

ODOR THRESHOLDS IN PASSERINES

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Abstract—1. Eight species of passerines were evaluated for their ability to form conditioned responses to odor stimuli. Only 5 species met training criteria and were tested for odor detection thresholds.

2. Detection thresholds were comparable to other passerines tested. Detection values were also similar to mammalian macrosmatic species, such as rats and rabbits. Thus, despite the poorly elaborated olfactory anatomy of passerines, these birds possess an adequate sense of smell.

3. Within the Passeriformes there is no correlation between olfactory acuity and relative size of the olfactory bulb. However, there is a correlation between acuity and olfactory bulb size across orders of birds. These latter observations are consistent with hypotheses correlating form and function.

INTRODUCTION

More information exists on the comparative anatomy of the olfactory system for Aves than for any other vertebrate taxa (Bang and Cobb, 1968; Bang, 1971). Yet information on olfactory abilities of birds is limited. Relying on anatomical information Bang (1971) and Wenzel (1971) suggested that the relative size of the olfactory bulb and degree of scrolling of the nasal conchae was positively related to olfactory ability in birds. Procellariiformes, cathartid vultures and kiwis all have well developed olfactory anatomies and are acutely sensitive to odors (Stager, 1964; Wenzel, 1972; Grubb, 1972). However, even species with moderately developed olfactory anatomies, e.g. pigeons, demonstrate odor detection capabilities that are on par with macrosmatic mammalian species (cf. Henton, 1969; Davis, 1973). Moreover, passerines, with their poorly developed olfactory anatomy, have been shown to attend to odor cues (Clark, 1991a).

Despite the apparent ubiquity of avian olfactory ability, it is premature to suggest that there is no relationship between olfactory performance and anatomical development. Still needed are quantitative evaluations of avian olfactory ability under standardized test conditions to match the detailed comparative anatomical data set. To this end, we continued our experiments documenting olfactory ability in birds (Clark and Mason, 1987, 1989; Clark and Smeraski, 1990; Clark, 1991a; Clark and Shah, 1991). Specifically, we set out to evaluate olfactory ability for 8 passerine species. Olfactory ability has two components, odor detection threshold (sensitivity) and discrimination capacity. Because discrimination

tasks are more rarely evaluated, references to olfactory ability are limited to threshold sensitivity for purposes of discussion. We also address the question whether olfactory performance and anatomical development is related within the order Passeriformes.

METHODS

Species

European goldfinches (*Carduelis carduelis*) and great tits (*Parus major*) were captured in funnel traps in a park district in Moscow, U.S.S.R. American goldfinches (*Spinus tristis*), song sparrows (*Melospiza melodia*), mockingbirds (*Mimus polyglottos*), catbirds (*Dumetella carolinensis*), eastern phoebes (*Sayornis phoebe*) and black-capped chickadees (*Parus atricapillus*) were captured in mist nets at the Vassar College Farm in Poughkeepsie, New York. All species were opportunistically selected.

Stimuli

We used HPLC-grade (Aldrich) cyclohexanone (CH) [$C_6H_{10}O$, *M*, 98.14, b.p. 155.6°C, d_4^{20}] as the standard odorant. This chemical is the standard reference used in our laboratory (Clark, 1991a). CH is without known biological significance to any of the species tested. Nonetheless, we justify its use because vertebrate olfactory receptors are sensitive to a range of reagents that are not encountered naturally (Fazzalari, 1978), suggesting that the functionality of receptors is for perception of volatile chemicals *per se*. Indeed, Mozell *et al.* (1984) showed that olfactory responding was most dramatically affected by access of molecules to the receptor field rather than the nature of the stimulus itself. However, this is not to ignore the possibility that some vertebrates may be

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odor specialists or that some species or individuals may exhibit hyposmias or anosmias. We do not consider these odor deficits a problem. Hyposmias and anosmias normally reflect polymorphic differences in detection and discrimination within populations as opposed to species-specific deficits for a given compound. In the absence of *a priori* knowledge as to what types of odors might be ecologically relevant to species we have chosen to test all species with a single standard for comparative purposes.

