Neurofilament Protein and Neuronal Activity Markers Define Regional Architectonic Parcellation in the Mouse Visual Cortex

This study was designed to assess the chemoarchitectural organization and extent of the mouse visual cortex. We used nonphosphorylated neurofilament protein, a neuronal marker that exhibits region-specific cellular and laminar patterns, to delineate cortical subdivisions. A comprehensive analysis demonstrated that pyramidal and nonpyramidal neurons expressing neurofilament proteins display striking laminar and regional patterns in the mouse visual cortex permitting the delineation of the primary visual cortex (V1) and its monocular and binocular zones, 2 lateral, and 5 medial extrastriate cortical areas with clear anatomical boundaries and providing evidence that the mouse medial extrastriate cortex is not homogeneous. We also investigated the expression profiles of 2 neuronal activity markers, the immediate early genes c-fos and zif-268, following deprivation paradigms to ascertain the visual nature of all subdivisions caudal, medial, and lateral to V1. The present data indicate that neurochemically identifiable subdivisions of the mouse visual cortex exist laterally and medially to V1 and reveal specific anatomical and functional characteristics at the cellular and regional levels.

Keywords: cortical organization, cytoarchitecture, immediate early genes, SMI-32, visual cortex, visual deprivation

Introduction

Neuroanatomical and physiological studies over the past 2 decades have greatly expanded our knowledge about the cortical organization of the visual system in the cat and a number of primate species (Felleman and Van Essen 1991; Payne 1993). However, similar information from the visual pathways in the mouse remains limited, no definite map of cortical visual areas being yet available for this species. The organization of the visual cortex in the mouse is likely to be comparably complex as in other species, as the different parcellation schemes that exist account for many separate visual areas (Caviness 1975; Dräger 1975; Wagor et al. 1980; Olavarria et al. 1982; Olavarria and Montero 1989; Schuett et al. 2002; Kalatsky and Stryker 2003; Wang et al. 2007). Although many functional and anatomical studies show that the extrastriate cortex lateral to the primary visual cortex (V1) is subdivided into several fields in mice, the subregional organization of extrastriate cortex medial to V1 has not been studied to the same extent. A few connectional and electrophysiological mapping studies indicate that the cortex medial to V1 contains some subdivisions (Wagor et al. 1980; Olavarria and Montero 1989; Schuett et al. 2002; Kalatsky and Stryker 2003; Wang et al. 2007), but these methods have not defined precise borders and locations of these cortical subfields.


Hence, this study was designed to assess the chemoarchitectural organization and extent of the mouse visual cortex, providing evidence that the mouse medial extrastriate cortex is not homogeneous. We also investigated the expression profiles of 2 neuronal activity markers, the immediate early genes c-fos and zif-268, following deprivation paradigms to ascertain the visual nature of all subdivisions caudal, medial, and lateral to V1. The present data indicate that neurochemically identifiable subdivisions of the mouse visual cortex exist laterally and medially to V1 and reveal specific anatomical and functional characteristics at the cellular and regional levels.

Keywords: cortical organization, cytoarchitecture, immediate early genes, SMI-32, visual cortex, visual deprivation

Materials and Methods

Animals and Visual Manipulation Paradigms

In the present study, 33 adult mice from the inbred C57Bl/6j strain were used. Animals were obtained from Janvier Elevage (Le Genest-St-Ise, France) and were maintained on standard laboratory diet and water ad libitum. All experimental manipulations were conducted in strict accordance to the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by an Institutional Laboratory Animal Use and Care Committee (Animal Facilities, Katholieke Universiteit Leuven, Leuven, Belgium).

Adult mice were monocularly deprived (MD, n = 2) or binocularly deprived (BD, n = 8) by enucleation of the left or both eyes, respectively.

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as described previously (Ceuppens et al. 2007). Subsequently, mice were placed back in an 11/13 h dark/light cycle for an additional 7 days before being sacrificed. Intact, control mice (CM, n = 23) were maintained in a similar light environment (11/13 h dark/light cycle). For in situ hybridization, the mice were put overnight in a dark room, subsequently exposed to a normal environment with ambient light for 45 min, and were immediately sacrificed thereafter. For Fos immunohistochemistry, mice were exposed to ambient light for 1 h on the morning following an overnight dark adaptation and were immediately sacrificed thereafter. Light exposure for 45 min after a period of darkness results in an increase in zif-268 mRNA expression levels in the mammalian visual system (Zangenehpour and Chaudhuri 2002), whereas light exposure for 1 h after a period of darkness results in a maximal increase in the expression levels of Fos in cortical neurons in the mammalian visual system (for review, see Kaczmarek and Chaudhuri 1997; Fairvar et al. 2004). All animals reported in this study were sacrificed between 9:00 and 11:00 AM to minimize variations in mRNA and Fos protein have labeling due to diurnal cycles of the animals.

