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Research report

Effects of lidocaine reversible inactivation of the median raphe nucleus on long-term potentiation and recurrent inhibition in the dentate gyrus of rat hippocampus

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Abstract

Considering the fact that median raphe nucleus (MRN) constitutes one of the inputs of the hippocampus, the effects of reversible inactivation of MRN on long-term potentiation (LTP) and recurrent inhibition in the dentate gyrus (DG) of rat hippocampus, in vivo, were examined. Rats were anesthetized with urethane (1.5 g/kg, i.p.). MRN was temporarily suppressed by intra-MRN injection of lidocaine (0.5 μl, 2%). For LTP induction, eight episodes of high frequency stimuli (100 Hz) were delivered to the perforant path (PP), each consisting of 10 stimuli at 100 Hz. Population spikes (PS) and population excitatory post synaptic potentials ($_{p}$EPSP) in DG were recorded 10 min before, and 5, 10, 20, 40, 60 and 120 min after tetanization. MRN inactivation itself had no effect on the amplitude of baseline responses. The PS amplitude and $_{p}$EPSP slope in rats, injected with intra-MRN lidocaine, 5 min before tetanization, were not different from the control group. However, at 120 min PS amplitude was significantly higher than control. Lidocaine injection 5 min after tetanic stimuli caused a significant decrease in PS amplitude (10, 20 and 60 min) and $_{p}$EPSP slope (20 and 40 min) after tetanization. The data showed that inactivation of MRN has no effect on LTP induction in the DG of hippocampus but it does affect its maintenance, and this effect depends on the pre- or post-tetanic inactivation. In the last part of this study, in order to investigate the effect of MRN on the efficacy of recurrent inhibition in the perforant–dentate synapses, paired pulse was applied to the PP at 10 and 20 ms interpulse intervals. Inactivation of MRN increased the amount of recurrent inhibition in the DG with 20 ms interpulse interval. This observation indicates that MRN inhibits the recurrent inhibition mechanism, which is in accordance with the suggested role of MRN neurons on inhibition of hippocampal GABAergic interneurons.

Theme: Neural basis of behavior

Topic: Neural plasticity

Keywords: Median raphe nucleus; Reversible inactivation; Synaptic plasticity; Long term potentiation; Recurrent inhibition

1. Introduction

The cell bodies of serotonergic afferents of the forebrain are in the dorsal and median raphe nuclei of the midbrain and upper pons [38]. Ascending projections of these two nuclei travel through the medial forebrain bundle [36]. Projections from MRN reach hypothalamic and thalamic nuclei, basal ganglia, septal area, cerebral cortex and the hippocampus [36]. The MRN projects predominantly to the hippocampus and medial septal area (MSA) via the fasciculus cinguli and the fimbria fornix [3,23,36]. In addition, a significant population of MRN neurons sends

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collateral projections to both the hippocampus and MSA [19].

Moreover, it has been demonstrated that the serotonergic system has a powerful influence on hippocampal electrical activity. Buspiron, a partial 5-HT_{1A} agonist, reduces theta activity following midbrain stimulation [7]. Working with anaesthetized rats, Richeter-Levin and Segal [27] showed that 5-HT suppresses spontaneous activity in dentate gyrus granule cells while enhancing their excitability to afferent stimulation. Lesions of the MRN facilitate hippocampal low frequency theta activity in freely moving rats [8].

Long-term potentiation (LTP) is characterized by a long-lasting increase in synaptic efficacy following a brief, high frequency (tetanic) electrical stimulation of afferent fibers. This phenomenon was first reported in the perforant path–dentate gyrus synapse [5], and has since been found in many other pathways in the hippocampal formation. The long duration of LTP, combined with the well-known involvement of the hippocampus in memory formation, has prompted its consideration as a model of long-term information storage in the brain [37].

To date, few studies have addressed the MRN effect on LTP in the hippocampus. Klancnick and Philips [17] have found that tetanic stimulation of MRN facilitates the induction of long-term potentiation in the dentate gyrus. This is consistent with results obtained by Bliss et al. [4] showing that depletion of 5-HT by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT) or *para*-chlorophenylalanine (PCPA) reduces the amount of LTP in the DG of rats anaesthetized with pentobarbitone. On the other hand, Stanton and Sarvey [34] have shown that depletion of 5-HT by 5,7-DHT had no effect on LTP in the DG of hippocampal slices. In the lesion studies, the serotonergic neurons were permanently lesioned and then their role in LTP was evaluated. It is impossible to demonstrate that a lesion affects induction of LTP or its maintenance. These difficulties can be avoided by temporary inactivation techniques that allow the examined structure to be eliminated during a specific phase for a short time. The aim of the present study was to examine the effect of MRN inactivation on induction and maintenance of LTP and recurrent inhibition in the dentate gyrus of the hippocampus.

