Changes in Mouse Barrel Synapses Consequent to Sensory Deprivation from Birth

YAIR SADAKA, ELIZABETH WEINFELD, DMITRI L. LEV, AND EDWARD L. WHITE*
Department of Morphology, Faculty of Health Sciences and Zlowtowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer Sheva, Israel

ABSTRACT

Neonatal sensory deprivation induced by whisker trimming affects significantly the functional organization of receptive fields in adult barrel cortex. In this study, the effects of deprivation on thalamocortical synapses and on asymmetrical and symmetrical synapses not of thalamic origin were examined. Thalamocortical synapses were labeled by lesion-induced degeneration in adult (postnatal day 60) mice subjected to whisker trimming from birth, other synaptic types were unlabeled. Brains were processed for electron microscopy, and numerical densities of synapses were evaluated by using stereologic approaches for whisker trimmed vs. control animals. Results demonstrated no change in nonthalamic, asymmetrical synapses; however, a decrease of 52% in the numerical density of symmetrical synapses (46.3 vs. 88.5 million per mm³; \( Z = -2.121; P < 0.05 \)) and a decrease of 43% in the numerical density of thalamocortical synapses (57.5 vs. 102.33 million per mm³; \( Z = -2.121; P < 0.05 \)) were observed after deprivation. Thus, experience-dependent plasticity of receptive fields in barrel cortex involves directly axons of both extrinsic and intracortical origin. The proportion of thalamocortical axospinous to axodendritic synapses was the same in control vs. deprived animals: in each instance, 80% of the synapses were axospinous (\( Z = 0.85; P = 0.2 \)). These results suggest that neither excitatory neurons, whose thalamocortical synapses are primarily axospinous, nor inhibitory neurons, whose thalamocortical synapses are mainly axodendritic (White [1989] Cortical Circuits. Synaptic Organization of the Cerebral Cortex; Structure, Function, and Theory. 1989; Boston: Birkhauser), are affected preferentially by the deprivation-associated decrease in thalamocortical synapses. J. Comp. Neurol. 457:75–86, 2003. ©2003 Wiley-Liss, Inc.

Indexing terms: thalamocortical; plasticity; receptive field; development; cortical representations; numerical density

The role of sensory experience in modeling cortical representation of peripheral organs has been the subject of intense study. The barrel cortex, which receives specific thalamocortical input associated with the large mystacial vibrissae, possesses certain important advantages for studying the plasticity of cortical representations. In rodents, this region of cortex contains a set of large barrels that are somatotopically and functionally related to the large mystacial vibrissae or whiskers (Woolsey and Van der Loos, 1970; Van der Loos and Woolsey, 1973; Woolsey, 1996). The large vibrissae are easily accessible to experimental manipulation, and the possible effects of these manipulations can be examined in posteromedial barrel subfield (PMBSF) barrels, which are consistently identifiable from one animal to the next (Kossut, 2001).

Whisker deprivation induces changes in cortical receptive fields: When all whiskers are trimmed during early postnatal development, stimulation of a regrown whisker causes reduced responses in neurons of layer II/III in the related barrel column, whereas stronger responses are recorded from layer II/III neurons within neighboring barrel columns (Lendvai et al., 2000; Stern et al., 2001). In...
like manner, when all whiskers but one or a few are plucked from birth, stimulation of spared whiskers causes weaker responses of neurons in layers II/III and IV of the related barrel column (Fox, 1992, 1994), but stronger responses in neurons within neighboring barrels (Simons and Land, 1987; Fox, 1992, 1994). Similar alterations in the responses of layer II/III barrel neurons have been observed in animals deprived as adults (Glazewski and Fox, 1996; Wallace and Fox, 1999a). The locus or loci of these experience-dependent changes has yet to be determined.

