

Phenotypic Diversity Is the Cornerstone of Evolution: Variation in Cortical Field Size within Short-Tailed Opossums

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ABSTRACT

Natural selection operates on phenotypic variation that exists within a population. Variable aspects of cortical organization, such as the size and connections of a cortical field, can generate differences in behavior, which is a target of natural selection. Yet studies pertaining to within-species variability in cortical organization are limited. In the present investigation, we examined variation in brain size, cortical sheet size, and primary sensory cortical field sizes in the adult short-tailed opossum (*Monodelphis domestica*). Within individuals, we found no significant difference between the right and left hemispheres in the overall size of the dorsolateral cortex or in primary cortical field sizes. Between individuals, we found relatively little intraspecies variation in brain weight, brain volume, and cortical sheet area for the dorsolateral neocortex and pyriform cortex; however, we observed a large degree of variability in body weight and primary sensory cortical field size, as defined by myeloarchitecture. Further, we found that the size of each cortical field correlated with the size of the other cortical fields as well as with the total size of the dorsolateral cortex. Here we discuss the possible sources of variation and examine the relationship between cortical field size and sensory processing abilities and behaviors across species. Since behavior is the target of natural selection, variation in cortical field size across individuals may supply the raw material necessary for cortical field evolution. *J. Comp. Neurol.* 499:990–999, 2006.

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Two of the fundamental tenets of the Darwinian Theory are that variation exists within a population and that natural selection operates on this variation. Thus, to understand how cortical field evolution proceeds it is necessary to identify the types of variation that exist in different cortical fields of individuals within a species or a population, to examine the mechanisms by which this variation arises, and to determine how selection operates on these mechanisms by selecting for specific aspects of behavior that are generated by cortical fields.

Before variability in aspects of cortical field organization can be appreciated, it is necessary to accurately and consistently identify cortical fields within and across species. The criteria used to define a cortical field have been described previously (Kaas, 1982, Kaas, 1983) and include a complete representation of the contralateral sensory surface (such as the body) or the contralateral visual field, often coincident with a unique architectonic appearance and pattern of connectivity with the thalamus and other cortical fields. Notably, the features used to define a cor-

tical field can be highly variable across species. For example, cortical fields can vary in their internal organization of sensory receptor representations, their architectonic appearance, their patterns of connectivity, and their size (Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). At a finer level of organization, variability in the response properties of neurons in a given location and synaptic connectivity within a cortical field may be different for different species. Thus, these differences in both the gross organization and connectivity of cortical fields and in the

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finer levels of organization are believed to account for the diversity of behaviors associated with the neocortex that are observed across species, such as differences in sensory, perceptual, and cognitive processing.

While the diversity of cortical organization that exists across species is appreciated, there are only a few studies on natural variation that exist in cortical organization within a population (e.g., Van Essen et al., 1984; Riddle and Purves, 1995; Dougherty et al., 2003). The goal of the current investigation was to examine the natural variation that exists within the neocortex of *Monodelphis domestica* by quantifying the size of the primary sensory cortical fields (primary auditory area (A1), primary somatosensory area (S1), and primary visual area (V1); see list for abbreviations), as defined architectonically, and two other well-defined cortical fields (frontal myelinated area (FM) and caudotemporal area (CT)), and by comparing the size of these fields across individuals. We chose to examine *M. domestica* because the overall size of the neocortex is relatively small and lissencephalic, and because architectonically defined cortical fields have been directly related to functional organization (Huffman et al., 1999; Catania et al., 2000; Frost et al., 2000; Kahn et al., 2000). Further, these animals have served as an important animal model for studies utilizing experimental manipulations early in development. We included cortical areas FM and CT since, in all *M. domestica* examined, these areas are readily identified architectonically and their relative positions on the cortical sheet are invariant. Electrophysiological recordings in CT indicate that it is involved in visual processing (Huffman et al., 1999); however, the function of FM is not known.

Ultimately, we plan to determine how differences in cortical field size, organization, and connectivity in both normal and experimental animals covary with quantifiable differences in behavior. This current study of normal variation is the first step toward understanding this relationship.

MATERIALS AND METHODS

All procedures were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, and conform to National Institutes of Health guidelines. Eleven female and 11 male adult short-tailed opossums (*Monodelphis domestica*) ranging in age from 6–32 months (average age, 13.9 ± 8 months) were used in these experiments. All of the animals were weighed prior to being euthanized. Weights ranged from 66–143 g (average, 97.6 ± 26 g).

Measuring brain size

Each animal was euthanized with an overdose of sodium pentobarbital (Beuthanasia, 250 mg/kg i.p.) and per-

fused transcardially with 0.9% NaCl in 0.1 M phosphate buffer (PB; pH 7.4), then with 4% paraformaldehyde (PA) in PB, followed by 4% PA + 10% sucrose in PB. Following perfusion, each brain was blocked behind the cerebellum and included everything anterior to the level of the spinal cord. Brains were then stored in 4% PA. The brains were photographed (RT Spot camera, Diagnostic Instruments, Sterling Heights, MI), weighed on a laboratory scale (A-250, Denver Instrument, Denver, CO), and the volume of each brain was measured.

Volume measurements were taken using fluid displacement (Scherle, 1970; Weibel, 1979; Mayhew et al., 1990; Howard and Reed, 2005), which relies on the Archimedeian principle of fluid displacement and is comparable to the Cavalieri method for measuring volume (Mayhew et al., 1990). Specifically, a beaker of 0.9% normal saline was placed on a laboratory scale, and a porous basket was suspended below the surface of the saline but above the bottom of the beaker by laboratory stand using a fine thread. The apparatus was tared and the brain was placed in the basket. The weight not borne by the thread, which is equal to the volume of the brain in cubic millimeters, was recorded and the procedure repeated five times. An average of five measurements was used as the final volume to increase the precision of the measurement.

