Fimbria-fornix lesion impairs long-term potentiation in the dentate gyrus of the rat

JA BERGADO, H MORENO and N NUÑEZ

Centro Internacional de Restauración Neurológica, La Habana, Cuba

Bilateral aspiration lesions of the fimbria-fornix were performed in 10 male Sprague Dawley rats weighing 240-300 g under chloral hydrate narcose (420 mg/kg). Another 9 animals were operated in the same way, but no aspiration was carried out to constitute a control group. A week after surgery recording and stimulation electrodes were lowered to the dentate gyrus and the perforant path respectively, using the same narcose. After tetanic stimulation (10 trains at 400 Hz) a potentiation of the population spike develops in both groups, but the slope of the excitatory postsynaptic potential showed no potentiation in the lesioned group. Acetylcholinesterase histochemistry confirmed a severe reduction of the cholinergic innervation to the hippocampal formation, suggesting a causal relationship to the deficits seen in long-term potentiation. This impaired potentiation could be related to the memory deficits reported for fimbria-fornix lesioned rats. Such pattern of potentiation deviates from what has been described for aged, memory deficient rats, but closely corresponds to the changes described in infantile rats.

Key terms: *dentate gyrus; fimbria; fornix; hippocampus; long-term potentiation; memory deficit.*

INTRODUCTION

According to the cholinergic hypothesis, the cognitive dysfunction which characterizes Alzheimer's Disease (AD) might be attributed mainly to a severe reduction of the cholinergic innervation to the hippocampus and cortex (Bartus *et al*, 1982). The fimbria-fornix (FF) fiber system provides the hippocampal formation with cholinergic afferents arising from cells located in the septal region, and the Broca's diagonal band (Moor *et al*, 1994; Smith *et al*, 1993). The FF lesion produces a severe reduction of the cholinergic activity in the hippocampus, as well as a reduction of the learning capacities (Francis *et al*, 1993; Shaw and Aggleton, 1993). Aged rats show also some reduction of the cholinergic activity in the hippocampus and of their mnemonic function (Fischer *et al*, 1989). The FF lesion appears, therefore as a plausible animal model of the cognitive disfunction associated with AD.

Long-term potentiation (LTP) is an endurable form of synaptic plasticity, first demonstrated at the perforant path-dentate granules synapses (Bliss and Lomo; 1973). Some evidence suggests that LTP could be an electrophysiological correlate of long-term memory (Eichenbaum and Otto, 1993; Krug *et al*, 1990; Matthies *et al*, 1986). Aged, memory deficient rats are unable to maintain LTP as long as young controls (Barnes and McNaughton, 1985), while LTP seems to be absent in FF-lesioned rats (Valjakka *et al*, 1991). Thus, the nature of the cognitive impairment in aged and FF-lesioned rats might not be the same.

Correspondence to: Dr Jorge A Bergado-Rosado, Laboratorio de Electrofisiología, Centro Internacional de Restauración Neurológica, Ave 25 N° 15805, Cubanacán Playa 12100, Ciudad de La Habana 21, Cuba. Phone: (53-7) 215-353. Fax: (53-7) 332-420. E-mail: neuro@cenia.cu.

MATERIALS AND METHODS

Nineteen male Sprague-Dawley rats (CENPALAB, Havana, Cuba) weighing 240-300 g by the time of surgery were used. A bilateral aspiration of the FF fibers and the overlaying cortex and corpus callosum (AP:-1.3 mm, L: 2.0 mm)was performed under chloral hydrate (420 mg/kg, ip.) anaesthesia in 10 animals to constitute the lesioned (L) group. Nine animals to which only the windows were opened, but in which no aspiration was performed, constituted the control (C) group.

A week after surgery the animals were reanesthetized with the same dose of chloral hydrate and mounted on a stereotaxic frame. A monopolar recording electrode was lowered to the internal blade of the right fascia dentata. Two miniscrews anchored to the frontal and parietal left bones served as earth and indifferent electrodes, respectively. A bipolar stimulating electrode was lowered to the perforant path. Recordings were made using a Neuropack 2 (Nihon Kohden) amplifier, and filtered within 1-10,000 Hz. Stimulation consisted of single pulses (0.1 msec) generated by a SNE-3301 electronic stimulator (Nihon-Kohden) connected through an isolator to the animal. The stimulus intensity for recordings was set at 50% of the intensity needed to evoke a maximal population spike (PS), and at 25 % of that for LTP induction.