Thalamocortical afferents provide the main ascending input to each of the primary sensory areas of the cerebral cortex (see review: White, 1979); in somatosensory and visual cortex, thalamocortical afferents have been shown to synapse in layer IV on neurons from layers III, IV, and V that have dendrites passing through this layer (see Table 3.2 of White, 1989b). Consequent to whisker deprivation, many of the observed changes in the responses of units in layer IV of barrel cortex are of short latency (Fox, 1992), an observation consistent with the suggestion that thalamocortical input underlies deprivation-associated changes in the receptive field properties of neurons in the barrel cortex (for review, see Feldman et al., 1999). Other results support the thesis that intracortical projections are a principal basis for changes in cortical receptive fields of animals deprived from birth (Keller and Carlson, 1999; cf., Wallace and Fox, 1999b).

One way to assess the relative influence of thalamocortical vs. intracortical input on cortical plasticity is to identify anatomic substrates for deprivation-associated changes by using approaches to label specific input pathways. In the present study, the numerical densities of several synaptic types in barrels, including thalamocortical and other synapses, were compared in normal and deprived mice containing labeled thalamocortical axon terminals. Axospinous thalamocortical synapses have been associated with excitatory neurons and axodendritic thalamocortical synapses with inhibitory cells (White, 1989a). To determine whether deprivation-associated changes in thalamocortical synapses involve preferentially excitatory or inhibitory cells, we quantified elements postsynaptic to thalamocortical synapses, i.e., dendritic spines and dendritic shafts.

**MATERIALS AND METHODS**

**Sensory deprivation**

All large mystacial vibrissae of the right whisker-pad of four CD1 male mice were trimmed to skin level from the day of birth (P0) to 60 days postnatal (P60) with the aid of a dissecting microscope. Until P18, trimming was performed once or twice daily (on Saturdays, whiskers were trimmed only once, but the trimming was timed such that, in all instances, vibrissa length was kept under 1 mm at all times) to maintain vibrissal length under 1 mm; at later ages, when vibrissa growth is slower, trimming was performed once a day under light ether anesthesia. Three male control animals were anesthetized and handled under identical conditions for the same duration. Two of the controls were littermates of and shared cages with the deprived mice; all the mice were perfusion-fixed at 2 months of age (P60).

**Lesion placement and tissue preparation**

Animals were anesthetized with sodium pentobarbitone (40 mg/kg). By using a posterior approach, lesions were made in the ventrobasal (VB) thalamic nucleus (VBrn nucleus of Van der Loos, 1976; VPM of Zantua et al., 1996; and see also Franklin and Paxinos, 1997), and the posterior thalamic nucleus (PO, Valverde, 1998), on the left, to induce anterograde degeneration of thalamocortical afferents in the PMBSF contralateral to the trimmed vibrissae (Woolsey and Van der Loos, 1970). Thalamic lesions included also the proximal portions of the cortical projections of these nuclei (Fig. 1) as well as other structures that are known to not be associated with the barrel neuraxis (White and DeAmicis, 1977). On the fourth day after the lesions, mice were deeply anesthetized by intraperitoneal injection of 0.4–0.5 ml of 3.5% chloral hydrate followed by intracardiac perfusion with weak and strong solutions of cacodylate buffered glutaraldehyde and formaldehyde (Reese and Karnovsky, 1967).

The left cerebral hemispheres containing the deprived barrels were removed, osmicated for 14 hours, surrounded with paraffin, and sectioned at 40 μm tangentially to the PMBSF (White, 1976). Sections containing barrel D4 throughout their entire thickness were embedded in araldite/Epon for electron microscopy. The extent and placement of the lesions were assessed with the light microscope in 60-μm-thick coronal, frozen sections through the thalamus.

