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Differences in paired-pulse inhibition and facilitation in the dentate gyrus and CA3 field between dorsal and ventral rat hippocampus

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ABSTRACT

We studied the processes of inhibition and facilitation in the dentate gyrus (DG) and the CA3 field by examining the effects of paired-pulse stimulation on the evoked population spike (PS) in dorsal (DH) and ventral (VH) hippocampal slices from the adult rat. The antidromic–orthodromic (A–O) and the orthodromic–orthodromic (O–O) paired-pulse stimulation protocols were used at varying inter-pulse intervals (IPI). In the DG, the A–O stimulation produced an early depression of PS lasting 30–40 ms which was significantly stronger in the VH compared with DH. The O–O stimulation produced a biphasic pattern of effects, in both dorsal and ventral DG, consisting of an early depression of PS followed by facilitation at relatively longer intervals. In the DH but not the VH the phase of facilitation was followed by a late depression of PS (>200 ms). In the CA3 field both A–O and O–O stimulation had a biphasic effect consisting of an early phase of strong depression of similar strength in DH and VH. The depression was followed by a phase of facilitation which was more pronounced with O–O stimulation. The facilitation observed with the O–O stimulation was much stronger in DH than VH and in DH only it was significantly reduced by the antagonist of $GABA_B$ receptors CGP52432. Furthermore, the facilitation was insensitive to changes in $[Ca^{2+}]_o$ in both hippocampal poles. These findings suggest that the dorsal compared with ventral DG is more amenable to fast-frequency input but filters out slow-frequency inputs more reliably while the gating and amplification of the excitatory input in the CA3 circuitry is more prominent in DH than in VH.

1. Introduction

The hippocampal formation can be divided into distinct segments along its long (i.e. dorso-ventral or septo-temporal) axis in virtue of their different functional implications and external connections (Fanselow and Dong, 2010; Small et al., 2011; Witter and Amaral, 2004). In addition, there is a growing body of data pointing to differences in the organization of the intrinsic circuitry between the distinct hippocampal segments, namely its dorsal and ventral poles. The organizational and functional

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differences in the local circuit which might importantly contribute to the functional segregation along the hippocampus, include synaptic transmission and plasticity (Colgin et al., 2004; Maggio and Segal, 2007; Maruki et al., 2001; Papatheodoropoulos and Kostopoulos, 2000a; Papatheodoropoulos and Kostopoulos, 2000b; Petrides et al., 2007), cell properties (Dougherty et al., 2012, 2013; Maggio and Segal, 2009), place cell firing (Kjelstrup et al., 2008; Maurer et al., 2005) and network activities (Derchansky et al., 2004; Gilbert et al., 1985; Papatheodoropoulos et al., 2005; Patel et al., 2012; Schmidt et al., 2013).

The activity in the local circuits is controlled by the interactions between synaptic excitation, inhibition and cell excitability. The balance between excitation and inhibition is fundamental for proper neuronal network activity and the excitation in hippocampal circuits is controlled by powerful inhibitory circuits (Alger, 1991). It has been previously shown that in the CA1 field both recurrent inhibition and synaptic facilitation are smaller in the ventral (VH) compared with the dorsal (DH) hippocampus (Papatheodoropoulos and Kostopoulos, 2000b; Papatheodoropoulos et al., 2002; Petrides et al., 2007). In the present study using field recordings we aimed to investigate and compare between DH and VH the effectiveness of paired-pulse stimulation in producing inhibition and facilitation of the synaptic response, in the dentate gyrus (DG) and the CA3. These two regions play distinct functional roles during the processing of information in the hippocampus (Kesner, 2007a; Kesner, 2007b) and differences in the phenomena of inhibition and facilitation in these fields between DH and VH might have important implications for the roles the two hippocampal poles play in behavior.

2. Results

PSs were recorded from 59 dorsal and 42 ventral hippocampal slices prepared from 38 animals. The values of maximal PS were similar between DH and VH and between DG and CA3. Specifically, the PS in the DG had maximal amplitude of 3.7 ± 0.4 mV and 3.65 ± 0.5 mV in DH and VH respectively. In CA3 the corresponding values in DH and VH were 4.12 ± 0.3 mV and 3.4 ± 0.3 mV respectively.

2.1. The effects of A–O and O–O stimulation

The excitation in the hippocampal neuronal circuits is controlled by powerful local inhibitory interneurons (Buhl and Whittington, 2007; Freund and Buzsaki, 1996) which are recurrently activated by principal cells. Accordingly, the action of the local inhibitory recurrent networks can be studied by activating these inhibitory networks though orthodromic (i.e. synaptic) or antidromic excitation of principal cells and observing the produced depression of firing during subsequent orthodromic activation of the same cells. Furthermore, by synaptically activating the local network twice, the phenomenon of facilitation can be also studied (Bekenstein and Lothman, 1991; Leung and Fu, 1994). As a general rule, the paired antidromic–orthodromic (A–O) stimulation may provide a more reliable tool than the double orthodromic stimulation (O–O) for measuring the strength and the duration of inhibition of PS since it is rather devoid of some factors that accompany paired orthodromic stimulation including synaptic facilitation. Conversely, the double orthodromic stimulation can reveal more accurately than A–O stimulation phenomena of facilitation. Here, in order to study inhibition and facilitation of the PS in the local neuronal circuits of DG and CA3 we used both A–O and O–O stimulation protocols.

In general, we found that at relatively short intervals both stimulation protocols produced depression of PS in both hippocampal subregions (DH and CA3) and both hippocampal poles (DH and VH). This paired-pulse inhibition was followed by facilitation at longer IPIs that was higher with the O–O compared with A–O stimulation and presented significant dorso-ventral differences.

