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Distribution of GABAergic Synapses and Their Targets in the Dentate Gyrus of Rat: a Quantitative Immunoelectron Microscopic Analysis

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With 4 Figures and 3 Tables

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Abstract: The dentate gyrus has been shown to receive a laminated and target selective GABAergic input (Han et al., 1993; Halasy and Somogyi, 1993), but the numerical parameters of this innervation are not known. In order to establish the relative weight of GABAergic inputs to the dendritic versus somatic regions of granule cells the numerical density and proportion of GABA-immunopositive and immunonegative synaptic boutons and their postsynaptic targets were determined in the molecular and granule cell layers of the dentate gyrus using the postembedding immunogold method.

The granule cell layer contained 9% of all synapses with the remaining 91% being in the molecular layer. Altogether 17% of all synaptic boutons were GABA-immunoreactive, and they formed either type 1 or type 2 synaptic junctions. About 88% of synaptic boutons in the granule cell layer and 7–8% in the molecular layer were GABA-positive. However, the numerical density (number of synapses per unit volume) of GABA-immunoreactive type 2 synapses was calculated to be only slightly less in the molecular layer than in the granule cell layer ($100 \times 10^6/mm^3$ tissue in the granule cell layer and about $86 \times 10^6/mm^3$ in the molecular layer). In addition, GABA-positive type 1 synapses were found in lower number at the border region of the two layers. The mean volume of the molecular layer of the dentate gyrus in the Wistar rat was calculated to be nearly 4 times larger than the volume of the granule cell layer (West and Andersen, 1980). This means that 25–26% of all GABAergic type 2 synapses are located in the granule cell layer, and 74–75% in the molecular layer. The mean postsynaptic targets of the GABA-immunoreactive boutons in the granule cell layer were granule cell somata (46–60%), followed by dendritic shafts (26–29%), spines (up to 14%), and axon initial segments (7–9%). In the molecular layer the dominant postsynaptic targets of GABAergic synapses were dendritic shafts (63–72%), followed by dendritic spines (26–37%). About 2–3% of the targets of all GABA-immunoreactive synapses were GABA-immunoreactive dendritic shafts or somata. Up to 98% of all GABA-immunonegative synaptic boutons were found in the molecular layer, most of them terminating on dendritic spines.

These results show that the dendritic region of the granule cells provides sites for GABAergic inhibition which in quantitative terms highly outnumber the somatic region in the dentate gyrus.

Key words: hippocampus, GABA, synapse, inhibition, immunocytochemistry, dentate gyrus

Introduction

Granule cells, the principal cells of the hippocampal dentate gyrus, are innervated by entorhinal, associational and commissural glutamatergic afferents and by local circuit neurones, many of which are thought to be inhibitory and use GABA as neurotransmitter. It is generally thought that the primary site of inhibition is the somatic region of principal cells, and the best known inhibitory cell types are the basket cells terminating on the somata and proximal dendrites (RAMON y CAJAL, 1893; LORENTE de NO, 1934; RIBAK et al., 1978; BUZSAKI, 1984; LUBBERS and FROTSCHER, 1987; LOPES da SILVA et al., 1990; SERESS and RIBAK, 1990; HAN et al., 1993). Axo-axonic cells, terminating exclusively on axon initial segments of principal neurones, have also been found in the dentate gyrus (KOSAKA, 1983; SORIANO and FROTSCHER, 1989; SORIANO et al., 1990; HAN et al., 1993). Recently, three additional types of inhibitory interneurones were revealed in the dentate gyrus of rat with extensive axonal arborizations in the dentate molecular layer, sparing the somatic region and establishing synapses with dendrites and dendritic spines of granule cells (HAN et al., 1993; HALASY and SOMOGYI, 1993). These cell types were shown to be associated with the termination zones of specific excitatory inputs to the granule cells and may have a significant role in the selective regulation of the effects of these pathways. Thus, the HICAP (hilar cell with axon associated with the commissural association pathway) cell terminated with the commissural and associational pathways in the inner molecular layer. In contrast the terminals of the HIPP (hilar

cell with axon associated with the perforant pathway) and MOPP (molecular layer cell with axon associated with the perforant pathway) cells were associated with the perforant path input in the outer molecular layer. The postsynaptic targets of the HICAP and HIPP cells were shared between dendritic shafts (70%) and dendritic spines (30%), whereas the targets of the MOPP cell were exclusively dendritic shafts.

The existence of these specific, presumably inhibitory cell types suggests that not only the somatic but also the dendritic region might be an important target of inhibitory input in the rat dentate gyrus. Indeed a previous postembedding immunogold study reported that GABA-containing boutons contact spines and dendritic shafts in the outer molecular layer of dentate gyrus and the convergence of type 1 GABA-negative and type 2 GABA-positive synapses on the same spine was also found (FIFKOVA et al., 1992). Quantitative studies on other cortical areas such as cat and monkey visual cortex (BEAULIEU and SOMOGYI, 1990; BEAULIEU et al., 1992) established that the proportion of GABA-immunoreactive axo-dendritic and axo-spinous synapses greatly outnumbers the axo-somatic ones in these areas.

To have a more accurate picture of the relative weights of the somatic and dendritic regions of granule cells in GABA-mediated synaptic events, we carried out a quantitative study in the dentate gyrus by combining the postembedding immunogold method for the demonstration of GABA with a stereological method. This allowed us to determine the number of GABAergic synapses and the distribution of their postsynaptic targets in the molecular layer, containing mostly granule cell dendrites, and in the cell body layer containing somata, proximal dendrites and axon initial segments.

Materials and methods

Tissue preparation

Two female Wistar rats each weighing 150 g were deeply anaesthetized with sodium pentobarbital (Sagatal), perfused through the left ventricle of the heart first with saline, then with a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After perfusion the hippocampus was removed, washed in 0.1 M PB, 60–80 µm thick transverse sections were cut with vibratome and processed for electron microscopy. The sections were postfixed in 1% osmium tetroxide (dissolved in PB) for one hour, contrasted with uranyl acetate dissolved in 70% ethanol for one hour during dehydration, then completely dehydrated and flat-embedded on slides in Durcupan ACM (Fluka) resin.