**Measurements of numerical densities of synapses**

The numerical densities of different populations of synapses were estimated in thin sections through the mid-layer IV level of barrel D4 in all animals. Only barrel D4 was selected for electron microscopy to exclude any variability in the numerical density of synapses in different
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barrels. Identifying the boundaries between barrel hol-

ows, sides, and septa in thin sections was facilitated by

relating electron microscopic images to drawings of blood

vessels and cell bodies made with the aid of the light

microscope in which barrel boundaries are more clearly

visible. In addition, barrel sides can be recognized in thin

sections by their content of closely spaced strings of cell

bodies (White, 1976). As observed previously (White,

1976), no distinct line demarcates neuropil in the barrel

septum from neuropil in the barrel side or side neuropil

from that in the hollow; these boundaries were delimited

by making imaginary lines connecting the strings of cell

bodies that form the barrel sides.

For each animal, three series of serial thin sections were

selected, 10 in areas identified as barrel hollow and 10 in

areas identified as barrel septa. Electron photomicro-

graphs were taken at an initial magnification of 8,000×

(see Sadaka et al., 2000). All synapses were identified by a

single observer who did not know the type of animal nor

the specific barrel region examined. The numerical densi-

ties (i.e., number per unit of volume) of thalamocortical,
asymmetrical (but not thalamocortical) and symmetrical

synapses were estimated for experimental and control

animals using the physical double disector method of Ste-

rio (1984). This method provides an unbiased estimation

of the numerical density of particles in three-dimensional

space (Braendgaard and Gundersen, 1986; Coggeshall and

Lekan, 1996; West, 1999). The first section for each set of

serial sections was used as a “reference” section. Sampling

frames were drawn to include the central regions of each
electron photomicrograph taken of the “reference” section,

and all synapses within the frames were identified; syn-
aspes not observed in corresponding areas in the second

section, i.e., “the look-up” section, were counted. “Refer-

cence” and “look-up” sections were then switched, and this

process was repeated. The volume of the disector was

obtained by multiplying the total area examined (parts

of the sampling frames containing cell bodies and blood ves-
sels were excluded) for each animal by the combined thick-

ness of the “reference” and “look-up” sections. Standard-

ization of section thickness was effected by performing all

thin sectioning with a single ultramicrotome (Porter-Blum

MT2-B) under identical conditions of temperature, humid-

ity, lighting, and viewing angle. Sections of constant

silver/gold interference color, estimated to be 80 nm thick

(Williams and Meek, 1966) were obtained.

All statistical data were performed by using the Mann-

Whitney test unless mentioned otherwise. Microsoft Excel

was used for graphic presentation of data.

Size of barrel D4

Changes in the size of barrel D4 after deprivation were
determined to examine the effect of deprivation on the
absolute number of synapses per barrel. The areas of barrel
hollows containing the mid-layer IV region of barrel
D4 examined with the electron microscope were measured
for “trimmed” and control animals by scanning the draw-
ings of the 40-μm-thick sections made with the light mi-
croscope into a Macintosh computer and measuring them
with NIH Image 1.62. Changes in the heights of PMBSF
barrels (not limited to D4) were determined by using 50-

μm-thick Nissl-stained coronal sections in four deprived

mice that were not used for electron microscopy. Hemi-

spheres contralateral to the trimmed side of the snout
(deprived) were compared with ipsilateral (nondeprived)
hemispheres and with hemispheres of two additional an-
imals that were not trimmed.

Diameters of postsynaptic densities and identification of postsynaptic elements

By using the approaches outlined by Braendgaard and

Gundersen (1986), the diameters of postsynaptic densities

were estimated for nontalamic, asymmetrical synapses.

Synapses sampled with the physical disector method were

identified in subsequent sections, and the diameters of

their postsynaptic densities (PSD) recorded in the section

in which the PSD appears at its longest (see Sadaka et al.,

2000). Diameters of postsynaptic densities associated with

thalamocortical synapses were not evaluated in view of

the likelihood that degeneration-associated changes in the

shapes of these synaptic junctions (Benshalom and White,

1988) would produce unreliable measurements.