Following weight and volume measurements for the whole brain, the cortical hemispheres were removed from the thalamus and brainstem using a procedure that has been performed in a variety of animals, including mice (Welker and Woolsey, 1974), rats (Welker, 1976), star-nosed moles (Catania and Kaas, 1995), and opossums (Huffman et al., 1999). Briefly, the anterior commissure was cut and the cortex was gently peeled away from the thalamus, breaking the axons in the corona radiata adjacent to the thalamus. The hippocampus was not separated from the cortex and was included in the cortical measurement. The weight and volume of each hemisphere and the remaining thalamus and brainstem were taken as described above.

Histological processing

Cortical hemispheres were manually flattened, cryoprotected in 30% sucrose, and sectioned tangentially on a freezing microtome at 20 μ m. In this preparation we did not undercut the white matter and retract the medial wall because the necessary cuts in the cortex needed to perform this technique would be different for each animal, and the resulting differential thickness across the cortical sheet that this method induced would have generated additional variability.

Myeloarchitecture was used to identify cortical fields. Myelin stains were done using the protocol described by Gallyas (1979). Since darkly myelinated regions are coextensive with functional boundaries (Huffman et al., 1999; Catania et al., 2000; Frost et al., 2000; Kahn et al., 2000), cortical fields were defined based on myelin density (Fig. 1A). Specifically, V1 (Area 17; striate cortex) was defined as a darkly myelinated field located caudomedially on the neocortex; A1 was defined as a darkly myelinated field located laterally on the neocortex, adjacent to the rhinal sulcus; S1 was identified as a darkly myelinated field located just rostral to V1 and A1; FM was defined as a darkly myelinated field located at the most rostral pole of the neocortex, adjacent to the rhinal sulcus; and CT was defined as a moderately myelinated, triangular field located at the caudolateral pole of the neocortex (see list for abbreviations).

Abbreviations

A1	Primary auditory area
CT	Caudotemporal area
CV	Coefficient of variance
FM	Frontal myelinated area
OB	Olfactory bulb
PYR	Pyramidal cortex
S1	Primary somatosensory area
V1	Primary visual area