Olfactometry

Birds were tested at field laboratories within 1 day of capture. Odor detection thresholds were evaluated using a cardiac conditioning paradigm and a field portable olfactometer, similar in design to that described by Clark and Mason (1989). The methods for preparing birds and the cardiac conditioning paradigm for determining odor thresholds were similar to those previously described (Clark and Smeraski, 1990). Briefly, birds were restrained and placed within a darkened sound-attenuating chamber with their nares placed at the exit port of a dilution olfactometer. Heart rate was monitored with a Type II ECG lead configuration via a high impedance probe, amplifier and oscilloscope. The frequency of heart beats was counted by processing the "R" component of the amplified ECG signal to a TTL pulse via a Schmitt trigger circuit, and recording the timed pulses via software to computer.

Newly captured birds are more excitable than those maintained in captivity, thus making cardiac conditioning more difficult. To ensure that reliable thresholds were obtained, we monitored heart rate (HR) until it stabilized (Fig. 1A). After heart rate stabilization, birds were presented with clean air from alternating sources to acclimate the birds to small vibrational and sound cues that might have been present during solenoid switching (Fig. 1B). When it appeared that birds no longer responded to switches in the air lines we proceeded to the next step in the acclimation/training process. Because there was some uncertainty about the olfactory ability of birds, especially passerines, it was important to evaluate the trainability of subjects relative to a nonodor cue. Birds were acclimated to a randomly initiated light cue (L_0) (12 V DC bulb placed over the roof of the chamber) to control for neophobic responses to a visual cue. Subsequently, the light was paired with a small electric shock (L^+) applied through the leg electrodes. The applied voltage and duration of the unconditioned stimulus varied as a function of body size. Smaller species were given 2 V DC for 2.5 sec and larger species were given 10 V DC for 8 sec. L^+ tested whether the birds could form a conditioned response. If a bird formed a conditioned response to light, it then was conditioned to respond to a strong odor cue, 5% vapor saturation (VS) of cyclohexanone (CH^+). Birds were presented with humidified air (A_0) as a control. Higher odor concentrations

(> 10% VS) were not used because of the possibility of involving trigeminal receptors in the perception of volatiles (Mason and Silver, 1983; Walker *et al.*, 1986). Throughout training baseline heart rate was monitored to determine whether the bird experienced undue stress. We assumed that birds experienced undue stress when the intertrial heart rate consistently exceeded the baseline acclimation heart rate plus 1 SD. Unless otherwise noted tests were carried out on birds that did not experience undue stress.

The precise timing of stimulus presentation was determined randomly by computer, with the minimum time between stimulus presentations set at 60 sec and the maximum time between stimuli set at 300 sec. During the presentation of air and CH the order of presentation within pairs was determined randomly by the computer. Heart rate was monitored for 10 sec prior to stimulus presentation to determine the intertrial heart rate, and during the 10 sec of stimulus presentation. A response was considered to have occurred if the heart rate during treatment presentation exceeded the mean intertrial heart rate (for the 5 previous consecutive intertrial samples) plus 1 SE. A bird was considered to have been trained to respond to a stimulus if the ratio of positive responses for $L^+ : L_0$ or $CH^+ : A_0$ was at least 2:1.

Thresholds were estimated first by using a least squares fit to the 4 parameter nonlinear regression, $(a - d) / [1 + (x - i)^b + d]$ to describe the concentration response profile, where a is the upper asymptote, b is the slope, i is the inflection point and d is the lower asymptote. A control response rate was defined as the proportion of positive responses occurring during presentations of A_0 . The point where the concentration response curve intersected the upper 95% confidence limit for the control response rate was interpreted as the detection threshold.

RESULTS

Training

We were unable to use three species for odor threshold experiments. Neither song sparrows ($N = 3$) nor American goldfinches ($N = 3$) acclimated to experimental conditions. Heart rate for these individuals remained high in the absence of any stimulatory cue, and showed no signs of decreasing even after 2.5 hr of monitoring. Attempts to increase heart rate, either through noise or strong odor stimuli, failed to produce an increase in heart rate, indicating that the observed heart rate was most likely the maximum achievable by the species. The heart rate for the 2 juvenile mockingbirds tested stabilized after 2–2.5 hr of acclimation. In our experience this was an unusually long acclimation time. Furthermore, we had poor success at training birds to differentially respond between S^+ and S_0 stimuli for both light and odor (Fig. 2A). The large error depicted in the figure reflects the tendency for one of the birds to respond to all changes in the experimental conditions