2. Materials and methods

2.1. Animals

Naive adult male albino rats weighing 350–400 g were obtained from the breeding colony of our institute. They were housed five per cage and maintained at constant temperature on a standard 12:12 h light/dark cycle with lights on at 07:00 h. Food and water were freely available while the animals adapted to the laboratory.

2.2. Surgery

Animals were deprived of food and water for 12 h prior to surgery. The animals were anesthetized with urethane (1.5 g/kg, i.p.). A guide cannula was implanted (15 mm, 23 gauge) at a site immediately above the MRN. A hole was drilled in the skull coordinates, AP, −7.6 mm; ML, 4.2. The cannula was inserted in the coronal plane at a 30° angle with respect to the vertical plane to a depth of 7.5 mm below the dura mater [24]. The cannula and two anchoring screws were fixed to the skull with dental cement. For electrophysiological recording, a bipolar Teflon-coated stainless-steel (125 μm diameter) in the PP (coordinates: AP, −8.1 mm; ML, −4.3 mm; DV, −3.3 mm, from skull surface) and a recording electrode (glass micropipette, 1.5–2 MΩ filled with physiological saline) in the DG granule cell layer (coordinates: AP, −3.8; ML, 2.4; DV, 2.7 to 3.2 from skull surface) were implanted.

2.3. Electrophysiological recordings

The stimulating and recording electrodes were adjusted to produce maximum field potentials by applying a single pulse stimulus (0.2 ms duration) and field potentials in the DG granule cell layer were recorded. To achieve this, it was at times necessary to reposition the stimulating and/or recording electrodes until the highest potential could be obtained. A test stimulus of intensity sufficient to produce a population spike (PS) 1.5–3.0 mV in amplitude was selected. This corresponded to response at approximately one third of the maximum. The evoked response was amplified, filtered (band-pass: 1 Hz–10 kHz), and sampled at a rate of 10 kHz and stored on the hard disk. The amplitude of the PS was calculated as the vertical distance between the peak negativity and a tangent constructed between the onset and termination of the spike, and then averaged (10 responses) off-line using a computer program. The population EPSP slope was measured as the maximum slope between baseline potential and the peak of the first positive wave [17].

For LTP induction (after the response became stable) a primed burst (PB) stimulation was delivered to the PP at the same stimulus intensity through the same electrode as used for test stimulation. PB consists of one single priming pulse followed 170 ms later by a burst of 10 pulses delivered at 100 Hz. This pattern was repeated eight times at 10-s intervals. The magnitude of potentiation was evaluated as the percentage change in the population spike amplitude at 5, 10, 20, 40, 60, and 120 min after tetanic stimulation relative to the pre-tetanus test value. Saline or lidocaine was injected during the baseline recording (experiment 1), 5 min before tetanus (experiment 2), and 5 min after tetanus (experiment 3).

In the paired-pulse stimulation experiment (experiment 4), 10 paired-pulses with 10- and 20-ms intervals were delivered to the PP at a frequency of 0.1 Hz. After these

baseline stimulations, lidocaine was injected into the MRN and the same stimulation pattern was repeated 5 min later. The efficacy of recurrent inhibition was evaluated by comparing percentage changes of the PS amplitude during each individual paired-pulse stimulation experiment.

2.4. Micro-injection procedure

Neural activity in the MRN was temporarily inactivated by microinjection of 0.5 μl 2% lidocaine. Lidocaine is a local anaesthetic which induces reversible block of impulse generation and conduction by reducing the membrane permeability to sodium ions [28,30]. The spatial extent of block induced by 0.5 μl of 2% lidocaine [18,25,30] suggests that inactivation was effective within a sphere 1 mm in diameter and thus was restricted to the MRN. The blocking effect lasted for a period of up to 30 min and the MRN returned to the pre-injection level in less than 1 h [25,30].

The cannula stylet was removed and replaced with a 30 gauge injection needle which was connected to the Hamilton syringe by a short piece of polyethylene tubing. The needle was inserted 0.5 mm beyond the tip of the cannula and 0.5 μl of saline or 2% lidocaine hydrochloride (Bayer) was injected within 1 min. The needle was left in place for another 60 s before it was slowly withdrawn.

