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SYNAPTIC STRENGTH AT THE TEMPOROAMMONIC INPUT TO THE HIPPOCAMPAL CA1 REGION *IN VIVO* IS REGULATED BY NMDA RECEPTORS, METABOTROPIC GLUTAMATE RECEPTORS AND VOLTAGE-GATED CALCIUM CHANNELS

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Abstract—The hippocampal CA1 region receives cortical information via two main inputs: directly via the perforant (temporoammonic) path (pp-CA1 synapse) and indirectly via the tri-synaptic pathway. Although synaptic plasticity has been reported at the pp-CA1 synapse of freely behaving animals, the mechanisms underlying this phenomenon have not been investigated. Here, we explored whether long-term potentiation (LTP) at the pp-CA1 synapse in freely behaving rats requires activation of N-methyl-D-aspartate receptors (NMDAR) and L-type voltage-gated calcium channels (VGCCs). As group II metabotropic glutamate (mGlu) receptors are densely localized on presynaptic terminals of the perforant path, and are important for certain forms of hippocampal synaptic plasticity, we also explored whether group II mGlu receptors affect LTP at the pp-CA1 synapse and/or regulate basal synaptic transmission at this synapse *in vivo*. In adult male rats, high-frequency stimulation (200 Hz) given as 3, or 10 trains, resulted in robust LTP that lasted for at least 4 h in pp-CA1 or pp-dentate gyrus (DG) synapses, respectively. Pre-treatment with the NMDAR antagonist D-(−)-2-amino-5-phosphopentanoic acid (D-AP5) partially inhibited LTP at pp-CA1, and completely prevented LTP at pp-DG synapses. Combined antagonism of NMDAR using D-AP5 and the VGCC inhibitor, (−)-methoxyverapamil hydrochloride elicited a further inhibition of the LTP response at pp-CA1 synapses. Whereas activation of group II mGlu receptors using (1R,2R)-3-((1S)-1-amino-2-hydroxy-2-oxoethyl) cyclopropane-1,2-dicarboxylic acid (DCG-IV) dose-dependently reduced basal synaptic transmission elicited by test-pulse stimulation, DCG-IV did not affect LTP in a dose that inhibited LTP at pp-DG synapses *in vivo*. These data indicate that LTP at the pp-CA1 synapse of freely behaving animals is dually dependent on NMDAR and VGCCs, whereby group II mGlu receptor activation affect basal synaptic tonus, but not LTP. The lower frequency-dependency of NMDA-VGCC LTP at pp-CA1 synapses compared to pp-DG synapses may comprise a mechanism to prioritize information processing at this synapse.

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Key words: perforant path, temporoammonic, synaptic plasticity, hippocampus, CA1, *in vivo*.

INTRODUCTION

The hippocampal formation is a highly plastic structure that is reciprocally connected to cortical brain regions, and engages in information processing and storage (Martin et al., 2000) by means of processes such as long-term potentiation (LTP) and long-term depression (LTD) (Kemp and Manahan-Vaughan, 2007). The main cortical input to the hippocampus originates from the entorhinal cortex (EC), where layer II neurons give rise to the tri-synaptic pathway, comprising the dentate gyrus (DG), CA3 and CA1 regions. In addition, EC layer III neurons directly connect to the CA1 region via the temporoammonic path, often referred as temporoammonic or perforant path to CA1 (pp-CA1) synapse (Witter et al., 1988). Thus, the CA1 region encounters pre-processed information arising from the CA3 region that is transferred via the Schaffer collateral-CA1 (Sc-CA1) synapse, as well as *un-edited* information that reaches it directly via the pp-CA1 synapse. This information might serve as an efference copy for newly stored information in the CA1 region (Lisman and Otmakhova, 2001) or subserve mismatch and/or error detection (Izumi and Zorumski, 2008).

Various learning and memory forms are thought to be encoded via differential involvement of persistent synaptic plasticity mechanisms that require activation of specific molecular cascades (Martin and Morris, 2002; Kemp and Manahan-Vaughan, 2004; Lisman et al., 2012). Induction of LTP in the CA1 region typically involves

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Abbreviations: ANOVA, analysis of variance; AP, anterioposterior; D-AP5, D-(−)-2-amino-5-phosphopentanoic acid; DCG-IV, (1R,2R)-3-((1S)-1-amino-2-hydroxy-2-oxoethyl) cyclopropane-1,2-dicarboxylic acid; DG, dentate gyrus; EC, entorhinal cortex; fEPSP, field excitatory post-synaptic potential; HFS, high-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; mGlu, metabotropic glutamate; ML, mediolateral; NMDAR, N-methyl-D-aspartate receptors; pp, perforant path; Sc-CA1, Schaffer collateral-CA1; STP, short-term potentiation; VGCCs, voltage-gated calcium channels.

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postsynaptic calcium ion entry via the ionotropic, glutamatergic N-methyl-D-aspartate receptors (NMDAR) and subsequent activation of protein kinases (Luscher and Malenka, 2012). However, depending on the stimulation strength Sc-CA1 and pp-DG synapses are capable of expressing LTP in the presence of NMDAR-antagonists, in a process that involves L-type voltage-gated calcium channels (VGCC) (Grover and Teyler, 1990; Manahan-Vaughan et al., 1998; Freir and Herron, 2003). In hippocampal slices it was also shown that pp-CA1 synapses express NMDAR-dependent (Remondes and Schuman, 2002) as well as VGCC-dependent LTP (Remondes and Schuman, 2003). However, very little data are available that describes the processes that underlie LTP induction at the pp-CA1 synapse in the intact brain of freely behaving rats (Aksoy-Aksel and Manahan-Vaughan, 2013). Recently, we reported that compared to Sc-CA1 synapses, the threshold for induction of LTP is lower at pp-CA1 synapses *in vivo* and these synapses are comparatively resistant to strong stimulation protocols (Aksoy-Aksel and Manahan-Vaughan, 2013).

