8 Scornik, O.A., and Paladini, A.C., Am. J. Pysiol. *206* (1964) 553.
9 Fuerstein, G., and Cohen, S., Eur. J. Pharmac. *55* (1979) 203.
10 Peart, W.S., Pharmac. Rev. *17* (1965) 143.
11 Otsuka, K., Assaykeen, T.A., Ganong, W.F., and Tu, W.H., Proc. Soc. exp. Biol. Med. *127* (1968) 704.
12 Rubin, B., Laffan, R.J., Kotler, D.G., O'Keefe, E.H., Demaio, D.A., and Goldberg, M.E., J. Pharmac. exp. Ther. *204* (1978) 271.
13 Hamilton, A.S., and Cohen, S., Am. J. med. Sci. *202* (1941) 914.
14 Fuerstein, G., Boonyaviroj, P., and Gutma, Y., Eur. J. Pharmac. *44* (1977) 131.
15 Laycock, J.F., Penn, W., Shirley, D.J., and Walter, S.J., J. Physiol., Lond. *296* (1979) 267.
16 Cowley, J.A., Schwitzer, S.A., and Guinn, M.M., Circulation Res. *46* (1980) 58.
17 Schwartz, J., and Reid, I.A., Endocrinology *109* (1981) 1778.

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Afferent fibers from the septum terminate on gamma-aminobutyric acid (GABA-) interneurons and granule cells in the area dentata of the rat

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Summary. Interneurons in the area dentata of the rat were immunostained with an antibody to gamma-aminobutyric acid. After septal lesions, degenerating terminals were found in asymmetric synaptic contact with granule cell somata and dendritic elements of immunoreactive and nonreactive cells in the supragranular part of the molecular layer.
Key words. Hippocampus; area dentata; GABA; interneurons; septal afferents.

The septo-hippocampal projection has been reported to terminate in virtually all areas of the hippocampus. In the area dentata, septal fibers end in the supragranular part of the molecular layer and in the hilus[2]. A greater part of this projection is cholinergic[3,4], but probably GABAergic fibers[5] and substance P-fibers[6] are also included. The cholinergic septal fibers are believed to act in an excitatory way on the granular and pyramidal cells of the hippocampus in a pacemaker action to generate the atropine-sensitive (type 2) type of the theta or rhythmic slow activity[7] which may be correlated with sensory processing in the hippocampus[8]. However, it is not yet clear whether the targets of the septo-hippocampal projections are only projection neurons (pyramidal and granular cells), or also interneurons, which is of interest in view of the proposition that part of the effect of acetylcholine is mediated by interneurons[9,10]. In the present study, immunocytochemical investigation of GABA-interneurons was performed in the area dentata of the rat in combination with degeneration studies after midseptal lesions.

Materials and methods. Under deep Nembutal anesthesia, lesions were made in the medial septum of adult Wistar rats by electrocoagulation with anodal current (5 mA, 20 s) applied through the tip of an insulated stainless steel electrode. Electrodes were positioned in the midsagittal plane, thus avoiding the neocortex. Inevitably, electrodes penetrated the corpus callosum. However, this additional damage should not constitute a problem in this case; so far, we do not know of any hippocampal afferent pathway passing via the corpus callosum. Two days later, the animals were injected with 50 µg colchicine in 5 µl 0.9% saline[11] in the neocortex overlying the hippocampus to inhibit fast intraaxonal transport[12]. The injections were not given directly into the hippocampus, to avoid additional lesioning. On the following day the animals were sacrificed by transcardial perfusion with 50 ml saline followed by fixative consisting of 4% paraformaldehyde and 0.09% glutaraldehyde, in 0.1 M phosphate buffer (pH 7.3). Hippocampi were removed, cut into small blocks and left for 3 h in the same fixative. After overnight immersion in phosphate buffer with 30% sucrose, blocks were rapidly frozen by plunging into liquid nitrogen[13], thawed by returning them to 30% sucrose, and cut into 40 µm thick sections on a vibratome. Sections were stored in test tubes containing phosphate buffered saline (PBS).