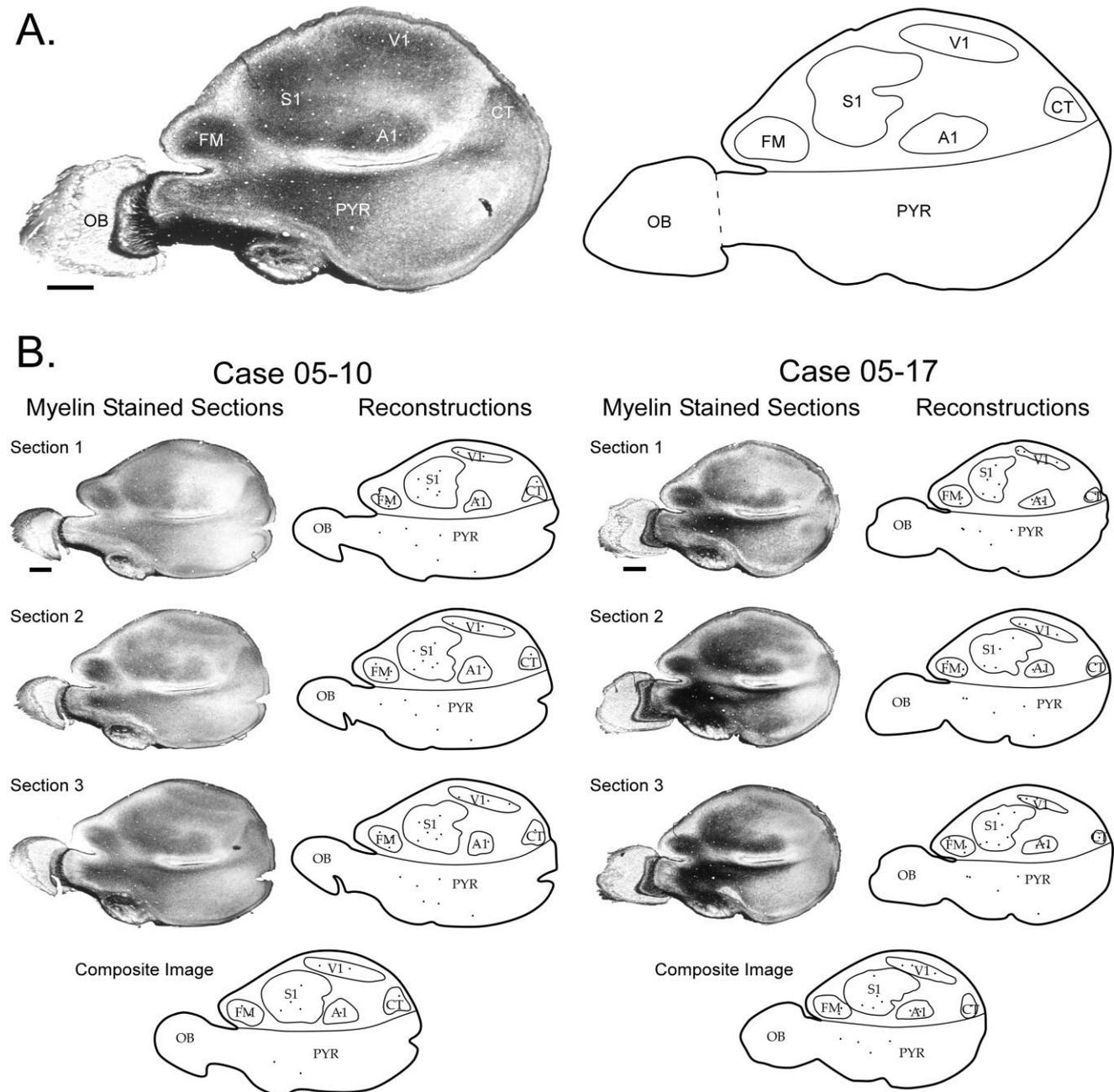


Fig. 1. Myeloarchitecture in flattened cortical sections. Primary sensory areas (V1, S1, A1) are darkly myelinated and their boundaries are coextensive with functional regions of the neocortex, defined using multiunit electrophysiological recording techniques (Huffman et al., 1999; Frost et al., 2000; Kahn et al., 2000). **A:** A myelin-stained section for a single cortical section taken from the middle cortical layers. A camera lucida was used to trace the outline of the section, the rhinal sulcus, and borders of the cortical areas (thin lines demarcate V1, S1, A1, FM, CT). Since no single section accurately represents all of the cortical field boundaries, the entire series of sections

were reconstructed from each case and the boundaries from those sections were compiled to make one comprehensive drawing to represent each hemisphere. **B:** Three sections from two different cases. Photographs of the sections are shown in the left column and camera lucida tracings are shown in the right column for each case. The camera lucida tracings were aligned using blood vessels. Comprehensive drawings were made by combining all of the individual drawings and using the largest outline from each tracing as the outer border. Rostral is left; medial is up; abbreviations defined in list. Scale bar = 1 mm.

Quantification of cortical field size

To quantify cortical field sizes, myelin boundaries were drawn for every section containing cortical fields of inter-

est using a camera lucida (Stemi SV6, Zeiss, Thornwood, NY). Since no single section accurately represents all of the cortical field boundaries, boundaries from several sections were compiled to make one comprehensive drawing

TABLE 1. Mean, Standard Deviation, and Coefficient of Variance

	Mean	Standard deviation	Coefficient of variance
Body weight (g)	97.6	25.9	26.49
Brain weight (mg)	828.0	67.6	8.17
Left hemisphere weight	217.2	18.5	8.50
Right hemisphere weight	216.8	15.5	7.17
Thalamus/brainstem weight	354.1	33.6	9.50
Brain volume (mm ³)	768.0	53.4	6.96
Left hemisphere volume	213.1	18.4	8.62
Right hemisphere volume	212.5	12.8	6.04
Thalamus/brainstem volume	349.3	31.8	9.11
% Cortical sheet devoted to:			
Neocortex	43.5	2.8	6.40
Pyriiform cortex	41.7	2.5	6.07
Olfactory bulb	14.8	2.5	16.87
% Neocortex devoted to:			
Primary auditory area (A1)	6.9	1.1	16.43
Primary somatosensory area (S1)	18.9	3.3	17.16
Primary visual area (V1)	9.1	2.1	23.17
Frontal myelinated area (FM)	5.6	1.3	23.40
Caudotemporal area (CT)	3.5	0.9	26.44

to represent each hemisphere (Fig. 1B). By matching blood vessels and tissue artifacts, a composite reconstruction was made, scanned into a computer, and area measurements were acquired using the NIH Image 1.62 program (Rasband, 1997–2006). This program has been used to measure cortical areas in previous studies (Huffman et al., 1999; Wahlsten et al., 2003; Airey et al., 2005; Hunt et al., 2006), and a similar method has been used by Riddle and Purves (1995).

Statistical analysis

Descriptive statistics of each measurement are presented. Differences between the two hemispheres were evaluated using paired *t*-tests. A Levene's test for equality of variances determined that there were no significant differences in the variances between the two hemispheres for any of the fields, so equal variances were assumed in all of the *t*-tests. To quantify the amount of variance, the coefficient of variance (CV) was calculated for each measurement using the formula: Coefficient of Variance = (Standard Deviation ÷ Mean) * 100. Pearson's correlation tests were used to determine the correlation between factors.

RESULTS

In this study we measured body weight, brain weight and volume, cortical sheet area, and the relative area of primary sensory cortical fields (defined as the cortical field area divided by the neocortical area) to determine the amount of intraspecies variation that exists within our colony. Means and standard deviations were calculated for body weight, brain weight and volume, cortical sheet area, and for the area of individual cortical fields, as defined by myeloarchitecture (Fig. 1).

Quantification of body size and brain size

Body weight and brain weight were measured in adult animals. The average body weight was 97.6 ± 25.8 g (mean \pm standard deviation, SD). Body weight varied by 26.5% (Table 1) and was the most variable of all measurements taken. Although the opossums ranged from 6–32

months of age, there was no correlation between body weight and age ($r(22) = 0.286$, $P = 0.197$).

Brain size was determined by two measurements, weight and volume. Brain weights are summarized in Figure 2A. The average brain weight was 828 ± 68 mg. Once the left and right hemispheres were separated from the thalamus, each hemisphere was weighed independently. On average, the left hemisphere weighed 217 ± 18 mg; the right hemisphere weighed 217 ± 16 mg; and the remaining thalamus and brainstem weighed 354 ± 34 mg. There was no significant difference between the weights of the two hemispheres ($t(20) = 0.091$, $P = 0.928$), and there was no correlation between brain weight and age ($r(22) = 0.169$, $P = 0.451$). Brain weight varied relatively little; the CV ranged from 7.17–9.50% (Table 1).

Brain volumes are summarized in Figure 2B. The average brain volume was 768 ± 53 mm³. The average volume of the left hemisphere was 213 ± 18 mm³; the average volume of the right hemisphere was 212 ± 13 mm³; and the average volume of the remaining thalamus and brainstem was 349 ± 32 mm³. There was no significant difference between the volumes of the two hemispheres ($t(20) = 0.149$, $P = 0.883$) and there was no correlation between brain volume and age ($r(20) = 0.248$, $P = 0.292$). Brain volume varied relatively little; the CV ranged from 6.04–9.11% (Table 1).

