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Effects of increased neural activity on brain growth

(neocortex/rat/development/somatosensory system/primary somatic sensory cortex)

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ABSTRACT **We have measured the effects of regionally increased metabolic activity—and by inference electrical activity—on cortical growth in the developing rat brain. Cortical growth is significantly and specifically greater in regions of chronically increased activity. This effect of activity on cortical growth may help explain the permanent storage of early experience in the developing nervous system.**

The mammalian brain grows progressively in postnatal life, increasing in weight about 4-fold from birth to maturity in man and mouse (1–3). In the work reported here we have tested the hypothesis that this gradual enlargement is modulated by experience, regional growth being increased by locally augmented neural activity (4–6).

Using the somatosensory cortex of the rat, we have taken advantage of previous studies that showed that the growth of one sensory system in the rodent brain can be enhanced by depriving the animal of a different sensory modality (7–9). Thus, neonatal eye removal in mice causes an enlargement of the cortical barrels (and neurons) that represent the mystacial vibrissae. These studies did not, however, examine levels of neural activity in the affected cortex or relate neural activity to cortical growth. Accordingly, we have asked whether metabolic activity, measured by the index of blood vessel density (4, 10, 11), is increased in the somatic sensory cortex of the enucleated animals and if changes in activity are associated with regionally specific enhancement of cortical growth during development. Since most of the energy demand on neurons is generated by synaptic signaling, local levels of metabolism reflect regional differences in the brain's electrical activity (12–15).

Our results show that bilateral eye removal at birth increased capillary density in the primary somatic sensory cortex (S1) and its component parts. Moreover, each of the cortical areas with chronically augmented levels of metabolic activity grew larger than its counterpart in control animals, whereas adjacent cortical areas with normal levels of activity did not.

MATERIALS AND METHODS

Six litters of male Long Evans (pigmented) rats from Charles River Laboratories were used in these experiments. Half of the pups in each litter underwent bilateral enucleation within 24 hr of birth; the other half served as controls. After inducing surgical anesthesia by cooling (16), both globes were removed. The remaining (control) animals in the litter were anesthetized only. Each litter was kept together until weaning; subsequently, experimental and control animals were fostered three or four per cage until 10–12 weeks of age. The animals were then anesthetized with pentobarbital (250 mg/kg) and perfused as described (4). Serial tangential sections (35 μm) were prepared for cytochrome oxidase histochemistry to demonstrate the primary somatic sensory cortex, the primary auditory cortex, the primary visual cortex, and the striatum. All slides were coded so that measurements were made without knowledge of an animal's history. Images of the ink-filled blood vessels were captured with a charge-coupled device videocamera and digitized by using an Image 1 analysis system (Universal Imaging, Media, PA). Blood vessel density was defined as the percentage of the imaged area occupied by vascular profiles and was measured in layer IV in and around S1 by an automated scanning technique (4). To examine other primary sensory cortical regions, 10 samples of 0.25 mm^2 each were measured in layer IV of the primary auditory and visual cortices. Vascular density was also examined in this same way in the dorsal striatum.

RESULTS

Blood vessel density in layer IV of S1 and the surrounding cortex was measured in 10 normal and 10 enucleated rats in the early adulthood to assess changes resulting from bilateral eye removal at birth (Fig. 1). The average blood vessel density in S1 as a whole was 11% greater in the enucleated rats than in littermate controls (Table 1). In contrast, no significant difference was observed in the cortical areas immediately surrounding S1. This effect on blood vessel density was due primarily to changes in the major S1 representations (see Fig. 1*B*), each of which was more heavily vascularized in the experimental animals.

To determine whether the increased vascularization of S1 in enucleated rats was present uniformly in S1 cortex or limited to barrels, we measured vascular density separately in each of the 36 whisker pad barrels and in the adjacent interbarrel cortex (Fig. 2). The average microvessel density in barrels was 16% greater in the enucleated rats than in littermate controls. When individual barrels representing particular whiskers were compared, each of the 36 whisker pad barrels showed increased vascularization, with percentage differences ranging from 7% to 23%. In contrast, blood vessel density in interbarrel regions was not significantly different in the two groups. Thus, the ongoing metabolic activity of these specialized functional units in S1 is enhanced as a consequence of visual deprivation during development. For the reasons stated, we take the cause of this metabolic change to be chronically increased electrical activity in the barrel neuropil.

