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Effects of phencyclidines on signal transfer from the entorhinal cortex to the hippocampus in rats

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Abstract

The information transfer from the superficial layers of the entorhinal cortex (EC) to the hippocampus is regulated in a frequency dependent manner. Phencyclidine and related compounds such as MK-801 produce psychotic symptoms that closely resemble schizophrenia. We studied the effects of systemic administration of MK-801 on the signal transfer from the EC layer III to the hippocampal area CA1. High frequency (above 10 Hz) activation of the bi-synaptic entorhinal input in control animals results in a strong suppression of the field potentials in the stratum lacunosum-moleculare of the area CA1. In contrast, in MK-801 pretreated rats the field response was less reduced. The field potential responses evoked in these two groups of animals by high-frequency activation of the monosynaptic input were similar suggesting selective alterations in layer III of the medial EC. We suggest, that MK-801 causes disinhibition of layer III projection cells and, therefore, may cause strong, pathological activation of direct layer III-CA1 pathway.

Keywords: Entorhinal cortex; Field potential; MK-801; Hippocampus; Rats; High frequency stimulation

The entorhinal cortex (EC) occupies a key position in the limbic system functioning as a relay station between the hippocampus and neocortex. The EC is a major gateway for sensory information from different association cortices and the amygdale into the hippocampal formation. Cells in the superficial layers of the EC form two branches of the perforant path: layer II cells project to the dentate gyrus and area CA3, whereas layer III cells project to area CA1 and the subiculum [9]. Interestingly, transfer of information in these two pathways is regulated in a frequency dependent manner and layer III cells stop action potential generation when synaptically activated with frequencies above 10 Hz [5]. Schizophrenia is a very common disorder affecting 1% of the world population (for review see ref. [1]). Phencyclidine and related compounds such as ketamine and MK-801 (here referred to as PCPs) produce both positive and negative psychotic symptoms in normal humans that closely resemble schizophrenia [7]. PCPs are non-competitive antagonists of the *N*-methyl-D-aspartate subtype of glutamate receptors and protect cortical neurons against ischemia. Paradoxically, PCPs produce neurotoxic effects in corticolimbic regions, including neurons of the EC [8]. The mechanism of these paradoxical effects is still unknown. Previous electrophysiological data had demonstrated that systemic administration of PCPs selectively alters the field potential evoked in layer III of the mEC [4]. It is however unclear whether and how the PCPs influence the interaction between the EC and the hippocampal formation.

Using electrophysiological techniques, we investigated the effects of systemic administration of MK-801 on the interaction between the EC and the hippocampus in an in vitro slice preparation. Combined hippocampal-entorhinal cortex slices (400 μm thick) were obtained from adult female Wistar rats. Rats received 6 mg/kg (i.p.) (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (Research Biochemicals, Natick, MA, USA), MK-801, and were killed 4 h later by decapitation. The control animals received (i.p.) the same volume of artificial cerebrospinal fluid (ACSF) as the pretreated rats and were decapitated 4 h later. The slices

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were cut with a Campden Vibratome and were transferred into an interface chamber where they were continuously perfused with oxygenated (95% O_2, 5% CO_2) ACSF containing (in mM): NaCl, 124; KCl, 3; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 1.6; $MgSO_4$, 1.8; glucose, 10; (pH 7.4). Stimulus-induced field potentials were recorded in the stratum lacunosum-moleculare of area CA1 (Fig. 1A). Field potentials (FP) were recorded following single or repetitive (3, 10, 20 and 40 Hz; 20 stimuli) electrical stimulation (0.1 ms, 3–8 V) of the lateral EC and perforant path by a bipolar insulated stimulation electrode. The slices were cut between the dentate gyrus (DG) and EC, and between CA3 and CA1 areas in order to exclude the passage of tri-synaptic input from the EC to the CA1 (Fig. 1A). To study the signal transfer from the EC to the hippocampus we employed low and high frequency synaptic activation of the mono-synaptic (electrical stimulation of the perforant path, PP) and bi-synaptic (stimulation of the lateral EC, lEC) pathways to the CA1 area, and the first (e_1) and last field potentials (e_{20}) elicited by the train of repetitive stimulation were compared. We used a stimulation intensity of 70–80% of that required to evoke a maximal amplitude response, unless indicated otherwise. P values of significance ($P < 0.05$ or $P < 0.001$) were determined using Student's t-test. Data are reported as mean ± SEM.