2.5. Experimental protocol

2.5.1. Experiment 1

The aim of this experiment was to examine the effects of lidocaine injection into MRN on baseline activity of the dentate gyrus cells of the hippocampus. Seven rats were implanted with cannula aimed at MRN. During baseline recording lidocaine was injected into the MRN and population spikes and $_{p}$EPSP were recorded for 120 min after injection. Control rats ($n=7$) received saline instead of lidocaine.

2.5.2. Experiment 2

The effect of MRN inactivation on LTP induction in the dentate gyrus was determined in this experiment. Baseline was recorded for 40 min and saline ($n=7$) or lidocaine ($n=8$) were injected into the MRN, 5 min prior to tetanus. Then population spikes and $_{p}$EPSP were recorded 5, 10, 20, 40, 60 and 120 min after tetanus.

2.5.3. Experiment 3

The effects of MRN inactivation on LTP maintenance in the dentate gyrus was examined in this experiment. Baseline was recorded for 40 min, then tetanic stimulation was applied, population spikes and $_{p}$EPSP were recorded 5 min later and LTP induction was determined. Then, saline ($n=7$) or lidocaine ($n=8$) was injected (5 min after tetanus). Population spikes and $_{p}$EPSP were recorded 10, 20, 40, 60 and 120 min after tetanus.

2.5.4. Experiment 4

The aim of this experiment was to examine the effects of MRN inactivation on recurrent inhibition in the dentate gyrus of the hippocampus. Eight rats were used in this experiment. After saline injection, 20 paired-pulses at 10 and 20 ms intervals were delivered to the PP at a frequency of 0.1 Hz. After these baseline stimulations, lidocaine was injected into the MRN and the same stimulation pattern was repeated 5 min later. The efficacy of recurrent inhibition was evaluated using PS2/PS1 or paired pulse index (PPI) by comparing percentage changes of the PS amplitude during each individual paired-pulse stimulation experiment.

2.6. Histological procedures

At the end of each experiment, injection, stimulation and recording sites were verified histologically. The perfusion–fixation was performed intracardially with saline followed by 10% formalin/phosphate buffer solution. Then the brains were removed and post-fixed in the same fixative. Paraffin sections were prepared and were stained (H&E) for histological examination. The location of the cannula was verified by examining enlarged projections of the slides. The volume of lidocaine injected into the MRN in these experiments has been reported to spread from 0.5 to 1.5 mm from the site of injection [18,30]. Therefore, a cannula positioned more than 0.5 mm from the intended site of injection was not included in the statistical analysis. We used 55 rats in our four experiments. In three cases, two from experiment 2 (from lidocaine group) and one from experiment 3 (from lidocaine group) cannula tips deviated by more than 0.5 mm from the target structure and were excluded from statistical analysis. In the remaining animals all MRN cannula tips were just above the MRN (Fig. 1B). A representative photomicrograph illustrating the location of the cannula aimed at the MRN is shown in Fig. 1A.

2.7. Statistical analysis

The data were analyzed by completely randomized analyses of variance (ANOVA) with repeated measures followed by Tukey's test for multiple comparisons. Data of paired-pulse were analysed by Mann–Whitney *U*-test to compare the two conditions. $P<0.05$ level was accepted as significant.

3. Results

3.1. Experiment 1

ANOVA on data of experiment 1 showed no significant difference between baseline recording in saline and lidocaine groups. This indicates that MRN inactivation has no

