THE JOURNAL OF COMPARATIVE NEUROLOGY 330:455–463 (1993)

Changes in Synaptic Density After Developmental Compression or Expansion of Retinal Input to the Superior Colliculus

MEIJUAN XIONG AND BARBARA L. FINLAY
Department of Psychology, Cornell University, Ithaca, New York 14853

ABSTRACT

The retinal projection to the superior colliculus can be made abnormally dense by inducing a "compressed" retinal projection into a subnormal tectal volume, or abnormally sparse by monocular enucleation early in development. Any or all of the features of cell number, axonal arbor, dendritic arbor, and synaptic density could potentially be adjusted to compensate for such variations in the convergence of one cell population on another. We have examined the consequences of neonatal partial tectal ablation or monocular enucleation for synaptic length, density, and relative numbers of synapse classes in the superficial gray layer of the hamster superior colliculus.

Monocular enucleation resulted in a reduction of synaptic density in the superficial gray layer of the colliculus ipsilateral to the remaining eye. This decrease in density was entirely accounted for by a reduction of the number of synapses with round vesicles, large asymmetric terminal specializations, and pale mitochondria characteristic of retinocollicular terminals (RLP synapses). There was no compensatory increase in any other synaptic class. RLP synapses were larger in monocular enucleates.

Partial tectal ablation had no effect on synaptic density, nor on the relative proportions of different synaptic types. Synapses of the RLP class were slightly smaller than normal. These results suggest that synaptic density is normally at a maximum that cannot be altered by increases in potential input. However, density may be reduced by decreasing the number of inputs. Terminal classes do not appear to compete with each other within the collicular volume, suggesting that postsynaptic cells control both the classes and numbers of their potential inputs. © 1993 Wiley-Liss, Inc.

Key words: retinotectal projection, hamster, monocular enucleation, partial tectum lesion, topographical mapping

When one population of neurons projects topographically upon another, two levels of their convergence must be regulated. At the level of the population, the entire input array should be represented, or representatively sampled. At the level of the single neuron, convergence must be regulated to produce receptive fields of usable size and structure. Cell number in either the afferent or target population, the axon arbor of the projecting population, the dendritic arbor of the target population, and their synaptic interface are all features that could regulate convergence during development. Experimental manipulation of the normal convergence ratio of an afferent onto a target population is one way of investigating the respective contributions of these sources of regulation of population mapping.

"Compression" of projections into experimentally reduced targets has been shown in several species. The retina is able to compress its projection into a smaller than normal tectal volume in the regenerating retinotectal projection of goldfish (Gaze and Sharma, '70; Sharma, '71, '72) and of frogs (Udin, '77). Retinotectal compression in development occurs after early partial lesions of the superior colliculus in hamsters (Jhaveri and Schneider, '74; Finlay et al., '79a). How is this compression accomplished? At a physiological level, although the entire visual field is represented in the partial tectum, visual receptive fields of single neurons are of normal size (Pallas and Finlay, '89). Retinal ganglion cell death shows only a minor change (8%) in the face of collicular ablations as large as 50% (Wikler et al., '86). More retinal ganglion cells must then project to a defined collicular volume, as can be demonstrated by retrograde transport

Accepted December 14, 1992.

Address reprint requests to Barbara L. Finlay, Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853.

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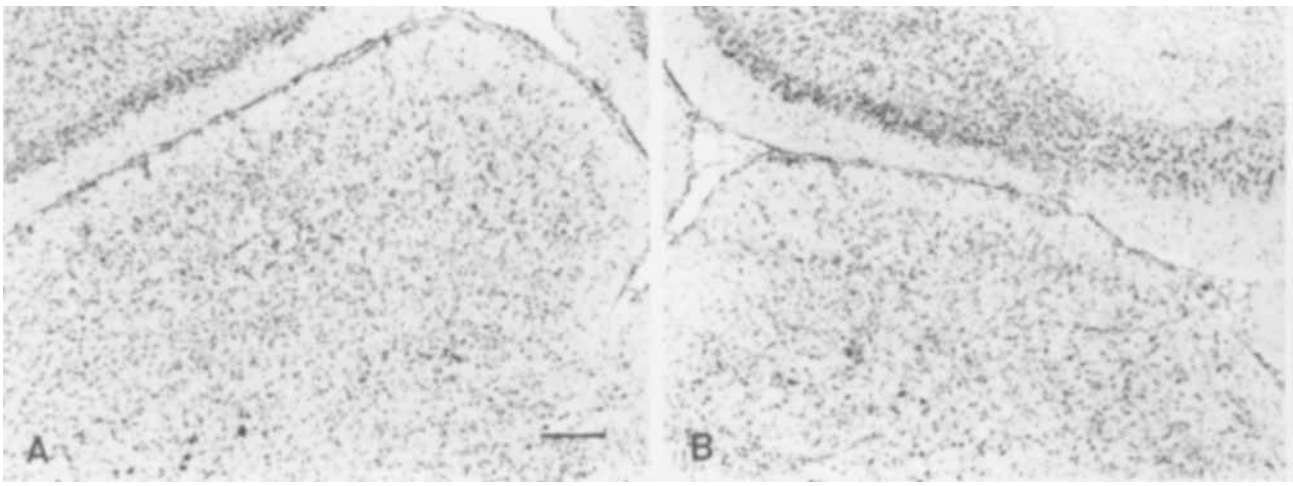