Control conditions regarding the nature of the visual stimuli and their effects on the induction pattern of zif-268 mRNA and Fos protein have been previously reported in detail in the mammalian visual cortex (Van der Gucht et al. 2002, 2005 and references therein). Briefly, we analyzed in cats the effect of 2 monocular deprivation conditions on the induction patterns of both genes (Van der Gucht et al. 2000) or following retinal lesions resulting in deprived and surrounding non-deprived zones in area 17 and higher order areas (Arckens et al. 2000; Qu et al. 2003; Van der Gucht, Massie, et al. 2003).

**Tissue Preparation**

For in situ hybridization, all BD (n = 6) and CM (n = 6) animals were killed by cervical dislocation and the brains were rapidly removed and postfixed for 24 h at 4°C in buffered saline (0.01 M Tris, 0.9% NaCl, 0.3% Triton-X 100, pH 7.6). Staining and method specificity (positive and negative control experiments) were carefully performed as described in detail in previous publications (Van der Gucht et al. 2001, 2002, 2005, 2006; Van der Gucht, Jacobs, et al. 2003). To avoid possible variability in staining patterns and intensity among the different paradigms and different immunocytochemical experiments, the sections were processed at the same time, including cross-experiment controls, using the same staining solutions.

**Microscopy and Data Analysis**

We used atlases of the mouse brain (Hof et al. 2000; Paxinos and Franklin 2001) and rat brain (Paxinos and Watson 1986; Swanson 1992; Paxinos et al. 1999), as well as cortical maps and area descriptions from the literature, to assist in defining the spatial extent and location of visual and nonvisual cortical areas in the mouse brain in determining anatomical boundaries among adjacent areas. Slides were observed using a Zeiss Axiophot 2 under brightfield microscopy conditions (Zeiss Microimaging, Oberkochen, Germany), and photographs (see Figs. 1, 2, and 4-7 [mouse CM1406] and Fig. 11 [mouse MD11, mouse BD09]) were obtained using a digital camera (Optronics MicroFire, Optronics, Goleta, CA) and the NeuroLucida and VirtualMicroscopy and Data Analysis (MicroBrightfield, Williston, VT). High-power micrographs of SMI-32-immunoreactive neurons (see Fig. 8 [mouse CM03] and Fig. 9 [mice CM03, CM130, CM1-406]) were taken with a Kodak 35-mm camera mounted on a Leitz DM RB2 microscope (Leica, Leitz Instruments, Heidelberg, Germany). Digital files of the films were assembled and edited with Adobe Photoshop version 10. The specific neurofilament protein immunoreactivity patterns of each visual cortex region recognized in the present study were used to produce a schematic surface map of the boundaries and extent of these cortical domains in one animal (see Fig. 3 [mouse CM1406]). Slight deformations due to the curvature of the cortex were not corrected in this 2-dimensional rendering.

**Results**

**Heterogeneity of Neurofilament Protein Staining Patterns**

The distribution of neurofilament protein-immunoreactive neurons in the mouse visual cortex is shown on coronal sections caudally to rostrally in Figure 1. The laminar pattern of neurofilament protein distribution is bistratified with immunoreactivity present in layers III and V, whereas layer VI is characterized by a loose meshwork of immunoreactive processes together with a heterogeneous population of smaller
neurons. Layer I is devoid of SMI-32-immunoreactive cells, whereas layers II and IV occasionally contain labeled neurons. The expression patterns of neurofilament protein in oblique parasagittal overviews are illustrated in Figure 2 from lateral levels in the hemisphere (Fig. 2A, B), and a section through the medial visual areas (Fig. 2C), to levels close to the midline (Fig. 2D, E).

The present observations provide a revised parcellation of the cortical region medial to V1. Our data are consistent with the presence of a V2M posteriorly (Figs 1A–C and 2B–D) and suggest the existence of 4 RM extrastriate areas (RM1–4) situated more anteriorly in the visual cortex of the mouse (Figs 1A–C and 2B, C). All topographic borders among these neighboring medial areas and the adjacent visual and retrosplenial areas can be reliably defined by variations in neurofilament protein expression, such as dendritic arborization patterns, cell typology, apparent laminar cellular density and distribution profiles, and staining intensity (Figs 1 and 2 and see Figs 4–9 for detailed description). In the following description, we focus on the observations of neurofilament protein expression in the visual cortex, but patterns in the adjacent auditory, somatosensory, and other nonvisual regions are not described, except for changes in immunoreactivity that involve the abutting retrosplenial agranular cortex (RSA). Finally, the serial