2.2. Dentate gyrus

In a first set of experiments the electrode for antidromic stimulation of granule cells was positioned at the hilus (S2 in Fig. 1, close stimulation). We found that in both dorsal and ventral DG the A–O stimulation produced significant depression of PS at IPIs of 5–30 ms in DH (from $-77.4\pm4.3\%$ at 5 ms to $-18.2\pm8.6\%$ at 30 ms, $n=11$, Wilcoxon test at each individual IPI, $P<0.05$) and 5–40 ms in VH (from $-91.1\pm3.6\%$ at 5 ms to $-22.6\pm4.4\%$ at 40 ms, $n=11$, Wilcoxon test at each individual IPI, $P<0.05$) (Fig. 2A). Furthermore, at IPIs between 5–20 ms the depression was significantly stronger in VH than in DH ($-81.55\pm3.32\%$ vs $-63.61\pm3\%$ respectively, univariate ANOVA $F(1, 54)=16.10$, $P<0.001$. The A–O stimulation did not produce any significant change at IPIs longer than 30 ms in DH and 40 ms in VH, (Wilcoxon test at each individual IPI in DH and VH, $P>0.05$). Due to the fact that stimulation at the hilus, in addition to mossy fibers, might directly excite interneurons

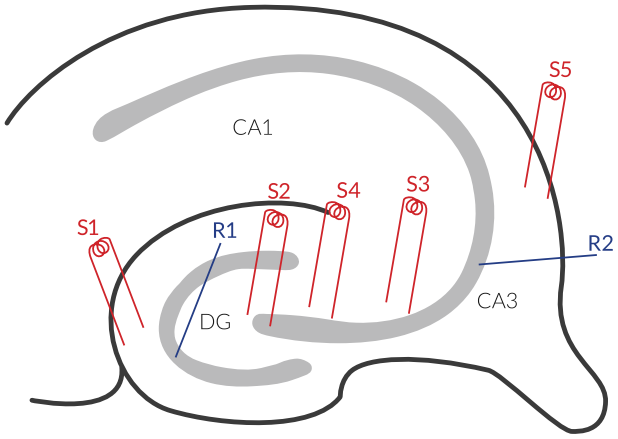

Fig. 1 – Drawing of a hippocampal slice illustrating the positions for the stimulation (S, red colored) and recording (R, blue colored) electrodes used in this study. S1 and S4 are the positions used for orthodromic activation of granule cells and CA3 pyramidal cells respectively. The positions used for close and remote antidromic activation of granule cells are indicated by S2 and S3 respectively. The position for antidromic (alvear) activation of CA3 pyramidal cells is shown by S5. R1 and R2 indicate the positions of electrodes for recording the field potentials from the granule cell layer and the CA3b pyramidal layer respectively.