Three control recordings were made before LTP induction with one minute intervals. Each record consisted of three consecutive potentials at 0.2 Hz stored on floppy disk for the off-line analysis.

LTP-induction was achieved applying ten trains of ten impulses (400 Hz) each at ten seconds intervals. Subsequent recordings were made at 2, 5, 15, 30, 60, and 120 minutes after the beginning of LTP-induction using the same paradigm as for the control records. In each averaged record the amplitude of the PS was measured and the slope of the excitatory post-synaptic potential (EPSP) calculated (slope function: SF). For qualitative analysis we considered as positively potentiated a PS value which rose over 150% of its control value. An SF value over 110% of control was also taken as potentiated.

The rectal temperature was continuously monitored and kept between 36 and 37° C using a radiant lamp. One third of the initial chloral hydrate dose was supplied to the rats two hours after the first injection, and repeated each hour from then on to maintain the anaesthesia.

Two days after the electrophysiological evaluation, all animals were anesthetized and transcardially perfused with 10% formaldehyde. Twenty µm sections were cut using a freezing microtome, and processed histochemically for acetylcholinesterase (AChE) as described by Thomas (1981).

RESULTS

An impaired potentiation of the EPSP slope function in the FF-lesioned animals is the main finding reported here.

The histological examination showed that the lesion affected completely and bilaterally the fornix, as well as the overlaying corpus callosum and cortex in all animals in the L group, the lesion extending sometimes anteriorly to the septal area. The AChE-histochemistry showed a strong and bilateral reduction of the enzyme, over the whole hippocampus and dentate gyrus in the lesioned rats, when compared to the controls (fig 1). Only one animal in the lesioned group showed some residual AChE activity.

A tendency to increased epileptogenesis has been reported to occur in FF-lesioned animals. We have monitored the hippocampal EEG during the recording session, and noticed in three lesioned rats the presence of frequent, irregularly spaced spikes, but ictal activity was not seen in any case before, during or after tetanic stimulation.

The comparison of the initial, pretetanization, control values for both groups demonstrated no differences in the PS-amplitude. The SF, however, was slightly but significantly smaller in the lesioned rats (*U*-test; $p < 0.05$). According to the established criteria, all the animals in the control group developed, and maintained a PS-potentiation over the whole recording time after tetanization (Wilcoxon test; $p < 0.05$). The same holds true for the SF, despite the fact that 2 animals were below the 110% level in some