Fig. 1. **A:** Normal left superior colliculus contralateral to a colliculus with a neonatal partial lesion. **B:** Right superior colliculus in the same animal with a neonatal lesion of its caudal half, taken at the approximate midpoint of its remaining rostrocaudal extent. Scale bar, 200 μm for A and B.

of horseradish peroxidase (HRP) to the retina from injections of equivalent size into normal and partial colliculi (Pallas and Finlay, '91).

An increase in synaptic density is one way that excess retinal axons could be accommodated into a limited tectal volume. Are potential synaptic sites sensitive to an increase in the number of available retinal terminals? In the case of compressed projections in goldfish, initial reports were contradictory (Marotte, '81; Murray et al., '82). More recently, conservation of total synaptic number was described for half ablated goldfish tecta: neuropil was increased, presumably due to the increased volume of afferent fibers, but synaptic density was reduced compensatorily (Hayes and Meyer, '88a,b). This result was interpreted as a competition of retinal axons for a fixed number of postsynaptic sites on the tectum. The developing mammalian retinotectal system differs markedly in several features important for convergence, principally, the presence of substantial developmental cell death (reviewed in Pallas and Finlay, '89), and a limited period for synaptic rearrangement and plasticity (So and Schneider, '78). Will the same developmental constraint hold for both systems?

In contrast to the compression case, the early removal of one eye in the hamster results in the loss of the normal massive contralateral projection to the superior colliculus, and an expanded ipsilateral projection from the remaining eye (Finlay et al., '79b). The expanded ipsilateral projection derives both from an increased projection from the temporal retina, and a stabilized exuberant projection from the nasal retina (Windrem et al., '83). Though this projection is much larger than the normal ipsilateral projection, retinal terminal density, as observed in light microscopy, is still much less than the normal contralateral projection. The absolute number of retinal synapses in the tectum is thus reduced, but the consequences for local density and the effect on other synaptic classes is unknown. A prior study examining the effect of monocular enucleation in rats found that the relative proportion of synapses containing spherical vesicles (S terminals), primarily retinal in origin, compared to flattened vesicles (F terminals) were reduced after adult removals, but that relative proportions were roughly normal after neonatal removals (Lund and Lund, '71). Synaptic density was not assessed. These authors suggest that the presence of an S terminal might induce the formation of an appropriate proportion of F terminals. Alternatively, density of each terminal class might be controlled independently, or all terminals could compete for a limited number of terminal sites independent of type.

In this paper, synaptic densities in the superior colliculus of normal hamsters, hamsters with early partial collicular lesions, and hamsters with monocular enucleation are compared. The relative densities of various terminal classes are also analyzed. A preliminary report of this investigation has been made (Wikler et al., '84).

MATERIALS AND METHODS

Neonatal surgery

Within 24 hours of birth, hamsters received unilateral lesions of the caudal half of the right superior colliculus, or monocular enucleation. Hamster pups were taken from the mother and anesthetized by induction of mild hypothermia. For partial tectum ablation, the skin on the head was cut and retracted to reveal the superior colliculi through the translucent skull (PT group). A heated probe was applied directly to the skull above the caudal superior colliculus. This procedure effectively removes the caudal part of the superior colliculus without the need to open the cranium and results in orderly compression of the retinal projection into the remaining superior colliculus (Finlay et al., '79a; Pallas and Finlay, '89). One eye was enucleated by making a small slit under the prospective eyelid, and withdrawing the eye with fine forceps (ENUC group). Hamsters were then rewarmed and returned to the mother for normal rearing.

Histological procedures for assessment of neuronal density by light microscopy

Four brains with partial tectal ablations of the desired size (rostrocaudal length between 40 and 60% of normal, mean = 48%; Fig. 1) were culled from available brains from prior studies whose tissue preparation was optimal for counting of neurons under light microscopy (paraffin-embedded; sectioned at 10 μm and stained with cresylecht

violet from Wikler et al., '86; Pallas et al., '88). A column of tissue 280 μm across and of variable depth, extending from the dorsal surface of the superficial gray layer of the superior colliculus to the midpoint of the stratum opticum, was counted for total neurons in the same four sampling locations used for electron microscopy described below. A mean density of cells per sample column of the four animals was calculated and compared to the animal's unlesioned side.