Electron microscopy and postembedding immunogold reaction for GABA

For each animal a part of the suprapyramidal blade of dentate gyrus of the dorsal hippocampus containing the whole thickness of both molecular and granule cell layers was selected under light microscope and re-embedded for ultrathin sectioning (blocks 91–320 a and 91–321 d resp.). Serial sections were cut and mounted on single-slot, Formvar-coated, copper or nickel grids. Sections mounted on nickel grids were processed to reveal GABA-immunoreactivity with the postembedding immunogold method (SOMOGYI and HODGSON, 1985). The reaction was carried out on droplets in humidified petri dishes. The resin was etched from the surface of the sections with 1% periodic acid for 8 minutes, and the osmium was removed with 2% sodium metaperiodate for 10 minutes. After washing in distilled water and Tris-buffered saline (TBS, pH 7.4), the grids were placed on drops of 1% ovalbumin, primary antiserum to GABA (Code No. 9, HODGSON et al., 1985) at a dilution of 1 : 2000 for 2 hours at room temperature. After thorough washing in TBS and Tris-buffer containing 1% bovine serum albumin and 0.5% Tween 20 (pH 7.4), 15 nm colloidal gold coated with goat anti-rabbit IgG (Bioclin) was applied as secondary antibody at a dilution of 1 : 20 for 2 hours. Following immunostaining the sections were washed in distilled water and contrasted with a saturated aqueous solution of uranyl acetate for 30 minutes then with lead citrate for 2 minutes.

Counting of synapses

Consecutive sections of non-reacted and GABA-immunoreacted pairs were chosen for the quantitative evaluation. A strip of tissue was photographed from the hilar border of the granule cell layer up to the top of the molecular layer from both sections at a magnification of × 14 000. In order to obtain comparable areas from both studied layers, further continuous areas adjacent to the strip were photographed from the granule cell layer. The final magnification of the prints was × 34 000. The number of synapses was counted on the prints and GABA-immunoreactivity of the pre- and postsynaptic profiles was established. Pre- and postsynaptic profiles were considered as GABA-positive if the density of gold particles over them was higher than over the surrounding neuropil elements. An "unidentified" category was set up for profiles which could not be classified with regard of their GABA-immunoreactivity. Somata were identified on the basis of their size and presence of nucleus or other cell organelles, such as Golgi apparatus. Dendritic shafts were recognized by the presence of microtubules and mitochondria, whereas dendritic spines did not contain these and were of small diameter. In the granule cell layer the "spine" category may include postsynaptic somatic, axonal as well as dendritic spines. Axon initial segments were identified by their microtubule fascicles and the undercoating attached to the inner surface of their membrane. Some small diameter profiles could not be classified and are presented as "unknown origin".

The numerical density of synapses in a unit volume (N_V) of tissue was calculated using the method of size-frequency distribution:

$$N_V = N_a/d$$

where N_a is the number of synaptic contacts per unit area and d is the mean trace length of the synaptic membranes which was measured on a digitizing tablet. This simple formula was used, because it was found to be as accurate as the dissector method (BEAULIEU and COLONNIER, 1985; COLONNIER and BEAULIEU,

1985; BEAULIEU and SOMOGYI, 1992). The extent of tissue shrinkage was not directly estimated. A shrinkage value of 15% has been published using similar tissue processing conditions (O'KUSKY and COLONNIER, 1982: BEAULIEU and COLONNIER, 1983) and this value was used for the correction of the absolute number of synapses.

Results

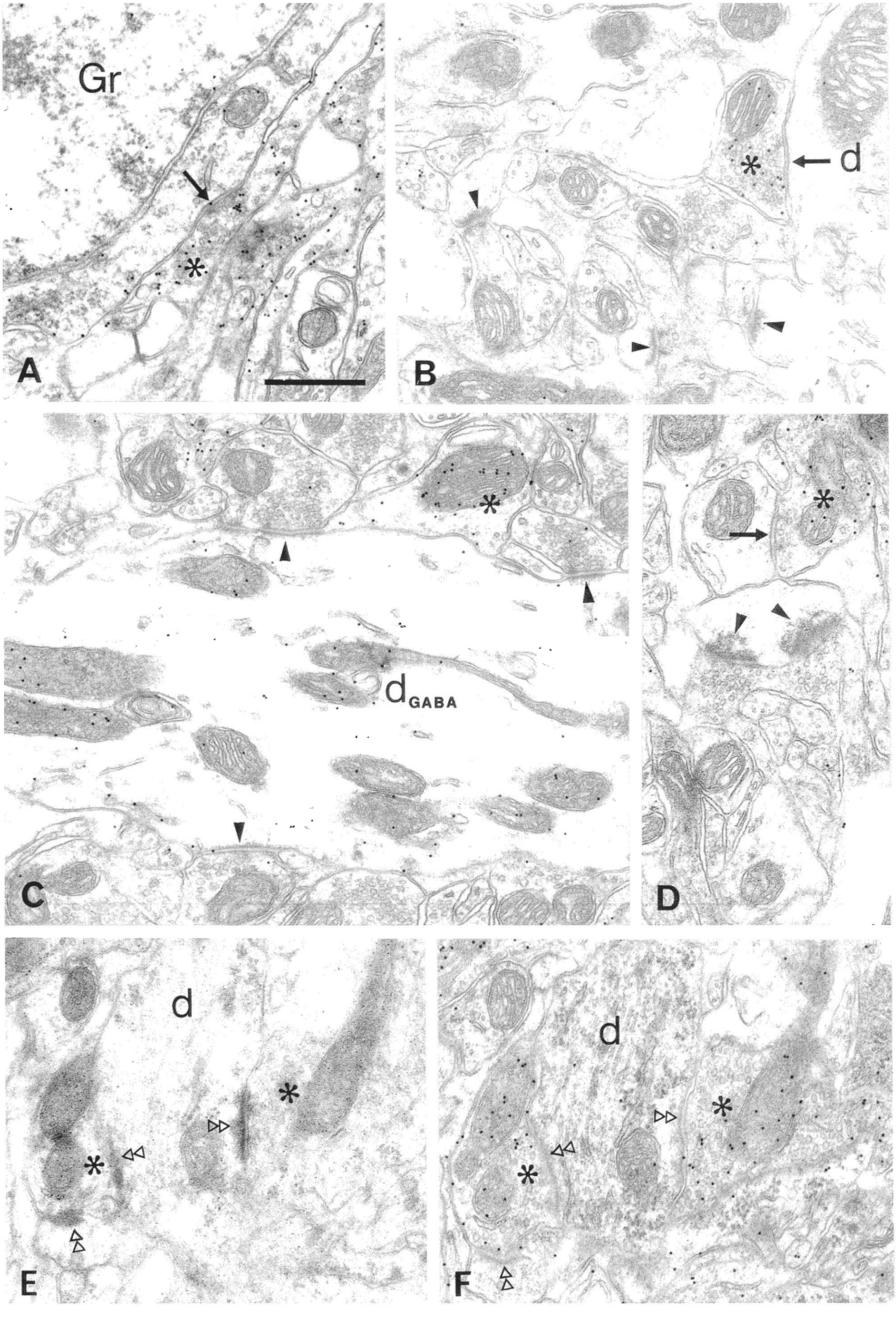
Number and proportion of different types of synapses

