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Bicuculline-resistant paired-pulse inhibition in the rat hippocampal slice

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1 An initial observation that paired-pulse inhibition in hippocampal slices was increased rather than decreased by bicuculline prompted the present study to explore the mechanism underlying bicuculline-resistant inhibition.

2 In the presence of bicuculline, paired-pulse interactions were dependent on the interpulse interval (i.p.i.) but a medium-latency inhibition was consistently observed at an i.p.i. of 300 to 500 ms.

3 The medium-latency (300 ms) bicuculline-resistant inhibition produced by paired orthodromic stimuli was substantially reduced by 2-hydroxysaclofen and was probably largely mediated by $GABA_B$-receptor activation. Paired-pulse inhibition produced by an orthodromic/antidromic stimulation sequence was not affected by 2-hydroxysaclofen suggesting the possibility that the $GABA_B$-receptors involved in orthodromic inhibition may be located presynaptically on the Schaffer collateral terminals rather than on the postsynaptic surface. The medium latency inhibition was also reduced by baclofen and under some conditions, by adenosine.

4 In addition to the $GABA_B$-component, a hydroxysaclofen-resistant depression of postsynaptic excitability contributed to bicuculline-resistant paired-pulse inhibition at the 300 ms latency.

Keywords: Paired-pulse facilitation; paired-pulse inhibition; bicuculline; hippocampus; adenosine; 2-hydroxysaclofen; baclofen

Introduction

Paired-pulse effects in the hippocampus (facilitation and inhibition) between extracellularly-recorded field potentials (single population spikes or the corresponding field e.p.s.ps) have been well characterized both *in vivo* and *in vitro*. In the CA1 region, in the hippocampal slice preparation facilitation may be seen when both conditioning and test stimuli activate the same Schaffer collateral/CA1 dendritic synapses and the inter-pulse interval (i.p.i.) between conditioning and test stimuli is greater than about 40 ms. Paired-pulse facilitation can sometimes be seen up to an i.p.i. of several seconds (Creager *et al.*, 1980; Dunwiddie *et al.*, 1980). Conversely, inhibition is most easily demonstrated at i.p.is less than 40 ms (Creager *et al.*, 1980; Dunwiddie *et al.*, 1980; Lynch *et al.*, 1981).

Paired-pulse inhibition is thought to be mainly or completely mediated by γ-aminobutyric acid (GABA)-releasing feedback and feedforward interneurones and it was therefore surprising to find, as part of another investigation, that the $GABA_A$ antagonist, bicuculline, increased paired-pulse inhibition in the CA1 area at moderately long (> 300 ms) i.p.is. In the presence of bicuculline, repetitive firing of pyramidal cells occurs following a single stimulation to the Schaffer collateral fibres. However, the prolonged population potential that reflects this multiple firing is still only about 30 ms in duration and seems unlikely to account for increased inhibition at a latency of 300 ms. Although there is a good deal of relevant information on inhibitory potentials, both in the normal and the disinhibited hippocampus, from work using intracellular recording techniques, paired-pulse interactions between extracellularly recorded potentials in the disinhibited hippocampus do not seem to have been studied systematically. The present study was therefore designed to characterize the action of bicuculline on paired-pulse interactions between these extracellular potentials in more detail and in particular to investigate the mechanisms of this medium-latency inhibition.

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Methods

Male Wistar rats (170 to 220 g) were anaesthetized with urethane and cooled on ice whilst breathing oxygen enriched air (Newman *et al.*, 1992). When their rectal temperatures reached 30°C (about 15 min) they were killed by cervical dislocation.

Hippocampal slices were prepared 450 μm thick in artificial CSF (ACSF) of composition (mmol l^{-1}): KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10, saturated with 95% O_2:5% CO_2.

For recording, individual slices were submerged and superfused at 30°C. Drugs were added to the superfusing ACSF. Extracellular population potentials were recorded by single glass microelectrodes in the CA1 pyramidal cell layer. Concentric bipolar stimulating electrodes were placed in the stratum radiatum around the CA1/CA2 junction for orthodromic activation of pyramidal cells and in the alveus for antidromic activation. When the paired stimuli were both orthodromic they were delivered through the same electrode. Evoked responses were amplified, displayed on digital storage oscilloscopes and averaged (8 sweeps) before being plotted onto a chart recorder.

