Thalamocortical Synapses Involving Identified Neurons in Mouse Primary Somatosensory Cortex: A Terminal Degeneration and Golgi/EM Study

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ABSTRACT Synapses involving thalamocortical afferents and hitherto unexamined neuron types of the posteromedial barrel subfield (PMBSF) of the mouse have been identified using a combined degeneration and Golgi/EM technique (Peters et al., '77). Degeneration of thalamocortical axon terminals was produced with electrolytic lesions of the nucleus ventralis posterior, pars lateralis thalami, and the nucleus posterior thalami. Four days after receiving lesions, the animals were perfused, and blocks of cortex containing the PMBSF were processed by the Golgi method. The blocks were tissue-chopped at 125 μm and examined with the light microscope. Sections containing neurons of interest were gold-toned and deimpregnated in preparation for electron microscopy (Fairen et al., '77). Portions of selected neurons contained in layers III–V were serially thin-sectioned and examined with an electron microscope to determine if they were involved in synapses with degenerating thalamocortical axon terminals. Results showed thalamocortical synapses on the apical dendrites of five different sized pyramidal cells whose somata occurred in layers V and VI, and on dendrites of one spiny bitufted neuron and one non-spiny multipolar neuron with somata in layer V. A non-spiny bitufted neuron of layer IV which was not impregnated also received thalamocortical synapses.

Although every neuron examined formed at least one thalamocortical synapse, some formed very few, whereas others formed many. Of the pyramidal cells, small layer V and VI pyramidal cells and a large deep layer V pyramidal cell were involved in small numbers of thalamocortical synapses, while a medium superficial layer V pyramidal cell and a large layer VI pyramidal cell each formed many. The spiny bitufted neuron formed a small number of thalamocortical synapses, while the non-spiny bitufted neuron formed very many. The non-spiny multipolar neuron was involved in a moderate number of thalamocortical synapses. The findings suggest that, whereas any type of neuron with a dendrite in layer IV likely receives some synaptic input from the thalamus, individual neurons were involved in very different quantities of thalamocortical synapses.

The bulk of the thalamic projections to the primary areas of sensory cortex terminates in layer IV (see review by White, '79). In the mouse, layer IV of primary somatosensory cortex contains about 200 multicellular units known as barrels (Woolsey and Van der Loos, '70), and the barrels which contain the cortical representation of the mystacial vibrissae comprise the posteromedial barrel subfield (PMBSF). By combining the Golgi/EM method of Fairen et al., ('77) with the technique of anterograde degeneration, White ('78) showed that thalamocortical synapses in mouse PMBSF involve the basal dendrites of layer III pyramidal cells, spiny and non-spiny layer IV nonpyramidal cells, and the apical dendrites of small superficial layer V pyramidal cells. In fact, this study and a similar one of rat visual
cortex (Peters et al., '79) suggested that every postsynaptic surface present in layer IV capable of forming asymmetrical synapses is postsynaptic to thalamic axon terminals (see Peters, '79; White, '79). However, many types of pyramidal and nonpyramidal cells with dendrites contained in layer IV have yet to be examined. Particularly interesting in this regard are the pyramidal cells with perikarya in the deeper layers of sensory cortex. These pyramidal cells have been shown by light microscopy of Golgi-impregnated tissue to possess spine populations that are very sensitive to both perinatal deafferentation and sensory manipulation (for example, Globus and Scheibel, '67; Valverde, '68), and the findings of such studies have been interpreted as evidence that nearly one third of the spines of pyramidal cell apical dendrites passing through layer IV receive thalamocortical input. The present study is a survey of the thalamocortical connectivity of hitherto unexamined neuron types in mouse PMBSF cortex, with an emphasis on the thalamic synapses involving pyramidal cells of layers V and VI. Our intention is to further test the hypothesis that all neurons with dendrites contained in layer IV form thalamocortical synapses, and to learn if individual cells are involved in very different numbers of thalamocortical synapses. An indication that this might be true is provided by a light microscopic Golgi study that showed superficial and deep layer V pyramidal cells to be differentially sensitive to postnatal deafferentation (Ryugo et al., '75a,b).