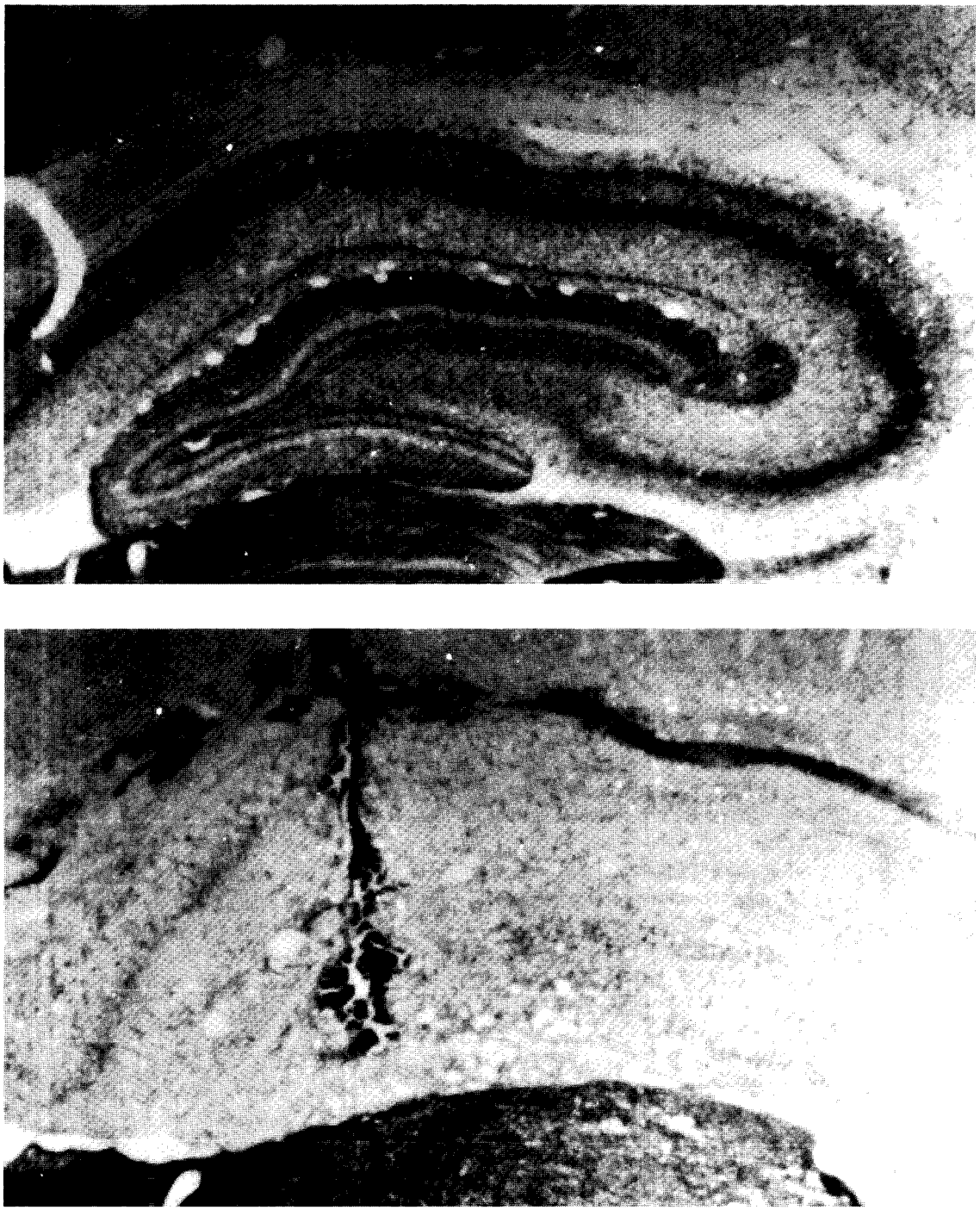

Fig 1. AChE histochemistry in hippocampal formation slices taken from a control (above) and a lesioned rat (below). Note the intense reduction of the AChE activity in the lesioned rat. The tract in B shows the position of the recording electrode.

intermediate recording times. The lesioned group showed a similar change in the PS-amplitude. When compared with their initial control values, 9 out of 10 rats increased this parameter over 150% after tetanic stimulation, and kept it above the potentiation criteria up to the end of the period (Wilcoxon test; $p < 0.05$). The SF variations after LTP induction differed drastically in this group. Six of 10 rats showed SF values over the criteria two minutes after tetanization. However, the lesioned animals failed to maintain

the SF potentiation. Only 3 rats were over the potentiation criteria in some of the remaining records, and one of these was the one in which some residual AChE was histochemically detected. Instead, some depression of the SF tends to occur in about the half of the animals in this group. The Wilcoxon test showed no significant changes in the SF at any time after LTP induction. The relative change in the PS-amplitude was not different between both groups (*U*-test; $p < 0.05$). Between groups, significant differences in the relative SF-potentiation could be demonstrated 2, 15, 60 and 120 min after tetanization (fig 2) (*U*-test; $p < 0.05$).