Blood vessel density was also examined in the primary auditory cortex, the primary visual cortex, and the striatum to evaluate the generality of the activity changes induced by enucleation (Fig. 3). Vascular density within the primary auditory cortex was also increased significantly in the enucleated animals (10% compared to controls). In contrast, there was a 14% decrease in blood vessel density in the primary visual cortex of the enucleated rats; this opposite effect on the visual cortex is expected since this region has been deprived of its normal input. Measurements of vascular density in the striatum showed no difference between normal and experimental animals, indicating that bilateral enucleation does not

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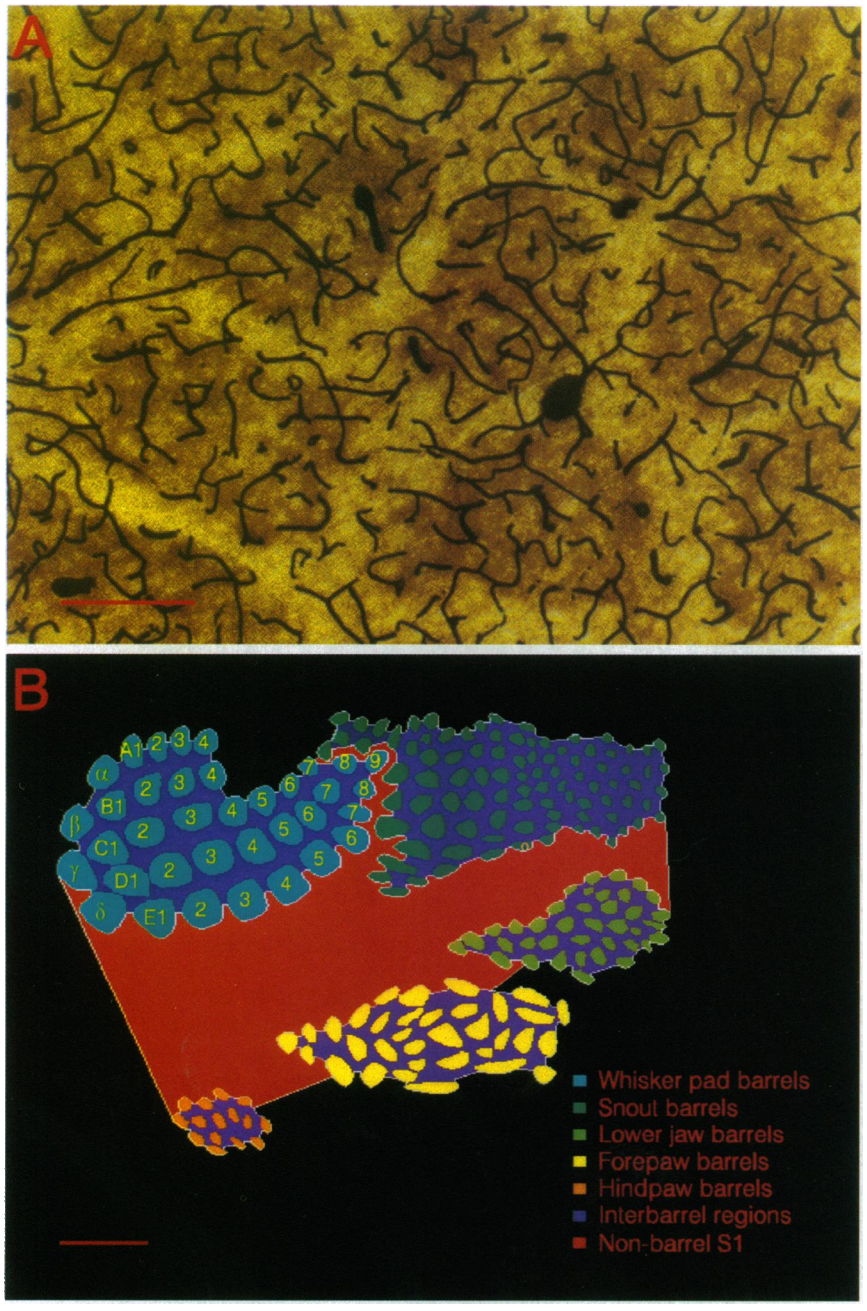