The numerical density of synapses, their distribution in the dentate molecular and granule cell layers and the distribution of their postsynaptic targets was established in the two rats (91-320 a and 91-321 d).

The total number of synapses counted in the test areas was 635 in one animal and 600 in the other, the majority (around 90%) being confined to the molecular layer (Table 1). The proportion of all GABA-immunoreactive synaptic boutons was 16.7% and 17.2% respectively in the two animals (Table 1). Three categories of presynaptic terminals were found: GABA-immunopositive boutons making type 2 (symmetric) junctions, GABA-immunopositive boutons making type 1 (asymmetric) junctions and GABA-immunonegative boutons making type 1 (asymmetric) junctions (Fig. 1 B, C, D). The vast majority of boutons (99%) making type 2 synapses were GABA-immunoreactive (Fig. 1 A, B, D). The GABA-positive boutons giving type 1 synapses were in the border region of the granule cell layer and the adjoining region of the molecular layer (Fig. 1 E, F). The numerical density of all GABA-positive synapses was about 120 and $124 \times 10^6/mm^3$ tissue in the granule cell layers of the two animals (all values in this section corrected for shrinkage). This value was lower (87 and $95 \times 10^6/mm^3$) in the molecular layer (Table 1; Fig. 2). The numerical density of GABA-immunoreactive type 2 synapses was $100 \times 10^6/mm^3$ in the granule cell layer of both rats and slightly lower (83 and $89 \times 10^6/mm^3$) in the molecular layer. The total volume of the molecular layer is nearly 4 times of that of the granule cell layer (West and

Table 1. Numerical density of GABA-immunopositive and immunonegative synaptic boutons in the dentate gyrus of two rats.

Animal No.	91-320 a			91-321 d		
Layers	Granule cell layer	Molecular layer	Total	Granule cell layer	Molecular layer	Total
Measured area (μm^2)	1586.82	1694.25	3281.07	1411.08	1420.88	2831.96
Number of synaptic boutons	73	562	635	71	529	600
GABA+	65 (89%)	41 (7.3%)	106 (16.7%)	62 (87%)	41 (7.8%)	103 (17.2%)
GABA-	7 (9.6%)	521 (92.7%)	528 (83.1%)	9 (13%)	487 (92%)	496 (82.7%)
unidentified	1 (1.4%)	-	1 (0.2%)	-	1 (0.2%)	1 (0.1%)
Measured synaptic length ± S.D. (μm)						
GABA+ type 2 (sym.)	0.300 ± 0.057	0.235 ± 0.036		0.313 ± 0.055	0.248 ± 0.055	
GABA+ type 1 (asym.)	0.237 ± 0.047	0.258 ± 0.016		0.251 ± 0.093	0.377 ± 0.090	
GABA- type 1 (asym.)	0.306 ± 0.025	0.203 ± 0.072		0.305 ± 0.022	0.227 ± 0.062	
Numerical density of synapses (N_a; No/$\mu m^2 \times 10^{-2}$)						
GABA+ type 2 (sym)	3.529	2.301		3.685	2.604	
GABA+ type 1 (asym)	0.567	0.118		0.709	0.282	
GABA- type 1 (asym)	0.441	30.751		0.638	34.275	
Numerical density of synapses (N_V; No in 1 mm^3 tissue $\times 10^6$						
GABA+ type 2 (sym)	118	98	216	118	105	223
corrected for shrinkage	100	83	183	100	89	189
GABA+ type 1 (asym)	24	5	29	28	7	35
corrected for shrinkage	20	4	24	24	6	30
GABA- type 1 (asym)	14	1518	1532	21	1511	1532
corrected for shrinkage	12	1290	1302	18	1284	1302
Total (corrected)	132	1377	1509	142	1379	1521


Gr
A
B
d
C
d_{GABA}
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d
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F

Table 2. Total number and proportions of GABA-immunopositive synaptic boutons in an entire dentate gyrus.