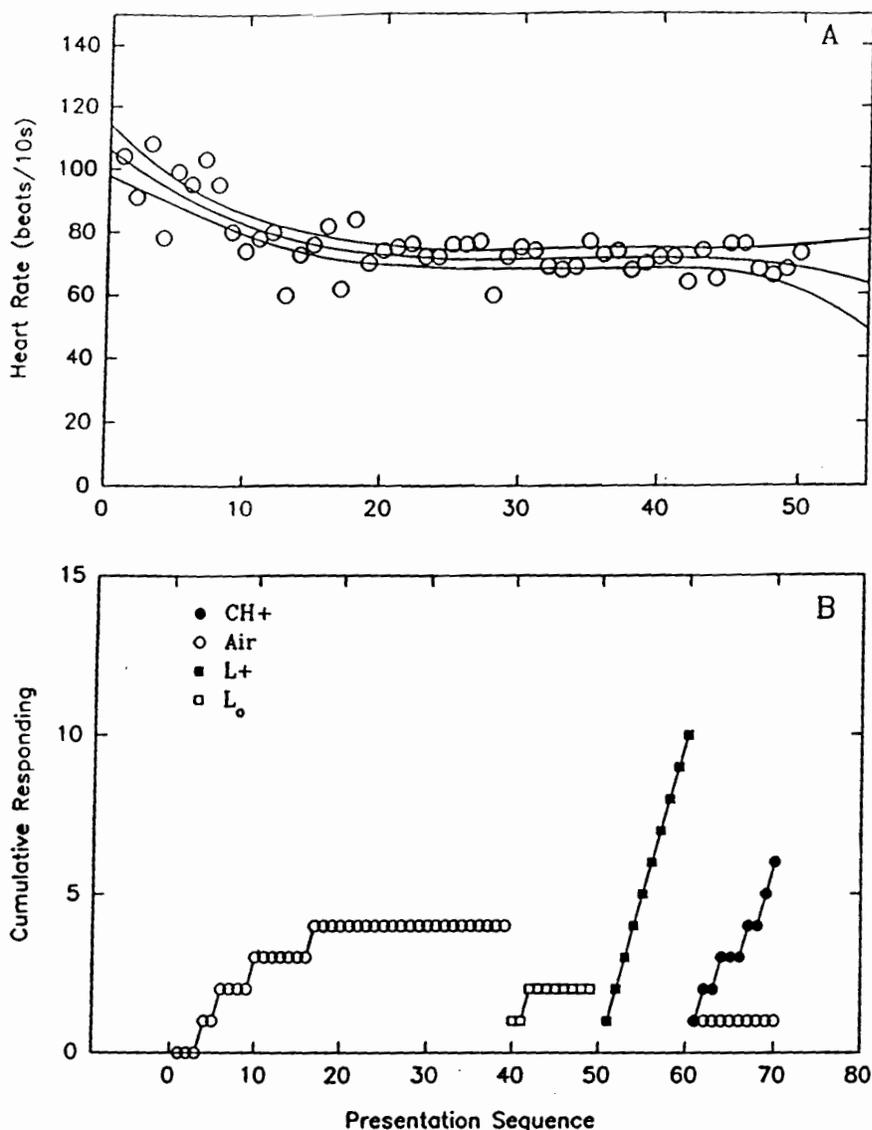


Fig. 1. (A) An example of the acclimation of heart rate for a catbird exposed to experimental conditions as a function of sampling sequence. Intervals between samples was randomly determined and fell between 60 and 300 sec. Middle line depicts mean heart rate. Upper and lower lines depict 95% confidence limits. (B) An example of training sequence for a single catbird as a function of trial sequence. Cumulative responding depicts the cumulative number of positive responses to a given stimulus. Codes for stimuli are defined methods.

and for the second bird to hardly ever respond to changes in experimental conditions. These data suggested that mockingbirds were not particularly good subjects.

We were able to train the five remaining passerine species to criteria. Two of the three gray catbirds acclimated to test conditions after 60 min. We were able to train the two acclimated birds to respond to light and odor stimuli (Fig. 2B), but only after lengthy training sessions (>50 trials for the S+). We were unable to train the third catbird, hence it was not used in subsequent studies. The two eastern phoebes quickly acclimated to experimental conditions, achieving stable heart rate after 10–20 min. Training also proceeded rapidly (Fig. 2C).