Figure 1. Low-power photomicrographs of the mouse brain showing 4 levels through the posterior to anterior extent of the visual cortex and surrounding cortical areas in coronal neurofilament protein–labeled sections; (A) – 4.00 mm from Bregma, (B) – 3.00 mm, (C) – 2.40 mm, and (D) – 1.95 mm. Arrowheads mark the topographic boundaries of adjacent cortical areas. The asterisk on B and C shows the transition area between RSA and the medial extrastriate cortex. Scale bar = 800 µm. RSG, granular retrosplenial cortex; S1BF, somatosensory area 1, barrel cortex.
sections stained for neurofilament protein along the entire visual cortex (see Figs 1 and 4–7) were used to prepare a reconstruction of the mouse visual cortex. Figure 3 shows a rendering of the anteroposterior and mediolateral extent of all of the visual areas described in the present study on the hemispheric surface of a mouse brain.

**Boundaries of Striate and Extrastriate Visual Areas**

**Area V1**
The cellular and laminar staining patterns observed in the posterior part of V1 (Figs 4A,C and 5A,D) appear quite different compared with more anterior levels of V1 (Fig. 6A,E), owing to the progressive curvature of the brain posteriorly that results in a more compressed organization of the cortical layers, as well as to a different orientation of the fiber patterns. However, along the entire posterior–anterior axis of area V1, neurofilament protein immunoreactivity is located mainly in the deeper part of both layers III and V and less so in layer IV (Figs 4A,C, 5A,D, 6A,E and 9A–C). In layer IV, only small, scattered, multipolar cells are found. Occasional immunoreactive neurons occur in upper layer VI. Also, layer VI contains stained fibers oriented perpendicular to the pial surface. The border of deep layer VI with the external capsule is characterized by a meshwork of horizontally stained curly processes (Fig. 8G). Interestingly, clear differential labeling for neurofilament protein is observed between the monocular and binocular segment of V1 along the posterior–anterior extent of the visual cortex (white arrowheads on Figs 5A, 6A, and 10A,D). A lower staining intensity and density profile in layers III and V characterizes the medial, monocular zone of V1 (Figs 5A,D and 6A,E). In contrast, layer V in the binocular segment of V1 exhibits a darker labeling of pyramidal neurons with longer apical dendrites penetrating layer IV (Figs 5A and 6A,E). Layer III in the lateral, binocular zone of V1, and especially at the most anterior level of V1, contains more tightly packed SMI-32–immunolabeled pyramidal and multipolar neurons (Figs 5A and 6A,E).

**Area V2l**
The border between V1 and V2L can be established on subtle SMI-32 staining differences in layers III, IV, and V. At about Bregma level –4.60 mm, the most posterior part of V2L (area
V2PL) occupies only a narrow strip of cortex difficult to discern from posterior V1 (Fig. 4A), yet the lower signal for neurofilament protein in layer IV cells indicates its border with V1. Figures 5A and 6A illustrate V2PL near −3.65 mm and −3.10 mm from Bregma, respectively. The most obvious difference between V1 and V2PL is a decrease in the presence of labeled multipolar cells at the border between layers II and III and in layer IV, as well as a change in layer V that tends to be narrower but contains many, intensely stained pyramidal cells with darkly labeled, thick apical dendrites (Figs 5E and 6F). The lateral border of V2PL with the temporal association cortex (TEA) at more posterior levels (Fig. 4C) is distinctive (Figs 4E and 6E) or with the auditory cortex (AUD) more anteriorly (Figs 7A,D,E) compared with the staining pattern in V2PL. Both layers become broader and have a higher density of immunoreactive pyramidal neurons (Fig. 9D,E). Finally, the upper part of layer VI is characterized by a compact meshwork of immunoreactive processes that are oriented randomly, whereas the deeper part of layer VI contains a dense meshwork of horizontally stained processes. Altogether, these parameters define a clear margin between V2AL and its neighboring areas V1 medially and the primary somatosensory cortex (S1) laterally. At far anterior levels, obvious changes in the overall density and neuropil staining intensity are found at the border from V2AL with the lateral parietal area (PARL) medially (Fig. 7A,D). PARL shows a much lower neuropil staining intensity, contains a lower density of layer III and V immunoreactive neurons, and a lower degree of the clustering of apical dendrites from layer V crossing layer IV (Fig. 7C). Finally, the lateral border of V2AL with the barrel field cortex (S1BF) is clearly visible and is formed by a higher density of labeled neurons in layer III and by the broadening of layer IV, although the barrel fields are not themselves discernible by neurofilament protein (Fig. 7A,E).