MATERIALS AND METHODS

Electrolytic lesions were made in the nucleus ventralis posterior pars lateralis thalami of male CD/1 mice ranging in age from 55-80 days. A transcollicular approach was used to avoid passing the lesion electrode through neocortex. Four days after the lesion, the mice were perfused intracardially with an initial weak fixative containing 1.0% paraformaldehyde and 1.25% glutaraldehyde, followed by a fixative containing 2.0% of each of these aldehydes. Both fixatives were buffered with 0.1 M sodium cacodylate and adjusted to pH 7.2. The thalami were removed, frozen-sectioned at 40 μm, mounted on glass slides, and then stained with cresyl violet to assess the placement and extent of the lesions. Blocks containing all of PMBSF cortex ipsilateral to the lesion were then processed by the rapid Golgi method (Valverde, '70). After impregnation, the tissue was dehydrated through graded glycerin solutions to 100% glycerin and tissue-chopped in the coronal plane at 125 μm with a Sorvall tissue chopper. Sections were wet mounted in 100% glycerin and, using a microscope equipped with a drawing tube, they were compared to drawings of similar Nissl-stained sections to identify the thalamocortical barrel subfield. Sections containing Golgi-impregnated PMBSF neurons were then prepared for electron microscopy using a procedure based on the Golgi/EM method of Fairen et al. ('77): Tissue-chopped sections in 100% glycerin were passed into 40% and 20% glycerin solutions for 1 minute each, then into 0.5% gold chloride for 15–20 minutes. The gold was then reduced with 0.5% oxalic acid and the precipitate removed with 1.0% sodium thiosulfate. The result of this procedure is to replace the dense Golgi precipitate (silver chromate) with a deposit of fine gold particles. Gold-toned neurons are still visible in the light microscope, and the gold deposit does not obscure their fine structure (Fairen et al., '77). Sections containing selected gold-toned neurons were subsequently postfixed for 30 minutes with 2.0% osmium tetroxide in 0.1 M cacodylate buffer, block-stained for 2 hours in 1.0% uranyl acetate in 70% methanol, and embedded in a thin layer of Epon-Araldite.

The selected neurons were then photographed or drawn using a light microscope equipped with a drawing tube, and mounted on Epon-Araldite cylinders for thin sectioning. Using a MTB-2B ultramicrotome, unbroken series of thin sections were taken through the portions of the neurons contained in layers III-V, subsequently mounted on formvar-coated slot grids, and stained with lead citrate. All sections through each neuron were then examined with an AEI Corinth electron microscope, and all synapses occurring between gold-toned processes and degenerating thalamocortical axon terminals were photographed at × 15,000.

In addition to the gold-toned neurons, an unimpregnated neuron was also examined. On the basis of the form of its cell body and dendrites in a three-dimensional reconstruction, this neuron was identified as a layer IV non-spiny bitufted cell (White and Rock, '80). Its thalamocortical connections were assessed by tracing its dendrites through the series and photographing its synapses with degenerating thalamocortical axon terminals at × 15,000.

To determine if the dendrites of each neuron examined passed through fields containing comparable numbers of degenerating thalamo-
cortical axon terminals, counts were made in layer IV of all degenerating and normal presynaptic elements occurring in small volumes of neuropil surrounding each dendrite.

RESULTS

Lesions

Thalamic lesions involved the entire nucleus ventralis thalami, pars lateralis (VB). The nucleus posterior thalami (PO), which also projects to mouse PMBSF cortex (White and DeAmicis, '77), was not always lesioned, but, in each instance, the lesions included the projection from PO to PMBSF. Parts of other thalamic nuclei were occasionally lesioned, but none of them are known to project to layer IV of mouse PMBSF.

Electron microscopy

Profiles of the deimpregnated gold-toned neurons were easily recognized in the electron microscope by virtue of the deposit of fine gold particles contained within their cytoplasm. Spines of gold-toned neurons displayed a particularly heavy deposit of gold (Fig. 1), and, as a result, they could always be traced back to their parent shafts. There was no evidence, in the electron microscope, for the existence of unimpregnated spines connected to the impregnated dendrites.

