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Rapid Communication

The L-type voltage-dependent calcium channel long-term potentiation is higher in the dorsal compared with the ventral associational/commissural CA3 hippocampal synapses

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ABSTRACT

The diversification between dorsal (DH) and ventral (VH) hippocampus includes the different ability to support NMDA receptor-dependent long-term synaptic potentiation (LTP). In this study, we assessed the ability of associational/commissural connections in the CA3 hippocampal field to show NMDA receptor-independent LTP. We found that high-frequency stimulation under blockade of NMDA receptors induced greater LTP in DH (40.7 ± 8.5%) than in VH (17.1 ± 4.6%). The blocker of L-type voltage-dependent calcium channels (VDCC) nifedipine prevented the induction of LTP. We hypothesize that the different ability for VDCC-LTP between DH and VH might have important implications in the memory-related information processing performed by the circuits of the two hippocampal segments.

The recently emerging body of evidence that demonstrates differences in the functional organization of the intrinsic neuronal circuitry between the dorsal (DH) and the ventral (VH) hippocampus includes the prominent difference in NMDA receptor-dependent long-term potentiation (LTP) (Colgin et al., 2004; Maruki et al., 2001; Papatheodoropoulos and Kostopoulos, 2000a). LTP is thought to fundamentally contribute to learning and memory processes (Morris, 2003) and consists of a collection of mechanistically distinct types of plastic changes (Blundon and Zakharenko, 2008). Accordingly, LTP can be induced by calcium entry into the postsynaptic cell through either NMDARs or voltage-dependent calcium channels (VDCC) (Blundon and Zakharenko, 2008; Grover and Teyler, 1990; Morgan and Teyler, 2001; Raymond and Redman, 2002).

In the CA3 hippocampal field recurrent associational fibers and commissural fibers (A/C) from other CA3 cells give rise to a dense excitatory input in the field (Amaral and Witter, 1989). It is thought that the CA3 network plays important functional roles in learning and memory especially of spatial information (Gilbert and Brushfield, 2009; Kesner, 2007). As occurs in the synapses between CA3 Schaffer collaterals and CA1 neurons, NMDAR-dependent LTP can be induced at A/C connections (Harris and Cotman, 1986; Hernandez et al., 1994; Kakegawa et al., 2004). In addition, mechanisms that do not depend on NMDARs are importantly involved in long-term changes associated to epileptogenesis in the CA3 network (Moschovos et al., 2008). In the present study we investigated whether NMDAR-independent LTP can be induced at the CA3 A/C connections and whether there are differences between the two poles of the hippocampus.

Twenty adult male Wistar rats were used in this study. All animal treatment and experimental procedures were conducted in accordance with the Directive Guidelines for the care and use of Laboratory animals of the European Communities Council (European Communities Council Directive Guidelines 86/609/EEC) and they were approved by the Prefectural (Achaia) Animal Care and Use Committee (No: EL 13BIO04). Hippocampal slices were prepared as previously described (Papatheodoropoulos and

Abbreviations: A/C, associational/commissural; DH, dorsal hippocampus; fEPSP, field postsynaptic potential; HFS, high-frequency stimulation; VH, ventral hippocampus.

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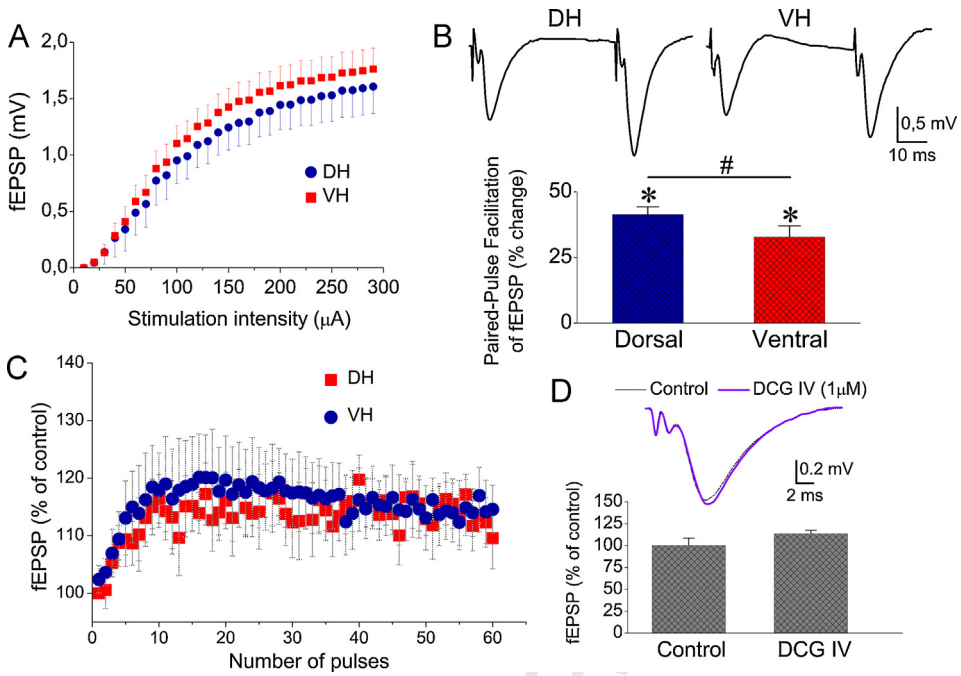