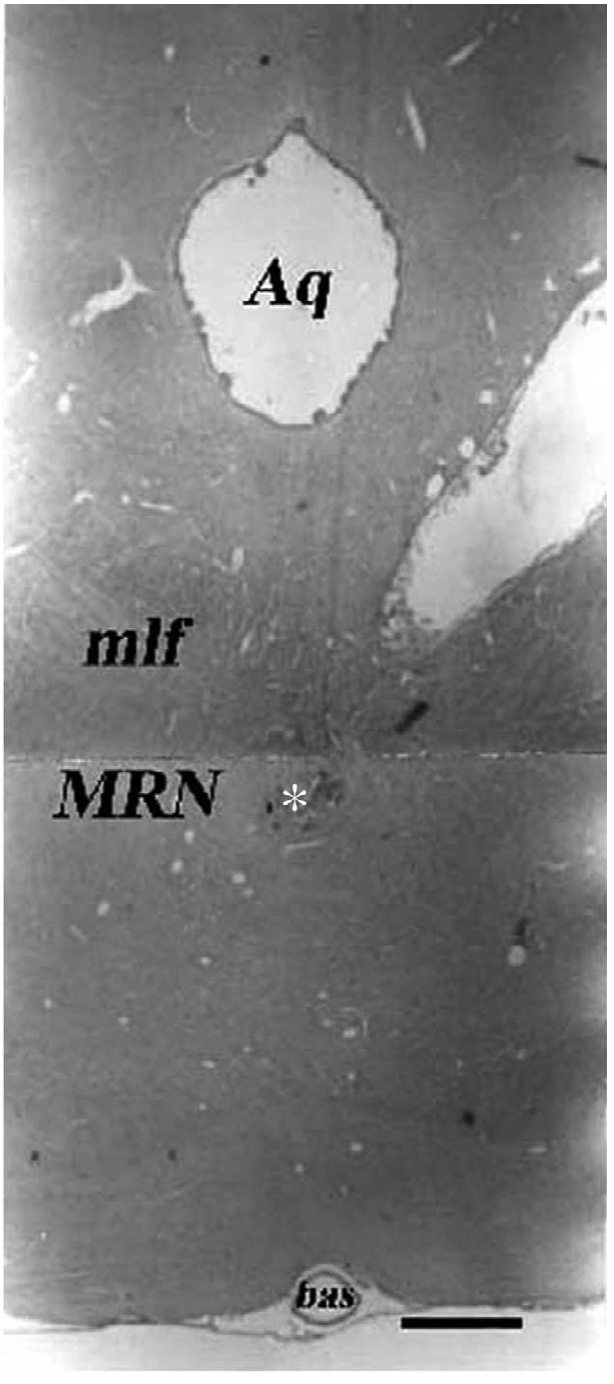

Fig. 1. (A) A typical photomicrograph of a coronal section of the median raphe nucleus through the injection site (asterisk). The trace of a cannula can be seen at the right side. Aq, aqueduct; bas, basilar artery; mlf, medial longitudinal fasciculus. Scale bar=300 μm. (B) Schematic drawing of the coronal plane through the MRN has been adapted from the atlas of Paxinos and Watson [24]. Hatched lines reveal the location of the cannula tips in the experimental cases with acceptable cannula placements. DR, dorsal raphe nucleus; PMR, paramedian raphe nucleus; scale bar=4 mm.

effect on baseline activity of granular cells of the dentate gyrus.

3.2. Experiment 2

A typical example of the effects of intra-MRN injection of lidocaine or saline, 5 min before tetanic stimulation, on the magnitude of LTP is illustrated in Fig. 2. The results of several similar experiments are summarized in Figs. 3 and 5. In the lidocaine-treated animals the PS amplitude was not significantly different from the control group at 5, 10, 20, 40, 60 min after tetanization but it was significantly higher at 120 min after tetanization ($P<0.01$). In addition, within the lidocaine group there was a significant difference between different intervals after LTP induction ($F_{(5,31)}=4.7$, $P=0.0026$) but not in the control group ($F_{(5,35)}=1.15$, $P=0.3$) (Fig. 3). The slope of $_{P}$EPSP in the lidocaine group was not significantly different from the control group at all time intervals. However, there was an increase in 120 min after tetanization which was not significant (Fig. 5). These results indicate that MRN inactivation before tetanus has no effect on LTP induction in the dentate gyrus but that it enhances PS amplitude when this area returns to its normal activity.

3.3. Experiment 3

The short lasting effect of lidocaine also makes it possible to assess the effects of MRN inactivation on the maintenance of LTP. Thus the aim of experiment 3 was to test the effect of post-tetanic inactivation of MRN by lidocaine on LTP maintenance. A typical example of the effects of post-tetanus intra-MRN injection of lidocaine or saline on the time course of LTP at 5, 10, 20, 40, 60 and 120 min after the tetanic stimulation is illustrated in Fig. 4. The results of several similar experiments are summarized in Figs. 3 and 5. ANOVA on PS amplitude data showed a significant difference between the two groups ($F_{(1,11)}=48.6$, $P<0.0001$). With respect to the control group, the PS amplitude was significantly lower in the lidocaine group at 10, 20 and 60 min after tetanization ($P<0.01$) but not at 40 and 120 min after tetanus (Fig. 3). ANOVA on $_{P}$EPSP slope showed a significant difference between the two groups ($F_{(1,11)}=54.3$, $P<0.0001$). There was a significant difference between the two groups at 20 and 40 min after tetanization, $P<0.01$ and $P<0.001$, respectively, but not at 5, 10, 60 and 120 min after tetanic stimulation (Fig. 5). As it is shown in Figs. 3 and 5, the shape and changes in the $_{P}$EPSP slope curve are similar to the PS curve. These results indicate that post-tetanus inactivation of MRN enhances LTP decay but this effect is eliminated when neuronal activity of MRN returns to the normal level.