Area V2M

Area V2M is located dorsomedially to V1 (Figs 4A,B and 5A,C). As for V1, the progressive curvature of the brain also affects its cellular and laminar staining patterns and the orientation of the fiber patterns in V2M resulting in a more compact assembly of the cortical layers in posterior V2M compared with the more anteriorly located V2M (Figs 4A,C and 5A,D). In its most caudal portion, V2M curves around the dorsal surface of the hemisphere, whereas in further consecutive posterior--anterior sections, V2M occupies progressively a more lateral position. Along the posterior--anterior axis, V2M joins medially the parasubiculum (PAS) (Fig. 4A) and more anteriorly RM3 (Fig. 5A). Changes in the arborization pattern and packing of SMI-32-immunoreactive cells, together with a decrease in neuropil staining in layers II–VI, clearly demarcate the border between V2M and the PAS (Fig. 4A) and between V2M and RM3 (Fig. 5A), whereas in contrast the lateral margin of V2M is primarily defined by a decrease in the density of the darkly stained multipolar neurons seen in layers III and IV of V1 (Fig. 4A) and by an overall less dense network of the SMI-32-immunoreactive fibers across all layers in V1 compared with the immunoreactive fiber network in V2M (Figs 4A and 5A). V2M contains several multipolar neurons immunoreactive for neurofilament protein at the border between layers I and II (Fig. 4A,B). Layer IV is densely populated by small, darkly stained nonpyramidal neurons and contains no stained parallel fiber bundles arising from the infragranular layers (Figs 4A,B and 5A,C). Numerous immunoreactive multipolar cells are also present in upper and lower layers (Figs 5C and 9F,G), whereas only a paucity of pyramidal neurons shows immunoreactivity for neurofilament protein in these layers (Fig. 4B). Layer VI demonstrates a low density of neurons and is characterized by a loose meshwork of lightly stained fibers and a low neuropil staining for neurofilament protein (Fig. 5A,B).

Areas RM1–4

Adjacent Nissl-stained sections in combination with neurofilament protein patterns were helpful in interpreting various aspects of the architectonic organization in the 4 RM areas (RM1–4; Fig. 8). Nissl staining clearly indicated a laminar architectonic transition, especially in RM4. A gradual broadening of supragranular layers in the medial-lateral direction characterizes the extent of RM4 in contrast to a condensed laminar zone of small neurons spanning cortical layer II in the adjacent RSA (Fig. 8A). Moreover, the cortical width of layers V

Figure 3. Schematic dorsal view of the mouse brain showing a topographic rendering of the striate and extrastriate cortical areas based on SMI-32 immunostaining patterns (case CM1406). The borders among visual cortical areas are labeled with fine dashed lines, but the delineations with other cortical nonvisual areas are shown with coarse dashed lines. The anterior extent of areas PARM and PARL was not fully assessed in the present study. For comparison, see Wagor et al. (1980, Fig. 8), Schuett et al. (2002, Fig. 3), Kalatsky and Stryker (2003, Fig. 4), and Wang et al. (2007, Fig. 2). M2, secondary motor cortex; PARM, parietal cortex, medial part.

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and VI in RM4 is gradually thinner compared with the thickness of infragranular layers in RSA, which are composed of small to large pyramidal, widely spaced cells in a wavy appearance (Fig. 8A). Also, like RSA, area RM4 contains no clearly defined granular layer IV. In RM3, supra- and infragranular and granular layers also differ markedly in their cell packing and laminar organization pattern from RM4. In the 2 other areas RM1 and RM2, another striking laminar architectonic change occurs: a granular layer IV is clearly present, forming a densely packed, thin layer, resulting in a typical 6-layered isocortical configuration comparable with that of V1 (Fig. 8A). However, in spite of these variable patterns, the topographic relationships among the 4 RM areas cannot be defined with precision based solely on the cytoarchitectonic features of a Nissl staining.