To aid penetration of the antibody into the sections, all the following reactions were made with the sections floating free. Prior to the antibody incubation, all sections were immersed in a solution of 3% H_2O_2 in methanol to inactivate endogenous peroxidases. Sections were incubated overnight at 4 °C with an antibody to gamma-aminobutyric acid (GABA) raised in rabbits (Immunonuclear Corp., USA) diluted 1:2000 in PBS containing 1% normal goat serum. Control sections incubated in nonimmune serum later showed no reaction product. The avidin-biotin-complex (ABC)-method[14] was used for the visualization of the antibody-antigen complex. In short, sections were washed in 0.1 M phosphate-buffered saline (PBS) and incubated for 1 h with biotin-coupled goat anti-rabbit IgG (Vector Labs., USA), washed again and incubated for another hour with avidin and biotinylated peroxidase (Vector Labs., USA). After washing, the sections were reacted with a freshly filtered solution of 0.05% 3,3'-diaminobenzidene (Sigma) and 0.04% H_2O_2 in PBS. Sections were postfixed in OsO_4, processed for electron microscopy and embedded in a thin layer of Durcupan between two transparent plastic foils. After polymerization of the medium, the preparations were checked. Cells with visible processes were cut out and glued onto plastic blocks for photographing (fig. 1) and final ultra-thin sectioning.

Results. Septal lesions led to dark degeneration of axon terminals in the stratum moleculare, stratum granulare, and hilus of the area dentata. Terminals ranged in size between about 0.5 µm and 2 µm and had round or ovoid profiles. They contained spherical synaptic vesicles and formed asymmetric (Gray I) synaptic contacts within a narrow zone of about 50 µm in the supragranular part of the molecular layer, mostly with dendritic spines (fig. 2), to a lesser extent also with dendritic shafts; very few boutons were found in synaptic contact with somata of granule cells in the granular layer, which were identified by the regular shapes of their somata and nuclei.

Asymmetric contacts were easy to identify by a clear synaptic cleft and a prominent dark postsynaptic dense area. Some degenerating terminals formed contacts with more than one postsynaptic element (fig. 2a); sometimes more than one terminal was connected with the same dendritic spine (fig. 2b). Most of the degenerating boutons found were in contact with postsynaptic elements which lacked immunostaining. In contrast to the

axosomatic contacts established with granule cell somata, the nature of these postsynaptic elements remained unclear. Granule cell dendrites are one candidate, but other non-reactive cells may also extend dendrites into the molecular layer.

After immunostaining, GABA-immunoreactive neurons were seen, colored dark brown in the vibratome sections, in the molecular layer, granular layer and hilus of the area dentata. Their dendrites lacked spines but possessed many varicosity-like swellings. The perikarya of these cells could not be classified into one homogeneous cell population, but appeared rather different. Probably, most of the cells were pyramidal basket cells, molecular layer basket cells, and fusiform basket cells as described by Ribak and Seress[15]. Electron microscopic investigation of the morphology of GABA-positive cells revealed intense cytoplasmic labeling (fig. 1). Most of the reaction product was bound to membranous structures. In general, nuclei and mitochondria appeared to be free from immunostaining except for their outer membranes. The cells possessed some cytological characteristics of basket cells[15]; deeply infolded nuclei, intranuclear rods or sheets of filaments, a well developed perikaryon with prominent granular endoplasmic reticulum and numerous mitochondria. Two putative pyramidal basket cells, the processes of which could be followed into the molecular and hilar layers (one shown in fig. 1), were selected for further electron microscopic study and sectioned serially. Their dendrites and somata bore numerous synaptic contacts. While most of these contacts were established with intact axon terminals, several of them were found also with degenerating boutons. Of these, the majority were axodendritic contacts in the molecular layer (fig. 3b), very few were axosomatic (fig. 3a). Again, these terminals contained spherical vesicles, and contacts were of the asymmetric type.