3.4. Experiment 4

The effect of MRN inactivation on recurrent inhibition

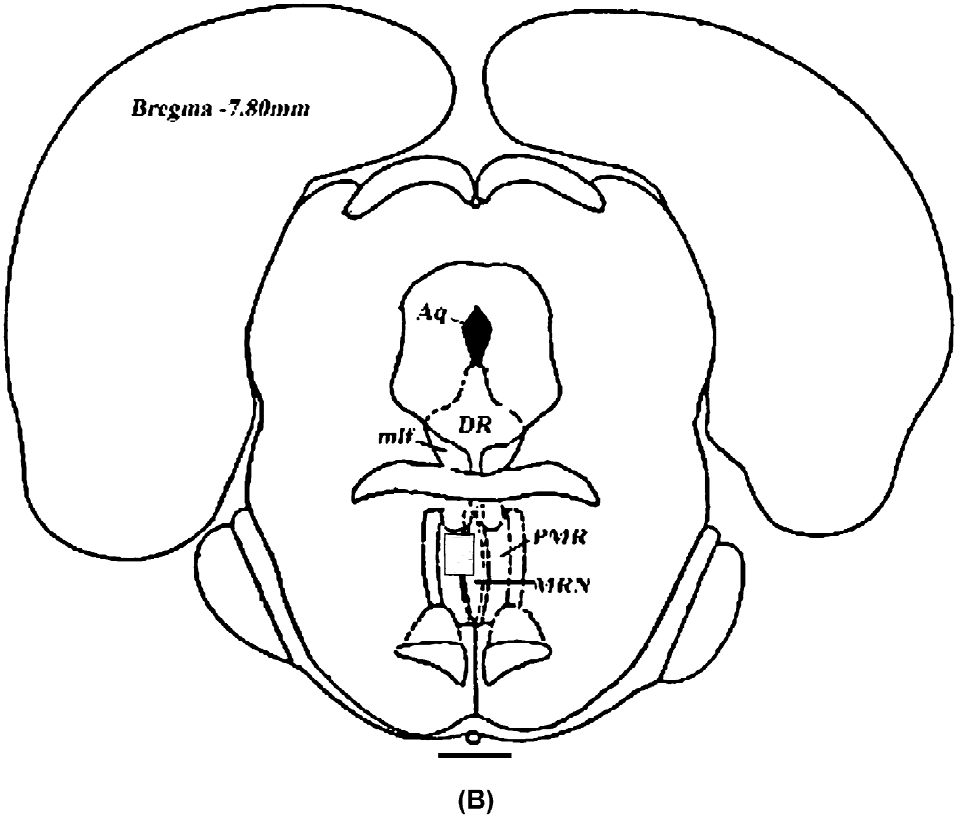

Fig. 1. (*continued*)