Animal	Layer	Type of bouton	Numerical density ($\times 10^6/mm^3$)	Total volume of layer (mm^3) (West and Andersen, 1980)	Total number of GABA+ synapses $\times 10^6$	Proportion
91-320 a	Granule cell layer	All GABA+	120	2.27	272	26%
		GABA+ type 2	100		227	25%
	Molecular layer	All GABA+	87	8.94	778	74%
		GABA+ type 2	83		742	77%
91-321 d	Granule cell layer	All GABA+	124	2.27	281	23%
		GABA+ type 2	100		227	22%
	Molecular layer	All GABA+	95	8.94	849	77%
		GABA+ type 2	89		796	78%

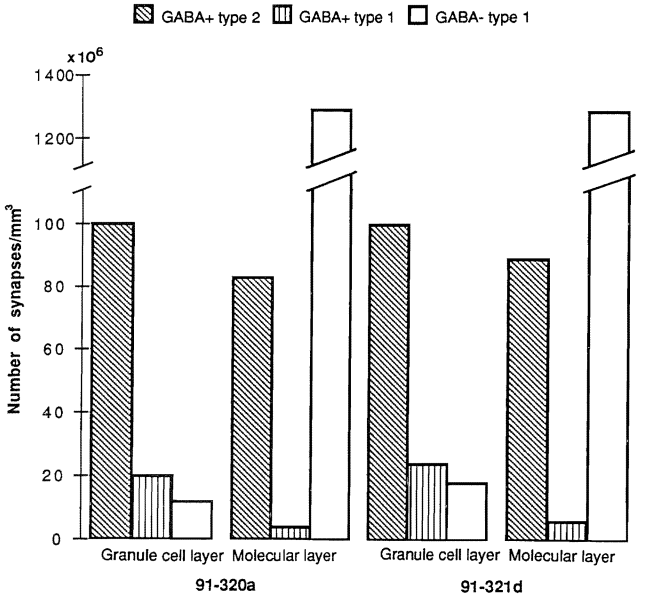

Fig. 2. Numerical density of different types of synapses in the dentate gyrus of two rats.

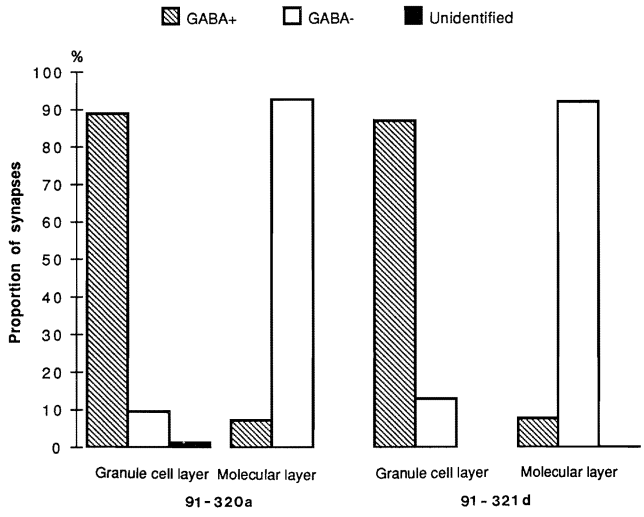

Fig. 3. Distribution of GABA-immunopositive and GABA-immunonegative synapses in the dentate gyrus of two rats.

Andersen, 1980). So, in absolute terms about 800×10^6 GABA synapses are located in the molecular layer, whereas this value is about $270\text{–}280 \times 10^6$ in the granule cell layer of one dentate gyrus of the rat. Thus roughly 75% of all GABA-positive boutons can be found in the molecular layer and 25% in the granule cell layer (Table 2).

The numerical density of GABA-immunopositive boutons making type 1 synapses was around $27 \times 10^6/mm^3$ (Table 1; Fig. 2) in the cell body layer. In the molecular layer, this type of synapse was found in very low numbers and was confined to the border with the granule cell layer.

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Fig. 1. Electron micrographs of synaptic boutons and their postsynaptic targets in the granule cell layer (**A**) and in the molecular layer (**B–F**) of the dentate gyrus after postembedding immunogold reaction for GABA. The neuronal profiles containing high density of gold particles are considered immunopositive. **A.** Type 2 synaptic contact (arrow) between a GABA-immunoreactive axon profile (asterisk) and a granule cell (Gr) soma. **B.** GABA-immunoreactive bouton (asterisk) establishing a type 2 synapse (arrow) with a dendritic shaft (d) in the molecular layer. Arrowheads indicate type 1 synapses established by GABA-negative boutons on spines. **C.** A large GABA-immunoreactive dendritic shaft (d_{GABA}) and numerous GABA-negative boutons forming type 1 synaptic contacts (arrowheads). Asterisk labels a GABA-immunoreacitve preterminal bouton. **D.** Type 2 synapse (arrow) between a GABA-immunoreactive bouton and a GABA-negative dendrite. Arrowheads show a perforated type 1 synapse made by a GABA-negative bouton with a large spine. **E.** Detail of a non-reacted section from the border zone of the molecular and granule cell layers showing type 1 synapses on a dendritic shaft (d) and a spine (double arrowheads). **F.** The same area in a GABA-reacted serial section reveals that the presynaptic boutons (asterisks) are GABA-immunoreactive.

The numerical density of GABA-negative boutons making type 1 synapses was found to be low in the granule cell layer ($12-18 \times 10^6/mm^3$), but very high in the molecular layer ($1\,377-1\,379 \times 10^6/mm^3$) (Table 1, Fig. 2).

The distribution of GABAergic synapses between the two layers (Fig. 3) shows that the majority of synapses are GABA-positive in the granule cell layer of both animals (87–89 %), whereas only 7.3–7.8 % are GABA-posititve in the molecular layer. This low proportion is due to the high density of GABA-negative type 1 synapses in the molecular layer.