Fig. 1. (A) Input/output curves between the stimulation current and fEPSP evoked after stimulation of CA3 A/C connections. Curves were constructed from twelve dorsal and eighteen ventral slices. (B) Paired-pulse stimulation at the inter-pulse interval of 50 ms induced facilitation in both hippocampal poles ($^{*}p < 0.05$, Wilcoxon test) and was significantly higher in DH than in VH ($^{\#}p < 0.05$, Mann–Whitney *U*-test). Traces on the top represent examples of facilitated responses. (C) Frequency facilitation induced by stimulation at 1 Hz was similar between DH and VH. (D) The agonist of mGluR II DCG IV did not affect fEPSP. Superimposed traces on the top were obtained before (thin line) and during application of DCG IV (thick line). The graph on the bottom shows collective results obtained from three slices.

Kostopoulos, 2000a). Briefly, animals were sacrificed under deep anesthesia with diethyl-ether. The brain was removed, submerged in cool (2–4 °C) artificial cerebrospinal fluid (ACSF) containing, in mM: 124 NaCl, 5 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, at pH 7.4. The two hippocampi were excised free and 550 μm thick transverse slices were prepared from the dorsal and ventral segment of the structure using a McIlwain tissue chopper. Slices were immediately transferred to an interface type chamber and kept at a constant temperature of 31 ± 0.5 °C. They were continuously supplied with a humidified mixed gas 95% O_2 and 5% CO_2 and perfused with ACSF. Evoked field excitatory postsynaptic potentials (fEPSPs) were obtained from the stratum radiatum of the CA3 field using carbon fiber electrodes (10 μm of diameter, WPI or Kation Scientific, USA). A bipolar stimulation electrode made of platinum/iridium wire of 25 μm diameter was placed at the stratum radiatum of the proximal CA3 field (i.e. near hilus) to activate recurrent associational connections originating from other CA3 pyramidal cells and commissural fibers (Pofantis et al., 2015). Potentials were amplified and filtered at 0.5 Hz–2 kHz using a Neurolog system (Digitimer Limited, UK), digitized at 4–5 kHz and stored on a computer for off-line analysis using the CED 1401-plus interface and the Signal software (Cambridge Electronic Design, Cambridge, UK). Baseline stimulation was adjusted to evoke a half-maximum response and delivered one every minute. fEPSP was quantified by its amplitude. For the induction of long-term potentiation (LTP) high-frequency stimulation (HFS) consisting of two trains of 100 pulses at 100 Hz, separated by 5 s was delivered at A/C connections using stimulation strength that produced maximum response. Paired-pulse facilitation of EPSP was accessed by delivering two pulses, 50 ms apart, and measuring as the percent ratio between the second and the first response ([EPSP2/EPSP1] × 100). Frequency facilitation was also examined by delivering sixty pulses at the frequency of 1 Hz. In the present study the following drugs were used: the competitive antagonist of NMDA receptor 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10 μM), the agonist of group II metabotropic glutamate receptors (mGluR II) (2S,2′R,3′R)-2-(2′,3′-Dicarboxycyclopropyl)glycine (DCG IV, 1 μM) and the blocker of L-type VDCC nifedipine (40 μM). Drugs were first prepared as stock solutions and then solved either in water (CPP, DCG IV) or dimethylsulfoxide (nifedipine). The percentage by volume of dimethylsulfoxide in the final solution of nifedipine did not exceed 0.005%. The non parametric Wilcoxon test and Mann–Whitney *U*-test and the analysis of variance were used for statistical comparisons. Values throughout the text express the mean ± S.E.M. while "*n*" indicates the number of slices.

Fig. 1A shows the input/output relationship between stimulation strength and fEPSP for the two hippocampal poles. fEPSP did not significantly differ between DH ($n = 10$) and VH ($n = 18$) at any stimulation intensity used. As shown in Fig. 1B, paired-pulse stimulation induced significant facilitation of fEPSP in both DH (change by 41.36 ± 2.9%, $n = 25$, Wilcoxon test, $p < 0.05$) and VH (change by 32.8 ± 4.2%, $n = 25$, Wilcoxon test, $p < 0.05$). In addition, paired-pulse facilitation was significantly greater in DH than in VH (Mann Whitney *U*-test, $p < 0.05$). The existence of dorsoventral difference in paired-pulse facilitation at A/C connections reminds that found at the Schaffer collateral-to-CA1 synapses (Papatheodoropoulos and Kostopoulos, 2000b). Low frequency stimulation consisting of sixty pulses at 1 Hz produced a relatively small enhancement of fEPSP either in DH or VH (Fig. 1C). The so produced frequency facilitation maximized during the first ten to eighteen pulses in both hippocampal poles and slightly declined and stabilized during the last thirty pulses. Specifically, 1 Hz stimulation reached a maximum of about 20% between the sixteenth and eighteenth pulse in DH and about 10% at the thirteenth pulse in VH (Fig. 1C). The values of paired-pulse facilitation and frequency facilitation at A/C synapses were similar to those found in previous studies and they were much smaller than those reported for the adjacently passing mossy fibers (Kobayashi and Poo, 2004; Nicoll and Schmitz, 2005; Salin et al., 1996). The insensitivity of fEPSP to the activation of group II mGlu receptors by DCG IV (1 μM) (Fig. 1D), a procedure that effectively

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