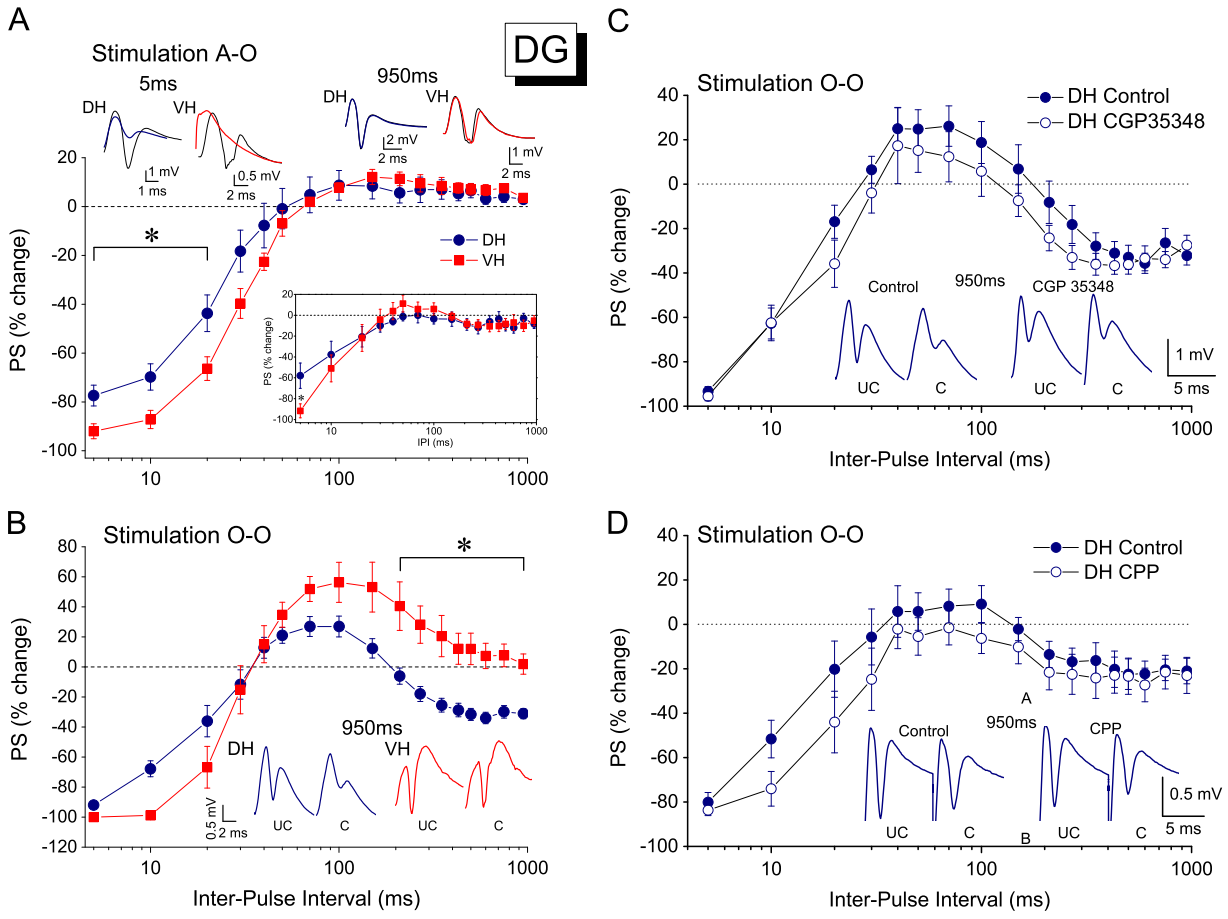

Fig. 2 – Effects of antidromic–orthodromic (A–O) and orthodromic–orthodromic (O–O) paired-pulse stimulation on the PS recorded from the dentate gyrus of DH and VH. A. Diagram of paired-pulse depression of PS in DG following the A–O stimulation paradigm (close stimulation, see Methods and Fig. 1) illustrating the stronger and longer inhibition in VH compared with DH. Depression of PS was statistically significant up to 30 ms in DH (Wilcoxon test, $P<0.05$) and 40 ms in VH ($P<0.05$). Asterisk denotes the range of IPIs (5–20 ms) where differences between DH and VH were statistically significant (Univariate ANOVA, $F(1, 54)=16.10$, $P<0.001$). The bottom right graph shows the results of A–O protocol with remote stimulation. Asterisk denotes statistically significant difference between DH and VH (Mann Whitney test, $P<0.05$). Examples of unconditioned and conditioned responses (superimposed thin and thick lines respectively) for the IPI indicated are also shown. Note that at 5 ms PS was completely abolished in the ventral slices. B. Diagram showing the effect of O–O paired-pulse stimulation of perforant path on PS in DG. Asterisk denotes the range of IPIs (210–950 ms) where differences between DH and VH were statistically significant (Univariate ANOVA, $F(1, 54)=16.10$, $P<0.001$). Symbols for DH and VH are as in "A". Examples of unconditioned (UC) and conditioned responses (C) for DH and VH at the interval of 950 ms are shown in the insert at the bottom. C-D. Diagrams showing the absence of effects of the antagonist of $GABAB_B$ receptors CGP 35348 (200–500 μM, $n=11$) (C) and the antagonist of NMDA receptors CPP (10 μM, $n=6$), (D) on the late inhibition observed with the O–O paired-pulse stimulation in the DG of dorsal hippocampal slices. Examples of unconditioned and conditioned responses (UC and C respectively) evoked at the IPI indicated are shown for DH and VH (left and right traces in each panel) on the bottom of the graphs. Artifacts in all examples are truncated.

that innervate and inhibit granule cells (Freund and Buzsaki, 1996) contributing thus to the observed dorsoventral differences, we performed a second set of experiments where the electrode for antidromic stimulation of granule cells was positioned at the stratum lucidum of CA3b-CA3c subfield (S3 in Fig. 1, remote stimulation). As shown in the insert of Fig. 2A, the results followed a similar pattern to that obtained with the close (i.e. hilar) antidromic stimulation. Specifically, the suppression of PS at the IPI of 5 ms was significantly greater in VH (91.7±6.7%, $n=9$) than DH (58.0±12.0%, $n=9$), (Mann Whitney, $P<0.05$). The depression of PS was greater in VH (51.0±13.0%) than DH (37.7±12.8%) also at the interval of 10 ms but not significantly so.

Using the O–O stimulation paradigm we found that its effect on granule cell population firing consisted of distinct phases of inhibition and facilitation (Fig. 2B). This is in line with previous in vivo (Bekenstein and Lothman, 1991; Gilbert and Burdette, 1996) and in vitro studies (DiScenna and Teyler, 1994; Rich-Bennett et al., 1993). Specifically, the O–O stimulation produced an early depression, between IPIs 5–20 ms, of similar strength in DH ($n=23$) and VH ($n=10$), $F(2, 28)=5.87$, Univariate ANOVA, $P>0.05$. In both DH and VH this initial

depression was followed by a phase of facilitation which spanned from 50 ms to 150 ms in DH (Wilcoxon test at each individual IPI, $P<0.05$) and from 50 ms to 270 ms in VH (Wilcoxon test at each individual IPI, $P<0.05$), reaching a maximum of 27.0±6.9% and 56.3±13.3% respectively at 100 ms. In addition, in the dorsal but not ventral DG the phase of facilitation was succeeded by a phase of late depression (at IPIs of 350–950 ms, Wilcoxon test at each individual IPI, $P<0.05$) with maximum values observed at 600 ms (34±3.8%). In contrast, in the ventral DG paired-pulse stimulation did not produce any significant change at IPIs greater than 270 ms (Fig. 2B). Taking into account that the time course of this late depression matches the time course of $GABA_B$ receptor-mediated potential, we examined the effect of the antagonist of $GABA_B$ receptors CGP35348 (200–500 μM). Fig. 2C shows that the drug produced no significant change at any IPI, ($n=11$, Univariate ANOVA, $F(1, 16)=0.41$, $P>0.05$). This is in accordance with previous observations (Albertson and Joy, 1987; Rich-Bennett et al., 1993). Then, taken into account that NMDA receptor-dependent actions of slow after hyperpolarization (Gilbert and Burdette, 1996) might underlie late depression, we applied the antagonist of NMDA receptors CPP (10 μM). Fig. 2D shows that the drug had no significant effect neither on the late depression or at any other inter-pulse interval (Univariate ANOVA, $F(1, 16)=0.363$, $P>0.05$).

2.3. CA3

As shown in Fig. 3A, the A–O stimulation in CA3 produced an early significant depression of PS which lasted 40 ms in DH ($n=11$) and 50 ms in VH ($n=11$) (Wilcoxon test at each individual IPI and at each hippocampal pole, $P<0.05$). However, in contrast to the difference between DH and VH observed in DG the inhibition of PS in CA3 was similar between DH and VH, (Mixed Model Analysis, $F(1, 21.84)=1.18$, $P>0.05$). The phase of depression was followed by a facilitation which peaked at 100–150 ms and it was statistically significant at the intervals of 70–600 ms in DH and 150–430 ms in VH (Wilcoxon test at each individual IPI and at each pole, $P<0.01$). In addition, at the range of 70–270 ms the facilitation was significantly higher in DH (50.84±12.78%) than in VH (6.58±13.4%), (Mixed Model Analysis, $F(1, 19.1)=5.71$, $P<0.05$). For instance, the facilitation at the IPIs of 150 ms and 210 ms was five-fold and two-fold greater in DH than in VH respectively.