FIG. 1. Analysis of microvessel distribution in and around the primary somatic sensory cortex of adult rats. (*A*) Photomicrograph of a cytochrome oxidase-stained tangential section showing a portion of S1 (the anterior snout representation; see *B*) after ink perfusion. The darker patches are barrels, each of which represents a special peripheral sensor, such as a whisker. (Bar = 100 μm.) (*B*) Digitized map of the entire primary somatic sensory cortex after cytochrome oxidase staining showing the component parts of S1. The primary somatic sensory cortex in the rat is delineated by about 200 barrel and barrel-like structures in layer IV that can be visualized by several histological techniques (17–20). Five major somatic representations are apparent: the whisker pad (also called the postero-medial barrel subfield), anterior snout, lower jaw, forepaw, and hindpaw. Blood vessel density and regional growth were determined in these five representations; the 36 barrels in the whisker pad representation can be identified individually and were therefore used for more detailed comparisons of enucleated and control animals (see Figs. 2 and 4). (Bar = 1 mm.)

Table 1. Regional differences of blood vessel density in S1 and the surrounding cortex in normal and enucleated rats

Region examined	Blood vessel density, % of sampled area: Normal (n = 10)	Blood vessel density, % of sampled area: Enucleated (n = 10)	% difference*	P value†
Whisker pad	19.3 ± 0.8	21.7 ± 0.7	13	<0.05
Anterior snout	19.3 ± 0.9	21.8 ± 0.7	13	<0.05
Lower jaw	19.2 ± 0.8	22.3 ± 0.8	16	<0.05
Forepaw	18.7 ± 0.8	21.9 ± 0.9	17	<0.05
Hindpaw	18.3 ± 0.6	20.8 ± 0.6	14	<0.01
Non-barrel S1	17.1 ± 0.8	17.7 ± 0.5	3	0.56
S1 as a whole‡	18.1 ± 0.8	20.2 ± 0.6	11	<0.05
Cortex surrounding S1	15.9 ± 0.8	15.9 ± 0.4	0	0.24

Data are expressed as mean ± SEM.
*% difference = [(enucleated − normal)/normal] × 100.
†Student's t test.
‡S1 as a whole is the sum of the major representations plus the non-barrel portion of S1; see Fig. 1*B*.