The proportions of axospinous vs. axodendritic thalamo-
cortical synapses were determined for deprived and con-

trol animals by tracing postsynaptic elements in the series

of thin sections used for stereologic analysis until the

identity of the postsynaptic element was determined. An-

dimal handing and care were consistent with guidelines of

the US National Institutes of Health and were approved

by the University Animal Care and Use Committee.

RESULTS

Identification of synapses in thin sections

Synapses were identified by using commonly accepted
criteria, including the presence of thickened, parallel pre-

and postsynaptic membranes, a cluster of synaptic vesicles

to the cytoplasmic side of the presynaptic membrane,

a synaptic cleft larger than the usual extracellular space,

and the presence of a band of postsynaptic dense material

adherent to the cytoplasmic surface of the postsynaptic

membrane. Synapses containing a relatively thick band of

postsynaptic dense material (~40 nm) were classified

asymmetrical, and those containing a relatively thin

postsynaptic specialization (~10 nm) were identified as

symmetrical (Colonnier, 1968). Thalamocortical synapses

labeled by anterograde degeneration consequent to lesion-

ing the ipsilateral thalamus were identified by character-

istic degenerative changes in axonal fine structure (Col-

onnier, 1964). These changes included swelling and

disruption of mitochondria and a marked increase in the
electron density of the axoplasm (Fig. 2).

Numerical density of synapses

Thalamocortical and nontalamicortical asymmet-
rical synapses. Synaptic density was analyzed in ap-
ximately 2,000 electron photomicrographs; the volume
sampled per animal was 735 μm³ with an average volume
of neuropil equal to 634.1 μm³ (SD = 16.89 μm³) of which
321.9 μm³ (SD = 10.62 μm³) belonged to barrel hollows
and 312.2 μm³ (SD = 10.61 μm³) to barrel septa. Num-

erical densities for the different populations of synapses

examined are shown in Table 1.

After deprivation, no significant changes were observed

in the numerical densities of all asymmetrical synapses

taken together (417.5 ± 47.99 in deprived vs. 481.67 ±
Figure 2 (Continued)
Fig. 2. Electron photomicrographs of four consecutive serial thin sections (A–D), showing the three different types of synapses identified in this study: Thalamocortical synapse between an electron dense, degenerating thalamocortical axon terminal (TC) that makes an asymmetrical synapse onto a spine (S1). Further to the right, is shown an asymmetrical synapse of a non-TC axon terminal (A1) with dendritic spine (S2); note the relatively thick band of postsynaptic dense material and wide cleft compared with the thinner band and narrower cleft associated with a symmetrical synapse made by axon terminal A2 onto dendritic shaft (D). Scale bar = 500 nm in D (applies to A–D).
To examine whether the decrease in thalamocortical synapses after deprivation was associated preferentially with dendritic spines or shafts, elements postsynaptic to thalamocortical synapses were identified by tracing them in serial thin sections. Examples of axospinous and axodendritic thalamocortical synapses are shown in Figures 2 and 4. Results for 208 thalamocortical synapses from deprived mice and 273 from control animals showed that, in each instance, approximately 80% were made with dendritic spines (Fig. 5). The proportion of axospinous to axodendritic synapses observed is consistent with previous reports concerning the proportions of spines and shafts postsynaptic to thalamocortical synapses in mouse barrels (Elhanany and White, 1990; cf., pp 192–196 [White, 1989b]). This finding indicates that the decrease in thalamocortical synapses observed in deprived animals is not associated preferentially with axospinous or with axodendritic synapses.

**Symmetrical synapses.** Table 1 includes data on the numerical densities of symmetrical synapses in deprived vs. control animals. The data show that, consequent to trimming all whiskers on the contralateral side from birth, a significant reduction occurred in the numerical density of symmetrical synapses (Fig. 6, 46.3 ± 4.58 in deprived vs. 88.47 ± 12.92 in controls; Mann-Whitney: Z = −2.312; P = 0.034).