Fig. 3B shows that the O–O stimulation protocol produced inhibition of similar strength in DH ($n=21$) and VH ($n=18$) at the range of 5–20 ms IPIs (Mixed Model Analysis, $F(1, 40.9)=0.55$, $P>0.05$), although it was shorter than that observed with the A–O stimulation. In line with the results obtained with the A–O stimulation, the O–O protocol produced facilitation of PS which followed the early depression; however, the facilitation seen with O–O protocol had a much higher magnitude than that observed with the A–O stimulation. In particular, we observed strong facilitation in the dorsal hippocampus at all intervals greater than 30 ms (Wilcoxon test at individual IPIs, $P<0.001$, $n=21$) and at 40–430 ms in the ventral hippocampus (Wilcoxon test at individual IPIs, $P<0.05$, $n=18$). What is more, facilitation was several times higher in DH than in VH. In particular, the difference was three times higher at all IPIs longer than 40 ms reaching a ten-fold difference at 50 ms (Mann–Whitney test, $P<0.001$). Such high values of facilitation have been observed at the synapses between granule cells mossy fibers and CA3 pyramidal neurons (Salin et al., 1996). Considering the possibility that stimulation at the area between hilus and CA3c subfield (see Fig. 1) might activate both mossy fibers and associational fibers of CA3 pyramidal cells, we examined the relative contribution of each synaptic input to the large difference in facilitation of PS observed between DH and VH. Thus, we performed the O–O stimulation paradigm in a different set of dorsal and ventral slices perfused with solution containing the agonist of group II metabotropic glutamate receptors (mGluR II) DCG IV (2 μM), which effectively suppresses neurotransmitter release from mossy fiber terminals (Weisskopf and Nicoll, 1995). As shown in Fig. 4A, DCG IV had no significant action on the effects of O–O stimulation in the dorsal ($n=9$, Univariate ANOVA, $F(16, 136)=0.633$, $P>0.05$) and ventral slices ($n=7$, Univariate ANOVA, $F(16, 102)=0.827$, $P>0.05$). Therefore, mossy fibers did not contribute to the effects of paired-pulse

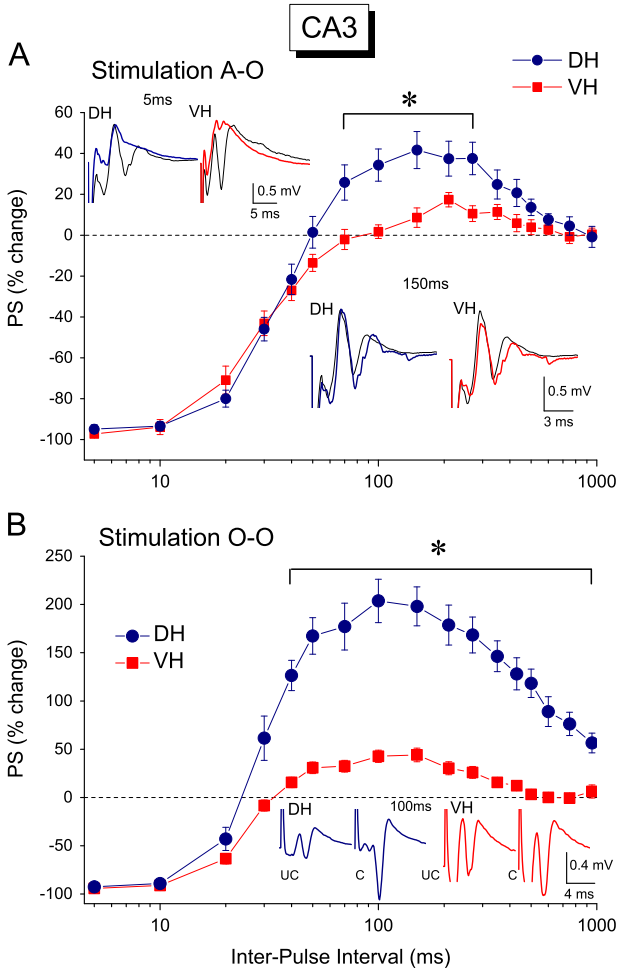

Fig. 3 – Effects of A–O (A) and O–O (B) stimulation protocols on the PS recorded from the dorsal and ventral CA3 pyramidal layer. Examples of unconditioned and conditioned responses (superimposed thin and thick lines respectively) for the IPI indicated are shown as insets in "A". Representative examples in "B" are indicated in the bottom of the diagram. Asterisks denote the range of IPIs where differences between DH and VH were statistically significant in the A–O (70–270 ms, Mixed Model Analysis, $F(1, 19.1)=5.71$, $P<0.05$) and O–O protocol (40–950 ms, Univariate ANOVA, $F(1, 12)=3.78$, $P<0.001$).