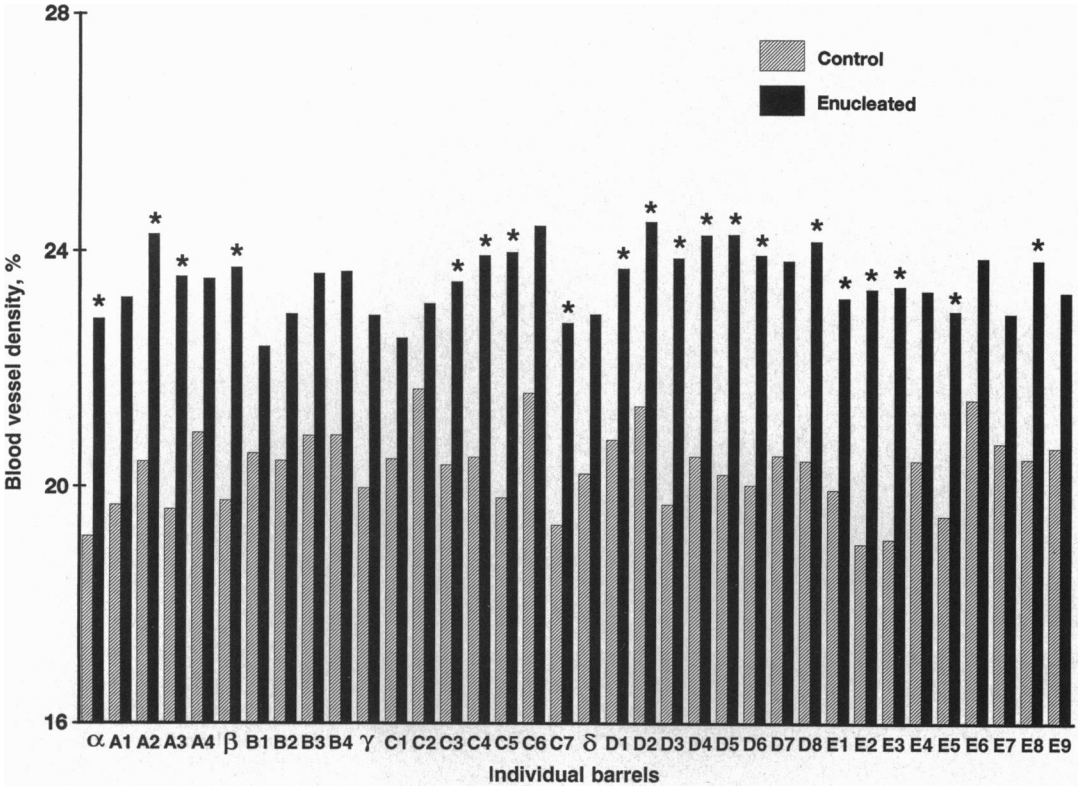

FIG. 2. Blood vessel density in the 36 barrels that represent the whisker pad. The average vascular density was consistently higher in barrels of the bilaterally enucleated rats than in littermate controls. Asterisks indicate that the difference between the experimental and control animals for a specific barrel is statistically significant ($n = 10, P < 0.05$; Student's t test). Barrels are identified here according to the standard nomenclature indicated in Fig. 1*B*.

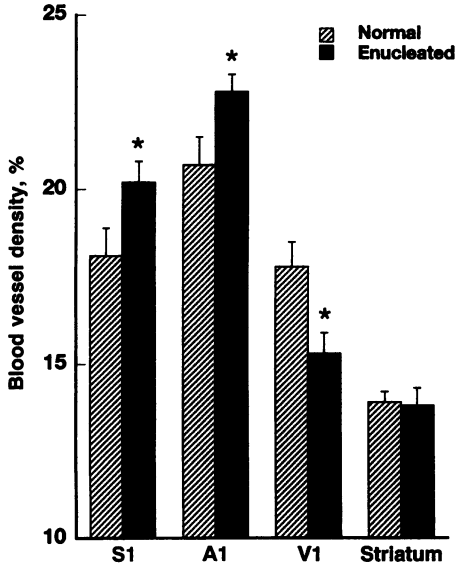

FIG. 3. Blood vessel density in different brain regions of normal and enucleated rats. Values are means ± SEM; asterisks indicate that the difference between the control and experimental animals is statistically significant ($n = 10, P < 0.05$; Student's t test). S1, primary somatic sensory cortex; A1, primary auditory cortex; V1, primary visual cortex. The metabolic activity of S1 and A1 is increased by enucleation, whereas the activity of V1 is decreased. The activity of the striatum is unchanged, indicating that enucleation does not produce a nonspecific alteration in the vascularization of the brain. Since the cortical areas devoted to A1 and V1 cannot be measured precisely (their boundaries in the rat are not sufficiently well defined), our analysis of the relation of activity and cortical growth is necessarily limited to S1.

induce a nonspecific change in vascular density throughout the brain.

We next asked whether the increased metabolic activity engendered in the somatic sensory cortex by neonatal enucleation specifically affects the growth of the involved cortical regions. To evaluate cortical growth, the area of S1 and its component parts was measured in the same rats in which we evaluated vascular density. The average size of the primary somatic sensory map was 11% greater in the enucleated rats (Table 2). However, we found no appreciable difference in the growth of non-barrel S1 regions in the experimental and control groups. Thus, the increase in S1 area arises from the greater growth of the major S1 representations in the visually deprived animals.

At a more detailed level, individual barrels in the whisker pad representation were consistently larger in the enucleated rats compared to the corresponding barrels in littermate controls (Fig. 4; see also refs. 7 and 8). The average increase in barrel size in the enucleated rats was 20%, the enlargement of individual barrels ranging from 4% to 46%. We also measured the average size of barrels in the other major representations of S1 to determine whether the greater growth of barrels in the experimental rats was restricted to the whisker pad representation (Table 3). Overall, the barrels and barrel-like structures in S1 were 31% larger in the enucleated animals. The greatest increase in barrel size occurred in the lower jaw and anterior snout representations, the regions in which normal postnatal growth is also greatest (17). In contrast, the area occupied by interbarrel regions in each representation was not significantly different among enucleated and control animals; nor was there any difference in the average number of barrels in S1 and its representations in the two groups of animals.