Distribution of postsynaptic targets

The distribution of GABA-positive and GABA-negative synapses on somata, dendritic shafts, spines, and axon initial segments is given in Table 3 and Fig. 4. In the granule cell layer the main targets of GABA-immunoreactive synapses are granule cell somata (46–60 %) followed by dendritic shafts (26–28 %), spines of somatic, axonal, or dendritic origin (6.5–13.8 %), and axon initial segments (6.5–9.2 %). In the molecular layer, dendritic shafts comprise 63.4 to 71 % of targets postsynaptic to GABAergic synapses with the remaining 27–36 % terminating on dendritic spines. Averaging the values obtained from two animals, and adding type 1 and type 2 synapses made by GABA-immunopositive boutons together gives in 1 mm^3 of granule cell layer tissue 66×10^6 synapses contacting somata, 33.7×10^6 dendritic shafts, 12.4×10^6 spines and 9.8×10^6 axon initial segments. In each mm^3 of the molecular layer 61.7×10^6 GABA-positive synapses terminate on dendritic shafts and 29.3×10^6 on dendritic spines. Only 2–3 % of the targets of GABA-positive synapses were also GABA-immunoreactive (3 dendritic shafts and 2 synapses on the same GABA-positive soma). The majority of the postsynaptic targets of GABA-negative synapses are spines exceeding any other target by one or two orders of magnitude (Table 3). The GABA-positive targets (13 in total) postsynaptic to GABA-negative synaptic boutons were dendritic shafts in our sample making up 1–2 % of all postsynaptic targets.

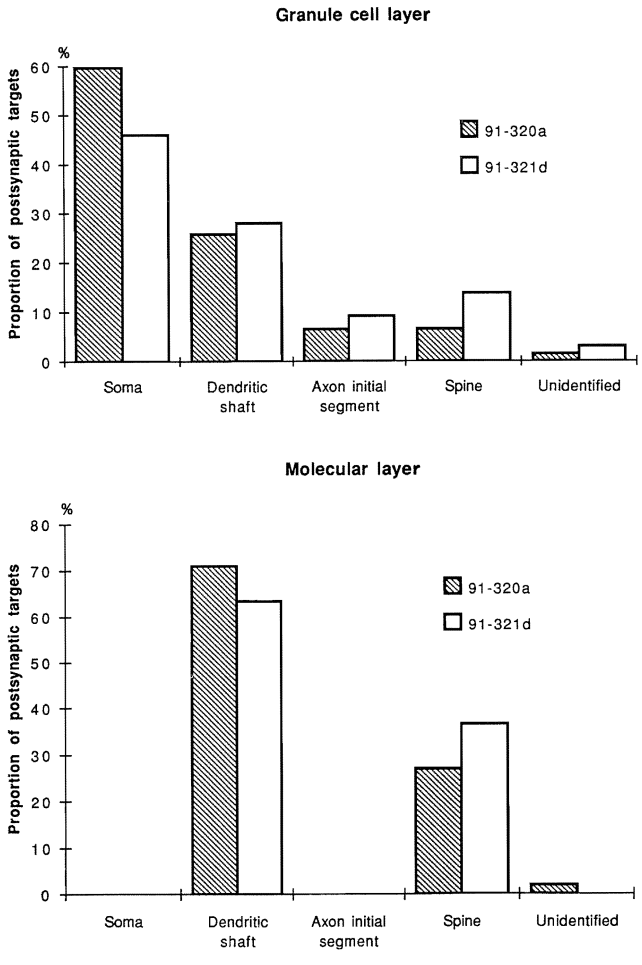

Fig. 4. Distribution of the postsynaptic targets of GABA-immunoreactive terminals in the granule cell layer and molecular layer of two rats.

Discussion

Location of GABAergic synapses in cortical circuits

The results show that both the somatic and dendritic regions of dentate granule cells have a similar numerical density of GABAergic synapses. However, in absolute numbers there are about three times more GABAergic synaptic boutons in the molecular layer than in the cell body layer. In spite of the higher absolute number of GABAergic synapses in the molecular layer the boutons are distributed over a much larger plasma membrane surface than in the cell body layer. Therefore the effect of synaptic GABA release is probably very different in the two layers and there are no simple ways to predict it.

Previous qualitative anatomical studies emphasized the concentration of the GABAergic system in the granule cell layer of the dentate gyrus (Kosaka et al., 1984; Ribak et al., 1978; Seress and Ribak, 1983; Storm-Mathisen, 1976). The present quantitative study shows that when synaptic contacts are counted using stereological methods, comparable density of GABAergic synapses can be found in the two layers. However, most of the synaptic boutons in the granule cell layer are GABAergic, whereas the opposite holds true for the molecular layer. As a result the proportion of GABAergic synapses in the total population is much lower in the molecular layer. According to previous estimates between 1 %

Table 3. Postsynaptic targets of GABA-immunopositive and immunonegative synaptic boutons in the dentate gyrus of two rats.

Animal	Layer	Presynaptic bouton	Postsynaptic targets				
			Soma	Axon initial segment	Dendritic shaft	Spine	Unidentified
91-320 a	Granule cell layer	GABA-positive	30 (46%)	6 (9.2%)	18 (28%)	9 (13.8%)	2 (3%)
		GABA-negative	3 (43%)	-	-	4 (57%)	-
		Unidentified	-	-	1	-	-
	Molecular layer	GABA-positive	-	-	29 (71%)	11 (27%)	1 (2%)
		GABA-negative	-	-	18 (3.5%)	501 (96.1%)	2 (0.4%)
		Unidentified	-	-	-	-	-
	Total	GABA-positive	30 (28.3%)	6 (5.7%)	47 (44.3%)	20 (18.9%)	3 (2.8%)
		GABA-negative	3 (0.6%)	-	18 (3.4%)	505 (95.6%)	2 (0.4%)
		Unidentified	-	-	1	-	-
91-321 d	Granule cell layer	GABA-positive	37 (59.7%)	4 (6.5%)	16 (25.8%)	4 (6.5%)	1 (1.5%)
		GABA-negative	2 (22.2%)	-	4 (44.5%)	3 (33.3%)	-
		Unidentified	-	-	-	-	-
	Molecular layer	GABA-positive	-	-	26 (63.4%)	15 (36.6%)	-
		GABA-negative	-	-	17 (3.5%)	469 (96.3%)	1 (0.2%)
		Unidentified	-	-	1	-	-
	Total	GABA-positive	37 (36%)	4 (4%)	42 (41%)	19 (18%)	1 (1%)
		GABA-negative	2 (0.4%)	-	21 (4.2%)	472 (95.2%)	1 (0.2%)
		Unidentified	-	-	1	-	-