**Hollow and septum separately.** Because neuropil in barrel sides cannot be clearly demarcated from neuropil in the hollows or septa (see Materials and Methods section), we focused our efforts on the latter two regions. The synaptic composition of hollow neuropil differs from that in the septa. For example, the hollow neuropil contains thalamocortical synapses mainly of VB, but also of PO origin, whereas the barrel wall, composed of barrel sides and sepsa (Woolsey and Van der Loos, 1970), contains thalamocortical synapses only or primarily from PO (Koralek et al., 1988; Lu and Lin, 1993). Establishing that specific subregions of the barrel respond differently to sensory deprivation is beyond the scope of the present study, as is the elucidation of possible deprivation-induced changes in the projection patterns of VB or PO neurons. A breakdown of numerical densities for barrel hollows and septa separately is presented in Table 2. These results show a tendency toward a decrease in numerical densities of thalamocortical and symmetrical synapses in both barrel hollows and septa after sensory deprivation. This finding suggests that the effects of sensory deprivation on synaptic reorganization are not localized preferentially to hollows or septa; a comprehensive analysis of this issue must await the assessment of deprivation-induced effects on specific, identified components of barrel synapses.

**Barrel size.** To determine whether deprivation affects the total number of synapses, the size of barrel D4 was estimated by using measurements of hollow area at the mid-layer IV level, and by taking into account the heights of barrels in layer IV as observed in coronal sections (see Materials and Methods section). No significant differences were observed in the areas of barrels in deprived (45,270 ± 2,323 μm²) vs. control (46,562 ± 4,210 μm²; Z = −0.22, p = 0.83) mice. Similar results have been reported previously (Simons and Land, 1987; Fox, 1992; Micheva and Beaulieu, 1995a, b). The heights of PMBSF barrels did not differ significantly in deprived vs. intact hemispheres in the deprived animals, or compared with hemispheres of non-deprived control animals (Kruskal Wallis test: Chi² = 1.718; P = 0.424). These results, and our previous findings (Sadaka et al., 2000) indicate that the size of barrel D4 is unaffected by the deprivation paradigm. Therefore, alterations in the numerical densities of thalamocortical and symmetrical synapses presumably reflect changes in the total numbers of these synapses.
Diameters of PSDs

The average diameters of PSDs for nonthalamic, asymmetrical synapses in deprived (470 synapses) vs. control animals (370 synapses) is shown in Table 3. A shift to the left in the distribution of the diameters of PSDs in the deprived animals (Fig. 7) suggests that a tendency toward shorter PSDs is associated with deprivation. However, a statistically significant difference in the average PSD diameters for deprived vs. control animals was not observed (Mann-Whitney Z = 1.414; P = 0.157).

DISCUSSION
Quantification of thalamocortical synapses

Lesion-induced degeneration of the VB and PO thalamic nuclei was used to identify thalamocortical axon terminals in layer IV of mouse barrel cortex. These terminals are known to degenerate simultaneously such that, at any postlesion survival time, all affected terminals are at the
same stage of degeneration, enabling quantification of the entire population of degenerating thalamocortical axon terminals (e.g., White, 1978, 1984). In contrast, in other systems, the degeneration of thalamocortical axons proceeds in a nonsimultaneous manner that complicates the quantification of thalamocortical synapses at any postlesion survival time (e.g., Jones and Powell, 1970; Peters and Feldman, 1976). At the 4-day survival time used in this study, approximately 20% of the asymmetrical synapses in PMBSF barrel hollows are identified as thalamocortical (cf., White, 1979). Similarly, an alternative labeling method using the anterograde transport of Phaseolus vulgaris-leucoagglutinin (PHA-L) also showed that thalamocortical synapses form roughly 20% of the asymmetrical synapses in mouse barrels (Keller et al., 1985).