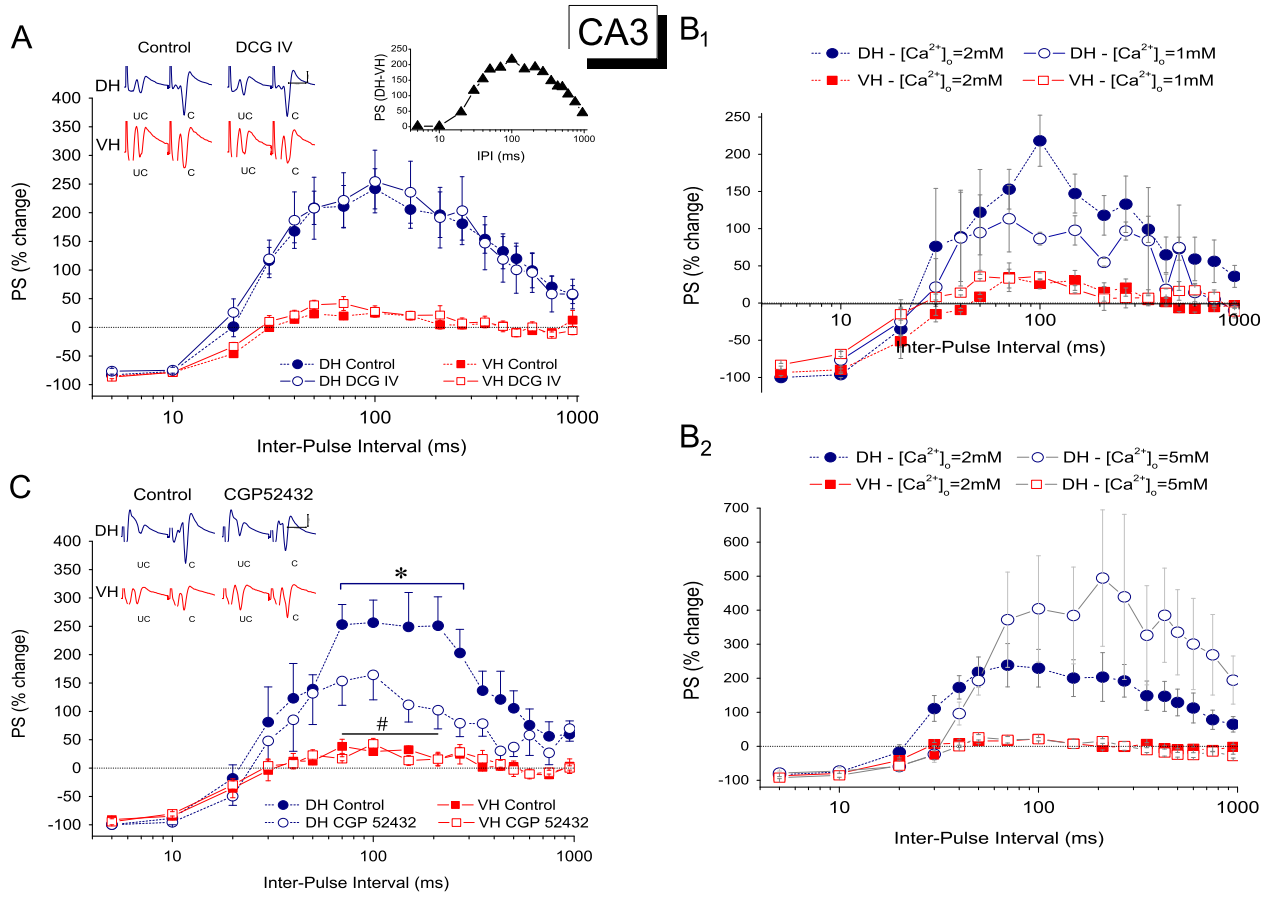

Fig. 4 – The effects of DCG IV (A), $[Ca^{2+}]_o$ (B) and CGP52432 (C) on the O–O paired-pulse facilitation of the PS recorded from the CA3 field of DH and VH are shown. A. Activation of mGluR II by DCG IV (2 μM) did not produce any significant change in the effects of O–O stimulation either in DH or in VH. Examples of pairs of unconditioned and conditioned responses (left and right traces in each condition) for the IPI of 100 ms are shown in the top-left inset. The graph in top right inset represents the difference in facilitation between DH and VH under normal conditions. B. Changing $[Ca^{2+}]_o$ from 2 mM to 1 mM and from 2 mM to 5 mM (B_1 and B_2 respectively) did not significantly affect paired pulse facilitation or inhibition either in DH or VH. C. The antagonist of $GABA_BRs$ CGP52432 (10 μM) significantly reduced paired-pulse facilitation of PS in DH but not VH. Statistically significant drug effects on the facilitation of PS in DH (*, Wilcoxon test, $P<0.05$) as well as significant differences in facilitation between DH and VH under blockade of $GABA_BRs$ (#, Mann–Whitney U test, at $P<0.05$) are shown. Traces in the top-left panel are examples of pairs of unconditioned and conditioned responses (left and right traces in each condition) for the IPI of 100 ms.

stimulation on PS in the CA3 circuit and the effects could be essentially attributed to the activation of the associational fibers of CA3 pyramidal cells only.

Several possible mechanisms could contribute to the dorsoventral differences in facilitation observed in the CA3 circuitry including synaptic facilitation and GABAergic postsynaptic inhibition. It has been previously shown that Schaffer collateral-to-CA1 synapses display higher facilitation in DH than VH suggesting that ventral synapses have higher probability of transmitter release than their dorsal counterparts (Papatheodoropoulos and Kostopoulos, 2000b). We tested this hypothesis by applying the O–O stimulation protocol in dorsal and ventral slices under conditions of normal (2.0 mM), reduced (0.5 mM and 1.0 mM) and increased (5.0 mM) extracellular calcium concentration (Fig. 4B). At the calcium concentration of 0.5 mM the unconditioned response was eliminated and therefore no measurements were possible. Either reducing or increasing $[Ca^{2+}]_o$ produced no statistically significant change in facilitation both in DH and VH, though in the dorsal hippocampus a tendency for increased facilitation at IPIs longer than 70 ms was seen at 5 mM (Fig. 4B_2).

It has been previously shown in CA1 and dentate gyrus that paired-pulse stimulation leads to a depression in the inhibitory postsynaptic potentials which are evoked with the test stimulus as compared with those evoked with the first, conditioning stimulus. The effectiveness of this paired-pulse depression of postsynaptic inhibition maximizes at the time intervals of 100–150 ms and is apparently based on the activity of presynaptic $GABA_BRs$ (Davies et al., 1990; Mott et al., 1993; Nathan and Lambert, 1991). In the present study we found that both the facilitation of PS in the dorsal hippocampus and the dorsoventral difference in facilitation also maximized around the interval of 100 ms. Thus, we examined the possibility that such a mechanism based on $GABA_BRs$ activity could participate to the large dorsoventral difference in the facilitation of PS. We then applied the antagonist of $GABA_BR$ CGP52432

(10 μM) in dorsal and ventral slices. As shown in Fig. 4C, blockade of $GABA_BRs$ produced a remarkable and reversible reduction in the facilitation of PS in dorsal hippocampal slices at the intervals of 70–270 ms, with a minimum of 92±30% at 100 ms and a maximum of 148±23% at 210 ms ($P<0.05$ and $P<0.01$ respectively, Wilcoxon test). Strikingly, the drug did not produce any effect in the ventral slices. Paired-pulse facilitation remained significantly higher in dorsal compared with ventral hippocampus under blockade of $GABA_BRs$. CGP52432 did not produce any significant change in the first (unconditioned) PS of DH (1.44±0.13 mV vs 1.49±0.13 mV in naive and treated slices respectively) or VH (1.38±0.07 mV vs 1.61±0.07 mV in naive and treated slices respectively).

It is interesting that we observed differences also when comparing inhibition and facilitation between DH and CA3. In Fig. 5 we summarized the results obtained with the A–O and O–O stimulation protocols in DG and CA3 of dorsal and ventral hippocampus. Both inhibition and facilitation were similar between the DG and the CA3 of VH whereas in DH both inhibition and facilitation were stronger in the CA3 than in the DG, with facilitation being several times higher in CA3 than DG.