Electron microscopy

Four normal, 4 PT, and 3 ENUC animals of at least 3 months of age were used for ultrastructural assessment of synaptic density. Animals were deeply anesthetized by intraperitoneal injection of pentobarbital and perfused transcardially with 0.9% saline in 0.1 M phosphate buffer followed by 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and postfixed in the same solution for 2.5–3 hours. The midbrain was dissected out, and the partial tectal ablations were verified by measuring the rostrocaudal extent of the remaining colliculus under a dissecting microscope. The four animals selected had rostrocaudal lengths that were 40–60% of the normal unoperated side. Completeness of the enucleation was also verified by absence of the optic nerve at the time of perfusion.

Four areas of the superficial gray layer of the superior colliculus were sampled from both the retinotopic center and periphery of the rostral and caudal half of the colliculus in the following manner. After gross dissection, sections of the superficial gray layer of the superior colliculus were cut at 100 μm on a vibratome. A representative section was taken from the midpoint of the rostral half and the caudal half of the colliculus on each side. Each section was further divided into thirds, from medial to lateral, and one trapezoidal block each was taken from the superficial gray layer of the middle and most lateral division. The long face of the trapezoidal block was cut at the superficial aspect of the superficial gray, and the short face at the dorsal aspect of the stratum opticum. Although all samples thus fell within the superficial gray, sampling locations within the superficial gray were not marked and samples were chosen from any dorsoventral location on the basis of tissue and staining quality.

Animals with partial tectal lesions were divided in the same proportions, on the assumption, therefore, that "central" and "peripheral" locations in the colliculus are best located relatively and not absolutely with respect to a colliculus of normal size. Both right and left colliculi were sampled in the experimental animals, thus giving a within-animal control for all of the experimental procedures as well as a comparison with the normal animals.

The blocks were then washed in chilled buffer, postfixed in 2% osmium tetroxide in buffer for 2 hours, stained in 2% uranyl acetate in chilled distilled water for 30 minutes, washed, dehydrated and embedded in plastic resin. Ultrathin sections were photographed at 5,586× and printed at a final magnification of 22,000×. At least two, and as many as four photomicrographs were analyzed at each sampling location depending on availability of appropriate fields. With an acetate grid for registration, each electron micrograph was scanned for all synapses.

Classification of synapses

Synapses were minimally identified by the presence of a synaptic cleft and a postsynaptic membrane specialization. Additional ultrastructural characteristics of each labelled terminal were also determined as possible: size and shape of vesicles, appearance of mitochondria, type of synaptic junction (asymmetric or symmetric) and the type of postsynaptic process contacted.

In accord with the extensive literature on mammalian retinocollicular terminals, synapses were classed as one of four types, principally to distinguish retinal synapses from nonretinal synapses in the tectum. The "RLP" type has round vesicles, large profiles with an asymmetric terminal specialization, and pale mitochondria (Fig. 2A and B). These ultrastructural features are characteristic of retinal terminals in mammals (Lund and Lund, '71; Lund, '72; Laufer and Vanegas, '74; Vrensen and De Groot, '77; Graham and Casagrande, '80; Behan, '81; Mize, '83; Huerta and Harting, '84; Hofbauer and Holländer, '86). A recent labelling study of retinal terminals in the superior colliculus of the hamster (Carter et al., '91) showed that 96% of labelled retinal terminals had paler mitochondria than their nearest neighbors, and 97% of retinal synaptic contacts were asymmetric. While nearly all retinal terminals have these characteristics, however, some terminals with these characteristics are not retinal in origin, notably cholinergic terminals (Hall et al., '89; Henderson, '89).

Nonretinal terminals are those with pleomorphic vesicles or small round vesicles or dark mitochondria (Meek, '81a,b). The "F" type has flat or pleomorphic vesicles, symmetric contacts, and dark mitochondria (Fig. 2C and D). The "RSD" type has round vesicles, usually smaller than those of the RLP class, a symmetric membrane contact, and dark mitochondria (Fig. 2E and F). RSD type terminals may be cortical in origin, as suggested by their degeneration after cortical lesions (Lund and Cunningham, '72; Lieberman and Webster, '74). The final category "Q" contained identifiable synapses whose type was questionable in either of two ways. The majority in this group did not have enough characteristics for unambiguous classification (for example, a synaptic thickening only). The remainder had conflicting features for the above classification scheme, such as pale mitochondria and an asymmetric contact.

Lengths of terminal contacts and area of neuropil were measured with the aid of a morphometry program (SigmaScan). Any break in the postsynaptic membrane thickening defined an individual synapse for this measurement, and thus perforated synapses would be counted as multiple synapses. The number of synapses per counted area was adjusted by the measured synaptic diameter, section thickness and an "uncounted caps" correction for each synaptic class for each animal (Floderus, '44; Abercrombie, '46; Weibel, '79; Colonnier and Beaulieu, '85). The density of synapses per unit neuropil volume was then calculated: the intent was not to obtain an absolute count, but to correct for possible sampling errors caused by treatment-induced changes in synaptic length and enable a comparison of relative densities between experiment and control populations. The area of neuropil was defined as the compartment of tissue that contains exclusively axons, small tertiary and secondary dendrites, synaptic boutons, and small glial processes (Cooper and Rakic, '83; Zecevic et al., '89). Somas, myelinated processes, and vascular elements were excluded.