Discussion. These results show that a projection from the medial septum reaches granule cells and GABAergic cells in the area dentata. Gamma-aminobutyric acid has been postulated as a major inhibitory neurotransmitter of hippocampal inter-

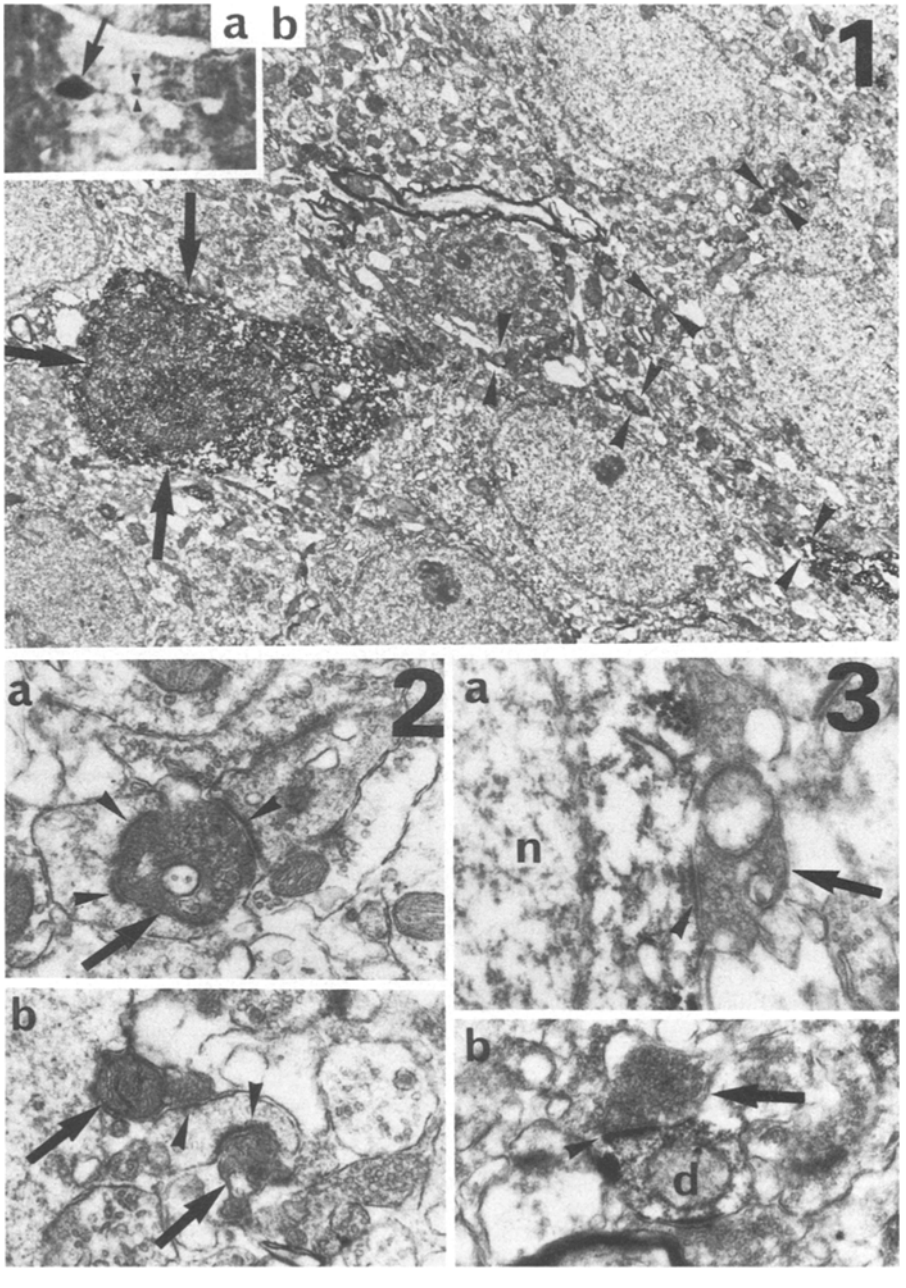

Figure 1. GABA-immunoreactive neuron (arrows) at the inner margin of the granular layer of the area dentata. a) light micrograph from Durcupan block, b) lower power electron micrograph. Arrowheads in a) mark bifurcation node of dendritic process passing through the granular layer, arrowheads in b) mark profiles of the same process. a) × 540, b) × 5400.

Figure 2. Dark degenerating terminal boutons (arrows) after midseptal lesions forming asymmetric synaptic contacts (arrowheads) with unstained dendritic elements. ×32,400.

Figure 3. Dark degenerating boutons (arrows) in asymmetric contact (arrowheads) with GABA-immunoreactive soma, a), and dendritic process (d) in b). n, nucleus. a) × 45,000, b) × 27,000.

neurons[16,17,18]. Ben-Ari et al.[9] and Krnjevic et al.[10] proposed that part of the effect of acetylcholine in the hippocampus is mediated by interneurons. Ashwood et al.[19] have described several putative interneurons of the rat hippocampus and discussed the possibility that they mediate a feed-forward inhibition. Afferent excitation of area dentata interneurons has been described by Buzsáki and Eidelberg[20]; electrophysiological evidence has been found that the septum may be one source of such excitation[21,22]. The results presented here support these findings, as well as the observation of Chandler and Crutcher[2] who found by anterograde tracing of horseradish peroxidase transport after injections in the septum, that HRP-reactive terminals made synapses with granule cells and putative pyramidal basket cells. Hence, the results offer an anatomical basis for direct excitation of granule cells as well as for feed-forward inhibition, mediated by interneurons, from an *extrahippocampal* structure. Anatomical evidence for *intrahippocampal* feed-forward inhibition of area dentata interneurons by contralateral hilar neurons has been described by Seress and Ribak[23]. Feed-forward inhibition is not only a rather economical mode of influencing large populations of principal neurons by exciting a relatively small number of interneurons. A parallel afferent projection to both interneurons and granular cells would allow selective excitation of some of the latter, while the other part is inhibited. This would be optimal, in terms of information theory, for increasing the 'signal-to-noise ratio'; feed-back inhibition may be too slow for this purpose[24]. It is possible that other hippocampal afferents will be described in the future which allow the generation of feed-forward *and* feed-back inhibition; moreover, the coexistence of both effects may be a widespread principle in brain organization.