3. Discussion

In this study we examined inhibition and facilitation of the principal cell firing in the dorsal and ventral hippocampal local networks of DG and CA3 by employing two variations of the paired-pulse stimulation, the antidromic–orthodromic (A–O) and the double orhtodromic stimulation (O–O). We found higher early inhibition in the ventral than in the dorsal DG and late inhibition only in the dorsal DG. In addition, the inhibition in the CA3 circuit was similar between DH and VH but facilitation was many folds higher in the dorsal compared with the ventral CA3.

3.1. *Inhibition in DG and CA3*

One of the main findings of the present study was that in the dentate gyrus the early inhibition was lower in DH than in VH while late inhibition appeared only in DH. Furthermore, the strength of inhibition in the CA3 network was fairly similar between DH and VH.

The output of dentate granule cells is controlled by powerful inhibition (Andersen et al., 1966; Scharfman et al., 1990) mediated by local circuits formed between granule cells and GABAergic basket interneurons (Freund and Buzsaki, 1996; Kosaka et al., 1984) either directly or indirectly (Scharfman et al., 1990; Scharfman, 1995). Control of principal cell output may be also provided from axo-axonic cells (Kosaka, 1980). Presumably most of the basket and axo-axonic cells belong to parvalbumin-containing neurons (Freund and Buzsaki, 1996) and participate in powerful inhibitory circuits in the CA3 field as well (Freund and Buzsaki, 1996). Our finding of higher early

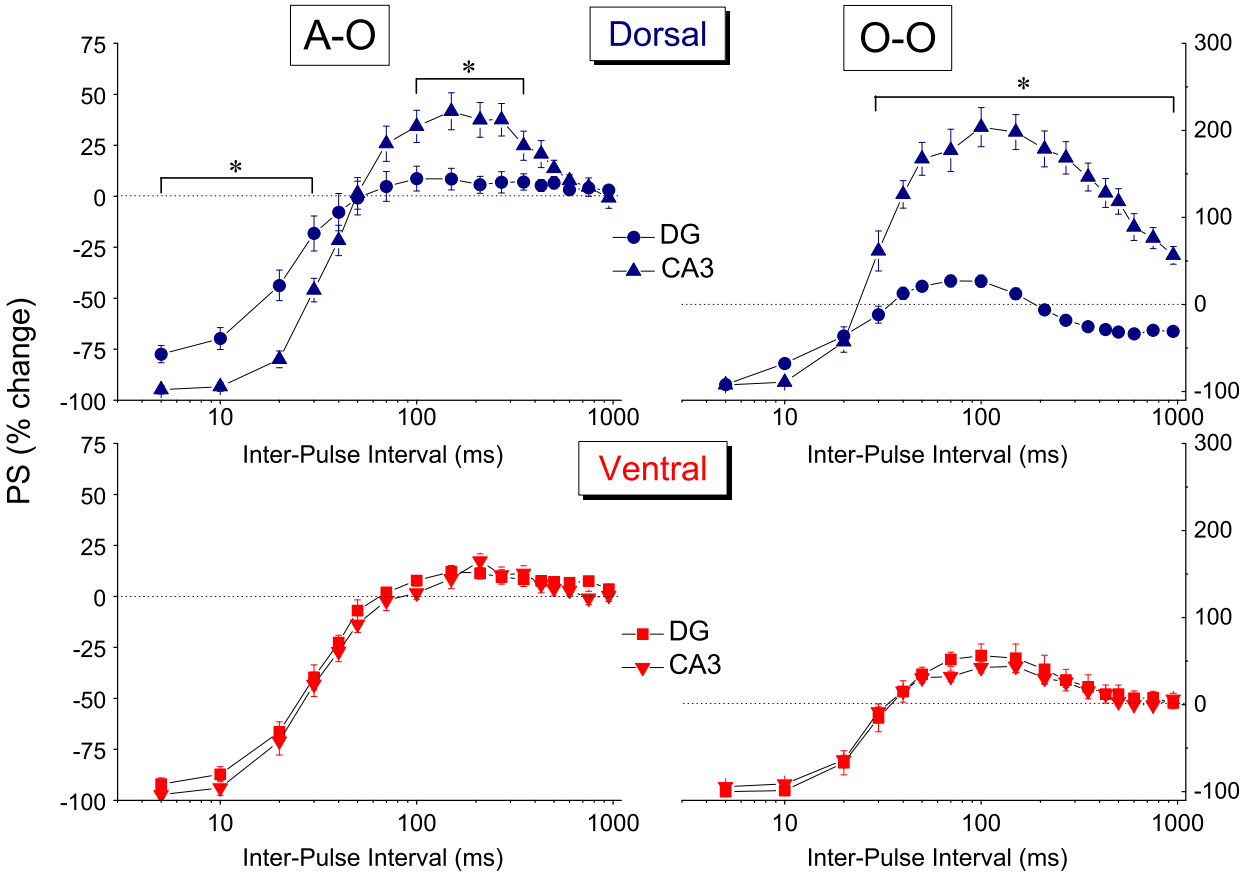

Fig. 5 – Comparisons of the effects of paired-pulse stimulation between DG and CA3, in bnoth DH and VH are shown. Bars with asterisks denote the intervals of statistically significant difference between DG and CA3 (at $P<0.05$). For comparison reasons, similar scales have been used in the graphs for DH and VH.

inhibition in the DG of VH compared with DH is in line with the higher densities as well as absolute numbers of GABAergic neurons in all layers of the ventral compared with the dorsal DG, especially of those neurons that belong to parvalbumin-expressing cells (Jinno and Kosaka, 2006). In addition, in the dorsal DG and in keeping with previous reports (Albertson and Joy, 1987; Bekenstein and Lothman, 1991; DiScenna and Teyler, 1994; Gilbert and Burdette, 1996; Rich-Bennett et al., 1993) we found $GABA_B$ receptor-independent (Albertson and Joy, 1987; Rich-Bennett et al., 1993) late depression. Though it has been suggested that late inhibition in DG might result from the action of NMDA receptor-dependent slow after hyperpolarization (Gilbert and Burdette, 1996) we did not observe any change in late inhibition following blockade of NMDA receptors.