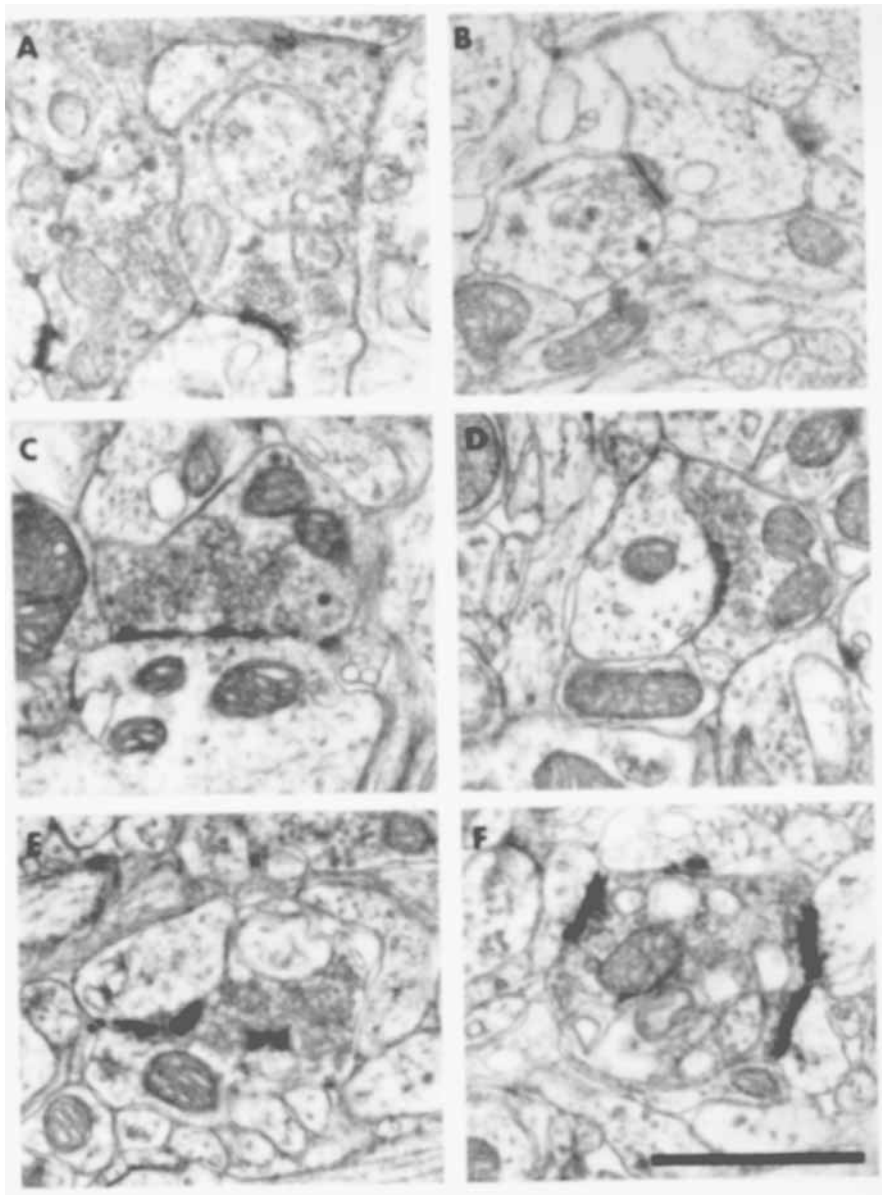

Fig. 2. **A, B:** Examples of the "RLP" terminal class. **C, D:** Examples of the "F" class. **E, F:** Examples of the "RSD" class. Scale bar, 1 μm. Magnification 22,000×.

Statistical analysis

The terminal density per unit volume of neuropil among control and experimental sides (lesioned sides) of colliculi in the PT group, and experimental sides (contralateral sides to the enucleation) of colliculi in the ENUC group was compared by a two-factor ANOVA analysis, in which treatments (3 levels) were one factor and synaptic types (4 levels, RLP, RSD, F, and Q) were the other. Planned comparisons among treatments (such as normal colliculi vs. experimental side of colliculi of PT animals) for each subclass of synaptic types were performed to determine the source of the significant variance. For a second control, the differences among normal colliculi, nonexperimental sides (unablated sides) of colliculi of the PT group, and nonexperimental sides (ipsilateral to the enucleation) of colliculi of the ENUC group were also analyzed in a two-factor ANOVA. Comparisons of synaptic length among different treatment groups were analyzed in the same way as the synaptic density data.

RESULTS

Overall ultrastructural characteristics of synapses

All types of synapses previously described for the rodent superior colliculus were found, with no remarkable deviations (Fig. 2A–F). After enucleation, only the retinocollicular terminal class RLP was reduced in relative density (Fig. 5), consistent with the many prior reports identifying this synaptic class with retinal terminals.