The boundary between V1 and RM1 is not abrupt, but the transition is obvious due to a decrease in the density of neurofilament protein-immunoreactive neurons in layer III, the absence of multipolar cells at the border between layers II and III, and the clustering of the dendritic bundles crossing layer IV (Fig. 8B,F). Layer VI shows a neurofilament protein staining pattern comparable with that in V1. The RM1–RM2 border is clearly identifiable (Fig. 8B,E) and can be reliably delineated by specific SMI-32 staining patterns in RM2 (Fig. 6A,D). Neurofilament protein immunocytochemistry only shows clear lamination and neuropil staining in layer V, which is densely stained, whereas layer III is less densely stained in RM2 compared with RM1. A dense pattern of basal and apical dendrites immunoreactive for neurofilament protein is obvious in layer V, whereas layer III is characterized by irregularly shaped and scattered cell bodies surrounded by a low neuropil labeling (Fig. 9H–N). Layer V contains a few SMI-32-immunoreactive neurons displaying apical dendrites and lightly stained pyramidal perikarya. RM3 and RM4 are both characterized by a very different immunoreactivity pattern (Figs 7 and 8). In RM3, the distribution of neurofilament protein immunoreactivity in layers III and V resembles that of RM2, but an alteration in the laminar architecture of layers III and V demarcates RM3 (Fig. 8A,B). As in RM2, the outer part of layer III bordering layer II is also populated by SMI-32-immunoreactive nonpyramidal cells (Fig. 9O,P), whereas layer V bends up toward layer III and has a differential staining profile because of a very low neuropil staining pattern and is nearly lacking labeled neurons (Fig. 8). In the most medially located area RM4, layers III and V contact each other as this is a dysgranular area (Fig. 8A,C). The neuropil staining as well as the intensity and apparent density of neurofilament protein-immunoreactive cells in layer V of RM4 (Fig. 9Q) are strongly reduced in comparison with RM3. Pyramidal cells showing neurofilament protein immunoreactivity are notably absent and only a few multipolar neurons and some fibers being scattered throughout RM4.

In the abutting RSA, the overall neurofilament protein immunoreactivity is drastically increased in all its layers except layer I in comparison with the 4 RM areas (Figs 5A, 6A, and 7A). Despite this clear difference, the medio-lateral aspect of RM4 does not form a clear-cut border with RSA. A distinct, small transition area in-between RSA and RM4 can be recognized (see asterisks in Figs 1, 5A, 6A, and 7A). As can be seen in Figures 5B, 6B, and 7B, this transition area contains no labeled large pyramidal neurons.
or arising dendrites of SMI-32-immunoreactive cells from its deep layers, unlike in RSA. Layers II, III, and especially the lower part of layer V are populated by small to medium size non-pyramidal neurons darkly stained by SMI-32 (Fig. 7B). Layer VI is only penetrated by a few labeled fibers from the cingulum bundle. Finally, the immunoreactive band corresponding to layer V in this transition area terminates abruptly in the corresponding layer of RM4 (Fig. 6B). The lateral border of this transition zone with RM4 is clearly illustrated in Figure 7A,B.

Effects of Visual Deprivation and Spatial Extent of the Mouse Visual Cortex

To assess the visual nature of the cortical areas reported in this study, adjacent sections of monocularly and binocularly enucleated mice were examined for Fos and neurofilament protein expression levels. In mice with the left eye enucleated (Fig. 10A-C), the visual cortex contralateral to the enucleated eye shows a low density of Fos immunoreactivity delineating the monocular zone from the binocular zone in V1 (Fig. 10A,C), matching the SMI-32 patterns (white arrowheads in Fig. 10A). In addition, removal of the visual input also results in a comparably low density of Fos-positive nuclei in area V2M (Fig. 10A,C). The visual cortex contralateral to the intact eye exhibits a high density of Fos expressing cells, most conspicuously in the supragranular layers, along the entire visual cortex (Fig. 10A,B). Animals with binocular enucleation show a low Fos expression pattern throughout the visual cortex of both hemispheres (Fig. 10E-G). Compared with the high densities of Fos-immunoreactive cells in nonvisually driven cortex, Fos expression is especially low in the supragranular layers of the visual areas (Fig. 10D,H). In BD animals, the medial region spanning areas RM1–4 also displays a decrease in Fos immunoreactivity (Fig. 10E–G) compared with the nondeprived situation shown in Figure 10A,B or with nonvisual regions (e.g., parietal cortex, medial part; Fig. 10H), revealing the extent of visual cortex that becomes unresponsive to light stimulation under this condition (Fig. 10E–G).