In general, single and double stimuli were alternated: test stimuli, orthodromic or antidromic, adjusted to evoke a population spike between 45% and 75% of maximum, were delivered at 0.1 Hz or 0.05 Hz and conditioning stimuli were delivered before alternate test stimuli. Responses to test stimuli which were preceded by a conditioning stimulus are designated T_{pair}. Responses to unpaired test stimuli are designated T_{single} and responses to conditioning stimuli are designated S_{cond}.

A preliminary series of experiments was carried out to examine the effects of bicuculline on paired-pulse interactions at various interpulse intervals (i.p.is) using orthodromic stimuli only.

A second series of experiments, all at an i.p.i. of 300 ms, was then carried out to examine the effect of agents on the paired-pulse inhibition seen at this latency in the presence of bicuculline. In an attempt (see Discussion) to separate pre- and postsynaptic effects (i.e. on the pyramidal cells or on the Schaffer collateral terminals), for these second experiments, we used two different stimulation paradigms, the standard

(ortho/ortho) paradigm with both conditioning and test stimuli orthodromic and a second arrangement (ortho/anti) in which the conditioning stimulus was orthodromic and the test stimulus antidromic. When assessing the action of agents on paired-pulse inhibition at this latency stimuli were adjusted in the presence of the agent, if necessary, to return T_{single} and S_{cond} to their control size (all test stimuli were still submaximal). To be certain that changes in inhibition were not, nevertheless, due to small changes in the excitatory conditioning potential, inhibition was measured with two strengths of conditioning stimulus, supramaximal (1 mA) and submaximal – producing in bicuculline a potential approximately 50 to 70% of maximal size.

In all experiments facilitation or inhibition was expressed as the percentage change in T_{pair} compared with T_{single}. Response size was taken to be the size of the population spike measured from the peak positivity to the peak negativity. In the presence of bicuculline, response size was taken to be the peak to peak size of the first population spike in each burst. Unless stated otherwise statistical analysis was by paired t test with $P<0.05$ taken to indicate statistical significance.

Slices were only accepted for experiment if the evoked maximum orthodromic population spike in ACSF after 15 min in the recording chamber was at least 4 mV and free from secondary spikes.

Baclofen, and adenosine hemisulphate were obtained from Sigma Biochemicals Ltd., (−)-bicuculline methobromide, and 2-hydroxysaclofen from Research Biochemicals Inc.

Results

Effect of bicuculline on paired-pulse interactions

In the absence of bicuculline, orthodromic stimuli normally exhibited paired-pulse inhibition at short i.p.is (less than 50 ms) and facilitation at longer i.p.is. The i.p.i. at which inhibition changed to facilitation was quite variable between slices ranging from less than 30 ms to over 300 ms (Figure 1).

As expected, in the presence of bicuculline methobromide (10 μmol l^{-1} or greater) a short burst of several population spikes replaced the single population spike seen in response to a stimulus in the absence of drugs. Orthodromic paired-pulse interactions in bicuculline also depended on the interpulse interval, both facilitation and inhibition being demonstrable. With a supramaximal conditioning stimulus two temporally distinct phases of inhibition were seen (Figure 1, solid squares). At i.p.is less than 50 ms and between 300 and 900 ms bicuculline 10 μmol l^{-1} significantly increased inhibition or decreased facilitation compared with drug-free control in the same slices. However, when the i.p.i. was 100 ms the slices showed significantly increased facilitation in the presence of bicuculline (Figure 1). At i.p.is longer than 900 ms there was a second phase of facilitation.

Effect of 2-hydroxysaclofen, baclofen and adenosine on inhibition in the presence of bicuculline

Control values All slices which showed ortho/ortho inhibition at an i.p.i. of 300 ms with supramaximal conditioning stimuli in the presence of 10 μmol l^{-1} bicuculline also showed inhibition with submaximal conditioning stimuli and with all stimuli in the ortho/anti paradigm. Inhibition in the ortho/anti paradigm was generally much less than inhibition in the ortho/ortho paradigm at either submaximal or supramaximal conditioning stimulus strength (see Figures 3, 4 and 5). In the ortho/ortho sequence inhibition was significantly less when the conditioning stimulus was supramaximal rather than submaximal ($P<0.05$, $n=22$ slices).