To preclude the possibility that the measured increase in barrel growth is simply the result of angiogenesis, we calcu-

Table 2. Regional differences of cortical growth in S1 in normal and enucleated rats

Region examined	Area, mm² Normal (n = 10)	Area, mm² Enucleated (n = 10)	% difference	P value
Whisker pad	5.090 ± 0.239	5.537 ± 0.174	9	0.15
Anterior snout	3.993 ± 0.129	4.906 ± 0.147	23	<0.0005
Lower jaw	1.139 ± 0.060	1.713 ± 0.086	50	<0.0001
Forepaw	2.107 ± 0.132	2.439 ± 0.170	16	0.14
Hindpaw	0.585 ± 0.033	0.702 ± 0.048	20	0.06
Non-barrel S1	8.531 ± 0.475	8.475 ± 0.293	−1	0.92
S1 as a whole	21.446 ± 0.868	23.772 ± 0.614	11	<0.05

Data are expressed as mean ± SEM.

lated the size of the relevant cortical areas after excluding the blood vessels. When the area occupied by blood vessels was subtracted from the overall area of the barrels, the average size of the whisker pad barrels remained 15% greater in the enucleated rats (0.082 ± 0.003 mm² in normal animals versus 0.094 ± 0.003 mm² in the enucleated animals; $P < 0.001$). Thus the increased blood vessel density of barrels in the visually deprived animals is not itself the cause of the greater size of barrels in the experimental group; rather, the neuronal and/or glial elements within barrels must have grown more as a consequence of neonatal eye removal.

DISCUSSION

These results show that the regional increases of blood vessel density observed in the barrels of enucleated rats closely match the enhanced growth of these components of the developing cerebral cortex compared to littermate controls. In contrast, neither microvessel density nor the growth of the interbarrel and the non-barrel S1 cortex was appreciably different in the experimental and control groups. Since barrels delineated by cytochrome oxidase staining are congruent with the Nissl-stained barrels in adjacent sections (see, for example, figure 7 in ref. 17), our measurements of the size of S1 and its constituent parts should accurately represent these cortical structures. The fact that postnatal cortical growth normally and after enucleation is greatest in those regions that are most heavily vascularized implies that increased levels of postnatal metabolic activity promote cortical growth.

The enhanced growth of the 200 S1 barrels and barrel-like structures in enucleated rats is presumably based on the growth of the neurons and glia that make up these structures. In support of this interpretation, the cell body size of neurons in whisker pad barrels is enlarged after bilateral enucleation in mice (9). Moreover, in the auditory cortex, the size of pyramidal neurons and the density of spines on their apical dendrites increase following enucleation (21, 22). These effects of enucleation on neurons in S1 and A1 are consistent with the hypothesis that neural activity promotes growth.