Quantitative verification of the qualitative classification scheme was found: RLP synapses, in accord with their

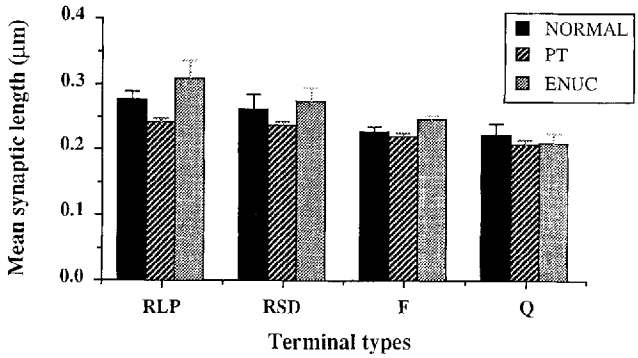

Fig. 3. Mean synaptic length for each synaptic class in each treatment group. PT, partial tectum ablation; ENUC, enucleated.

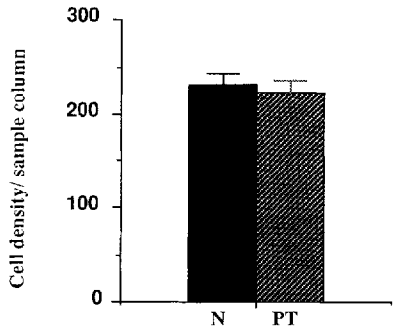

Fig. 4. Neuronal density in the superficial gray layer in PT animals, comparing the undamaged side to the partially ablated side (n = 4) in a column 280 μm wide, 10 μm deep, and of variable height corrected.

qualitative definition, did in fact have statistically longer synaptic thickenings than other synaptic classes (Fig. 3). Synaptic terminal types (RLP, F, RSD, and Q) differed significantly in length of their terminal specializations, across all treatment groups (PT, ENUC, and normal; two-factor ANOVA, $F = 5.99$, $P < 0.0023$). In normal animals, the RLP synapses measured 0.28 μm in length, the RSD synapses 0.26 μm, and the F and Q types 0.22 μm.

Synaptic length

The colliculi of normal animals did not differ on any measure of length from the within-animal controls (the unlesioned side of PT animals or the colliculus contralateral to the remaining eye in the ENUC group). Synaptic terminal lengths were not different between normal superior colliculi and the control sides of the ENUC and PT groups ($F = 0.27$, $P > 0.76$). No difference between the two types of controls was seen for this analysis nor for any of the following, and this contrast of control groups will not be further remarked upon.

Experimental treatment significantly affected synaptic length ($F = 6.9$, $df = 2$, $P < 0.0053$) (Fig. 3). Figure 3 shows that overall terminal lengths in PT animals are somewhat shorter than normal and that terminal lengths in ENUC animals are somewhat longer than normal (mean terminal length in normal 0.28 ± 0.013 μm; in PT, 0.24 ± 0.003 μm; in ENUC, 0.31 ± 0.026 μm). This effect is most pronounced for the RLP class of terminal, though this planned comparison of independent groups did not quite reach statistical significance (N vs. PT, $F = 3.57$, $P < 0.068$; N vs. ENUC, $F = 2.7$, $P < 0.1103$).

Neuronal density

Consistent with the results of a prior study that examined animals up to 10 days of age (Wikler et al., '86), neuronal density in the superficial gray layer of the remaining colliculus was unaltered by partial tectal ablation (t test, matched pairs, $df = 3$, $t = 0.49$, $P > 0.65$; Figs. 1 and 4). In the PT animals, lamination of the superior colliculus may often appear slightly disturbed compared to normal, as in Figure 1, but overall, there is no change in cell density.

Neuronal density after enucleation was not assessed quantitatively, due to multiple prior reports on this subject in our own laboratory and elsewhere (Tsang, '37; Finlay et al., '79a, '86; reviewed in Finlay and Pallas, '89): qualitatively, as in prior reports, the superior colliculus was reduced in size contralateral to the enucleation, due to decrease in both neuron numbers and neuropil. The neuropil is reduced more than neuronal number, resulting in an increase in neuronal density. Soma size of neurons in the superficial gray layer is also reduced.

Synaptic density

The total density of synaptic terminals per unit neuropil volume was calculated as described for all three groups of animals. There were no differences or noticeable trends in synaptic density between normal untreated animals and the control untreated colliculus of the experimental animals (PT control side vs. normal, $F = 0.05$, $P = 0.84$, mean 0.59 ± 0.030/μm^3 and 0.58 ± 0.05/μm^3, respectively; ENUC control side vs. normal, $F = 0.01$, $P = 0.94$, mean 0.57 ± 0.06/μm^3 and 0.58 ± 0.05/μm^3, respectively); the same is true for any subclass of retinal terminals.