Synapses were identified as symmetrical or asymmetrical according to the usual criteria for mammalian synapses (Colonnier, '68). Both types possessed a cluster of synaptic vesicles accumulated against the presynaptic membrane, a synaptic cleft, and a postsynaptic membrane. Asymmetrical synapses had a plaque of electron-dense material on the cytoplasmic surface of the postsynaptic membrane. All dendritic shafts formed both symmetrical and asymmetrical synapses, but most spines formed only asymmetrical synapses. Most frequently, spines formed only a single asymmetrical synapse, but a few had none, and a few had more than one. Occasionally, spine necks were involved in a symmetrical synapse near their point of origin from their parent dendrite.

At the 4-day postlesion survival time used in this study, all thalamocortical axon terminals appeared to be at the same stage of degeneration. Each one possessed very electron-dense cytoplasm in which organelles, including synaptic vesicles, were markedly disrupted or absent. However, synapses between identified neurons and degenerating thalamocortical axon terminals could be recognized by the presence of a synaptic cleft and the prominent postsynaptic density characteristic of asymmetrical synapses. On the average, synapses involving degenerating thalamocortical axon terminals accounted for 20% of all the synapses in the neuropil surrounding layer IV portions of the identified neurons. Thalamocortical synapses occurred on dendritic shafts (Fig. 2) and spines (Figs. 3, 4) of the examined dendrites, and, in a few instances, degenerating thalamocortical axon terminals synapsed with spines that received another synapsing axon terminal that was not degenerating (Fig. 4).

Pyramidal cells

The pyramidal cells that were examined are shown in Figure 5. Each pyramidal cell was characterized according to the layer in which its soma occurred. On the basis of the relative diameters of their somata, pyramidal cells were designated as being small, medium, or large. The apical dendrites of large pyramidal cells were thicker and possessed more spines than the apical dendrites of small pyramidal cells.

Layer V pyramidal cells. One example each of small, medium, and large layer V pyramidal cells were examined. The soma of the small layer V pyramidal cell (Fig. 5A) was located in the lower half of layer V. Its apical dendrite ascended as far as the superficial third of layer IV and gave rise to one small collateral branch within layer IV. One spine of the collateral branch and one spine of the apical dendritic shaft distal to the branch were postsynaptic to degenerating thalamocortical axon terminals.

The medium pyramidal cell (Fig. 5B), whose soma occurred in the superficial portion of layer V, and the large pyramidal cell (Fig. 5C), whose soma occurred in the deep portion of layer V, were present in the same block of tissue. The apical dendrites of each of these pyramidal cells proceeded, without branching, through layer IV and ended in terminal tufts (not shown) beginning near layer II. The medium superficial layer V pyramidal cell was involved in 42 thalamocortical synapses, which was more than any other pyramidal cell examined; but in contrast, the large deep layer V pyramidal cell was involved in only one thalamocortical synapse, which was unusual because it involved the dendritic shaft. In the electron microscope, spines from the deep layer V pyramidal cell could frequently be seen to approximate degenerating thalamocortical axon terminals, but they did not synapse with them.
By contrast, almost every time an apposition occurred between spines of the superficial layer V pyramidal cell and a degenerating terminal, a synapse was evident.

Layer VI pyramidal cells. One small and one large layer VI pyramidal cell were examined. The apical dendrite of the small layer VI pyramidal cell (Fig. 5D) entered layer IV, gave off two oblique branches, and terminated in the deep half of layer III. Six widely spaced spines of the apical dendrite and its branches formed thalamocortical synapses. The apical dendrite from the large layer VI pyramidal cell (Fig. 5E) ascended to the upper half of layer IV, where it trifurcated, and the three daughter branches proceeded towards the pial surface. Thirty-four spines of the large layer VI pyramidal cell synapsed with degenerating thalamocortical axon terminals.