The CA3 circuit is characterized by a dense network of axonal collaterals of pyramidal cells that innervate other pyramidal cells in the same network as well as local interneurons (Freund and Buzsaki, 1996). Thus, "antidromic" as well as "orthodromic" stimulation can excite local GABAergic interneurons that exert a powerful recurrently inhibitory action on pyramidal cell firing with a considerable contribution from parvalbumin-expressing cells (Freund and Buzsaki, 1996). This can explain the strong depression of PS at short intervals observed with both stimulation protocols. Furthermore, the similarity in the depression found between DH and VH is in keeping with previous data that have shown similar densities of parvalbumin-expressing cells in DH and VH CA3 field (Jinno and Kosaka, 2006).

3.2. Facilitation in DG and CA3

We found that paired-pulse facilitation in DG was similar between DH and VH. In sharp contrast however, the facilitation in the CA3 network was much higher in DH than in VH especially with the double orthodromic stimulation.

The facilitation of PS in CA3 and the accompanying dorsoventral differences, may result from a combination of presynaptic and postsynaptic mechanisms including those processes underlying synaptic facilitation as well as the mechanisms that control postsynaptic GABAergic inhibition (Leung and Fu, 1994). It is noted that in CA3 we observed considerable facilitation also with the A–O stimulation. Antidromic stimulation at the alveus might synaptically activate the local network through the abundant associational connections. Consequently, during the A–O stimulation mechanisms of heterosynaptic facilitation based on processes of summation of the postsynaptic potentials can be recruited and contribute to the total amount of facilitation observed.

The facilitation at a given synapse is inversely correlated with the preexisting probability of transmitter release which is strongly affected by the calcium extracellular concentration (Zucker and Regehr, 2002). Previous observations have suggested the probability of transmitter release as a possible mechanism underlying the dorsoventral differences in facilitation of the excitatory synaptic transmission in CA1 (Papatheodoropoulos and Kostopoulos, 2000b). However, the present results in CA3 obtained with the O–O stimulation showed that changes in extracellular calcium concentration do not contribute to the facilitation of PS and therefore differences in transmitter release probability at the associational excitatory synapses do not apparently participate to the differences in PS facilitation found between DH and VH.

Mechanistically, transient depression of postsynaptic inhibition during the O–O paired-pulse stimulation could potentially explain the facilitation of PS observed. Depression of postsynaptic inhibition might result from a reduction in GABA release. In the hippocampus it is well established that inhibition of GABA release occurs through activation of presynaptic $GABA_B$ autoreceptors (Gassmann and Bettler, 2012). Previous studies in CA1 have shown that during paired-pulse stimulation the $GABA_B$R-mediated reduction in GABA release induced by the conditioning stimulus, leads to depression of fast ($GABA_A$R-mediated) and slow ($GABA_B$R-mediated) inhibitory postsynaptic potentials (Davies et al., 1990; Nathan and Lambert, 1991). Interestingly, this depression is highest when the test stimulus is given 100–150 ms after the conditioning one. This range of intervals coincides with that of maximum PS facilitation in DH. The present finding of depressed facilitation induced by blockade of $GABA_B$ receptors suggest that depression of postsynaptic inhibition mediated by presynaptic $GABA_B$ receptor activity might significantly participate to the facilitation. Most importantly, we found that the $GABA_B$R-dependent mechanism contributed to the strong facilitation observed in the dorsal hippocampus but was not involved in the much lower facilitation of the ventral hippocampus suggesting that this mechanism is much more effective in the dorsal than the ventral CA3 associative circuit. Taking into account the powerful gating action of this mechanism, the consequences for the functional diversification of the intrinsic network between DH and VH become obvious. Apparently, information processing involving gating and amplification by the CA3 network are performed more efficiently by the dorsal than the ventral segment of the hippocampus.

The difference in facilitation of PS remaining after the blockade of $GABA_B$Rs between DH and VH could be attributed to differences in synaptic facilitation at the synapses of CA3 associational fibers similar to the dorsoventral difference in synaptic facilitation observed at Schaffer collateral-CA1 synapses (Papatheodoropoulos and Kostopoulos, 2000b).

3.3. Implications for the information processing in DH and VH

Functional differences in the intrinsic circuit between DH and VH include cell properties (Dougherty et al., 2012; Dougherty et al., 2013) synaptic transmission and plasticity (Colgin et al., 2004; Maggio and Segal, 2007; Maggio and Segal, 2009; Maruki et al., 2001; Mikroulis and Psarropoulou, 2012; Papatheodoropoulos and Kostopoulos, 2000a; Papatheodoropoulos and Kostopoulos, 2000b; Petrides et al., 2007), place cell firing (Kjelstrup et al., 2008; Maurer et al., 2005) and network oscillations (Derchansky et al., 2004; Gilbert et al., 1985; Patel et al., 2012; Schmidt et al., 2013). We hypothesize that the elucidation of the differences in the organization of the intrinsic network between DH and VH will promote the understanding of the three-dimensional spatiotemporal processing of information inside the hippocampus.

The DG is the gate of hippocampal formation for incoming cortical information subserving the storage and recall of episodic memories (Kesner, 2007b; Rolls, 2007). On the other hand, the CA3 field plays an important role in learning and memory acting as an autoassociation network that provides the

substrate for episodic memories to be formed, maintained and retrieved in the CA3 circuit (Kesner, 2007a; Rolls, 2007). This function of the CA3 circuit is based on the network of the extensive associational connections among its pyramidal cells, which also supports the retrieval of the whole memory through the process of pattern completion (Kesner, 2007a).

Inhibition importantly contributes to the functions supported by DG and CA3 networks (Gilbert and Brushfield, 2009). Taking into account that recurrent inhibition in DG is reduced during sensory activation (Herreras et al., 1988) the higher early inhibition in the ventral DG and the existence of late depression exclusively in the dorsal DG might imply that it is easier for fast-frequency inputs but more difficult for slow inputs to pass from the dorsal as compared with the ventral DG.