Similarly, low zif-268 mRNA expression levels in binocularly enucleated mice (BD in Fig. 11A) clearly demarcate the spatial extent of the visual cortex, compared with the high zif-268 mRNA hybridization signal in nonvisual cortical areas (Fig. 11B). In a visually intact CM (Fig. 11A), the high levels of zif-268 mRNA correspond to the high neuronal activity in the visual cortex in response to a binocular visual stimulation following an overnight dark adaptation period (Fig. 11A). As with Fos, the size of areas RM1–4 can also be demarcated from surrounding visual areas based on a clear difference in zif-268 mRNA intensity signals (Fig. 11A). The zif-268 in situ hybridization patterns demonstrate that the medial region spanning areas RM1–4 of BD animals shows a lesser decrease in hybridization levels.
signal compared with the very low zif-268 mRNA expression levels in the adjacent region corresponding to V2M, as well as in V1 and V2L laterally (see small arrows on Fig. 11A). Comparing these differential zif-268 mRNA expression levels along the mediolateral extent in the entire visual cortex of a BD mouse with the uniform zif-268 mRNA signal in a CM reveals that binocular deprivation does not affect equally the entire visual cortex, indicating that areas RM1--4 are not purely visually driven (Fig. 11A).

In summary, the outline of areas RM1--4 defined by in situ hybridization for zif-268 mRNA (Fig. 11A) coincides with the border of RM1--4 defined by the differential Fos protein expression levels in BD animals (Fig. 11D--H). Thus, these approaches permit to map the spatial extent of the mouse visual cortex (Figs 10 and 11) and confirm the results obtained with neurofilament protein immunocytochemistry.

**Discussion**

The present study provides new information and a comprehensive analysis about the cortical organization of the adult mouse visual cortex using SMI-32. The size, shape, laminar distribution, intensity, and density of neurofilament protein–immunoreactive neurons and their dendritic arborization pattern differed substantially among 8 cortical areas, and these profiles were used to parcellate the mouse visual cortex. We distinguished V1, posterior and anterior V2L lateral to V1, and 5 small areas medial to V1 termed V2M and RM1--4, which are each characterized by a specific neurofilament protein expression pattern and by a low SMI-32 immunoreactivity compared with V1 and the lateral visual areas. Detection of neuronal activity markers c-fos and zif-268 together with the observed neurofilament protein patterns confirmed the visual nature of all the extrastriate regions medial and lateral to V1.

**Neurofilament Protein Expression and Neuronal Typology**

Neurofilament protein immunolabeling throughout the mouse visual cortex showed generally a bilaminar pattern corresponding to layers III and V as previously observed in a number of other mammalian species (Hof and Morrison 1995; Van der Gucht et al. 2001, 2006; Kirkcaldie et al. 2002; Bourne and Rosa 2003; Baldauf 2005; Boire et al. 2005). A notable dissimilarity from the SMI-32 staining profiles of other species is that layers II and IV in the mouse visual cortex also contain neurofilament protein–immunoreactive neurons. In carnivores and primates, neurofilament protein immunoreactivity is restricted to a subset of pyramidal neurons known to be a class of excitatory projection neurons (Campbell et al. 1991; Hof and Morrison 1995; Hof et al. 1995; Van der Gucht et al. 2001, 2006; Bourne and Rosa 2003; Baldauf 2005; Boire et al. 2005). It is also interesting that c-fos expression has been reported in neurofilament protein–immunoreactive neurons in cats following dark adaptation and short-term light exposure (Van der Gucht et al. 2005), supporting the role of these particular
neurons in visual processing. In this context, it should be noted that the visual deprivation paradigms used in the present study did not alter the apparent expression of neurofilament protein in any of the visual areas at the light microscopy level.

In the mouse, our results also show that in addition to pyramidal neurons, a substantial population of nonpyramidal or multipolar neurons exhibiting neurofilament protein immunoreactivity has been documented in other rodents and marmoset monkeys (Kirkcaldie et al. 2002; Baldauf 2005; Boire et al. 2005), but not in carnivores or Old World monkeys (Hof and Morrison 1995; Hof, Bogaert, et al. 1996; Hof, Ungerleider, et al. 1996; Van der Gucht et al. 2001, 2006). Recent anatomical and physiological studies have shown that mouse V1 contains several morphologically distinct types of neurons with defined patterns of intrinsic connectivity (Brumberg et al. 2003; Voelker et al. 2004; Benavides-Piccione et al. 2006). Although the possible heterogeneity of different morphological and neurochemical cellular phenotypes in the mouse visual cortex is far less extensive compared with that in rat, carnivore, or primate visual cortex, the nonpyramidal cells showing immunoreactivity for neurofilament protein in our study include most probably star pyramids and some large multipolar cells, which are likely comparable with the spinous nonpyramidal cells lacking an apical dendrite described by Fairen and Valverde (1979).