Fig. 1. Electron micrograph of a section through the apical dendrite (D) of a small layer V pyramidal cell showing an axon terminal forming an asymmetrical synapse with one of the dendrite’s spines (S). × 40,000.

Fig. 2. Electron micrograph of a section through a dendrite (D) of a non-spiny multipolar neuron showing a synapse between it and a degenerating thalamocortical axon terminal (TA). × 90,000.

Fig. 3. Electron micrograph showing a synapse between a spine (S) of the apical dendrite of a large layer VI pyramidal cell and a degenerating thalamocortical axon terminal (TA). × 90,000.

Fig. 4. Electron micrograph of a cross section through a collateral dendritic branch (D) of the apical dendrite of a small layer V pyramidal cell. The spine (S) arising from the dendrite is involved in asymmetrical synapses with a degenerating thalamocortical axon terminal (TA) and with a normal axon terminal (A). × 65,000.
Nonpyramidal cells

Spiny and non-spiny bitufted cells. One spiny bitufted neuron with its soma in layer V (Fig. 5F) and one non-spiny bitufted neuron whose soma was in the deep portion of IV (Fig. 5G) were examined. Both cells had vertically oriented oval-shaped cell bodies, the poles of which gave rise to one or more main dendritic trunks. Each main trunk branched repeatedly, giving these cells fusiform dendritic fields. Since, in Golgi preparations of lesioned material, barrels often stand out due to light background staining (White, '78), it could be seen that the superficial tuft of the spiny bitufted cell was contained within a single barrel. One vertically oriented dendrite passed through the center of the barrel, and several branches passed along the barrel periphery. Two spines of the vertically oriented dendrite and one spine of a lateral dendritic branch were postsynaptic to degenerating thalamocortical

Fig. 5. Drawings of the neurons examined in the present study. Neurons A–F and H were impregnated by the Golgi method and then gold-toned and deimpregnated. Neuron G was reconstructed from serial thin sections. Representations of spines shown on the layer IV portions of dendrites were based on photomicrographs. Not shown on the pyramidal cells are spines in layers other than IV, basal dendrites, and apical tufts. Scale = 5 μm.
axon terminals. The smooth bitufted cell was not impregnated but was recognized from its appearance in thin sections (Fig. 6, and see Methods). In contrast to the small number of thalamocortical synapses formed by the spiny bitufted cell, the non-spiny bitufted cell formed 105 thalamocortical synapses on the surfaces of its superficial dendritic tuft (Fig. 7).

Layer V multipolar cell. Dendrites of the non-spiny multipolar neuron (Fig. 5H) extended from layer V to layer III. Two of its dendrites were examined, and together they formed only nine thalamocortical synapses. A detailed analysis of the thalamic connectivity of the non-spiny bitufted and multipolar neurons is presented by White and Rock ('80).

DISCUSSION

Based in part on their analysis of synapses between geniculocortical axon terminals and unidentified neurons in area 17 of the rat, Pe-
Fig. 6. Electron micrographs of a section through the cell body (CB) of the non-spiny bitufted cell. × 9,000. Inset is a high magnification view of one of the thalamocortical axon terminals (TA) synapsing with the cell body (arrow). × 80,000.
Fig. 7. Electron micrograph of a section through the proximal portion of a dendrite (DEN) of the superficial tuft of the non-spiny bitufted cell, which was postsynaptic to many degenerating thalamocortical axon terminals (TA). Also shown is an axon (A) forming an asymmetrical synapse with the dendrite. × 25,000.
terms and Feldman ('76) proposed that any postsynaptic element in layer IV capable of forming asymmetrical synapses is postsynaptic to thalamocortical axon terminals (also see Peters, '79, White, '79). Subsequent Golgi/EM studies of White ('78) and Peters et al. ('79) showed various neuronal types with dendrites in layer IV to be postsynaptic to thalamocortical axon terminals. The present study has extended the list of neurons involved in thalamocortical synapses to include non-spiny bitufted cells of layer IV, spiny bitufted cells of layer V, non-spiny multipolar cells of layer V, and five types of layer V and VI pyramidal cells.