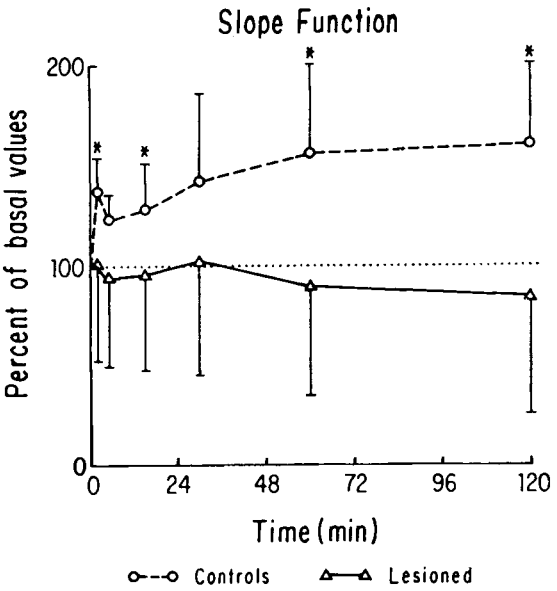

Fig 2. Temporal course of the EPSP's slope (mean ± SEM) function potentiation in both groups after LTP induction. *p < 0.05; Mann-Whitney's *U*-test.

DISCUSSION

It might seem contradictory that the absence of potentiation of the EPSP was accompanied by a normal PS-potentiation. The fact that a PS-potentiation can occur with a reduced, or absent EPSP-potentiation has been recognised since the first report describing LTP (Bliss and Lomo, 1973). Such a difference in the change of the PS and the EPSP after tetanization is a constant, yet unexplained feature of LTP. Modifications in the discharge of dendritic spikes (Fricke and Price, 1984), ephaptic interactions (Turner *et al*, 1984), and electrical coupling through gap junctions (Dermietzel and Spray, 1993; MacVicar and Dudek, 1982) represent mechanisms which might account for an increased population firing following a small, or even zero enhancement in the synaptic reactivity. There is evidence that such mechanisms can be modified by neurotransmitters (Dalkara *et al*, 1986), but their exact role on LTP remains to be established.

Previous reports on LTP in FF-lesioned animals describe an absence of potentiation of the PS (Valjakka *et al*, 1991) or both, the PS and the EPSP (Buzsaki and Gage, 1989; Czéh *et al*, 1992). More recently, however, a normal PS potentiation has been reported in the CA1 area after FF-lesion (Kleschevnikov *et al*, 1994) or destruction of the cholinergic septal population with AF64A (Abe *et al*, 1994), though the last two reports provide no data concerning the EPSP. The different time between surgery and the electrophysiological study - several weeks in the former and one week in the latter - could account for the differences.

An impaired memory function has been described in FF-lesioned rats (Fischer *et al*, 1989; Shaw and Aggleton, 1993) which was comparable to that produced by lesions to the hippocampus (Aggleton *et al*, 1992), or the septum (Kelsey and Vargas, 1993). An altered synaptic plasticity of the FF-lesioned rats may be responsible for their reduced learning ability.

The strong reduction of AChE in the hippocampus after FF-lesion suggests the existence of a link between the cholinergic deficit, and memory and LTP impairments. However, other transmitter systems arising from, or crossing through the septal areas should not be disregarded as they can also influence memory, synaptic transmission, and plasticity in the hippocampal formation . All these subcortical afferents play a crucial role in the regulation of intrinsic circuits which in turn modulate the excitability of the principal cells (Han *et al*, 1993; Ribak, 1992).

As mentioned above, the LTP impairments observed in FF-lesioned rats differ from those reported for aged animals. The main feature of LTP in senescent rats is

the faster decay rate (Barnes and McNaughton, 1985; De Toledo-Morrel *et al*, 1988; Deupree *et al*, 1993) while in the FF-lesioned rats no potentiation of the EPSP develops.