Typical recordings from the stratum lacunosum/moleculare (SLM) of the hippocampal CA1 area of control animals during low and high frequency electrical stimulation are shown in Fig. 1B. Single electrical stimulation of the lEC evoked stable, small amplitude negative-going extracellular responses (see also refs. [2,3]) that did not change shape and amplitude during the low frequency (3 and 10 Hz, Stm. lEC) stimulation. In contrast, the evoked responses were gradually and substantially reduced during high frequency stimulation (Fig. 1B, 40 Hz, Stm. lEC; Fig. 2B, Table 1). Likewise, monosynaptic activation of the cells of CA1 by low-frequency stimulation of the PP did not change the character of the responses (Fig. 1, Stm. PP, Fig. 2B, Table 1). During high frequency activation of this pathway the evoked potentials were also significantly reduced (Fig. 1, B, 40 Hz, Stm. PP, Fig. 2B). However, the observed reduction was substantially smaller in comparison to the one observed during bi-synaptic stimulation from the lEC ($P < 0.05$ and $P < 0.001$, respectively; Fig. 1B 40 Hz, Stm. lEC and Stm. PP and Fig. 2B, Table 1). Fig. 2A shows similar experiments carried out in MK-801 pretreated animals. The evoked field

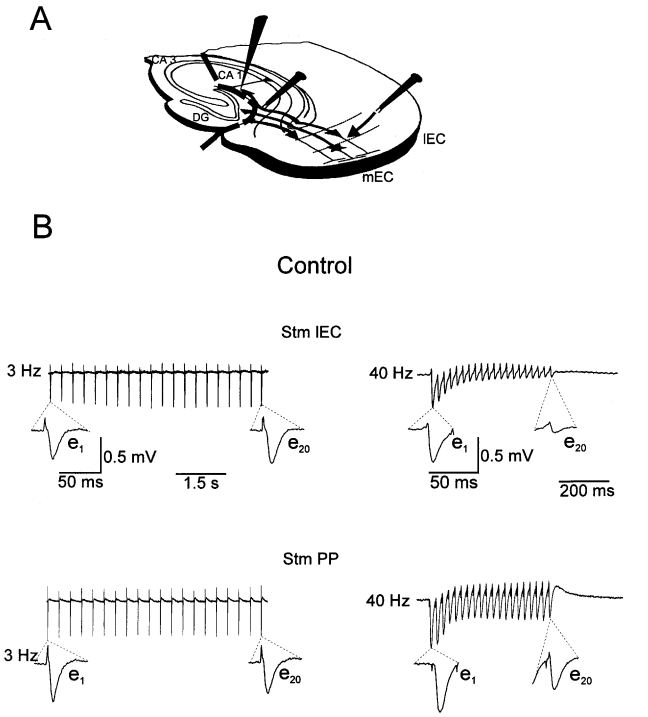

Fig. 1. (A) Schematic representation of the experimental paradigm showing location of the different stimulation and recording sites in hippocampal-entorhinal cortex combined slice. The slices were cut between the DG and EC, and between CA3 and CA1 areas. (B) Low (3 Hz) and high (40 Hz) frequency stimulus-induced bi- (Stm. lEC) and monosynaptic (Stm. PP) field potentials in stratum lacunosum-moleculare of the area CA1 from the control rats. Field potentials in response to 1 and 20 stimulus are shown in an expended time scale.

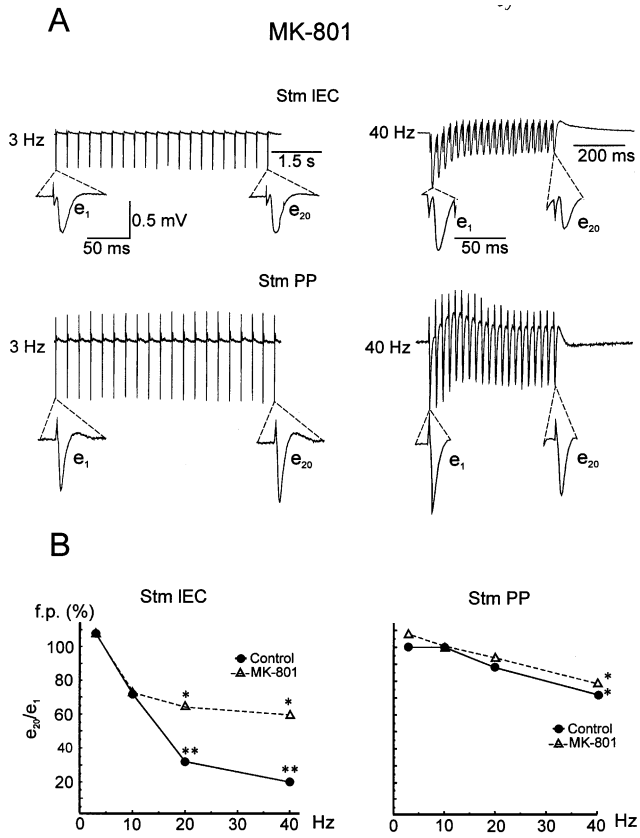

Fig. 2. (A) Representative recordings from stratum lacunosum-moleculare of the hippocampal area CA1 of MK-801 pretreated animals during low- (3 Hz) and high-frequency (40 Hz) electrical activation of bi- (Stm. lEC) and monosynaptic (Stm. PP) pathways. Field potential responses to first (1. Stm) and last stimulus (20. Stm) are shown in an expended time scale. (B) Diagrams summarizing the results of electrical stimulation of the lEC (bi-synaptic) and perforant path (monosynaptic) in control and MK-801 pretreated animals.