2-Hydroxysaclofen Superfusion with the $GABA_B$-antagonist 2-hydroxysaclofen 200 μmol l^{-1} (in the presence of bicuculline at i.p.i. = 300 ms) significantly reduced ortho/ortho

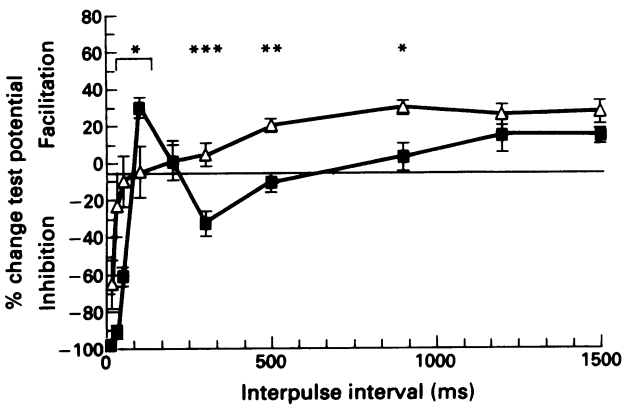

Figure 1 Influence of bicuculline methobromide 10 μmol l^{-1} on paired-pulse interactions at i.p.is up to 1500 ms, $n=9$ slices with each point being the mean of between 4 and 9 values. Paired pulse changes were recorded in the same slices in the absence (△) and presence (■) of bicuculline. The conditioning stimulus was supramaximal at 1 mA. The unpaired test stimulus was readjusted to give a response between roughly 50 and 70% of maximum (constant within each experiment) in the presence of bicuculline. All stimuli were orthodromic. Values are mean ± s.e.mean. $*P<0.05$; $**P<0.01$; $***P<0.001$ for difference between ACSF and bicuculline.

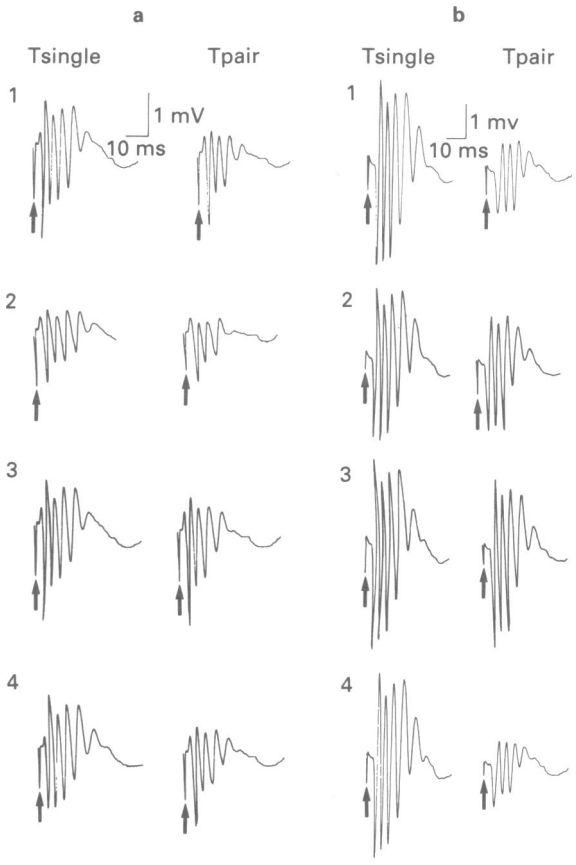

Figure 2 Paired-pulse inhibition between evoked potentials in bicuculline 10 μmol l^{-1}, i.p.i. = 300 ms. All stimuli are orthodromic. Conditioning stimuli and unpaired test stimuli are submaximal. Each potential is the average of eight responses. (a) Effect of baclofen 1 μmol l^{-1}. (b) Effect of 2-hydroxysaclofen 200 μmol l^{-1}. Sequence shows: (1) control values; (2) effect of drug with stimuli unadjusted from control; (3) effect of drug with stimuli adjusted to return S_{cond} and T_{single} to control size; (4) wash with stimulus strength readjusted again in (a). Arrows mark stimulus artifacts. Calibration bars 1 mV and 10 ms.

inhibition both when the conditioning stimulus was supramaximal ($P<0.05$) and submaximal ($P<0.01$, $n=6$ slices) (Figures 2 and 3). In some slices inhibition was reversed to facilitation in the presence of 2-hydroxysaclofen. The effect of 2-hydroxysaclofen partially reversed on washing the drug from the recording chamber.