The present results on network inhibition in DG and CA3 in combination with previous observations showing a higher inhibition in the dorsal as compared with the ventral CA1 circuit (Papatheodoropoulos et al., 2002; Petrides et al., 2007) reveal a pattern of gradual increase in the ratio of inhibition between DH and VH, along the trisynaptic circuit of the hippocampus. Thus, the DH/VH ratio of inhibition in DG, CA3 and CA1, calculated as the ratio of percentage depression of PS at the IPI of 10 ms, is 0.8, 1.0 and 1.4 respectively. It is thought that the connections in both transverse and longitudinal axes of the hippocampus (Witter and Amaral, 2004) participate in the information flow through the hippocampus. Taking into account that early depression displays a pattern of gradual changes along the dorso-ventral axis, it seems plausible that the entry and output of fast-frequency inputs into and from the hippocampus differ between its dorsal and ventral regions. This might have important implication in the way we think about information processing in the hippocampal formation. The higher facilitation of excitatory input in the dorsal compared with the ventral CA3 circuit in combination with the lower constrain of input from the corresponding DG could suggest that information in the DG-to-CA3 circuit is gated and short-term amplified more reliably in DH than in VH. In addition, the differences in inhibition and facilitation across the DG-to-CA3 circuit in the dorsal but not the ventral hippocampus suggest that the dorsal circuit might have a wider dynamic range of information processing.

In summary, the findings of the present study imply that the dorsal DG favors the passage of fast-frequency inputs ($\geq$ 50 Hz) whereas the ventral DG favors the passage of slow-frequency inputs ($\leq$ 3 Hz). In addition, the gating and amplification of excitatory input in the circuit of CA3 field through the process of facilitation that involves $GABA_BR$ activity is more prominent in the dorsal hippocampus.

4. Experimental procedure

4.1. Slice preparation

Slices were prepared from adult male Wistar rats (40–60 days-old). All experimental procedures were made in accordance to the European Communities Council Directive Guidelines (86/609/EEC) for the care and use of Laboratory animals and they have been approved from the animal subject review board of our institution. In addition, all efforts have been made to minimize the number of animals used and their suffering. Transverse slices were prepared from the dorsal (DH) and the ventral (VH) hippocampus extending more than 1.0 and less than 4.0 mm from either end of the structure as previously described (Papatheodoropoulos and Kostopoulos, 2000a; Petrides et al., 2007). Specifically, animals were decapitated, after deep anesthesia with diethyl-ether, their brains were removed and placed in chilled (2–4 °C) standard artificial cerebrospinal fluid containing (in mM): 124 NaCl; 4 KCl; 2 $MgSO_4$; 2 $CaCl_2$; 1.25 NaH_2PO_4; 26 $NaHCO_3$; 10 glucose, at pH 7.4 and equilibrated with 95% O2 and 5% CO2 gas mixture. Both hippocampi were excised free and 500–550 μm thick slices were prepared using a McIlwain tissue chopper. After preparation, the slices were immediately transferred to an interface recording chamber, continuously perfused with ACSF at a constant temperature of 32±0.2 °C and humidified with 95% O_2 and 5% CO_2 gas. Slices were left to equilibrate for at least one hour after their preparation before recordings started.

4.2. Electrophysiology

Evoked field potentials were recorded from the granule cell layer of the DG and from the pyramidal cell layer of the CA3b field using carbon fiber electrodes (diameter 7 μm, Kation Scientific, Minneapolis, USA). Orthodromic (i.e. synaptically evoked) population spikes (PS) in DG and CA3 were recorded after electrical stimulation in the middle of the molecular layer of the DG and the region between hilus and CA3c respectively (S1 and S4 in Fig. 1 respectively). Antidromic activation of the granule cells and CA3 pyramidal cells was achieved by positioning the stimulation electrode at the hilus and the alveus respectively (S2 and S5 in Fig. 1 respectively). In a set of experiments antidromic activation of granule cells was performed by a stimulation electrode positioned at the stratum lucidum of the CA3b subfield (S3 in Fig. 1). In order to distinguish between the two different stimulation electrode positions used for antidromic activation of granule cells we will refer to “close” and “remote” antidromic stimulation for electrode position at the hilus and st. lucidum respectively. Both orthodromic and antidromic population spikes were quantified by their amplitude. The amplitude of PS was measured as the length of the projection of the negative peak on the line connecting the two positive peaks of the PS waveform while the amplitude of the antidromically evoked population spike was measured as the difference between the negative peak of the waveform and the baseline. Stimuli (0.1 ms) were delivered at a frequency of 0.05 Hz using bipolar electrodes. We used two versions of the paired-pulse stimulation paradigm, the antidromic–orthodromic (A–O) and the orthodromic–orthodromic (O–O) stimulation (Georgopoulos et al., 2008). In the A–O protocol, the strength of the antidromic (conditioning) stimulus was set to evoke an antidromic spike at 75% of maximum amplitude and the strength of the orthodromic stimulus was adjusted to produce a half-maximum orthodromic PS. In the O–O protocol the strength of both stimuli was set to produce a PS at 75% of maximum amplitude. The effect of the antidromic conditioning stimulus to the orthodromic test response was quantified by the percentage of the test response with respect to the unconditioned orthodromic response (i.e. the response evoked without a conditioning stimulus). In the O–O protocol, the effect of the conditioning stimulus on the conditioned response was

quantified by the percent change of the conditioned in respect to the unconditioned response.

4.3. *Drugs*

In the present study the following drugs were used: the agonist of group II metabotropic glutamate receptors (mGluR II) (2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine (DCG IV, 2 μM), the antagonists of $GABA_B$ receptor (3-aminopropyl)(diethoxymethyl)phosphinic acid (CGP35348, 200–500 μM), 3-[[(3,4-Dichlorophenyl)methyl]amino]propyl] diethoxymethyl) phosphinic acid (CGP52432, 10 μM), and the competitive antagonist of NMDA receptor 3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10 μM). All substances were purchased from Tocris (Tocris Cookson Ltd, UK). Drugs were first prepared as stock solutions and then were dissolved in standard medium and bath applied to the tissue.

4.4. *Statistics*

The Student's independent and paired *t*-tests and the Mann–Whitney *U* test and Wilcoxon test were used to evaluate statistical significance of the differences between two groups. A Univariate Analysis of Variance (ANOVA), or a Linear Mixed Model Analysis when the Univariate ANOVA assumptions were violated, was employed when multiple comparisons were necessary. Values throughout the text are expressed as mean ± S.E.M. and "*n*" indicates the number of slices included in the analysis.

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