(MATTHEWS et al., 1976) and 10% (CRAIN et al., 1973) of the boutons form type 2 (symmetrical) synapses in the molecular layer of the dentate gyrus. Since the majority of such synapses were GABA-immunopositive in our study, the value of 7.3–7.6% GABAergic synapses is in line with the above estimates. The majority of boutons forming type 1 synapses in the molecular layer were GABA-negative and had a numerical density of $1.4 \times 10^9/mm^3$. This value is within one order of magnitude to the total number of spines calculated indirectly by AMARAL et al. (1992) to be 3.5×10^9 in the outer and 1.1×10^9 in the inner molecular layer of an entire dentate gyrus (about 11 mm^3 in the rat). In view of the high density of GABA-negative boutons it is not surprising that the GABA-immunopositive synapses are less obvious in the molecular layer than in the cell body layer, leading to the impression that the somata are the main sites of GABAergic influence.

Comparing the distribution of GABAergic synapses in the dentate gyrus to other cortical areas, it appears that the numerical density of GABAergic synapses is higher in the dentate gyrus than in the cat visual cortex (48 million/mm^3, BEAULIEU and SOMOGYI 1990), and closer to the value in the monkey visual cortex (50–118 million/mm^3, BEAULIEU et al. 1992). However, when the proportion of GABA-immunopositive synaptic boutons is examined, a surprising similarity becomes obvious. In all three areas the proportion of GABA-immunopositive synaptic boutons is 17%, although the density of neurones and synapses is different in the three areas. This similarity points to a fundamental characteristic of cortical circuits, namely that the ratio of GABAergic and non-GABAergic synapses remains constant irrespective of the functional differentiation of cortical areas. Since most of the GABA-immunonegative synaptic boutons use excitatory amino acids as transmitters, this ratio represents a constancy between GABAergic inhibitory and excitatory amino acid mediated synaptic influence.

Sources of GABAergic terminals

Most of the GABAergic terminals originate from local neurones of the dentate gyrus and a recent intracellular labelling study delineated 5 types of cells (HAN et al., 1993; HALASY and SOMOGYI, 1993). The proportion of GABA-positive cell bodies has been suggested to be only about 2% of neurones in the granule cell layer and 42% in the molecular layer, but somata are very sparse in the latter layer (WOODSON et al., 1989). However these cells have very extensive axonal arborizations (HAN et al., 1993; SORIANO and FROTSCHER, 1993). The somata of many GABAergic neurones innervating granule cells are located in the hilus, under the granule cell layer, where up to 60% of neurones were suggested to be GABAergic (SERESS and RIBAK, 1983; WOODSON et al., 1989; HAN et al., 1993). However, the place of these GABAergic neurones in the hippocampal network is impossible

to predict without the delineation of their axonal projections. Our recent study (HAN et al., 1993; HALASY and SOMOGYI, 1993) demonstrated that the GABAergic cells subdivide the surface of the granule cells as postsynaptic target. Thus, the basket cell mainly terminates on the somata and proximal dendrites, the axo-axonic cell on the axon initial segments (SORIANO and FROTSCHER, 1989; SORIANO et al., 1990), whereas the HICAP, HIPP and MOPP cells terminate exclusively in the molecular layer. Interestingly the axonal field of the HICAP cell is restricted to the inner one third of the molecular layer and the axons of the HIPP and MOPP cells are restricted to the outer two thirds of the molecular layer, corresponding to the division in glutamatergic innervation of the granule cell dendritic field.

In addition to the type 2 GABAergic synapses found throughout the cortex, the dentate gyrus also has a significant number of type 1 synapses made by GABA and GAD (KOSAKA et al., 1984) immunopositive boutons. At least one source of the GABAergic type 1 synapses found in the inner molecular layer is the so-called HICAP-cell (HAN et al., 1993), which preferentially innervates the inner molecular layer and establishes occasional type 1 synapses in addition to type 2 ones (HALASY and SOMOGYI, 1993). KOSAKA et al. (1984) also found GAD-immunoreactive type 1 axo-somatic synapses in the dentate gyrus of the rat, whereas the synapses made by the HICAP cell were on dendritic shafts and spines. Some of the GABA positive boutons may have originated from the local axon collaterals of granule cells, which are known to store GABA (SANDLER and Smith, 1991) but not GAD (RIBAK et al., 1978; SOMOGYI et al., 1983; BABB, 1988; FROTSCHER, 1989) in their terminals and to send collaterals to the granule cell and inner molecular layers (BLACKSTAD 1963; RIBAK and PETERSON, 1991). Granule cells make asymmetric or type 1 synapses so they would only contribute to the population of synapses estimated separately in the border region between the granule cell and molecular layers. The role of GABA in granule cell terminals is not known, but it has been suggested that GABA may not play a role as transmitter (STORM-MATHISEN and OTTERSEN, 1986; SANDLER and SMITH 1991) at granule cell synapses. Therefore some of the terminals in the category forming type 1 synapses may not represent sites of GABAergic neurotransmission.

There is a GABAergic projection from the septum to the hippocampal formation including the dentate gyrus (Freund and Antal, 1988; Freund, 1992). However these axons selectively innervate interneurones and are not thought to terminate on granule cells. Since, in our sample, only 2–3 % of the postsynaptic targets of GABAergic synapses were found to be GABA-immunoreactive, and some of the terminals providing these synapses may have originated from local hippocampal cells, the extrinsic GABAergic afferents can give only a minor contribution to the total number of GABAergic synapses.