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2 Chandler, J.P., and Crutcher, K.A., Neuroscience *10* (1983) 685.
3 Lynch, G., Rose, G., and Gall, C., Functions of the Septohippocampal System, p.5, Ciba Foundation Symposion *58*, Elsevier, Amsterdam 1978.
4 Storm-Mathisen, J., Prog. Neurobiol. *8* (1977) 119.
5 Köhler, C., Chan-Palay, V., and Wu, J.-Y., Anat. Embryol. *169* (1984) 41.
6 Baisden, R.H., Woodruff, M.L., and Hoover, D.B., Brain Res. *290* (1984) 146.
7 Swanson, L.W., Teyler, T.J., and Thompson, R.F., Neurosci. Res. Prog. Bull. *20* (1982).
8 Bland, B.H., Seto, M.G., Sinclair, B.R., and Fraser, S.M., Brain Res. *299* (1984) 121.
9 Ben-Ari, Y., Krnjevic, K., Reinhardt, W., and Ropert, N., Neuroscience *6* (1981) 2475.
10 Krnjevic, K., Reifenstein, R.J., and Ropert, N., Neuroscience *6* (1981) 2465.
11 Ribak, C.E., Vaughn, J.E., and Saito, K., Brain Res. *140* (1978) 315.
12 Hökfelt, T., and Dahlström, A., Z. Zellforsch. *119* (1971) 460.
13 Priestley, J.V., Immunolabelling in Electron Microscopy, p.37, Elsevier, Amsterdam 1984.
14 Hsu, S.-M., Raine, L., and Fanger, H., J. Histochem. Cytochem. *29* (1981).
15 Ribak, C.E., and Seress, L., J. Neurocyt. *12* (1983) 577.
16 Alger, B.E., and Nicoll, R.A., J. Physiol. *328* (1982) 125.
17 Andersen, P., Bie, B., Ganes, T., and Laursen, M., Iontophoresis and Transmitter Mechanisms of the Mammalian Central Nervous System, p.179, North Holland, Amsterdam 1978.
18 Storm-Mathisen, J., Brain Res. *40* (1972) 215.
19 Ashwood, T.J., Lancaster, B., and Wheal, H.V., Brain Res. *293* (1984) 279.
20 Buzsáki, G., and Eidelberg, E., Brain Res. *230* (1981) 346.
21 Buzsáki, G., and Eidelberg, E., J. Neurophysiol. *48* (1982) 597.
22 Rovira, C., Ben-Ari, Y., and Cherubini, E., Exp. Brain Res. *49* (1983) 151.
23 Seress, L., and Ribak, C.E., J. Neurocyt. *13* (1984) 215.
24 Buzsáki, G., Prog. Neurobiol. *22* (1984) 131.

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A comparative study of the innervation of the choroid plexus in amphibia

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Summary. The aminergic and cholinergic innervation of choroid plexuses in three species of amphibia was investigated. Plexuses of the Japanese toad and the bullfrog had poor innervation by adrenergic nerves of sympathetic origin, but in the clawed toad, these plexuses were heavily innervated by adrenergic axons from ganglion cells located in the plexus stroma. Nerve fibers positive for acetylcholinesterase were not found in the plexuses, except for a few fibers with very weak enzyme activity in the clawed toad.
Key words. Adrenergic nerves; cholinergic nerves; ganglion cells containing (nor)-adrenaline; choroid plexus; amphibia.

In the mammalian choroid plexuses, dual innervation by sympathetic adrenergic nerves that arise exclusively from the superior cervical ganglia and by parasympathetic cholinergic ones of unknown origin has been found histochemically and ultrastructurally[2-9]. Pharmacological experiments[2,5,9-14] showed that sympathetic nerves affect the two major functions of choroid plexuses; inhibition of the production of cerebrospinal fluid (CSF) by the epithelial cells, and regulation of transport exchange between the CSF compartment and the blood. For submammals, little is known about the innervation of these plexuses. Here, we report the distribution pattern of aminergic and cholinergic nerves in the choroid plexuses of three amphibia.

Materials and methods. 30 clawed toads *(Xenopus laevis)*, 20 Japanese toads *(Bufo bufo)*, and 30 bullfrogs *(Rana catesbeiana)* were used. The animals were anesthetized with ethyl ether, perfused through the aorta with Ringer solution and decapitated. For whole-mount preparations, choroid plexuses and cerebral arteries were carefully dissected out. Choroid plexuses are very undeveloped and sometimes absent in the lateral ventricles of clawed and Japanese toads, but are well-developed in the bullfrog. The materials were either stretched over nonfluorescent glass slides and transferred to a desiccator to be dried in vacuo over P_2O_5 for 1 h, or else fixed with 4% buffered formaldehyde (pH 7.2) for 30 min at 4 °C. Small blocks of the brains containing choroid plexuses were quickly frozen in isopentane chilled with dry ice. Then, they were freeze-dried[15], or else 15 µm sections were cut with a cryostat, mounted on glass slides, and fixed with 4% cold formaldehyde for 30 min.