The RLP class of terminals shows a significant decrease in density on the experimental (ipsilateral) side of enucleated animals, as shown in Figure 5 ($F = 6.48$, $P < 0.0159$, mean 0.17 ± 0.03/μm^3 in normal and 0.08 ± 0.03/μm^3 in ipsilateral colliculus of the ENUC group). The other classes of synapses showed no significant differences from normal. For PT animals, the density of the RLP terminal class was not different from normal ($F = 2.04$, $P > 0.1627$, 0.17 ± 0.03/μm^3 and 0.21 ± 0.03/μm^3 in normal and PT, respectively). No other synaptic class was significantly changed. Therefore, enucleation reduced synaptic density of the RLP (principally retinal) terminal class in the contralateral tectum, with no evidence of compensatory increase by any other synaptic class. Partial tectal ablation, though potentially increasing the number of retinal and other terminals available to a given tectal volume, did not induce a change in the density of retinal or any other terminal class.

Analysis of synaptic density by region

We include here a report of a spatial analysis that uncovered no significant differences in synaptic density, though we note trends that might guide future analyses. In general, the colliculus is quite uniform in its synaptic density. Sampling locations in the superior colliculus were chosen to span two regional divisions that might be suspected to have intrinsic differences in synaptic density. The ipsilateral retinal projection is principally to the rostral colliculus, so we compared rostral to caudal colliculus. In the normal development of the hamster retina and superior colliculus, there is significantly greater evidence of cell

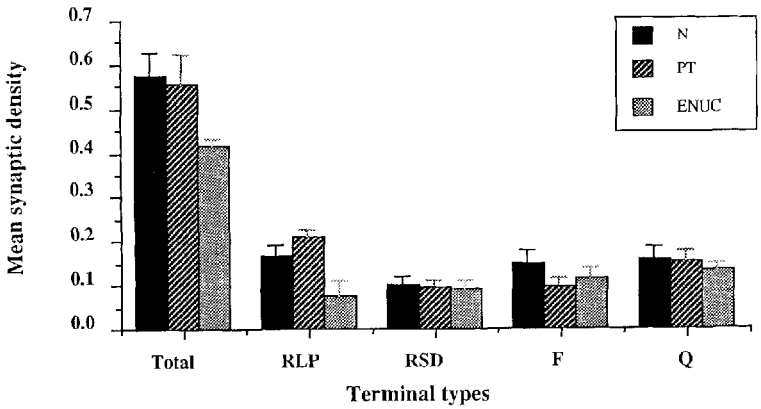

Fig. 5. Mean synaptic density of all synapses in each treatment group (Total), and for each subclass. Synaptic density is reduced in the ENUC group, due to the reduction of RLP class synapses.

degeneration and death in the retinotopically defined periphery of both structures (Sengelaub and Finlay, '82; Finlay et al., '82). We thought it possible that regional differences in synaptic site availability might produce this nonuniform cell loss and thus compared synaptic density between the retinotopic center and periphery. Although there were reliable small trends across all groups, no statistically significant regional differences in density were found. In normal animals, both the total density and the retinal terminal density were higher in the center of the superior colliculus (SC) than in the periphery of the SC, but this was not statistically significant (total, $t = 2.882$, $P > 0.0634$; RLP $t = 2.932$, $P > 0.0609$). This trend was similar in PT animals.

Although there was a trend for terminal density in the rostral SC to be greater than that in the caudal SC both for total density and retinal terminal density ($t = 0.24$, $P > 0.833$; $t = 0.858$, $P > 0.4811$), it also did not reach statistical significance. The same nonsignificant trend was true for the PT animals (for total, $t = 2.099$, $P > 0.1267$; for RLP, $t = 2.295$, $P > 0.1055$). We were particularly interested in this comparison for the enucleate animals, because there is an expanded ipsilateral projection from the remaining retina to the rostral part of the superior colliculus; therefore, a decrease in synaptic density compared to normal should be greatest in caudal tectum and intermediate in rostral tectum. However, no significant differences were observed.

DISCUSSION

The main findings of this study are the following: (1) monocular enucleation reduces synaptic density in the contralateral superior colliculus, with the effect confined to the terminals of characteristic retinotectal morphology; (2) no compensatory increase in density is seen in other terminal classes; (3) compression of projections into a smaller than normal tectal volume does not result in a significant increase in terminal density in the remaining neuropil, nor an increase in neuropil; and (4) the mean length of synaptic thickenings, particularly of retinal terminals, is altered by both manipulations.

Methodological considerations

Stereology. The quantitative technique we have used, simple sampling with a split profile correction, is appropriate for comparisons of synaptic density per volume between our control and experimental groups, but is inappropriate for calculation of the absolute number of synapses in the collicular neuropil, or synapses per neuron, and we have limited our interpretation accordingly. However, some inferences are possible. Since both neuronal density (neurons/neuropil) and synaptic density (synapses/neuropil) are unaltered in the PT group, we might reasonably infer that synapses/neuron are also unchanged, though a direct demonstration of this inference would be desirable.