Phoebes quickly learned to respond to light after only a few re-enforced trials. Learned responses to odor were also achieved quickly. Training for black-capped chickadees, great tits and European goldfinches took place under slightly different experimental conditions (Fig. 2D–F). We did not adapt then train these three species to light stimuli. However, the procedures used to train and test these three species with odor stimuli were the same as used for other species. We were able to train all three species to respond to an odor cue.

Odor detection thresholds

Detection thresholds were estimated for cyclohexanone as follows: catbird (0.69% VS), phoebe

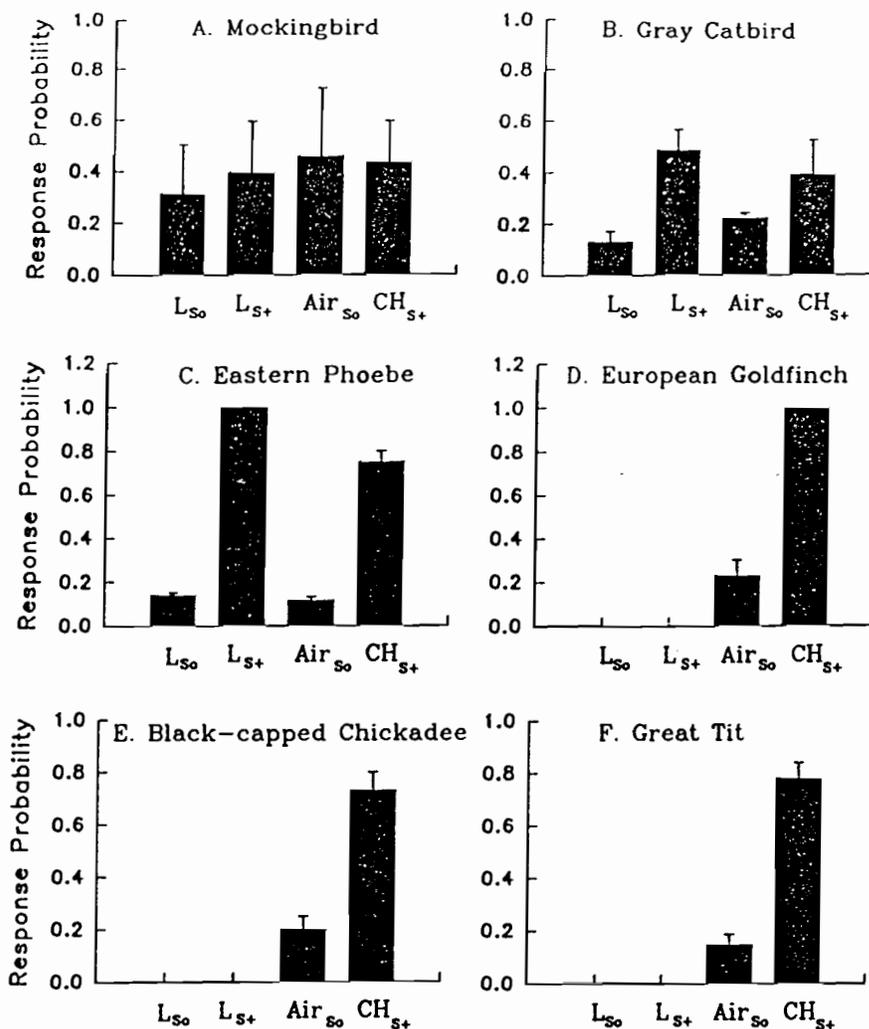


Fig. 2. The probability of a positive response for each of several stimulus conditions during acclimation and training light (L_{so}), light paired with shock (L_{s+}), air (A_{so}) and 5% VS cyclohexanone (CH_{s+}) for (A) catbird, (B) eastern phoebe, (C) European goldfinch, (D) great tit, (E) black-capped chickadee. Vertical lines depict +1 SE.

(0.7% VS), European goldfinch (0.3% VS), great tit (0.68% VS) and black-capped chickadee (1.1% VS) (Fig. 3).