By contrast, 2-hydroxysaclofen had no significant effect on postsynaptic ortho/anti inhibition in the same slices under the same conditions (Figure 3). As control inhibition in the two paradigms was different, the proportional change in inhibition caused by 2-hydroxysaclofen relative to the respective control was compared by the Wilcoxon matched pairs test between the two paradigms. The difference between the effect of 2-hydroxysaclofen on ortho/ortho and ortho/anti inhibition was significant ($P<0.05$) for both supramaximal and submaximal conditioning stimuli.

Baclofen Baclofen 1 μmol l^{-1}, a $GABA_B$-agonist, also markedly and significantly reduced inhibition in the ortho/ortho paradigm both when the conditioning stimulus was supramaximal ($P<0.01$) and submaximal ($P<0.001$, $n=7$ slices) (Figures 2 and 4). The effect of baclofen partially reversed when the drug was washed from the recording chamber.

Baclofen at this concentration had no significant effect on the ortho/anti inhibition. The difference between the effect of baclofen on ortho/ortho and ortho/anti inhibition was significant ($P<0.05$ Wilcoxon matched pairs test) for both maximal and submaximal conditioning stimuli when the proportional change in inhibition caused by baclofen relative to the respective control was compared between the two paradigms.

Although the effects of baclofen and 2-hydroxysaclofen were similar in some respects, baclofen initially decreased both T_{single} and T_{pair} (but T_{single} more than T_{pair}) before the stimulation strength was adjusted, whereas 2-hydroxysaclofen increased the size of T_{pair} (Figure 2).

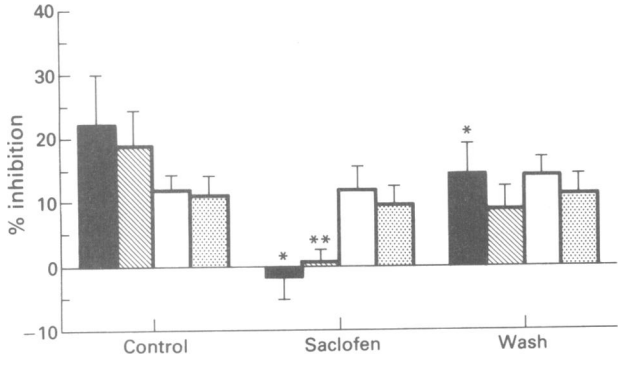

Figure 3 Effect of 2-hydroxysaclofen 200 μmol l^{-1} on paired-pulse inhibition at an interpulse interval of 300 ms in the presence of bicuculline methobromide 10 μmol l^{-1}. Both ortho/ortho and ortho/anti stimulation paradigms were used (see text) and each was repeated with a supramaximal (1 mA) conditioning stimulus and a submaximal conditioning stimulus set to give a response between 50 and 70% of maximum (constant within each experiment). The unpaired test stimuli were also set to give a response between 50 and 70% maximum. The unpaired test stimuli and the submaximal conditioning stimuli were re-adjusted if necessary in the presence of 2-hydroxysaclofen and after washout to keep them as constant as possible. Solid columns show ortho/ortho responses with conditioning stimulus supramaximal; cross hatched columns show ortho/ortho responses with conditioning stimulus submaximal; open columns show ortho/anti responses with conditioning stimulus supramaximal; stippled columns show ortho/anti responses with conditioning stimulus submaximal. $n=6$ slices. Values are mean ± s.e.mean. $*P<0.05$; $**P<0.01$ for difference between drug and control or wash and drug.