Since our method of synapse counting would class a sectioned perforated synapse as two or more synapses, our synaptic density measure is more properly a calculation of the sectioned synaptic length per neuropil volume, rather than an enumeration of the total number of physically continuous synapses per volume. Both measures would appear to be an interesting feature of synaptic activity in an identified tissue.

Use of neuropil as the denominator in synaptic density. The intent of the neuropil measure is to remove from the analysis such factors as changed soma size, changes in glial constituents, or changed total volume of entering and exiting myelinated processes, all of which change as a result of enucleation (Tsang, '37; Lund and Lund, '71), and which might change with partial tectal ablation. Our studies show that after an early period of excess neuropil after early partial tectal ablation, neuronal density returns to normal after the period of developmental cell death (Wikler et al., '86) and remains so until adulthood, which makes interpretation of changed synaptic density in this case uncomplicated. This result contrasts with observations in goldfish (Hayes and Meyer, '88a,b), which will be discussed below. In the case of enucleation, the number of retinal synapses is decreased, and the volume of neuropil accounted for by retinal terminals is also decreased. If synapse number and terminal volume decrease disproportionately—for example, if a 10% reduction in retinal synapse number is accompanied by a decrease in 20% of retinal terminal volume—relative density will be artifactually depressed. The opposite disproportion will produce artifactual elevation. Therefore, the apparent magnitude of the density change should be interpreted cautiously.

The identity of RLP terminals. The diagnostic ultrastructural characteristics of pale mitochondria for retinotectal terminals have been reported in a wide number of vertebrate species, including teleosts, anurans, birds, and

various mammals including primates (Lund, '69, '72; Lund and Lund, '71, '72; Valverde, '73; Szekely, '73; Hayes and Webster, '75; Reperant and Angant, '77; Vrensen and De Groot, '77; Ito et al., '80). In mammals, the terminals of retinal ganglion cell axons in the SC (in addition to pale mitochondria) have spherical vesicles and make asymmetric synapses almost exclusively onto dendrites (see Huerta and Harting, '84 for review; Carter et al., '91 for a review of these features in hamsters). As expected, this class of terminals is decreased after enucleation. The density of this class is decreased by more than half but not reduced to zero, due to at least two factors: after monocular enucleation, the remaining eye expands its projection throughout the SC, and second, some terminals of this type are not retinal in origin (Hall et al., '89; Henderson, '89).

Alteration of synaptic length

Retinotectal terminals in the tecta contralateral to enucleation formed longer synaptic contacts, while retinal terminals in partially ablated tecta form shorter contacts. There are several possible explanations for these observations. Thinning of terminal density may directly affect synapse size. Lengthening has been noted in other preparations where afferent fiber populations are decreased experimentally (Sotelo, '75; Hillman and Chen, '85). Regenerating retinotectal terminals in goldfish and hamsters are also longer (Radel and Yoon, '85; Carter et al., '89). Some studies of the collateral sprouting by intact neurons in partially denervated fields have shown larger terminals and longer, more frequent synapses per terminal (Raisman, '69; Matthews et al., '76; Chen and Hillman, '87; Murray et al., '87; Steward et al., '88). It is possible that crowding might decrease the length of the synaptic contacts, though we are not aware of any direct demonstration of this possibility.

Alternatively, the alteration in length may simply be due to an alteration in the ratio of classes of retinal neurons that innervate the tectum after enucleation and partial tectal ablation, rather than a direct influence of denervation on synapse length. These two manipulations alter the early patterns of cell death in the retina as well as the size distribution of retinal neurons in adulthood in contrasting ways (Sengelaub and Finlay, '82; Sengelaub et al., '83; Wikler et al., '86; Pallas and Finlay, '91). If subclasses of retinal neurons have different mean synaptic lengths within the general RLP class, the subtle changes observed in mean synaptic length could be accounted for.

One prior study of the effects of early monocular enucleation in rats (Lund and Lund, '71) found no change in synaptic length in S type terminals, a category which would span our RLP and RSD classes. It should be emphasized that the change in length we observed was small, 11%, which could easily be obscured by the inclusion of the RSD class, which showed no change.

Regulation of terminal differentiation

Some morphological features of synaptic complexes depend on the postsynaptic environment, and others on the identity of the presynaptic terminal (Kane, '73; Palay and Chan-Palay, '74; Campbell and Frost, '87, '88). For example, if retinal ganglion cell axons are rerouted into the primary somatosensory thalamic nucleus (ventrobasal nucleus, VB), terminals of this anomalous retino-VB projection have round vesicles and pale mitochondria like those formed by normal retino-dLGN (dorsal lateral geniculate nucleus) projections, but other features, such as glomerular position and glomerular organization as well as the size range of terminals and amount of appendages per axon terminal, are like the terminals of ascending somatosensory projections to VB (Campbell and Frost, '87, '88). Within the normal visual pathway, retinal terminals have different features in the dLGN and SC (Lieberman and Webster, '72, '74; Lund, '69). It is reasonable to speculate that changing afferent/target ratios might change the ultrastructural features of tectal terminals by producing a new microenvironment for retinal ganglion cell axons. In the present experiment, however (with the exception of synaptic length), constellations of synaptic morphology were unaltered; in particular, there is no alteration in the proportion of "Q" terminals with unclassifiable or conflicting features.