Relationship between acuity and olfactory anatomy

Olfactory bulb size is independent of brain size (Fig. 4, $r = 0.139$, $P > 0.1$). Thus, the proportion of brain devoted to the olfactory bulb is significantly larger in species with smaller brains (Fig. 5, $r = 0.514$, $P < 0.01$). In spite of this relationship, detection threshold and relative olfactory bulb size are not related (Fig. 6, $r = 0.14$, $P > 0.1$).

DISCUSSION

Passerine olfaction

Passerines are commonly assumed to lack a sense of smell (Bang and Cobb, 1968; Welty, 1972). The present experiments argue against this assumption: all those passerine species that could be trained to

respond to stimuli demonstrate an ability to detect odors. The threshold detection level for cyclohexanone was within the range 0.3–0.7 ppm. This range is comparable to that found for cyclohexanone in other passerines (Clark and Mason, 1989; Clark and Smeraski, 1990; Clark, 1991a), and for other reagents in pigeons, chickens and quail (Stattleman *et al.*, 1975). This range of sensitivity to reagents is similar to values of reagents reported for mammalian macrosmatic species such as rats and rabbits (Davis, 1973; Fazzalari, 1978).

There is a difference between an individual's capacity and predilection to attend to an odor cue. When an unconditioned response is used as the metric for odor detection, the biological relevance of an odor cue is important (e.g., Snyder and Peterson, 1978). But a difficulty arises in judging what is a biologically relevant cue, especially for taxa where there is no *a priori* knowledge as to what purpose species might be using their sense of smell. Reagent