We examined the relationship between brain weight and body weight by graphing the data on a log-log plot (Fig. 2C). Using the allometric equation: $\log y = a \log x + \log b$, where $y =$ brain weight; $x =$ body weight; $a =$ slope; and $b = y$ -intercept (West, 1990; Kruska, 2005), we found a slope of $a = 0.213$. The general range of slopes reported for intraspecies comparisons fall between 0.20 and 0.30 and for interspecies comparisons fall between 0.56 to 0.63 (Kruska, 2005). A slope of 0.213 demonstrates that our data are comparable to other intraspecies studies and is consistent with the idea that the slope of intraspecies comparisons is approximately half as steep as interspecies comparisons.

Quantification of cortical sheet size

The area of the flattened, dorsolateral cortical sheet, taken from the composite image (Fig. 1B), was measured using the NIH Image program. The average area of the cortical sheet was 97.5 ± 15.9 mm². Although adults ranged in age, we found no correlation between cortical sheet size and age ($r(19) = -0.061$, $P = 0.804$). Further, there was no significant difference in the area of the cortical sheet between the two hemispheres ($t(12) = -0.478$, $P = 0.641$); however, cortical sheet size varied by 16.33% across animals, primarily due to the high level of variability in olfactory bulb size (see below).

The cortical sheet was divided into three regions: the neocortex (defined as cortex medial to the rhinal sulcus; Fig. 1), pyriform cortex (PYR; defined as cortex lateral to the rhinal sulcus), and the olfactory bulb (OB; defined as the bulb-shaped projection on the rostral end of the cortical sheet, separated from pyriform cortex by a dense band of myelin). The area of each region was standardized as a percent of the cortical sheet. On average, the neocortex occupied $43.5 \pm 2.8\%$, PYR occupied $41.7 \pm 2.5\%$, and the OB occupied $14.8 \pm 2.5\%$ of the cortical sheet (Fig. 2D). There was no significant difference between the two hemispheres in the percent of the cortical sheet occupied by the neocortex ($t(12) = 0.386$, $P = 0.706$), the PYR ($t(12) =$

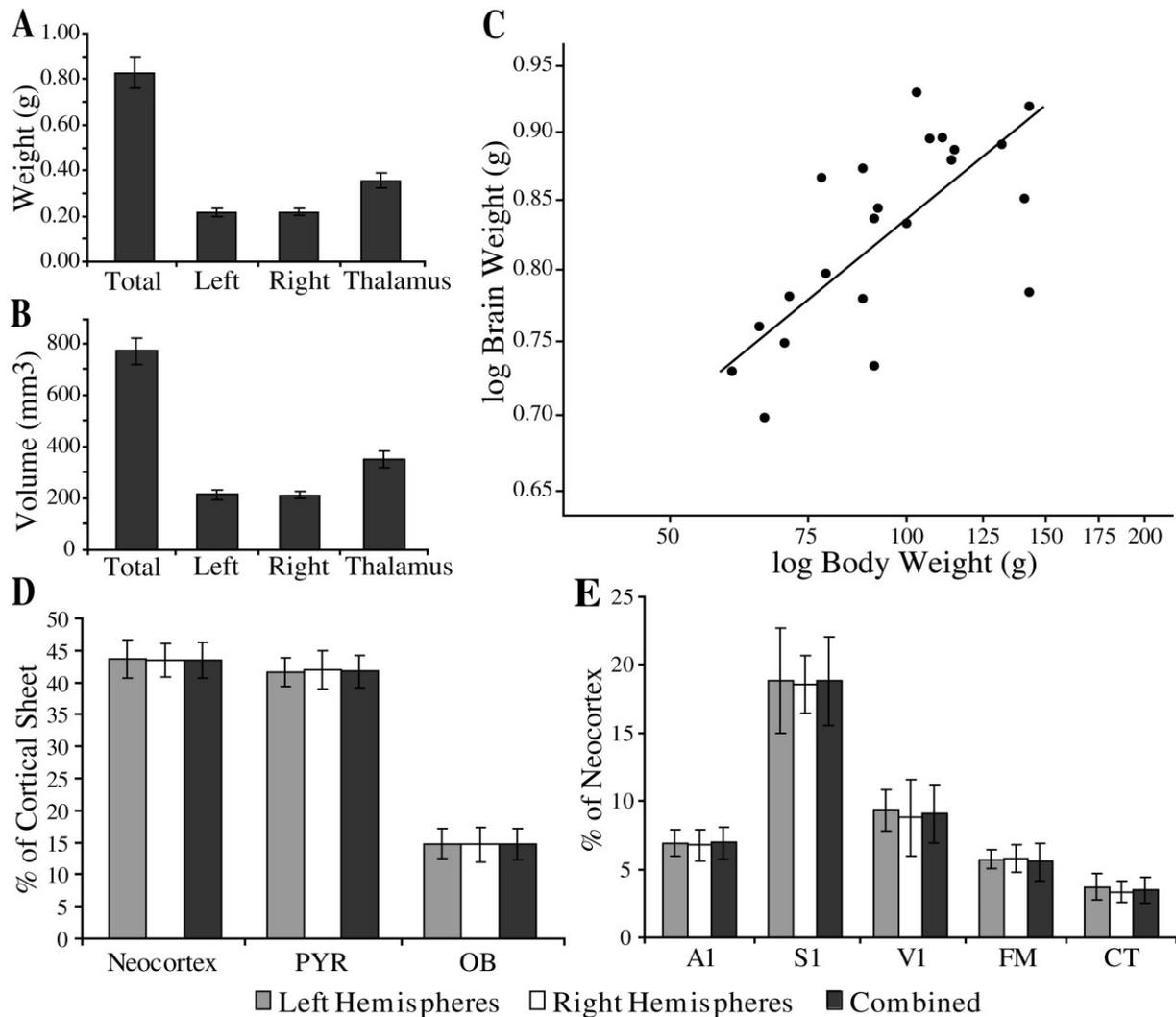


Fig. 2. Quantification of brain and cortical field size. Average measurements are given; error bars depict standard deviation. **A:** Brain weights for the whole brain, left hemisphere, right hemisphere, and remaining thalamus and brainstem. There was no significant difference between the left and right hemispheres. **B:** Brain volumes for the whole brain, left hemisphere, right hemisphere, and remaining thalamus and brainstem. There was no significant difference between the two hemispheres. **C:** Plot of brain weight versus body weight on a log-log scale. Each point represents an individual