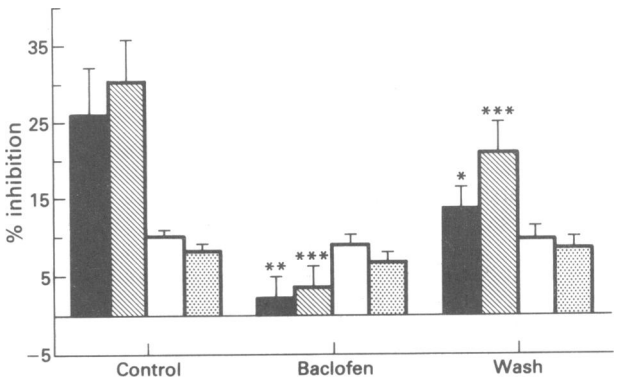

Figure 4 Effect of baclofen 1 μmol l^{-1} on paired-pulse inhibition at an interpulse interval of 300 ms in the presence of bicuculline methobromide 10 μmol l^{-1}, $n=7$ slices. See Figure 3 for details. Values are mean ± s.e.mean. $*P<0.05$; $**P<0.01$; $***P<0.001$ for difference between drug and control or wash and drug.

Adenosine The effect of adenosine 20 μmol l^{-1} on paired-pulse inhibition was examined at an i.p.i. of 300 ms in the presence of bicuculline 10 μmol l^{-1}. Adenosine (Figure 5) had no significant effect on ortho/anti inhibition with maximal or submaximal conditioning stimuli or on ortho/ortho inhibition when the conditioning stimulus was maximal (Figure 5).

With a submaximal conditioning stimulus adenosine significantly ($P<0.01$, $n=9$ slices) and reversibly reduced inhibition in the ortho/ortho paradigm (Figure 5). Adenosine, like baclofen, markedly reduced the size of both T_{single} and T_{pair} before the stimuli were readjusted to their control amplitude.

Discussion

In the presence of bicuculline, paired-pulse inhibition was markedly increased at short and intermediate i.p.is compared with the bicuculline-free control slices. In bicuculline, evoked potentials are prolonged to 30 ms or more as a result of repetitive firing of pyramidal cells (see Figure 2). However, the later inhibition cannot be explained by refractoriness of the postsynaptic membrane since it follows a period of facilitation at 100 ms.

In CA1 area of the normal hippocampus, pyramidal cell inhibitory postsynaptic potentials (i.p.s.ps) consist of two components, a short-latency $GABA_A$-mediated component

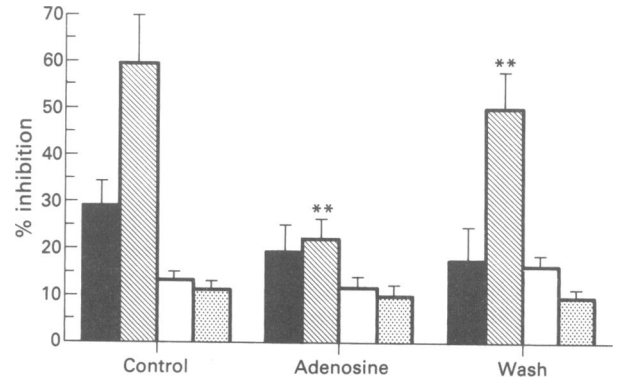

Figure 5 Effect of adenosine 20 μmol l^{-1} on paired-pulse inhibition at an interpulse interval of 300 ms in the presence of bicuculline methobromide 10 μmol l^{-1}, $n=9$ slices. See Figure 3 for details. Values are mean ± s.e.mean. $**P<0.01$ for difference between drug and control or wash and drug.

and a longer latency, longer duration $GABA_B$ component (Alger & Nicoll, 1982a,b; Newberry & Nicoll, 1984; 1985; Peet & McLennan, 1986; Dutar & Nicoll, 1988; Davies *et al.*, 1990; Lambert *et al.*, 1991a). Also, under certain conditions, a long-lasting $GABA_A$-mediated depolarizing inhibitory potential may be seen (Alger & Nicoll, 1982a,b; Newberry & Nicoll, 1985; Avoli & Perreault, 1987; Lambert *et al.*, 1991a). In the presence of $GABA_A$ antagonists $GABA_B$-i.p.s.ps recorded in the pyramidal cells are enhanced and merge with a prolonged K_{Ca}-mediated membrane after-hyperpolarization (Schwartzkroin & Stafstrom, 1980; Newberry & Nicoll, 1984). This is probably the result of increased activation of recurrent interneurones secondary to repetitive pyramidal cell firing as well as increased activation of feedforward interneurones which will be subject to decreased $GABA_A$-mediated inhibition (Lacaille, 1991).