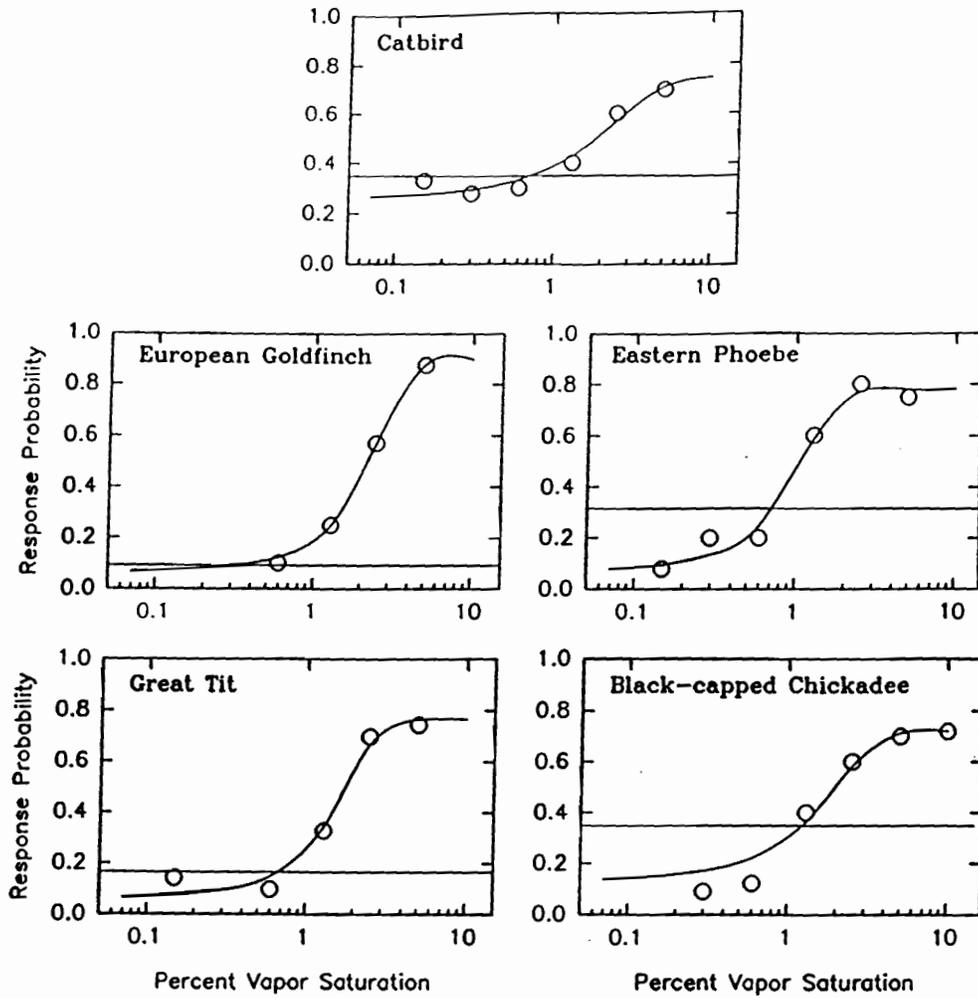


Fig. 3. The probability of responding to cyclohexanone as a function of concentration for (A) catbird, (B) eastern phoebe, (C) European goldfinch, (D) great tit, (E) black-capped chickadee. The horizontal line depicts the upper 95% confidence limit for positive responding to control odor (humidified air). The intersection between the fitted curve and the confidence limit is taken to be the detection threshold.

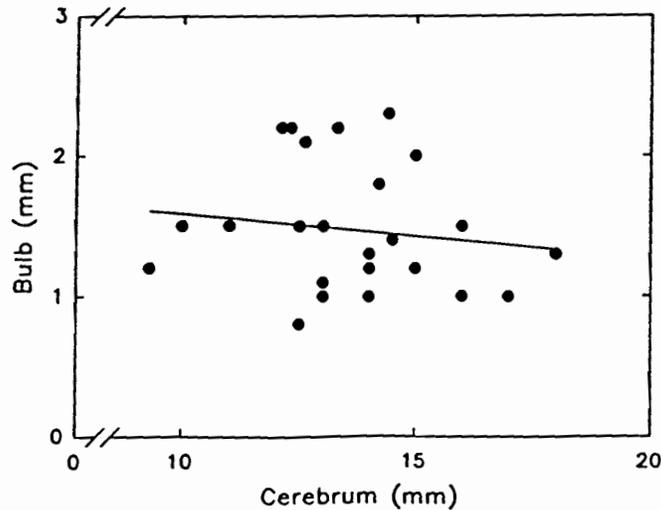


Fig. 4. The relationship between longest diameters of ipsilateral hemispheres of the olfactory bulb and cerebrum for passerines. Data derived from Bang and Cobb (1968).

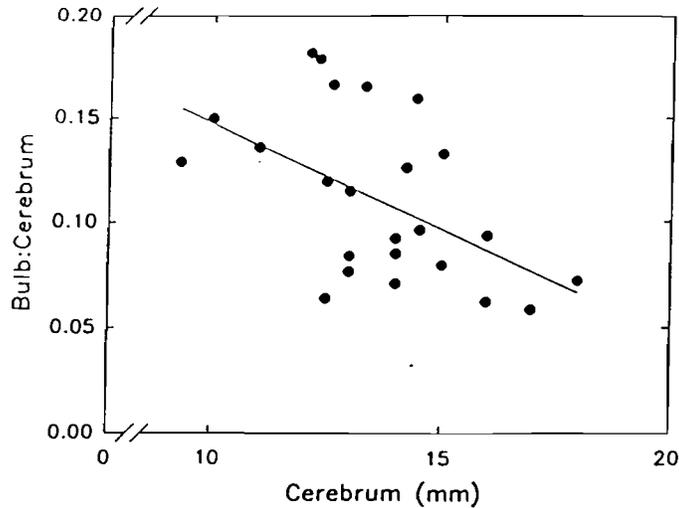


Fig. 5. The proportion of brain tissue allocated to the olfactory bulb relative to an index of total brain size for passerines. Data were derived from Bang and Cobb (1968).

probes are useful because they do not code for any biological information, their relevance is shaped entirely by the conditioning paradigm. Hence, we argue that for comparative purposes probe odor stimuli (i.e. reagents) are generally good indicators of overall olfactory ability.

Trends in form and function

Irrespective of taxonomic order, the minimum size of an avian olfactory bulb is approximately 1.0 mm in diameter (Bang and Cobb, 1968). The uniformly small size of passerine olfactory bulbs (1.5 mm), irrespective of brain size, suggests an evolutionary convergence toward a minimum bulb size within this order. A lower limit to olfactory bulb size most likely reflects the importance of critical nonolfactory functions of the bulb (Macrides and Davis, 1983).