animal. The slope of the line is 0.213, which is comparable to other intraspecies studies. **D:** The cortical sheet was divided into three regions: the neocortex, pyriform cortex, and olfactory bulb. We found no significant difference in the area of these regions between the two hemispheres. **E:** Myeloarchitecture was used to identify the cortical fields A1, S1, V1, FM, and CT. We found no significant difference in the area of any of these fields between the left and right hemispheres, but there was a large degree of variation in the area of individual cortical fields across animals.

-0.471 , $P = 0.646$), or the OB ($t(12) = -0.066$, $P = 0.948$), indicating that the two hemispheres were symmetrical.

The percent of the cortical sheet devoted to the neocortex and the PYR varied relatively little, CV = 6.40% and 6.07%, respectively. The percent of the cortical sheet devoted to the OB varied by 16.87%, more than twice as much as the variance found in the neocortex and the PYR. It is unclear whether this variation in OB size is due to variance between animals or is an artifact from inconsistencies in removing the OBs from the skull. Although brains were removed as carefully as possible, the OBs are sometimes difficult to remove, particularly the most rostral portion. To ensure that the variation in OB size did

not skew other area measurements, all comparisons of cortical field size were made by comparing cortical field size to neocortical area, as defined above, rather than to the total area of the dorsolateral cortical sheet.

Quantification of primary sensory cortical fields

Myeloarchitecture was used to identify the primary cortical fields and the area of each cortical field was measured using the NIH Image program. Cortical field measurements were standardized as a percent of neocortical area. On average, A1 occupied $6.9 \pm 1.1\%$, S1 occupied $18.9 \pm 3.2\%$, V1 occupied $9.1 \pm 2.1\%$, FM occupied $5.6 \pm 1.3\%$,

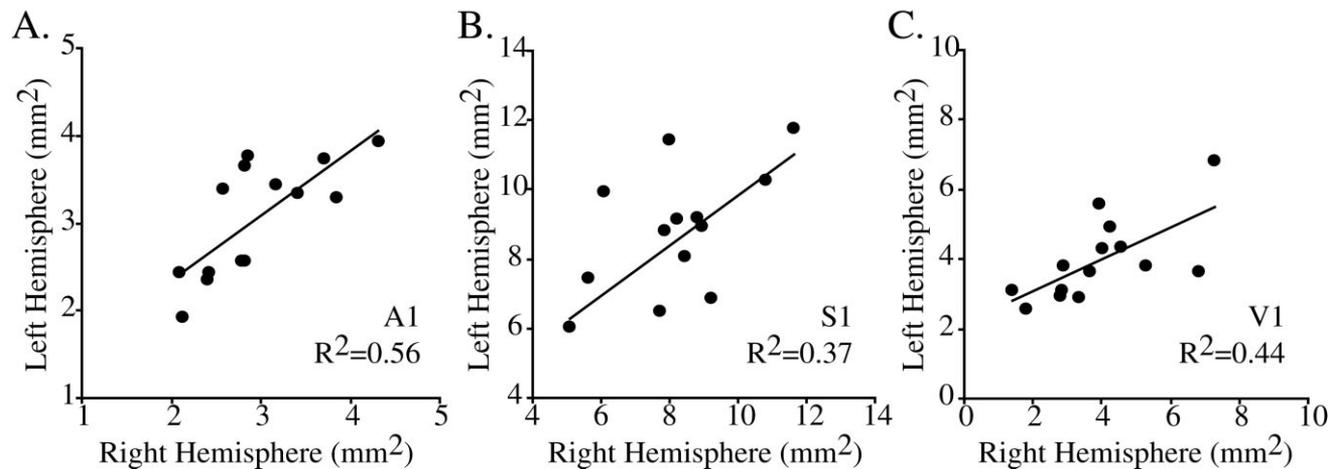


Fig. 3. The size of the primary cortical fields A1, S1, and V1 were highly correlated between the left and right hemispheres within individuals, as determined by Pearson correlation tests. Each point represents a single animal. **A:** The size of A1 was highly correlated

between the two hemispheres ($P < 0.05$). The line of best fit is shown ($R^2 = 0.56$). **B:** The size of S1 was highly correlated between the two hemispheres ($P < 0.05$; $R^2 = 0.37$). **C:** The size of V1 was highly correlated between the two hemispheres ($P < 0.05$; $R^2 = 0.44$).

and CT occupied $3.5 \pm 0.9\%$ of the neocortex (Fig. 2E). There was no significant difference between the two hemispheres in the percent of the neocortex occupied by A1 ($t(13) = 0.520$, $P = 0.612$), S1 ($t(13) = 0.844$, $P = 0.414$), V1 ($t(13) = 0.428$, $P = 0.676$), or FM ($t(13) = 1.327$, $P = 0.207$), indicating that these fields are symmetrical. There was a significant difference in the percent of the neocortex occupied by CT ($t(13) = 2.251$, $P = 0.042$), suggesting that CT may be slightly larger in the left hemisphere than in the right hemisphere.