The fact that 2-hydroxysaclofen caused a marked reduction in ortho/ortho inhibition in the present work would support the view that it was at least partially mediated by $GABA_B$ receptors. The time course of the inhibition is also consistent with $GABA_B$ activation. Davies *et al.* (1990) measured a latency to onset of 29 ms, with a peak at 135 ms and duration of 723 ± 135 ms for pure isolated $GABA_B$ i.p.s.ps on CA1 pyramidal cells.

The ortho/anti paradigm was used to examine pyramidal cell excitability 300 ms after an orthodromic conditioning stimulus. A small but consistent inhibition was demonstrated. Since this was not significantly reduced by 2-hydroxysaclofen (or by baclofen or adenosine which also reduced ortho/ortho inhibition), this postsynaptic decrease in excitability may be non-GABA-mediated. Furthermore this leaves unresolved the question of the location of the $GABA_B$ receptors which are involved in paired-pulse inhibition at this latency. In addition to $GABA_B$ receptors located postsynaptically on the CA1 pyramidal cells, there are known to be presynaptic $GABA_B$ receptors whose activation inhibits excitatory transmission at the Schaffer collateral terminals (Blaxter & Carlen, 1985; Harrison *et al.*, 1990). It is possible these mediate at least part of the 2-hydroxy-saclofen sensitive component.

The reduction in ortho/ortho inhibition produced by baclofen, a $GABA_B$ agonist also suggests that this inhibition is substantially mediated by GABAergic interneurones. In the absence of other drugs, baclofen is known to decrease paired-pulse inhibition of single population spikes in the CA1 area (Steffensen & Henriksen, 1991; Ault & Nadler, 1983). It acts at receptors on interneurone terminals to reduce GABA release (Davies *et al.*, 1990; Lambert *et al.*, 1991b; Nathan & Lambert, 1991; Thompson & Gahwiler, 1992) and depresses interneurone excitability by inducing hyperpolarization (Madison & Nicoll, 1988; Misgeld *et al.*, 1989). The receptors mediating this effect are also involved in synaptic transmission to the interneurones since slow, $GABA_B$-mediated i.p.s.ps have been recorded from hippocampal interneurones (Segal, 1990; Lacaille, 1991).

Adenosine, like baclofen, decreases excitatory synaptic transmission and hence the evoked orthodromically evoked population spike in the hippocampus, at least in part by a presynaptic mechanism (Okada & Ozawa, 1980; Corradetti *et al.*, 1984; Burke & Nadler, 1988; Yoon & Rothman, 1991; Thompson *et al.*, 1992). Adenosine, however, has virtually no presynaptic action to reduce GABA release from interneurone terminals: it does not affect monosynaptic i.p.s.ps resulting from the direct stimulation of interneurones (Lambert & Teyler, 1991; Yoon & Rothman, 1991; Thompson *et al.*, 1992) or the potassium-evoked release of GABA from cortical slices except at millimolar concentration (Hollins & Stone, 1980). In the absence of other drugs adenosine has been reported to increase paired-pulse facilitation of the field excitatory postsynaptic potential by a presynaptic action at the Schaffer collateral terminals (Dunwiddie & Haas, 1985). We were thus interested in the effect of adenosine on paired-pulse inhibition in the presence of bicuculline.

The effect of adenosine was less clear-cut than that of baclofen. Although it reversibly reduced ortho/ortho inhibition when the conditioning stimulus was submaximal it was apparently not effective when the conditioning stimulus was supramaximal (Figure 5). It is possible that adenosine is working through a different mechanism from baclofen.

In summary, following an observation in rat hippocampal slices that paired pulse inhibition at moderately long i.p.is was increased by the $GABA_A$ antagonist bicuculline, the mechanism of the inhibition has been examined in more detail. The medium latency (300–500 ms) inhibition was found to be substantially mediated by 2-hydroxysaclofen-sensitive $GABA_B$ receptors which did not influence antidromic excitability and may not therefore be located postsynaptically. A decrease in postsynaptic excitability which was resistant to both bicuculline and 2-hydroxysaclofen contributed to paired-pulse inhibition at this latency.

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