Trommer and Routtenberg (1990) studying LTP in infantile rats from days P14 to P28 found in all animals a constant PS potentiation up to the end of the 120 min follow up time. However, an EPSP-potentiation was rather infrequent occurring only in about one third of the animals. Furthermore, in some animals instead of a potentiation a prolonged EPSP depression develops. Such a result is quite similar to the findings reported here. Interestingly, the septo-hippocampal cholinergic system is immature at birth (Auburger *et al*, 1987; Nio *et al*, 1993) and its final adult functional level is reached only after the first postnatal month (Thal *et al*, 1991). It could be hypothesized that the observations described by Trommer and Routenberg (1990) might be caused, at least in part, by the immaturity of the septal cholinergic afferents to the hippocampal formation. Based on these considerations, and on our findings, we suggest that the fimbria-fornix lesion, a procedure applied to young and otherwise healthy animals, induces a functional state in the hippocampal formation, which is closer to immaturity than to senescence. Moreover, an increased tendency to hippocampal seizures has been described repeatedly in FF-lesioned rats (Buzsaki and Gage, 1989; Kleschenikov *et al*, 1994; Valjakka *et al*, 1991) a feature which characterizes infantile limbic structures in animals (Roper *et al*, 1993; Swann *et al*, 1992), and humans, but is extremely rare in aged subjects (De Toledo-Morrel and Morrel, 1991).

According to the above, the use of the FF-lesion as a model of AD should be treated with caution when trying to obtain information about the functional consequences of aging. Further comparative studies are required to assess how far FF-lesion resembles or differs from aging.

REFERENCES

ABE K, NAKATA A, MIZUTANI A, SAITO H (1994) Facilitatory but nonessential role of the muscarinic cholinergic system in the generation of long-term potentiation of population spikes in the dentate gyrus in vivo. Neuropharmacology 33: 847-852

AGGLETON JP, KEITH AB, RAWLINS JNP, HUNT PR, SAHGAL A (1992) Removal of the hippocampus and transection of the fornix produce comparable deficits on delayed non-matching to position by rats. Behav Brain Res 52: 61-71

AUBURGER G, HEUMANN R, KORSCHING S, THOENEN H (1987) Developmental changes of nerve growth factor and its mRNA in the rat hippocampus: comparison with choline acetyltransferase. Dev Biol 120: 322-328

BARNES CA, McNAUGHTON BL (1985) An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. Behav Neurosci 99: 1040-1048

BARTUS RT, DEAN III RL, BEER B, LIPPA AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217: 408-415

BLISS TVP, LOMO T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. J Physiol, London 232: 331-356

BUZSAKI G, GAGE FH (1989) Absence of long-term potentiation in the subcortically deafferented dentate gyrus. Brain Res 484: 94-101

CZEH G, HORVATH Z, CZOPF J (1992) Time course of changes in long-term potentiation of synaptic transmission following subcortical deafferentation on the rat hippocampus. Acta Physiol Hung 79: 77-93

DALKARA T, KRNJEVIC K, ROPERT N, YIM CY (1986) Chemical modulation of ephaptic activation of CA3 hippocampal pyramids. Neuroscience 17: 361-370

DE TOLEDO-MORREL L, GEINISMAN Y, MORREL F (1988) Age-dependent alterations in hippocampal synaptic plasticity: relation to memory disorders. Neurobiol Aging 9: 581-590

DE TOLEDO-MORREL L, MORREL F (1991) Age-related alterations in long-term potentiation and susceptibility to kindling. In: MORREL F (ed) Kindling and Synaptic Plasticity. The Legacy of Graham Goddard. Boston: Birkhauser Press. pp 160-176

DERMIETZEL R, SPRAY DC (1993) Gap junctions in the brain: where, what type, how many and why? Trends Neurosci 16: 186-192

DEUPREE DL, BRADLEY J, TURNER DA (1993) Age related alterations in potentiation in the CA1 region of F344 rats. Neurobiol Aging 14: 249-258

EICHENBAUM H, OTTO T (1993) LTP and memory: can we enhance the connection? Trends Neurosci 16: 163-164

FISCHER W, GAGE FH, BJORKLUND A (1989) Degenerative changes in forebrain cholinergic nuclei correlate with cognitive impairments in aged rats. Eur J Neurosci 1: 87-98

FRANCIS L, NUÑEZ N, CASTELLANOS O, MORENO H, FERNANDEZ I, DE LA CUETARA K, MACIAS R (1993) Spatial learning and memory following fimbria-fornix lesion and intrahippocampal grafts. In: BEREGI E, GERGEL IA, RAJCZI K (eds) Recent Advances in Aging Science. Bologna: Monduzzi Ed. pp 1245-1248