The percent of neocortex devoted to each cortical field varied considerably more than any other brain measurement we made (except OB, but see above), and this variance was surpassed only by the variance in body weight (Table 1). Specifically, A1 varied by 16.43%, S1 varied by 17.16%, V1 varied by 23.17%, FM varied by 23.40%, and CT varied by 26.44%. This amount of variation is two-to-three times higher than brain weight and volume measurements (6–9% vs. 16–26%; see Table 1), suggesting that the size of cortical fields varies considerably more across individuals than does brain size.

We ran a series of Pearson correlation tests to determine the relationship between cortical field area and neocortical area. As expected from the paired t -tests, we found that the size of A1 was highly correlated between the right and left hemispheres ($r(14) = 0.748$, $P = 0.002$; Fig. 3A); the size of S1 was highly correlated between the two hemispheres ($r(14) = 0.061$, $P = 0.022$; Fig. 3B); and the size of V1 was highly correlated between the two hemispheres ($r(14) = 0.664$, $P = 0.010$; Fig. 3C). Since the right and left hemisphere measurements could not be considered independent and were not significantly different, we averaged the values to obtain a single measurement for each animal. Using these values, we found no correlation between cortical field size and age (A1: $r(19) = 0.207$, $P = 0.395$; S1: $r(19) = -0.023$, $P = 0.925$; V1: $r(19) = -0.241$, $P = 0.321$; FM: $r(19) = -0.307$, $P = 0.200$; and CT: $r(19) = -0.418$, $P = 0.075$). Further, there was no correlation between the percent of the neocortex occupied by any cortical field with the percent of cortex occupied by any

other cortical field. This indicates that there is no relationship between the relative sizes of cortical fields, as measured by surface area; thus, if one field increases as a percentage of the neocortex, another field does not change its size in a dependent way. Nevertheless, the absolute size of cortical fields did correlate with overall neocortical sheet size as well as with the size of other cortical fields (Fig. 3). Specifically, we found that the area of A1 correlated with neocortical area ($r(19) = 0.722$, $P = 0.000$; Fig. 4A); the area of S1 correlated with neocortical area ($r(19) = 0.771$, $P = 0.000$; Fig. 4B); and the area of V1 correlated with neocortical area ($r(19) = 0.803$, $P = 0.000$; Fig. 4C). Further, the area of A1 correlated with S1 ($r(19) = 0.645$, $P = 0.003$; Fig. 4D), the area of A1 correlated with V1 ($r(19) = 0.480$, $P = 0.038$; Fig. 4E), and the area of S1 correlated with V1 ($r(19) = 0.587$, $P = 0.008$; Fig. 4F). Together, these data indicate that the absolute area of cortical fields scales positively with neocortical area as well as with the area of other cortical fields.

DISCUSSION

In the present investigation we examined the variation in brain size, cortical sheet size, and primary sensory cortical field sizes in adult short-tailed opossums. We observed that there was relatively little variability across animals in total brain size, as measured by weight and volume, and in the size of the neocortex and PYR, as measured by surface area. Likewise, the surface area of the cortical sheet and of primary cortical fields varied relatively little across hemispheres within an individual. In contrast, the surface area of individual cortical fields varied considerably more between animals than any other measurement, except for variation in body weight and OB size. Although the relative sizes of cortical fields were not correlated, the absolute size of cortical fields correlated with absolute cortical sheet size, as well as with the size of other cortical fields within the same hemisphere. Thus, the area of any given cortical field did not increase in size at the expense of another primary cortical field. In the