Likewise, the expression pattern of neurofilament proteins in mouse medial visual cortex is generally comparable with that in other rodents, but differs from nonrodents. Interestingly, recent investigations of neurofilament protein in rat and hamster reported a low expression pattern for SMI-32 immunoreactivity in the cortex located medial to V1 as seen in the present study (Kirkcaldie et al. 2002; Boire et al. 2005). This region medial to V1 is characterized by distinct and unique neurofilament protein staining patterns, such as a striking gradual decrease in cell density and the bundling of apical dendrites and processes, their progressive disappearance medially, and a clear gradient of decreasing intensity of neuropil labeling from area RM1 toward RM4. Previous investigations on the connectivity and myelin patterns of medial area 18b in the mouse and other rodents revealed that its projections to other regions of the visual cortex are very sparse (Caviness and Frost 1980; Cusick and Lund 1981; Simmons et al. 1982; Kirkcaldie et al. 2002; Boire

Figure 7. Neurofilament protein staining patterns in a coronal section about −1.90 mm from Bregma (A). This level is characteristic of the anterior most extent of the visual cortex adjacent to the parietal cortex. Panels (B–E) show details from the boundaries of the transition (*) between RSA and the anterior end of RM4 and PARM (B), the differential patterns in PARM and PARL (C), and the differences between PARL, V2AL, and the barrel field (S1BF, D, E). The arrows point to the same neurons for orientation. Scale bar = 350 μm (A) and 125 μm (B–E). CA1, hippocampal field 1; cing, cingulum bundle; DG, dentate gyrus; ec, external capsule; IG, indusium griseum; PARM, parietal cortex, medial part; RSG, granular retrosplenial cortex; S1BF, somatosensory area 1, barrel cortex.
et al. 2005). These anatomical observations in area 18b fit our morphological findings in this medial zone, regarding the low neuropil staining intensity and the reduced cell and fiber density for neurofilament protein. In this context, it has been shown that phylogenetically related species share many characteristics of neurofilament protein immunoreactivity (Van der Gucht et al. 2001, 2006; Hof and Sherwood 2005).

**Organization of the Mouse Visual Cortex**

Previous mapping studies in murid and nonmurid rodent visual cortices provided evidence for subdivision of the medial extrastriate region in the mouse (Dräger 1975; Wagor et al. 1980; Olavarria et al. 1982; Olavarria and Montero 1989; Schuett et al. 2002; Kalatsky and Stryker 2003; Wang et al. 2007), rat (Olavarria and Montero 1981; Malach 1989; Coogan and Burkhalter 1993; Rumberger et al. 2001), squirrel (Kaas et al. 1989), golden hamster (Tiao and Blakemore 1976; Olavarria and Montero 1990), guinea pig (Choudhury 1978; Spatz et al. 1991), and degu (Olavarria and Mendez 1979). However, there remains some uncertainty about the organization of the visual extrastriate areas in murid rodents. Based on cytoarchitectural and electrophysiological mapping studies, the mouse visual cortex was originally divided into 3 areas, the area 17 or V1 and 2 extrastriate areas, 18a laterally and area 18b on the medial surface (Caviness 1975; Dräger 1975; Wagor et al. 1980). The microelectrode mapping study of Wagor et al. (1980) subdivided area 18a into V2 and V3 and area 18b into 2 small medial fields Vm-r and Vm-c. These authors’ V2 and V3 are generally consistent with areas V2AL and V2PL of the present study. The higher power photomicrographs in panels (C–G) show the changes in the laminar profile of SMI-32 immunoreactivity in layer V (oblique arrows). Arrows on C–G point to different cellular features (see also Fig. 9) in layers III and V present in RM1–4 (C–F) and V1 (G). Asterisks on panels D–F indicate the differential pattern of fiber bundles crossing layer IV in RM3 (D), RM2 (E), and RM1 (F). Arrows on A and B indicate the boundaries between abutting areas. Scale bar = 160 μm (A, B) 90 μm (C–G). ec, external capsule.
physiological mapping and connectivity analyses provided a refined functional map including as many as 8 functional domains within the regions described here as V2AL and V2PL and at least 3 regions coextensive with V2M and part of RM1--4 (Wang et al. 2007). These results and our morphological data clearly demonstrate the occurrence in the medial extrastriate cortex of a comparable organizational trend as that seen in the lateral extrastriate cortex with small discrete areas (Schuett et al. 2002; Kalatsky and Stryker 2003; Wang et al. 2007), allowing for a refined definition of these regions.