In addition to ionotropic glutamate receptors, metabotropic glutamate (mGlu) receptors play an important role in hippocampal synaptic plasticity and in hippocampal basal synaptic tonus (Mukherjee and Manahan-Vaughan, 2013). Group II mGlu receptors (mGluR2/3) are G-protein receptors that are negatively coupled to adenylyl cyclase and are densely located in a perisynaptic manner on the perforant path and on mossy fiber boutons in the hippocampal formation (Shigemoto et al., 1997). Activation of group II mGlu receptors reduces basal synaptic transmission in the pp-DG and mossy fiber-CA3 synapses in a dose-dependent manner in freely behaving rats (Kulla et al., 1999; Hagena and Manahan-Vaughan, 2010). *In vitro*, the pp-CA1 synapse exhibits a higher sensitivity to the activation of group II mGlu receptors compared to the Sc-CA1 synapse (Speed and Dobrunz, 2009) suggesting that they play a role in the regulation of synaptic plasticity thresholds.

The capacity of a synapse to express different forms of potentiation offers insights into its role in behavioral phenomena such as learning and memory. Different demands for information encoding, retrieval or extinction, for example, might be mediated through differing requirements for NMDAR-dependent and VGCC-dependent LTP (Borroni et al., 2000; Bauer et al., 2002; Moosmang et al., 2005; Davis and Bauer, 2012). Furthermore, very long-lasting forms of synaptic plasticity are linked to the activation of mGlu receptors and to long-term spatial memory (Mukherjee and Manahan-Vaughan, 2013). Considering that the temporoammonic input to CA1 is believed to play an important role in spatial recognition memory (Brun, 2002) and long-term memory maintenance (Remondes and Schuman, 2004) we investigated whether LTP at pp-CA1 synapses is NMDAR, VGCC or group II mGlu receptor dependent, and whether synaptic transmission is regulated by group II mGlu receptors. We observed that robust LTP at the pp-CA1 synapse *in vivo* is induced with less afferent stimulation than LTP at pp-DG synapses. Furthermore, LTP at pp-CA1 synapses is only partially affected by NMDAR antagonism, whereas LTP at the pp-DG synapse is completely abolished. Moreover, NMDAR-independent LTP at the pp-CA1 synapse is sensitive to a VGCC antagonist. We also observed that prior agonist activation of group II mGlu receptors using a dose that does not affect basal synaptic transmission prevents persistent LTP at pp-DG synapses, but has had no effect on LTP at pp-CA1 synapses. These data suggest that synaptic plasticity at the pp-CA1 synapse is distinct to the pp-DG synapse. This may reflect its specific role in hippocampal information processing.

EXPERIMENTAL PROCEDURES

Surgical procedures

The present study was carried out in accordance with the European Communities Council Directive of September 22nd, 2010 (2010/63/EU) for care of laboratory animals and after approval of the local ethics committee (Bezirksamt Arnsberg, Germany). All efforts were made to reduce the number of animals used.

Male Wistar rats (7–8 weeks old at the time of surgery) were implanted with recording and stimulation electrodes (polyurethane-coated stainless steel wire; 1 mm in diameter) into the right hippocampus and a cannula (length: 5.6 mm, diameter: 0.8 mm, Gündel Biomedical Instruments, Germany) into the right lateral cerebral ventricle, as described previously (Aksoy-Aksel and Manahan-Vaughan, 2013). The stereotaxic coordinates for electrode and cannula placements, were referenced to the bregma for anteroposterior (AP) and to midline for mediolateral (ML) distance. The coordinates were as follows: guiding cannula (AP: −0.5 mm; ML: +1.6 mm; DV: ca. 4 mm); recording electrode (AP: −3.0; ML: +2.0 mm) and stimulating electrode (AP: −6.9; ML: +4.1 mm) (Fig. 1). The accuracy of the electrode implantations was verified as described previously (Aksoy-Aksel and Manahan-Vaughan, 2013). In addition, another group of animals was implanted with a recording electrode in the granule cell layer of the DG (AP: −3.1, ML: +1.9 mm) and a stimulating electrode in the perforant path. Here, the same stimulating electrode coordinates were used as for the pp-CA1 preparation. For all synapses the depth of the electrodes was determined during the surgery by giving test-pulse stimulation to generate an field excitatory post-synaptic potential (fEPSP) while the recording electrode was lowered in stepwise decrements until a typical fEPSP was observed (Aksoy-Aksel and Manahan-Vaughan, 2013).

Electrophysiological recordings

The *in vivo* experiments were carried out 7–10 days after the implantation of the electrodes. Synaptic transmission was recorded via test-pulse stimulation of the medial perforant path using a stimulation intensity that elicits ca. 40% of the maximum fEPSP obtained from a previously established input/output (I/O) relationship (100–900 μA) for each individual animal. Responses were evoked by stimulating at frequency of 0.025 Hz with a single biphasic square wave pulse of 0.2-ms stimulus duration and 10 kHz sample rate. For each time-point, five