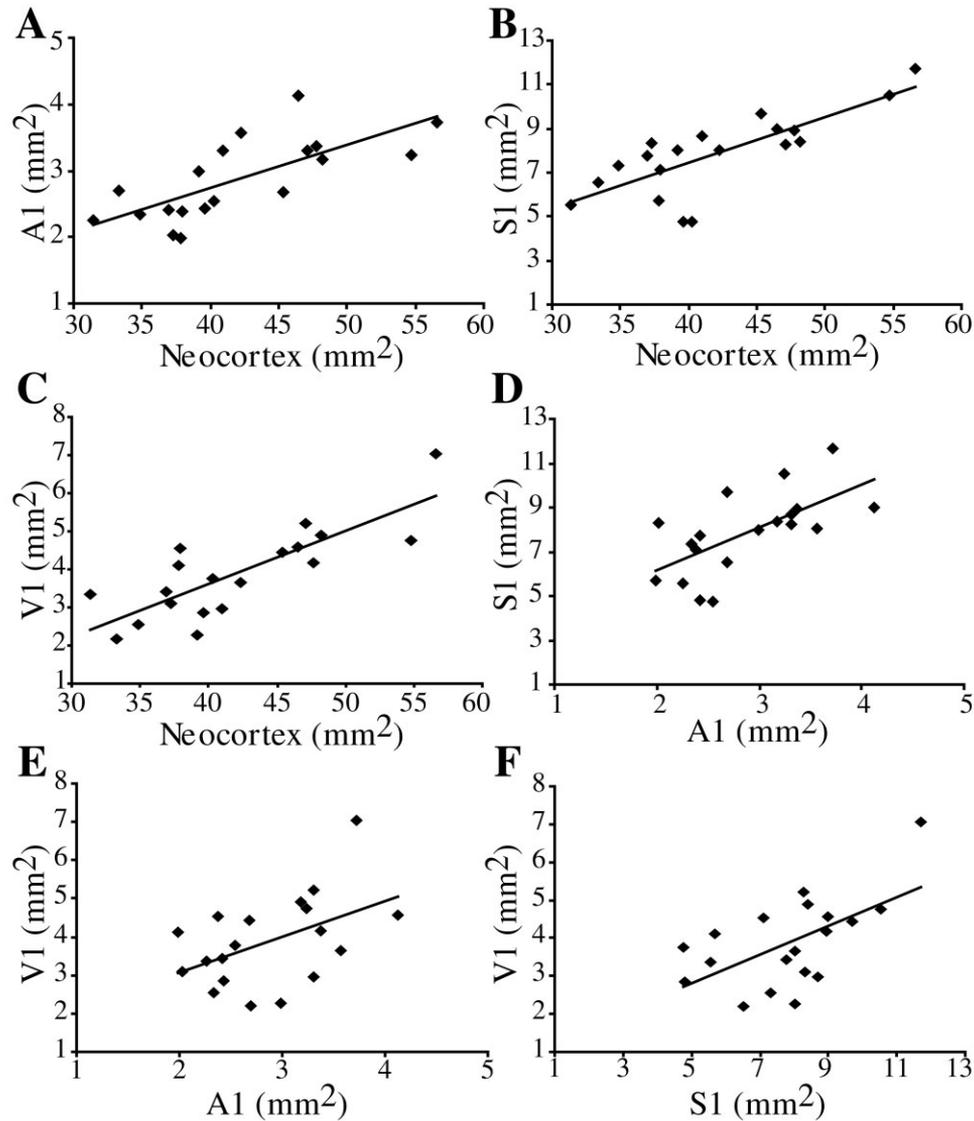


Fig. 4. The absolute size of cortical fields correlated with the overall size of the neocortex as well as with the size of other cortical fields, as determined by Pearson correlation tests. Each point represents a single animal; data points were obtained by averaging the right and left hemisphere values, since the two hemispheres could not be considered independent measurements and were not significantly different. **A:** The area of A1 correlated with neocortical area ($P <$

0.001). The line of best fit is shown ($R^2 = 0.52$). **B:** The area of S1 correlated with neocortical area ($P < 0.001$; $R^2 = 0.59$). **C:** The area of V1 correlated with neocortical area ($P < 0.001$; $R^2 = 0.64$). **D:** The area of S1 correlated with A1 ($P < 0.005$; $R^2 = 0.42$). **E:** The area of V1 correlated with A1 ($P < 0.05$; $R^2 = 0.23$). **F:** The area of V1 correlated with S1 ($P < 0.05$; $R^2 = 0.34$).

following discussion we examine other studies in which variation in cortical field size has been observed within a species, and we discuss the possible sources of variation in cortical field size and the relationship between the diversity in cortical field size and behavior.

Variation in cortical sheet and cortical field size

Although the surface area of the dorsolateral cortical sheet varied between individuals, there was relatively little variability across hemispheres within an individual. This type of interhemispheric consistency in size has been

observed for a variety of mammals including horses, pigs, goats, dogs, cats, rabbits (Mayhew et al., 1996), macaque monkeys (Van Essen et al., 1984), and humans (Henery and Mayhew, 1989; Hutsler et al., 1998). Similarly, cortical field area varied two-to-three times more between individuals than across hemispheres within an individual, a result similar to that observed in other mammals. For example, Riddle and Purves (1995) found that in rats the size of S1 and its individual isomorphic representations of body parts varied considerably across individuals. Furthermore, intraspecies variability in the size of V1 and several other extrastriate cortical fields has been de-

scribed in humans (Dougherty et al., 2003), as well as in several nonhuman primates (Van Essen et al., 1984; Krubitzer and Kaas, 1990; Purves and LaMantia, 1993). Interestingly, the magnitude of variability described in the size of V1 in these studies was relatively high for all species, as measured by the coefficient of variance (i.e., 13.2% in squirrel monkeys; 35.8% in owl monkeys; 13.7% in marmosets; 15.3% in galagos; 27.4% in humans; and 23.2% in the current study), and was not correlated with the overall size of the brain.

Sources of variation

In the present investigation we noted that the sizes of individual cortical fields, as determined by area, were more variable than the other measurements made. There are several possible sources of this variation. First, the variation could be genetic. Recent studies in which the size of cortical fields were shown to vary in different strains of mice support the hypothesis that the source of the variation is genetic (Wimer et al., 1969; Wahlsten et al., 2003; Airey et al., 2005). For example, Airey et al. (2005) demonstrated that the size of V1 and the barrel fields in S1 were significantly different between two strains of mice (C57BL/6J and DBA/2J). Because these animals were reared in almost identical environments, the source of the variation is most likely due to inherited differences between strains. Although a one-to-one correspondence between a specific gene and the emergence of a particular cortical field has yet to be established, recent studies in mice have demonstrated that it is possible to change the size of a cortical field by altering the spatial distribution of regulatory genes in the neocortex (Malamaci et al., 2000; Bishop et al., 2000; Fukuchi-Shimogori and Grove, 2001; Hamasaki et al., 2004; see O'Leary and Nakagawa, 2002, for review). For example, in mice that overexpress *Emx2* (Hamasaki et al., 2004), the size of V1 is markedly larger than in normal animals, and S1 is distinctly smaller. While this disproportionate increase in V1 and decrease in S1 is different than the types of variation observed in a normal population, this study supports the assertion that genes can play a critical role in regulating cortical field size. If *Emx2*, in combination with other genes, was involved in regulating cortical field size within a normal population, then one would expect the expression pattern of this gene to be variable within a population. Unfortunately, the precise role that this gene, or other genes, may play in cortical arealization is not known, nor is the variability of gene expression patterns appreciated across individuals within a population.