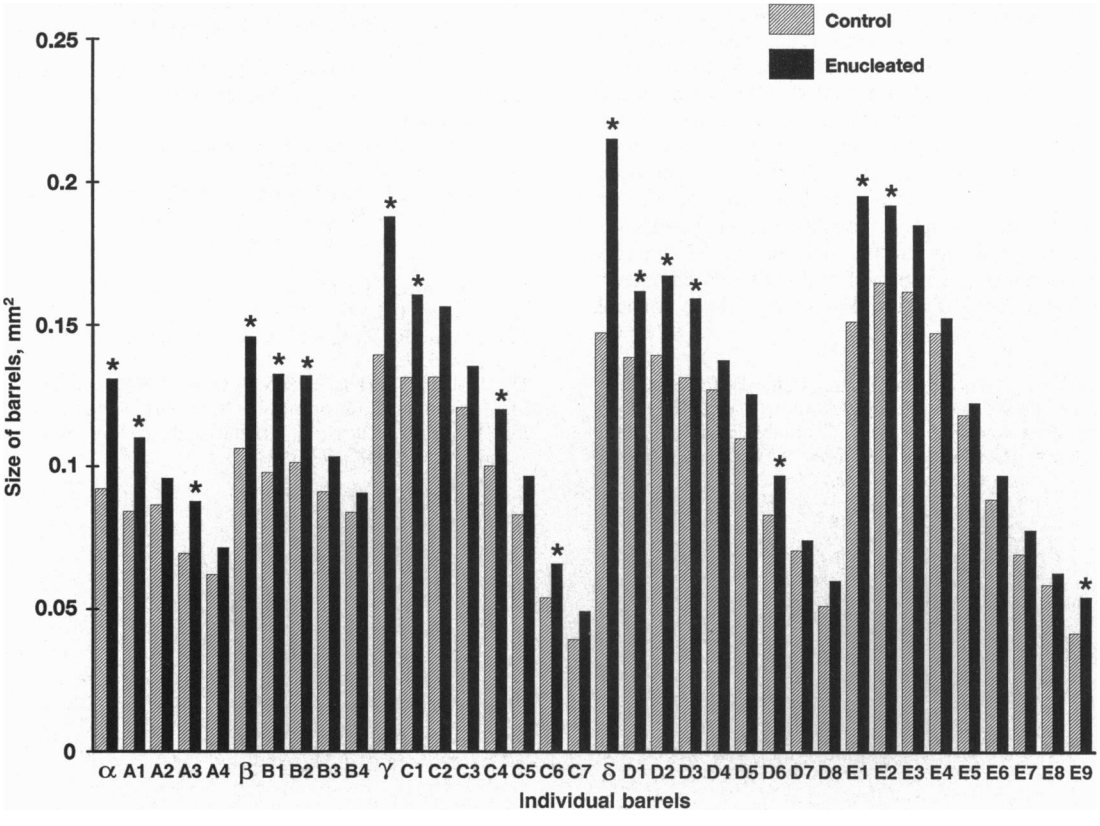

FIG. 4. Cortical growth in the whisker pad barrels of normal and enucleated rats. The adult size of the 36 barrels of the whisker pad representation was in every case greater in the experimental rats compared to controls, indicating greater growth of these cortical regions during maturation. Asterisks indicate that the difference between the experimental and control values for a specific barrel is statistically significant (n = 10, $P < 0.05$; Student's t test).

Table 3. Growth of barrels in S1 and its major representations in normal and enucleated rats

Region examined	Area, mm² Normal (n = 10)	Area, mm² Enucleated (n = 10)	% difference	P value
Whisker pad	0.102 ± 0.003	0.122 ± 0.004	20	<0.005
Anterior snout	0.025 ± 0.001	0.036 ± 0.002	44	<0.0005
Lower jaw	0.024 ± 0.001	0.036 ± 0.002	49	<0.0001
Forepaw	0.040 ± 0.003	0.052 ± 0.003	29	<0.01
Hindpaw	0.025 ± 0.001	0.033 ± 0.002	34	<0.005
All S1 barrels	0.040 ± 0.001	0.053 ± 0.002	31	<0.0001

Data are expressed as mean ± SEM.

The mechanism by which neonatal visual deprivation increases metabolic (and by inference electrical) activity in S1 (and A1) is not known. A possible explanation is increased use of the somatic sensory (and auditory) system in visually deprived animals. Enucleated rats are known to perform better than controls on maze tests, whereas acute loss of the whiskers diminishes maze performance in previously trained animals (23). Moreover, the facial whiskers, which are important for orientation and tactile discrimination, grow longer in visually deprived cats and mice, suggesting that enucleated animals use their whiskers more than normal (7). Alternatively, the increased activity of some cortical regions in enucleated animals could arise from abnormal connections. In anophthalmic mice, for example, somatic sensory axons from the dorsal column nuclei are found in the dorsal lateral geniculate body of adult animals; normally the lateral geniculate receives only visual input (24).

Although the magnitude of enhanced cortical growth in S1 and its component parts in these experiments is relatively small (≈10–50%; see Tables 2 and 3), the potential importance of activity-modulated growth should not be underestimated. Changes in cortical growth of even a few percent may be highly significant in the context of preferred behavior (25) or circuitry generated in response to experience (6). Since the human brain stops growing as an individual reaches maturity (2), any differential growth elicited by activity (i.e., differential use) should be permanently figured in brain structure. In this view, distinct patterns of neural activity generated by experience modulate the construction of brain circuitry, fomenting this process in regions of chronically high activity. Whatever the merits of this rather general speculation, the results we describe here show that metabolic activity in S1 barrels increases after neonatal eye removal and that this change is reflected in highly specific additional growth of these cortical regions.

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