Previous descriptions of degenerating thalamocortical afferents in mouse barrel cortex focus on synapses made by degenerating axon terminals, or more accurately, varicosities. Preterminal axonal segments are described only as being phagocytosed before the appearance of degenerative changes in the terminals. In the present study also, the use of lesion-induced degeneration precluded the identification of synapses, if any, made by preterminal segments of thalamocortical axons. Synapses made by preterminal segments also have not been noted in studies of thalamocortical synapses in preparations using PHA-L as a label (Keller et al., 1985; Lu and Lin, 1993). This latter finding may be related to the poor preservation of membranes in PHA-L processed tissue and to the uneven staining of labeled processes, which complicates the differentiation from the background neuropil of lightly labeled preterminal axon segments. Slim, cylindrically shaped segments of thalamocortical afferents labeled anterogradely by biotinylated dextran amine have been observed to form synapses during early postnatal development (Lev et al., 2002), but synapses made by preterminal segments of thalamocortical afferents have yet to be quantified for the adult. The inability to identify preterminal synapses in degenerating preparations is not considered to affect the validity of our finding showing a decrease in the numerical density of thalamocortical synapses, because results from both deprived and control animals were subjected to the same sources of bias.

Effect of sensory deprivation on thalamocortical afferents in the cortex

A significant decrease (43.8%; $P < 0.05$) in the numerical density of thalamocortical synapses was observed in barrels of mice that had their whiskers trimmed daily from birth to 60 days. Because no alterations were detected in barrel size, changes in the numerical density of thalamocortical synapses suggest an alteration in the total numbers of this synaptic population consequent to sensory deprivation. The reduction in thalamocortical synapses after whisker deprivation is consistent with the reduced response of barrel neurons to stimulus of the principal whisker. These findings may reflect the longer periods of whisker regrowth used by Simons and Land (3–15 weeks) vs. the much shorter periods of regrowth used by Fox (4–7 days).

No changes were observed in the numerical density of nonthalamic, asymmetrical synapses. A small decrease in the numerical density of asymmetrical synapses that did not attain statistical significance was observed when all asymmetrical synapses were considered together (i.e., including both thalamocortical and nonthalamocortical synapses). Because thalamocortical afferents form a relatively small proportion of the asymmetrical synapses in PMBSF, the reduction observed in thalamocortical synapses may be masked when examining the effect of sensory deprivation on all asymmetrical synapses.
The change in the numerical density of thalamocortical synapses after whisker deprivation is consistent with observations of activity-dependent morphologic plasticity of thalamocortical afferents. Damaging whisker follicles during the period of barrel formation disrupts the arrangement of barrels in layer IV (e.g., Van der Loos and Woolsey, 1973; Killackey et al., 1976; Woolsey and Wann, 1976; Killackey and Belford, 1979; Jeanmonod et al., 1981; Catalano et al., 1995), producing changes that are mirrored by abnormal patterns of arborization of thalamocortical afferents (Jensen and Killackey, 1987; Catalano et al., 1995; Higashi et al., 1999). However, lesioning the follicles induces changes in the nerves that innervate them (Li et al., 1995); therefore, it is difficult to determine whether the changes in the organization of thalamocortical afferents are due to lack of whisker stimulation or rather to some trophic effect related to peripheral nerve damage.

The implication of thalamocortical afferents in activity-dependent cortical plasticity is suggested also by the results of studies of the efficacy of thalamocortical synapses in vitro (for review, see Feldman et al., 1999). Mechanisms that may underlie activity-dependent plasticity, such as long-term potentiation (LTP) and long-term depression (LTD; Bear et al., 1987; Singer, 1995; Katz and Shatz, 1996), have been postulated for thalamocortical synapses in the barrel cortex (Crair and Malenka, 1995; Feldman et al., 1998). The ability to induce LTP and LTD in vitro is restricted to early postnatal days, similar to the critical period for experience-dependent plasticity of barrel neurons. The involvement of thalamocortical afferents in activity-dependent plasticity is suggested by the observed reduction in thalamocortical synapses.