A second source of variation in cortical field size may be due to differences in patterns of sensory-driven activity during development. Several studies have demonstrated that it is possible to alter the size of a cortical field by changing peripheral morphology early in development and thereby the relative activity between sensory systems. Both monkeys (Rakic et al., 1991) and opossums (Kahn and Krubitzer, 2002) that were bilaterally enucleated very early in development have a substantially smaller V1 than normal animals. Likewise, congenitally deaf mice have a smaller A1 than normal animals and a larger V1 (Hunt et al., 2006). These studies indicate that differences in sensory receptor arrays and associated activity may contribute to cortical field size differences in a normal population. Furthermore, cross-species comparisons indicate that such variation exists and is directly

related to specializations in peripheral morphology, which may themselves be genetically regulated (Krubitzer and Kahn, 2003).

Another source of variation is due to measurement error, which may arise from deformations and shrinkage from histological processing or extraction of the tissue from the cranium. For example, it is likely that the variation in OB size is an artifact arising from inconsistencies in removing the OBs from the skull. Although brains were removed as carefully as possible, the OBs are sometimes difficult to remove, particularly the most rostral portion. Since all comparisons of cortical field size in this study were made by comparing cortical field size to dorsolateral neocortical size rather than to total cortical sheet size (which would include the neocortex, the PYR, and the OB), variation in OB size did not confound the results.

We do not believe that the variability in cortical field size observed in this study was due to measurement error because if the variation found in the current study was experimentally induced, then one would expect to see the same degree of variation for cortical field area measurements across hemispheres within the same individual as one sees across individuals, since the techniques used to process tissue, draw cortical field boundaries, and measure cortical field area in the two conditions were identical. This is not the case for the present investigation. Indeed, the interhemispheric variation in cortical field size was negligible.

Finally, variation could be induced by selection pressure. When pressure is high, one would expect variability to be low, because slight alterations in phenotype in a rigid environment could adversely affect the viability of the individual. On the other hand, when selection pressure is low, as in animals bred and reared in captivity, one might expect variability to be high. However, one could also argue that such low selection pressure is countered in captivity by the decreased environmental variability, thereby decreasing variability in activity patterns across sensory receptor arrays, and thus phenotypic variability.

Relationship between cortical field size and behavior

Regardless of the source of variation in cortical field size within a population, a more important issue is what the specific relationship is between the size of a cortical field and the sensory discriminatory abilities and behaviors of an individual. Although not explicitly tested in this study, it has been suggested that intraspecies variability in cortical field size is correlated with some measure of performance, such as visual acuity in the case of V1 (Duncan and Boynton, 2003). Unfortunately, a direct relationship between cortical field size and some quantifiable measure of performance has yet to be established for individuals within a species. However, such a relationship has been established for cortical domain allocation, or the amount of total neocortex devoted to a particular sensory system, in congenitally deaf and blind humans.

Several studies have demonstrated an enhancement of sensory discriminatory abilities with the expansion of cortical space devoted to processing a particular input. Specifically, psychophysical studies have shown that congenitally deaf individuals have enhanced processing of visual motion (Armstrong et al., 2002) and peripheral vision as compared to normal individuals (Neville and Lawson, 1987), as measured by the amplitude of the event-related

potential's N1 component. Further, this enhancement is associated with an expansion of visual processing into what would normally be auditory cortex (Catalan-Ahumada et al., 1993; Finlay and Darlington, 1995; Levänen et al., 1998; Bavelier and Neville, 2002; Fine et al., 2005). Likewise, congenitally blind individuals have shorter detection times for auditory discrimination tasks, as measured using auditory event-related potentials (Roder et al., 1999). Blind individuals also process language faster than sighted individuals (Roder et al., 2000). This compensatory adaptation is likely due to an expansion of auditory processing into what would normally be visual cortex (Weeks et al., 2000).

Because these examples demonstrate a clear relationship between a measurable feature of cortical organization and a sensorimotor behavior, it is reasonable to assume that a similar relationship exists for more complex behaviors, such as perception and cognition. While it is unlikely that simply devoting more cortex to a complex process would be sufficient to explain the individual nuances of a number of sophisticated behaviors exhibited by humans, such differences are likely due to alterations in size, gene expression patterns, and cortical field connectivity along the entire neuroaxis. Thus, small differences at multiple levels of processing across the nervous system could be sufficient to explain the large differences in behaviors exhibited by individuals within a population.

Comparative studies have also shed some light on this issue. Both sensory domain allocation and the cortical magnification of behaviorally relevant sensory surfaces within a cortical field are well-documented phenomena for a wide variety of mammals in all sensory systems. For example, much of the primate neocortex is devoted to processing visual inputs or coordinating visuo-motor behavior, and within cortical fields such as V1 the representation of the fovea is greatly magnified (Azzopardi and Cowey, 1993). Similarly, the somatosensory system of the duck-billed platypus encompasses the majority of its cortex: ~75% of the entire cortical sheet is devoted to processing inputs from the bill, and the bill representation in S1 is greatly magnified (Krubitzer et al., 1995). Finally, in echolocating bats most of the neocortex is devoted to auditory domain allocation and the magnification of particular auditory frequencies within cortical fields (Suga, 1984; Suga, 1994; Fitzpatrick et al., 1998). Thus, there is a clear correlation between amount of cortical space devoted to a particular sensory system and an animal's ability to detect, perceive, and act appropriately on highly relevant stimuli.

CONCLUSIONS

In this study we found relatively less intraspecies variation in brain weight, brain volume, and cortical sheet area for the dorsolateral neocortex and pyriform cortex than in the body weight and the surface area of primary sensory cortical fields. There was little to no difference in cortical sheet size or cortical field size, as measured by area, between hemispheres within individuals. While the source of intraspecies variation is not well understood, developmental studies indicate that both genes and differences in sensory-driven activity could account for the variation we observed. Together, the examination of individuals with congenital loss and comparative analyses indicate that differences in cortical field size, or at least

sensory domain allocation, are related to differences in sensory processing ability and behavior. Since behavior is the target of natural selection, these types of variation in cortical field size across individuals, if genetically mediated, could supply the raw material necessary for cortical field evolution.

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