The classes of synaptic terminals we distinguished, RLP, F and RSD, appear to regulate their density independently, showing no evidence of between-class competition. In the case of expansion of retinal input, the decrease of RLP density is not compensated by other classes of terminals. Similarly, in the lateral geniculate nucleus of the mouse after enucleation, absent RLP terminals were replaced by large terminals, containing round vesicles with dark mitochondria, but were not replaced by RSD or other types of terminals (Lieberman and Webster, '72, '74). This may suggest that there are regional signals in the postsynaptic membrane surface, which control the ratios of the diverse terminal types potentially impinging on a single neuron.

In apparent conflict, Lund and Lund ('71) observed that the ratio of total synapses that are S type (comprising both RLP and RSD classes), compared to F type, stayed constant after neonatal monocular enucleation, and they hypothesized that S terminals induce the formation of F terminals in constant proportion. We recalculated our data as relative proportion in the manner of Lund and Lund ('71), and find that in our normal hamsters, the ratio of S to F synapses is 64%/35%, quite comparable to Lunds' 61%/39%, and that enucleates show a ratio change to 61%/39% compared to Lunds' 56%/44%. It is clear that the approximate magnitude and the direction of change (S% decreases while F% increases) is similar in the two studies. Synaptic density as opposed to synaptic ratio is perhaps a more sensitive measure of population change, as the measure of density of any one synaptic class is unconfounded by alteration of another synaptic class. We would argue that the choice of this measure allowed us to see a relative reduction of RLP synapses not seen in the Lunds' study. The interesting observation of the Lund and Lund ('71) study, however, that led to the conclusion of the mutual regulation of S and F synapses was that after adult enucleation, the ratio of S to F synapses falls to 26%/74%. This strongly suggests some form of developmental regulation or induction of the entire S/F synaptic complex; our observations suggest, however, that the regulation is not entirely compensatory.

Control of retinal terminal density in the SC

Two prior studies of compressed or abnormally convergent projections in regenerating nervous systems have reported conservation of the total number of synaptic sites (Murray et al., '82; Hayes and Meyer, '88a,b). Hayes and Meyer ('88a,b) report that while synaptic number remains constant in a halved tectum with a compressed retinotectal projection, synaptic density is decreased due to a retained number of incoming axon terminals and a resulting increase in the volume of neuropil. In this study, by contrast, axon arbor appears to be proportionate to termination sites.

We know that an increased number of retinal axons project to a defined tectal volume (Pallas and Finlay, '91) as in the goldfish, but this increased number of axons does not produce an increase in neuropil.

Thus, individual retinotectal axon arbors must be of reduced volume. Our preliminary studies show this to be the case (Xiong et al., '91). The contrast may lie between the resting state of regenerating and nonregenerating systems. Early in the development of a compressed mammalian retinotectal projection, before cell death and axon retraction, neuron density does become significantly reduced due to an increase in neuropil, returning to normal by the tenth postnatal day (Wikler et al., '86), and remaining at normal density to adulthood (this study). Hayes and Meyer ('88b) report that their cases of very long-term regeneration show lesser neuropil volume, suggesting that it may be reduced at a slow rate, perhaps to some optimal arbor/synapse ratio.

Since the volume of neuropil to neurons is unaltered, however, and synaptic density is constant, we also infer that the number of synaptic sites per postsynaptic neuron is constant, in accord with Hayes and Meyer ('88a,b). This observation further constrains the locus of plasticity in the mapping of populations of disparate numbers. Our prior studies have shown that cell death does not serve to stabilize the ratio of neurons between the retina and the tectum (Wikler et al., '86), and that greater numbers of axons are accommodated into a defined tectal volume, representing a larger spatial extent in the retina (Finlay et al., '79; Pallas and Finlay, '91). At the level of the single cell, however, spatial convergence from retina to tectum does not change, as assessed electrophysiologically (Pallas and Finlay, '89). If synaptic density is also unaltered, as argued here, the locus of plasticity for remapping these populations lies in the volume and conformation of the axonal arbor. Experiments to test this prediction are in progress.

ACKNOWLEDGMENTS

We thank Ken Wikler for his extensive pilot work and consultation on this project, Vincent Tamariz and Bryant Howard for technical assistance, and particularly Myra Liboff for electron microscopy. We also thank particularly Sarah Pallas and David Troilo for their helpful comments on this paper. Supported by NIH grants RO1-NS 19245 and KO4 NS 00783 to B. Finlay.

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