Changes in the numerical density of thalamocortical synapses have been suggested to play an important role in the plasticity of visual cortex (e.g., Tieman and Hirsch, 1982; Silver and Stryker, 1999). Reconstruction of single geniculocortical axonal arbors after either brief or prolonged monocular occlusion reduced the total length and the total number of branch points of deprived arbors (Antonini and Stryker, 1993, 1996). No change was observed in the density of synapses along deprived arbors, suggesting a substantial reduction in the total number of thalamocortical synapses that had occurred (Silver and Stryker, 1999). Our results, together with these observations, indicate that changes in the density of thalamocortical synapses may serve as a mechanism for experience-dependent plasticity that is common to different cortical areas.

**Effect of deprivation on thalamocortical synapses onto excitatory vs. inhibitory neurons**

Within the barrel hollows, thalamic afferents target all types of layer IV neurons including spiny stellate cells (White, 1978; White and Rock, 1979, 1980; Geffard et al., 1985; Benshalom and White, 1986), pyramidal cells (White and Hersch, 1981), and many morphologic subtypes of nonspiny nonpyramidal cells, including various γ-aminobutyric acid (GABA)ergic neurons (White, 1978; White and Rock, 1981; White et al., 1984; Keller and White, 1987, 1989; Aroniadou-Anderjaska and Keller, 1995). Neurons with somata in layers III, V, and VI of barrel cortex may have dendrites that enter layer IV and receive thalamocortical input within this layer (e.g., White, 1978; Hersch and White, 1981a, b, c; White and Hersch, 1982; White and Keller, 1987; Keller and White, 1988; Elhanany and White, 1990). A large body of evidence indicates that excitatory neurons, such as pyramidal and spiny stellate cells, form nearly all of their asymmetrical, presumed excitatory synapses with their spines, whereas inhibitory cells tend to be nonspiny and to form their excitatory synapses directly onto their dendritic shafts and cell bodies (White, 1989a). Data supporting this thesis have been derived from studies of thalamocortical synaptic connectivity across a broad spectrum of cortical areas and species, including mouse barrel cortex (e.g., White, 1978, 1989b; White and Rock, 1980; Hersch and White, 1981a; White et al., 1984; Keller and White, 1987). The association of thalamocortical axospinous synapses with excitatory cells and thalamocortical axospinous synapses with inhibitory cells means that the proportion of axospinous vs. axodendritic synapses formed by thalamocortical afferents can be considered an indication of the proportions of synapses impinging on excitatory vs. inhibitory neurons. Our finding of the same proportion of axospinous vs. axodendritic thalamocortical synapses in deprived vs. control animals (4:1) suggests that sensory deprivation decreases the quantity of thalamocortical synapses to the same extent for both excitatory and inhibitory neurons.

**Effect of deprivation on inhibitory circuits**

Deprivation reduces the numerical density of symmetrical, presumed inhibitory synapses in barrel neuropil by 52%. This finding is consistent with previous studies demonstrating fewer inhibitory components, i.e., GABAergic cell bodies, axosynaptic synaptic terminals immunoreactive for GABA, and fewer GABA receptors in the barrel cortex after whisker deprivation (Micheva and Beaulieu, 1995a, b, 1997; Fuchs and Salazar, 1998). A decrease in the complement of GABAergic components after deprivation was also observed in somatosensory cortex after deprivation of the hindlimb in rats (Warren et al., 1989), and in visual cortex of monocular enucleated rats (Ribak and Robertson, 1986), of dark-reared rats (Benevento et al., 1995), and of monkeys after monocular impulse blockade (Nie and Wong-Riley, 1996).