The action of GABA on granule cells

Considering the multiple sources of GABAergic terminals to different parts of granule cells, the possibility arises that GABA acts through different receptors at the different sites. Granule cells and hippocampal pyramidal cells are inhibited by GABA through both $GABA_A$ and $GABA_B$ receptors (THALMANN and AYALA, 1982; MISGELD et al., 1986; RAUSCHE et al., 1989; MULLER and MISGELD, 1990; 1991; MOTT and LEWIS, 1991; STEFFENSEN and HENRIKSEN, 1991; MISGELD et al., 1992). Furthermore granule cells are known to express at least 11 subunits of the $GABA_A$ receptor (WISDEN et al., 1992) providing ample opportunity for differential expression at the surface of the cell. The precise subcellular distribution of GABA receptors has not been examined in detail in the dentate gyrus, but from an immunohistochemical study it is apparent that the receptor immunoreactivity (and presumably the active $GABA_A$ receptor site as well) is more dense in the dendritic region (HOUSER et al., 1988). The somatic region and the axon initial segment have independent GABAergic innervation and, being closer to the action potential initiation site, may require fewer synaptic contacts and receptors for effective operation. The basket and axo-axonic cells terminating at these sites may control the overall output of granule cells. In contrast the GABAergic cells terminating in the dendritic domain interact with other inputs, the most numerous being the glutamatergic afferents. It was suggested that this association of GABAergic terminals with glutamatergic afferents, among other possible roles, probably serves the downward re-scaling of the EPSPs thereby extending the dynamic range of the postsynaptic cells (HALASY and SOMOGYI, 1993). The present results show that the dendritic GABAergic innervation comprises about three quarters of all GABAergic synapses on granule cells and it is very likely to be a significant contributor to the effects of GABA.

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References

AMARAL, D. G., N. ISHIZUKA, and B. CLAIBORNE: Neurons, numbers and the hippocampal network. In: STORM-MATHISEN, J., J. ZIMMER and O. P. OTTERSEN (Eds.): Understanding the brain through the hippocampus: the hippocampal region as a model for studying brain structure and function. Prog. Brain Res. 83, pp. 1–13. Elsevier, Amsterdam, New York, Oxford, 1990.

BABB, T. L., J. K. PRETORIUS, W. R. KUPFER, and W. J. BROWN: Distribution of glutamate-decarboxylase-immunoreactive neurons and synapses in the rat and monkey hippocampus: light and electron microscopy. J. Comp. Neurol. **278,** 121–138 (1988).

BEAULIEU, C. and M. COLONNIER: A laminar analysis of the number of round-asymmetrical and flat-symmetrical synapses on spines, dendritic trunks, and cell bodies in area 17 of the cat. J. Comp. Neurol. **231,** 180–189 (1985).

BEAULIEU, C. and M. COLONNIER: The number of neurons in the different laminae of the binocular and monocular regions of area 17 in the cat. J. Comp. Neurol. **217,** 337–344 (1983).

BEAULIEU, C., Z. KISVARDAY, P. SOMOGYI, M. CYNADER, and A. COWEY: Quantitative distribution of GABA-immunopositive and -immunonegative neurons and synapses in the monkey striate cortex (area 17). Cerebral Cortex **2,** 295–309 (1992).

BEAULIEU, C. and P. SOMOGYI: Targets and quantitative distribution of GABAergic synapses in the visual cortex of the cat. Eur. J. Neurosci. **2,** 296–303 (1990).

BLACKSTAD, T. W.: Ultrastructural studies on the hippocampal region. Prog. Brain Res. **3,** 122–148 (1963).

BUZSAKI, G.: Feed-forward inhibition in the hippocampal formation. Prog. Neurobiol. **22,** 131–153 (1984).

COLONNIER, M. and C. BEAULIEU: An empirical assessment of stereological formulae applied to the counting of synaptic disks in the cerebral cortex. J. Comp. Neurol. **231,** 175–179 (1985).

CRAIN, B., C. COTMAN, D. LYNCH, and G. TAYLOR: A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat. Brain Res. **63,** 195–204 (1973).

FIFKOVA, E., H. EASON, and P. SCHANER: Inhibitory contacts on dendritic spines of the dentate fascia. Brain Res. **577,** 331–336 (1992).

FREUND, T. F.: GABAergic septal and serotonergic median raphe afferents preferentially innervate inhibitory interneurons in the hippocampus and dentate gyrus. In: RIBAK, C. E., GALL, C. M. and I. MODY (Eds.): The Dentate Gyrus and Its Role in Seizures. Epilepsy Res. **Suppl. 7,** pp. 79–91. Elsevier, Amsterdam, New York, Oxford, 1992.

FREUND, T. F. and M. ANTAL: GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature **336,** 170–173 (1988).

FROTSCHER, M.: Mossy fiber synapses on glutamate decarboxylase-immunoreactive neurons: evidence for feed-forward inhibition in the CA3 region of the hippocampus. Exp. Brain Res. **75,** 441–445 (1989).

HALASY, K. and P. SOMOGYI: Subdivisions in the multiple GABAergic innervation of granule cells in the dentate gyrus of the rat hippocampus. Eur. J. Neurosci. 5, 411–429 (1993).

HAN, Z.-S., E. H. BUHL, Z. LORINCZI, and P. SOMOGYI: A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurones in the dentate gyrus of the rat hippocampus. Eur. J. Neurosci. 5, 395–410 (1993).

HODGSON, A. J., B. PENKE, A. ERDEI, I. W. CHUBB, and P. SOMOGYI: Antisera to γ-aminobutyric acid. I. Production and characterization using a new model system. J. Histochem. Cytochem. **33,** 229–239 (1985).

HOUSER, C. R., R. W. OLSEN, J. G. RICHARDS, and H. MOHLER: Immunohistochemical localization of benzodiazepine/$GABA_A$ receptors in the human hippocampal formation. J. Neurosci. **8,** 1370–1383 (1988).

KOSAKA, T.: Axon initial segments of the granule cell in the rat dentate gyrus: synaptic contacts on bundles of axon initial segments. Brain Res. **274,** 129–134 (1983).

KOSAKA, T., K. HAMA, and J.-Y. WU: GABAergic synaptic boutons in the granule cell layer of rat dentate gyrus. Brain Res. **293,** 353–359 (1984).