Thus, given that some olfactory tissue remains, it is not unexpected that some olfactory function should also remain. The lack of a relationship between bulb size and detection threshold suggests that simple olfactory tasks can be accommodated with even small amounts of olfactory tissue, and at least in passerines, the relative allocation of brain tissue to olfactory function has no bearing on olfactory acuity. What remains to be determined is how small amounts of tissue might influence other measures of olfactory performance, i.e. discrimination ability as a function of complexity of the task.

Focus on olfactory performance in other taxa would prove valuable in clarifying the relationship between form and function in the olfactory system. Comparison across taxa suggest relative allocation to olfactory tissue is related to olfactory acuity (Fig. 7). Relating patterns of form and function within taxa

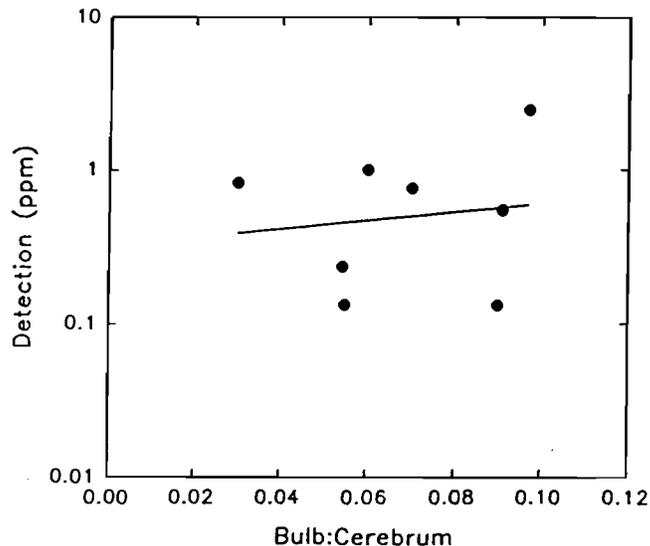


Fig. 6. The relationship between olfactory acuity and proportion of brain tissue allocated to the olfactory bulb for passerines. Data were derived from this study and from Clark and Mason (1989).

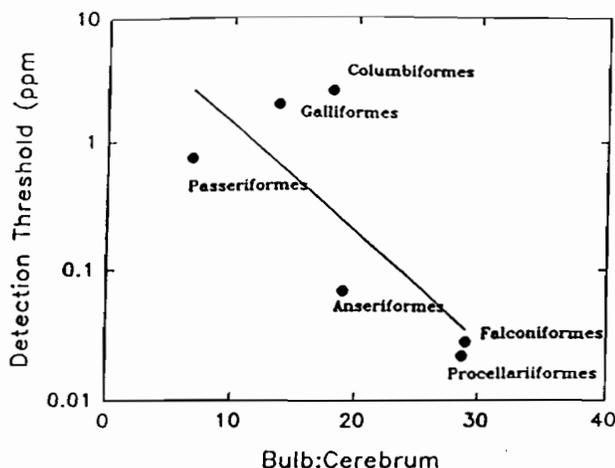


Fig. 7. The relationship between olfactory acuity and proportion of brain tissue allocated to the olfactory bulb for 6 orders of birds. Data were derived from this study, Clark and Mason (1989) and Wenzel and Sieck (1972).

would also be informative. For example, based on Bang and Cobb's (1968) data, olfactory bulbs of Procellariiformes increase as brain size increases ($r = 0.908$, $P < 0.01$), but the relative brain tissue allocated to olfactory function is constant across species ($r = 0.14$, $P > 0.1$). Thus, tests of detection and discrimination within this taxa would address questions of whether performance changes as a function of absolute bulb size, independent of the bulb to brain ratio. The size of the olfactory bulb also increases as brain size increases in Culculiformes ($r = 0.974$, $P < 0.01$), but the relative size of the bulb decreases ($r = 0.804$, $P < 0.05$). Thus, it may be possible to evaluate whether olfactory performance is more critically affected by changes in relative allocation of brain tissue vs absolute quantity of brain tissue devoted to olfaction.

Finally, given the diversity of olfactory structures in birds and the probable diversity in olfactory ability, it will be instructive to begin to consider how form and function may have constrained species' exploitation of resources (Wenzel, 1972; Clark, 1991b; Clark and Shah, 1991; Healy and Guilford, 1990), or molded social behavior (Balthazart and Schoefeniels, 1979) in a new synthesis of the evolutionary chemical ecology of birds.

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