FRICKE RA, PRINCE DA (1984) Electrophysiology of dentate gyrus granule cells. J Neurophysiol 51: 195-209

HAN ZS, BUHL EH, LORINCZI, SOMOGY P (1993) A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local circuit neurons in the dentate gyrus of the rat hippocampus. Eur J Neurosci 5: 395-410

KELSEY JE, VARGAS H (1993) Medial septal lesions disrupt spatial, but not nonspatial, working memory in rats. Behav Neurosci 107: 565-574

KLESCHEVNIKOV AM, SINDEN JD, MARCHBANKS R (1994) Fimbria-fornix lesions impair spatial performance and induce epileptic-like activity but do nor affect long-term potentiation in the CA1 region of rat hippocampal slices. Brain Res 656: 221-228

KRUG M, BERGADO J, RUETRHICH H (1990) Long-term potentiation and post-conditioning potentiation. The same mechanism? Biomed Biochim Acta 49: 273-279

MAC VICAR BA, DUDEK FE (1982) Electronic coupling between granule cells of the dentate gyrus: Physiological and anatomical evidence. J Neurophysiol 47: 579-592

MATTHIES H, RUETRHICH H, OTT T, MATTHIES HK, MATTHIES R (1986) Low frequency perforant path stimulation as a conditioned stimulus demonstrates correlations between long-term synaptic potentiation and learning. Physiol Behav 36: 811-821

MOOR E, DE BEER P, BELDHUIS HJA, WESTERINK BHC (1994) A novel approach for studying septo-hippocampal cholinergic neurons in freely moving rats: a microdialysis study with dual probe design. Brain Res 648: 32-38

NIO J, BESSON MJ, BRETON P (1993) Ontogenic distribution of muscarinic receptors and acetylcholinesterase in the rabbit hippocampus. Brain Res Bull 31: 723-732

RIBAK CE (1992) Local circuitry of GABAergic basket cells in the dentate gyrus. In RIBAK CE, GALL CM, MODY I (eds) The Dentate Gyrus and its Role in Seizures. Amsterdam: Elsevier. pp 29-47

ROPER SN, OBENAUS A, DUDEK FE (1993) Increased propensity for nonsynaptic epileptiform activity in immature rat hippocampus and dentate gyrus. J Neurophysiol 70: 857-862

SMITH ML, DEADWYLER SA, BONZE RM (1993) 3-D reconstruction of the cholinergic basal forebrain system in young and aged rats. Neurobiol Aging 14: 389-392

SWANN JW, SMITH KL, GOMEZ CM, BRADY RJ (1992) The ontogeny of hippocampal local circuits and focal epileptogenesis. In: ENGEL J, WATERLAIN C, CAVALHEIRO EA, HEINEMANN U, AVANZINI G (eds) Molecular Neurobiology of Epilepsy. Amsterdam: Elsevier. pp 115-126

SHAW C, AGGLETON JP (1993) The effects of fornix and medial prefrontal lesions on delayed non-matching-to-sample by rats. Behav Brain Res 54: 91-102

THAL LJ, GILBERTSON E, ARMSTRONG DM, GAGE FH (1991) Development of the basal forebrain cholinergic system: phenotype expression prior to target innervation. Neurobiol Aging 13: 67-72

THOMAS E (1981) Enzyme histochemistry of the nervous tissue. In: HEYM C, FORSMANN WG (eds) Techniques in Neuroanatomical Research. Berlin: Springer Verlag. pp 111-123

TROMMER BL, ROUTTENBERG A (1990) Long-term potentiation in intact infant rat hippocampus. Dev Brain Res 53: 288-290

TURNER RW, RICHARDSON TL, MILLER JJ (1984) Ephaptic interactions contribute to paired pulse and frequency potentiation of hippocampal field potentials. Exp Brain Res 54: 567-570

VALJAKKA A, LUKKARINEN K, KOIVISTO E, LAMMINTAUSTA R, AIRAKSINEN MM, RIEKKINEN P (1991) Evoked field responses, recurrent inhibition, long-term potentiation and immobility-related nonrhythmical EEG in the dentate gyrus of fimbria-fornix lesioned and control rats. Brain Res Bull 26: 525-532