The disruption of thalamocortical input to inhibitory cells consequent to the observed decrease in axodendritic thalamocortical synapses may underlie the reduction in inhibitory components after deprivation. In vitro studies have shown the ability of neuronal activity to adjust the strength of cortical inhibition (Rutherford et al., 1997; Marty et al., 2000). Moreover, developmental studies observed maturation of inhibitory components coincident with the completion of thalamocortical connections (Hendrickson et al., 1991; Alcantara and Ferrer, 1995). Finally, severing pathways extrinsic to the parietal cortex (Vogt Weisenhorn et al., 1998) or severing thalamocortical input to the barrel cortex (Alcantara et al., 1996) have been demonstrated to modulate the development of inhibitory circuits.

Results of physiological experiments suggest that thalamocortical input induces brief, presumed monosynaptic excitatory responses in both excitatory and inhibitory neurons, followed by disynaptic feed-forward inhibitory responses generated by the inhibitory cells (e.g., Ferster and Lindstrom, 1983; Simons and Carvell, 1989;
Swadlow, 1989, 1990; Agmon and Connors, 1992; Simons, 1995; Brumberg et al., 1996; Gil and Amitai, 1996; Zhu and Connors, 1999). The feed-forward inhibition of these inhibitory cells influences the cortical representation of the sensory environment (Sillito, 1975; Tsumoto et al., 1978; Sillito et al., 1980; Dykes et al., 1984; Kyriazi et al., 1996). A reduction in feed-forward inhibition induced by fewer thalamocortical synapses onto inhibitory cells, as well as the observed decrease in inhibitory synapses, are consistent with the substantial expansion of whisker representations in barrel cortex after sensory deprivation (Simons and Land, 1987; Fox, 1992, 1994; Lendvai et al., 2000; Stern et al., 2001).

**Effect of deprivation on asymmetrical synapses of nonthalamic origin**

No significant differences were found in the numerical density of nonthalamic, asymmetrical synapses of adult (P60) mice after sensory deprivation. Statistically significant alterations in the size of PSDs, associated with plastic alterations in synaptic efficacy (e.g., Greenough and Chang, 1988; Schikonski and Stevens, 1999; Murthy et al., 2001), were also not observed in nonthalamic, asymmetrical synapses. In a previous study, we reported a reduction in the size of PSDs at postnatal day (P) 20 associated with asymmetrical synapses after deprivation from P0; PSDs of all asymmetrical synapses, thalamocorticals and nonthalamocorticals, were included in those measurements, because thalamocortical axon terminals were not labeled (Sadaka et al., 2000). In the present study, thalamocortical synapses were not included in the measurements of PSDs, because we were unable to exclude possible effects of degeneration on PSD size. Thus, changes in the size of PSDs observed at P20 in the previous study may be associated primarily with alterations in thalamocortical synapses, or alternatively, these changes may appear in all asymmetrical synapses but at stages of development earlier than the 60 days examined in the present study.

In mouse barrels, axons that form asymmetrical synapses comprise a heterogeneous group, including the local axon collaterals of spiny stellate and pyramidal cells and afferents from other regions of the brain (Keller, 1995). Presumably, the various neurons whose axons provide asymmetrical synapses in barrels are affected differentially by sensory deprivation by virtue of the specific circuits in which they participate. It may well be that one or more excitatory pathways exhibits an increase in synapse density as a consequence of the reduction in inhibitory components in barrel cortex. Compensatory synaptogenesis associated with one or more nonthalamic pathways to the barrels could explain why deprivation-associated reduction of synapse density was not observed for the population composed of all nonthalamic, asymmetrical synapses. The resolution of this issue will require analyses of deprivation-associated alterations in synapse density for individual nonthalamic pathways of known origin.

**ACKNOWLEDGMENTS**

We thank the Israel Science Foundation, President Avishai Braverman, and Rena and Martin Blackman for providing the funds and environment that have enabled us to perform this study. Y.S. thanks the Kreitman family for establishing the Kreitman Foundation Fellowships that have enabled him to embark on a career of scientific research. E.L.W. receives funding from the Israel Science Foundation and from Ben Gurion University.

**LITERATURE CITED**


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