In addition, we have shown striking differences among pyramidal cells and nonpyramidal cells in the numbers of thalamocortical synapses they form. These findings indicate that, whereas any type of neuron with a dendrite in layer IV likely receives some synaptic input from the thalamus, individual neurons are involved in very different quantities of thalamocortical synapses.

To use lesion-induced degeneration to identify a synaptic connection and reliably compare the quantity of inputs received by different neurons, the axon terminals from the projection must be simultaneously identifiable, and terminals from other sources should not be involved. In many thalamocortical systems (for example, the visual system of the rat (Peters and Feldman, '76) and monkey (Garey and Powell, '71) and the somatosensory system of the cat (Jones and Powell, '70), degeneration appears to proceed with different rates in different axons, so that at a given survival time, some terminals may not have begun to degenerate, while others have been completely phagocytosed. In those systems, only a fraction of the total number of thalamocortical synapses can be identified at any single postlesion survival time. By contrast, in mouse PMBSF cortex, thalamocortical axon terminals degenerate simultaneously (White, '78), and thus they can be identified at one survival time. A similar simultaneity of degeneration has been reported in the corticothalamic projection of the cat (Jones and Powell, '89). A possible explanation for simultaneous degeneration is that the projection which has been lesioned is composed of axons having the same diameter, which degenerate at the same rate. Consistent with this explanation is the finding that the thalamocortical projection to layer IV of mouse primary somatosensory cortex is composed of axons having similar diameter (Caviness and Frost, '79). The large lesions that are necessary to satisfactorily ablate the VB projections no doubt interfered with the so-called "non-specific" thalamic projections to PMBSF, and, consequently, some of the degenerating synapses reported here are likely to be non-specific in origin. To separate specific and non-specific thalamocortical projections, it is necessary to use a relatively non-destructive labeling technique, such as EM autoradiography.

Following vibrissae removal or ocular enucleation in the newborn rat, Ryugo et al. ('75a,b) found that large deep layer V pyramidal cells had 26–30% fewer spines than controls, and that medium superficial layer V pyramidal cells remained unchanged. Based on these findings, the authors suggested that deep layer V pyramidal cells receive direct thalamocortical input, while the more superficial pyramidal cells do not. In the present study, a large deep layer V pyramidal cell was involved in almost no thalamocortical synapses while a medium superficial layer V pyramidal cell formed the most thalamocortical synapses of any pyramidal cell examined. This discrepancy indicates that conclusions about direct synaptic connectivity cannot be drawn from the effects of sensory manipulation on Golgi-impregnated neurons examined with the light microscope.

Although all pyramidal cells possess conical cell bodies and have apical and basal dendrites, the size and location of their cell bodies and dendrites, as well as the number of their dendritic spines, vary greatly (e.g., Lorente de Nó, '38; O'Leary, '41; Lund, '73). The morphological diversity of pyramidal cells is paralleled by diversity in their afferent and efferent connections. For example, Deschenes et al. ('79a,b) found in cat motor cortex that large layer V pyramidal cells with extensive dendritic fields and few spines were monosynaptically activated by the thalamus, whereas smaller layer V pyramidal cells with more confined dendritic fields and more spines were not. In the present study, the pyramidal cells examined formed varying numbers of thalamocortical synapses, and these variations might be related to differences in their efferent connections. In a detailed study of the cells of origin of corticofugal and commissural projections from rat somatic sensory cortex, which also contains barrels, Wise ('75) and Wise and Jones ('77) found that the pyramidal cells giving rise to different projections possess characteristic sizes and occur within particular sublaminae of layers V and VI. They found, for example, that medium-sized superficial layer V pyramidal cells project to the striatum and to the thalamus, and that
the larger deep layer V pyramidal cells project to the tectum, pons, medulla, and spinal cord. To determine whether pyramidal cells receiving different amounts of thalamocortical input project to different areas, we are currently comparing the thalamocortical relations of pyramidal cells identified by HRP transported retrogradely through different efferent pathways.

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LITERATURE CITED