Table 1
Field potentials in SLM of the CA1 area in response to mono-(Stim. PP) and bi-synaptic (Stim. lEC) stimulation in control and MK-801 pretreated rats

Stim./Rec.	Freq. (Hz)	Control		MK-801	
		e_1 (amp. mV)	e_{20} (amp. mV)	e_1 (amp. mV)	e_{20} (amp. mV)
Stim. lEC	3	0.26 ± 0.01	0.28 ± 0.01 ($n = 30$)	0.43 ± 0.02	0.46 ± 0.03 ($n = 24$)
Rec. CA1 (SLM)	10	0.29 ± 0.02	0.21 ± 0.03 ($n = 30$)	0.36 ± 0.04	0.27 ± 0.03 ($n = 24$)
	20	0.37 ± 0.03	0.10 ± 0.01 ($n = 30$)	0.50 ± 0.03	0.30 ± 0.01 ($n = 24$)
	40	0.34 ± 0.02	0.07 ± 0.01 ($n = 30$)	0.51 ± 0.03	0.28 ± 0.01 ($n = 24$)
Stim. PP	3	0.42 ± 0.07	0.40 ± 0.04 ($n = 28$)	0.80 ± 0.10	0.85 ± 0.02 ($n = 23$)
Rec. CA1 (SLM)	10	0.50 ± 0.05	0.50 ± 0.05 ($n = 28$)	0.90 ± 0.20	0.90 ± 0.30 ($n = 23$)
	20	0.37 ± 0.08	0.29 ± 0.05 ($n = 28$)	0.90 ± 0.79	0.79 ± 0.30 ($n = 23$)
	40	0.55 ± 0.04	0.37 ± 0.04 ($n = 28$)	0.88 ± 0.10	0.70 ± 0.10 ($n = 23$)

First (e_1) and last (e_2) field potentials in the train of repetitive stimulation were compared. Values are means ± SEM; n, number of slices are in parentheses.

potentials in SLM following single lEC and PP stimulation were significantly larger (0.43 ± 0.02 mV, $n = 19$ and 0.85 ± 0.1 mV, $n = 9$, respectively) than those from control animals (0.27 ± 0.04 mV, $n = 30$ and 0.49 ± 0.04 mV, $n = 30$, upon lEC and PP stimulation, respectively). Similar to the control animals, the low (3 and 10 Hz) frequency stimulation of both lEC and PP did not substantially change responses in SLM ($P > 0.05$). Higher frequency activation of PP was accompanied by a small but significant reduction of the amplitude of the field responses, similar to that seen in the control rats (Figs. 2A,B, Table 1). The key difference between these two groups of animals was, however, seen in the response to high frequency stimulation of the lEC. Whereas high-frequency lEC stimulation almost completely suppressed the field responses in control rats, use of the same experimental paradigm in pretreated rats did not abolish the field response, although a significant reduction ($P < 0.05$) was apparent. Similar results were found in MK-801 pretreated animals also with lower stimulation intensity which evoked smaller FPs, of similar size as those observed in control rats (data not shown). Fig. 2B and Table 1 summarize the results of electrical stimulation of both sites in the two groups of animals.

The present data show that, in response to high frequency stimulation of the lEC, field potentials in SLM of the area CA1 of MK-801 pretreated rats exhibit significantly weaker habituation than those of control rats. Field potentials evoked by low frequency stimulation however were unchanged. In contrast, field potential responses evoked in these two groups of animals by both low- and high-frequency activation of the PP reacted similarly. This suggests that altered responses to high frequency lEC stimulation in pretreated animals are a result of selective alterations in the EC. Interestingly, the evoked FPs, in MK-801 pretreated animals was significantly larger than those in control rats. One possible explanation of this could be a reduced inhibition in the hippocampus which can itself affect signal habituation during repetitive synaptic stimulation. However, a similar suppression of FPs was also found with reduced stimulation intensity in MK-801-pretreated animals suggesting direct alterations in the EC. Systemic administration of MK-801 affected the stimulus-induced field potential selectively in layer III [4]. In addition, intracellular recordings from layer III projection neurons in normal rats showed a propensity to fire action potentials during low frequency (3, 10 Hz) synaptic activation of the lEC, but developed a strong spike frequency habituation upon higher (20, 40 Hz) frequency stimulation [5]. These effects were not observed in MK-801 pretreated animals (J. Breustedt, U. Heinemann, T. Gloveli, unpublished observation). Therefore, one may argue that the strong suppression of field potentials in the SLM of control animals, observed in the present study after high-frequency activation of the bi-synaptic entorhinal input, is a result of strong inhibition of layer III projection cells under these conditions. Consequently, pretreatment of animals with MK-801 might cause disinhibition of layer III projection cells and cause a strong, pathological activation of direct layer III-CA1 pathways.

The present results suggest that PCP may have implications for the transfer of information from the EC to the hippocampus. In particular, the enhanced activation of the direct EC pathway to area CA1 may cause disturbances of cognitive function and of storage of information that are typically involved in psychosis [6].

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