain sensory papillae (clusters of mechanoreceptors), is highest in tactile foragers [3]. Similarly, bony pits in the bill tip organ of the mallard also contain sensory papillae [1,4]. However, the rest of their bill skin is also covered with corpuscles. Consistent with this, the density of corpuscles from skin of the rostral and caudal areas of the dorsal bill was strongly correlated (Fig. 9,11). However, our results did not show a strong correlation between bill bone pits and Herbst corpuscle density (r = 0.53). This could be a consistent feature of anseriform bills or depend on the developmental timepoint chosen for this study. The size of Herbst corpuscles differs slightly across species (Table 1).

Both the density of bill pits and corpuscles was surprisingly inconsistent with foraging strategy. As might be expected, mechanosensitive corpuscles are found more prevalent in tactile-foraging ducks of the genus Anas, such as the Pekin and Black duck. In contrast, the diving ducks such as Harlequin and Merganser had low densities of corpuscles in the bill skin. However, the Wood duck, known for its large eyes and preference for acorns, had some of the highest density of Grandry corpuscles in our dataset. Finally, the Ruddy duck, known for its foraging methods of diving down and "sweeping" for prey, has both a low number of bill pits and corpuscles, completely inconsistent with a tactile foraging strategy. Given the low density of corpuscles, perhaps a different mechanism, such as electroreception, is used by this species.

Future Work

These data show substantial variation in both mechanosensory pits in the bill bone and density of corpuscles at all locations measured in the bill and are not entirely consistent with foraging strategy. This suggests that adaptations that support tactile foraging in the sensory periphery may be evolutionarily constrained [5], whereas the relative size of brain regions encoding tactile information may be more free to vary. Future work in this area linking tactile foraging capability to anatomical and functional variation in the trigeminal system of a wider range of species is necessary.

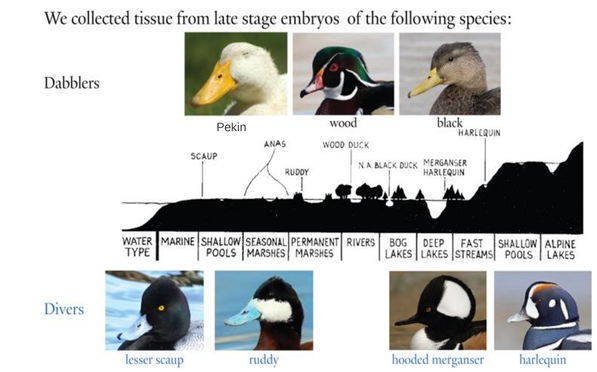

Figure 1: Various species of duck used throughout the experiment as well as their foraging method.

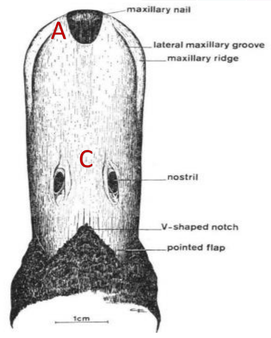

Figure 4: Different regions of bill skin where samples were collected. The regions were selected due to high bone pit density (region A) and no bone pits (region C) [1].

Figure 5: Whole section of American Black Duck 1A

Results

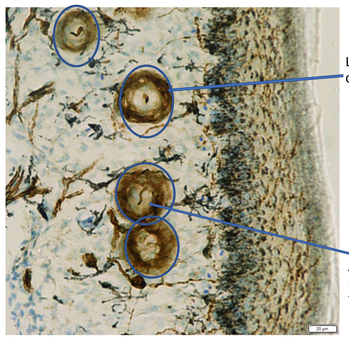

Figure 2: Herbst corpuscles and key characteristics labeled.

Figure 3: Grandry corpuscles and key characteristics labeled.

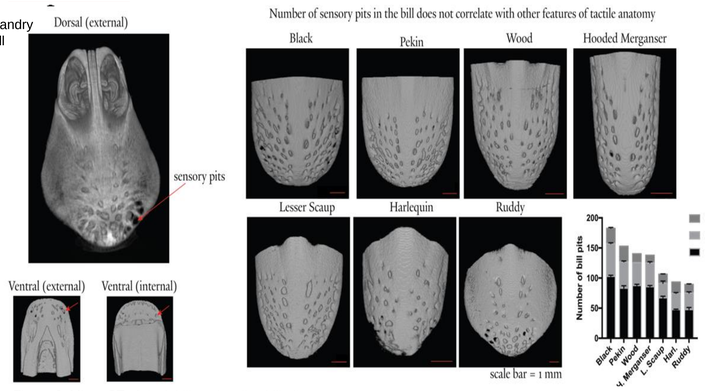

Figure 6: Micro-CT of duck bill to visualize bill pits.

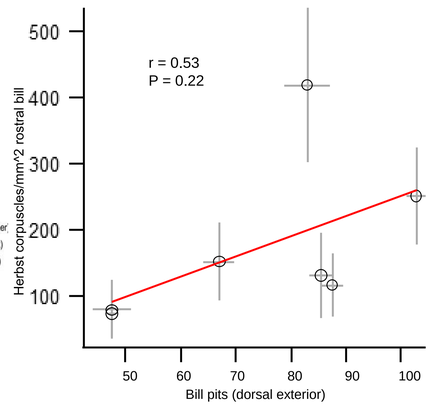

Figure 10: Correlational data between the density of Herbst corpuscles in the rostral bill and the number of bill bits found in the micro-CT.

References

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Acknowledgements

Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103436.

Thank you to Dr. Mirabito, Dr. Schneider, and my colleaques for their guidance and assistance.