LOPES DA SILVA, F. H., M. P. WITTER, P. H. BOEIJINGA, and A. H. M. LOHMAN: Anatomic organization and physiology of the limbic system. Physiol. Rev. **70,** 453–511 (1990).

LORENTE DE NO, R.: Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. J. Psychol. Neurol. **46,** 113–177 (1934).

LUBBERS, K. and M. FROTSCHER: Fine structure and synaptic connections of identified neurons in the rat fascia dentata. Anat. Embryol. **177,** 1–14 (1987).

MATTHEWS, D., C. COTMAN, and G. LYNCH: An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. I. Magnitude and time course of degeneration. Brain Res. **115,** 1–21 (1976).

MISGELD, U., M. BIJAK, H. BRUNNER, and K. DEMBOWSKY: K-dependent inhibition in the dentate-CA3 network of guinea pig hippocampal slices. J. Neurophysiol. **68,** 1548–1557 (1992).

MISGELD, U., R. A. DEISZ, H. U. DODT, and H. D. LUX: The role of chloride transport in postsynaptic inhibition of hippocampal neurons. Science **232,** 1413–1415 (1986).

MOTT, D. D. and D. V. LEWIS: Facilitation of the induction of longterm potentiation by $GABA_B$ receptors. Science **252,** 1718–1720 (1991).

MULLER, W. and U. MISGELD: Inhibitory role of dentate hilus neurons in guinea pig hippocampal slice. J. Neurophysiol. **64,** 46–56 (1990).

MULLER, W. and U. MISGELD: Picrotoxin- and 4-aminopyridine-induced activity in hilar neurons in the guinea pig hippocampal slice. J. Neurophysiol. **65,** 141–147 (1991).

O'KUSKY, J. and M. COLONNIER: A laminar analysis of the number of neurons, glia, and synapses in the visual cortex (area 17) of adult Macaque monkeys. J. Comp. Neurol. **210,** 278–290 (1982).

RAMON Y CAJAL, S: Estructura des asta de Ammon y fascia dentata. Anal. Soc. espan. Historia natural **22,** 53–114 (1893).

RAUSCHE, G., J. M. SARVEY, and U. HEINEMANN: Slow synaptic inhibition in relation to frequency habituation in dentate granule cells of rat hippocampal slices. Exp. Brain Res. **78,** 233–242 (1989).

RIBAK, C. E. and G. M. PETERSON: Intragranular mossy fibers in rats and gerbils form synapses with the somata and proximal dendrites of basket cells in the dentate gyrus. Hippocampus **1,** 355–364 (1991).

RIBAK, C. E., J. E. VAUGHN, and K. SAITO: Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. Brain Res. **140,** 315–332 (1978).

SANDLER, R. and A. D. SMITH: Coexistence of GABA and glutamate in mossy fiber terminals of the primate hippocampus: an ultrastructural study. J. Comp. Neurol. **303,** 177–192 (1991).

SERESS, L. and C. E. RIBAK: GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. Exp. Brain Res. **50,** 173–182 (1993).

SERESS, L. and C. E. RIBAK: The synaptic connections of basket cell axons in the developing rat hippocampal formation. Exp. Brain Res. **81,** 500–508 (1990).

SOMOGYI, P. and A. J. HODGSON: Antisera to γ-aminobutyric acid. III. Demonstration of GABA in Golgi-impregnated neurons and in conventional electron microscopic sections of cat striate cortex. J. Histochem. Cytochem. **33,** 249–257 (1985).

SOMOGYI, P., A. D. SMITH, M. G. NUNZI, A. GORIO, H. TAKAGI, and J.-Y. WU: Glutamate decarboxylase immunoreactivity in the hippocampus of the cat. Distribution of immunoreactive synaptic terminals with special reference to the axon initial segment of pyramidal neurons. J. Neurosci. **3,** 1450–1468 (1983).

SORIANO, E. and M. FROTSCHER: GABAergic innervation of the rat fascia dentata: a novel type of interneuron in the granule cell layer with extensive axonal arborization in the molecular layer. J. Comp. Neurol. In Press (1993).

SORIANO, E. and M. FROTSCHER: A GABAergic axo-axonic cell in the fascia dentata controls the main excitatory hippocampal pathway. Brain Res. **503,** 170–174 (1989).

SORIANO, E., R. NITSCH, and M. FROTSCHER: Axo-axonic chandelier cells in the rat fascia dentata: Golgi-electron microscopy and immunocytochemical studies. J. Comp. Neurol. **293,** 1–25 (1990).

STEFFENSEN, S. C. and S. J. HENRIKSEN: Effects of baclofen and bicuculline on inhibition in the fascia dentata and hippocampus regio superior. Brain Res. **538,** 46–53 (1991).

STORM-MATHISEN, J.: Distribution of the components of the GABA system in neuronal tissue: cerebellum and hippocampus – effects of axotomy. In: ROBERTS, E. CHASE, T. N. and D. B. TOWER (Eds.): GABA in Nervous System Function. pp. 149–168. Raven Press, New York, 1976.

STORM-MATHISEN, J. and O. P. OTTERSEN: Antibodies against amino acid neurotransmitters. In: PANULA, P. PAIVARINTA, H. and S. SOINILA (Eds.): Neurohistochemistry: Modern Methods and Applications. pp. 107–136. Alan R. Liss Inc., New York, 1986.

THALMANN, R. H. and G. F. AYALA: A late increase in potassium conductance follows synaptic stimulation of granule neurons of the dentate gyrus. Neurosci. Lett. **29,** 243–248 (1982).

WEST, M. J. and A. H. ANDERSEN: An allometric study of the area dentata in the rat and mouse. Brain Res. Rev. **2,** 317–348 (1980).

WISDEN, W., D. J. LAURIE, H. MONYER, and P. H. SEEBURG: The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. **12,** 1040–1062 (1992).

WOODSON, W., L. NITECKA, and Y. BEN-ARI: Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. J. Comp. Neurol. **280,** 254–271 (1989).

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