**Functional Implications**

The differences in chemoarchitectural organization in mouse extrastriate cortex reported here support the general notion that there are multiple visual areas between V1 and the midline convexity as well as between V1 and the more ventral aspect of the cortical surface in mammalian visual cortex (Rosa and Tweedale 2000; Kaas and Collins 2001; Casagrande et al. 2002). The observed regional variations in neurofilament protein expression in mouse medial and lateral visual cortices therefore point to a high degree of cytoarchitectural diversity and provide additional criteria for functional segmentation of the rodent visual cortex. This cortical organization scheme in mouse argues in favor of a considerable heterogeneity in the chemo- and cytoarchitecture of the visual cortex of murid rodents that finds parallels in sciurid rodents as well as in primates and carnivores (Kaas et al. 1989; Felleman and Van Essen 1991; Coogan and Burghalter 1993; Beck and Kaas 1999; Rosa and Tweedale 2001; Wagner et al. 2006; Wang et al. 2007).

The present data suggest that the neurochemical features of areas RM3 and RM4 represent the characteristics of a transitional region located between the visual and retrosplenial cortices, whereas areas V2M, RM1, and RM2 show an expression pattern of neurofilament protein more directly comparable with that in V1 and V2L. The medial extrastriate areas RM1--4 in the mouse visual cortex are in a position to contribute to a pathway through which visual regions may interact with the retrosplennial cortex (Caviness and Frost 1980; Kaas et al. 1989). Even though the role of this medial extrastriate cortex remains speculative in mice, it is worth noting that lesions in the anteromedial extrastriate cortex in rats result in deficits in visuospatial functions (Sa´nchez et al. 1997). Furthermore, Olavarria et al. (1982) reported the presence of a small, unnamed field adjacent to area AM but apparently located within Caviness‘ areas 29c and 29d of the retrosplenial cortex (Caviness 1975). They proposed that this field, which may include the present areas RM3--4, might in fact correspond to a striatocingulate projection that had been previously noted in the rat, squirrel, and rabbit (Montero 1981). Clearly, identification of the corticocortical and thalamocortical connections of each cortical domain of the mouse medial visual cortex will be required to understand fully the relationships between the limbic and visual components of this system.
Figure 10. Immunocytochemistry for the neuronal activity marker Fos in a MD (A–C) and a BD (D–H) mouse. Fos expression in the monocular zone of V1 (white arrowhead on A) is drastically reduced (C) in contrast to its counterpart in the nondeprived left hemisphere showing many Fos-immunoreactive nuclei throughout all layers of V1 (B). Note the differential Fos expression patterns between the monocular and binocular zone in V1 indicated with a white arrowhead on the corresponding neurofilament protein pattern. Following a binocular deprivation (D), the deprived visual cortex shows a lower Fos expression pattern in both hemispheres in RM3-4 (asterisks on E, F) and RM1-2 (G) compared with the high Fos expression levels in adjacent nonvisually deprived areas and nonvisual regions such as PARM and PARL (H). The extent of the visual cortex showing a reduced Fos expression pattern is demarcated with arrowheads (D). Scale bar = 700 μm (A, D) and 500 μm (B, C, E–H). PARM, parietal cortex, medial part.
Moreover, decreased Fos protein expression levels together with the in situ detection of very low zif-268 mRNA levels permitted the delineation of the extent of the entire cortex responsive to a visual stimulus that became inactivated following deprivation. Comparison of zif-268 activity expression patterns in intact mice with those in BD animals clearly showed that the neuronal activity along the mediolateral extent of the visual cortex is not homogeneous contrary to the complete deprivation of visual inputs. In fact, binocular deprivation was not maximally effective in the medial cortical region that corresponds to areas RM1–4, which may indicate that these areas are driven by multimodal inputs.

Whereas the mouse is becoming the animal of choice in many disciplines and the interest in the functional anatomy of the mouse cerebral cortex has increased since the introduction of many genetically modified mouse models of brain diseases, less attention has been paid to its detailed cellular organization. As new regional protein markers become available, much insight may be gained about important complex structure–function relationships in the cerebral cortex (Krubitzer and Huffman 2000; Watakabe et al. 2006; Yamamori and Rockland 2006). Also, such detailed analyses of cortical parcellation can serve as normative data sets for the study of genetically modified mouse models of cortical development (e.g., Hunt et al. 2006) and, in the particular case of neurofilament proteins, of many neurodegenerative disorders (for review, see Julien and Mushynski 1998).

In conclusion, neurofilament protein immunoreactivity in the mouse visual cortex not only reveal distinct staining patterns in each region but also characterize neuronal populations with neurochemical features possibly related to their function within a given area, the physiological role of the area itself, and their connections with other brain regions. Medial extrastriate areas are likely to play a prominent role in the processing of visuosaosensory interactions, inviting future studies to define their role and relationships to other cortical brain regions, and to compare further the functionality of this regional organization among rodents in relation to specific natural and behavioral patterns.
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