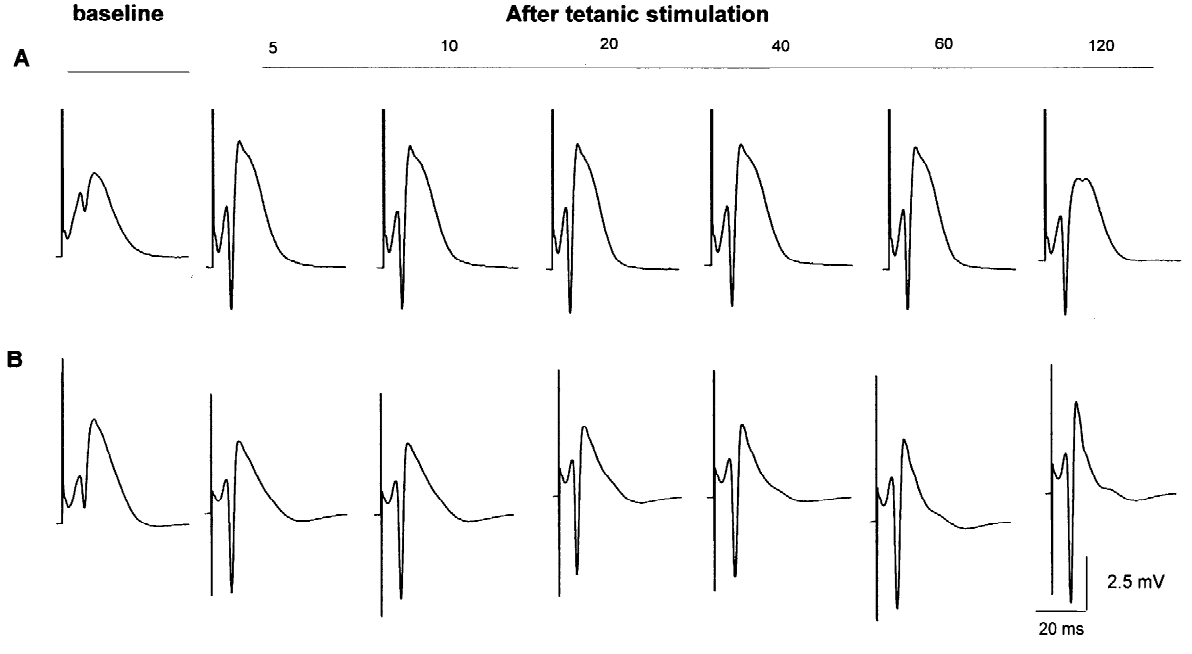

Fig. 2. A typical example of the effects of intra-MRN injection of saline (A) or lidocaine (B) on LTP induction in the dentate gyrus of the hippocampus. 5–120 min, time after the tetanic stimulation. Baseline, baseline recording for 60 min before PP tetanization. Saline or lidocaine were injected 5 min prior to the tetanic stimulation.

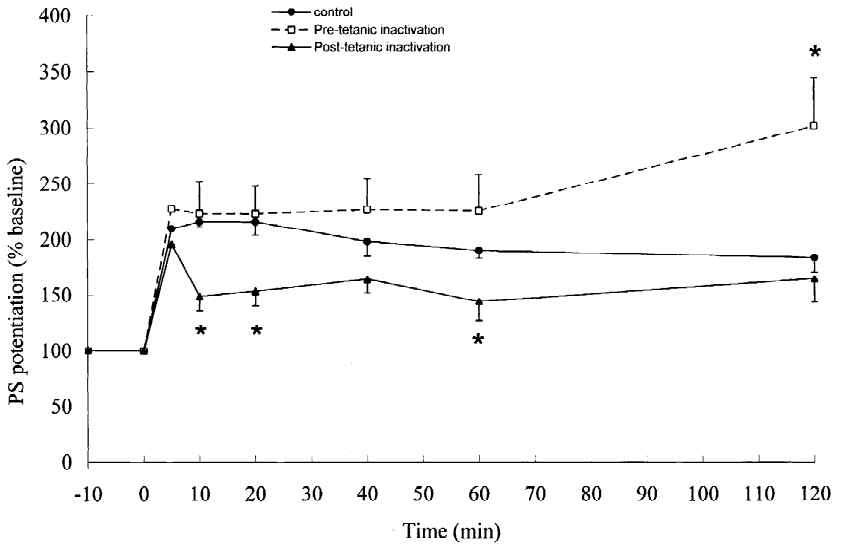

Fig. 3. The effects of intra-MRN injections of lidocaine before and after tetanic stimulation on induction and maintenance of LTP of the population spike (PS) amplitude in the dentate gyrus of the hippocampus. Data are plotted as an average of the percentage change from baseline responses. Values are mean+S.E.M. Control group received saline instead of lidocaine. $^{**}P<0.001$, $^{*}P<0.01$. $N=7$ for control group and $N=8$ for each test group.

in the dentate gyrus of the hippocampus was determined. A typical example of averaged extracellular evoked responses elicited from the dentate gyrus with paired-pulses (10 ms and 20 ms interstimulus intervals) using saline and 5 min after intra-MRN injection of lidocaine is illustrated in Fig. 6. The results of several similar experiments are summarized in Fig. 7. This figure shows that the paired pulse index (PPI) was lower under the lidocaine condition as compared to the control which was statistically significant at the 20-ms paired pulse interval ($P<0.05$), but not at the

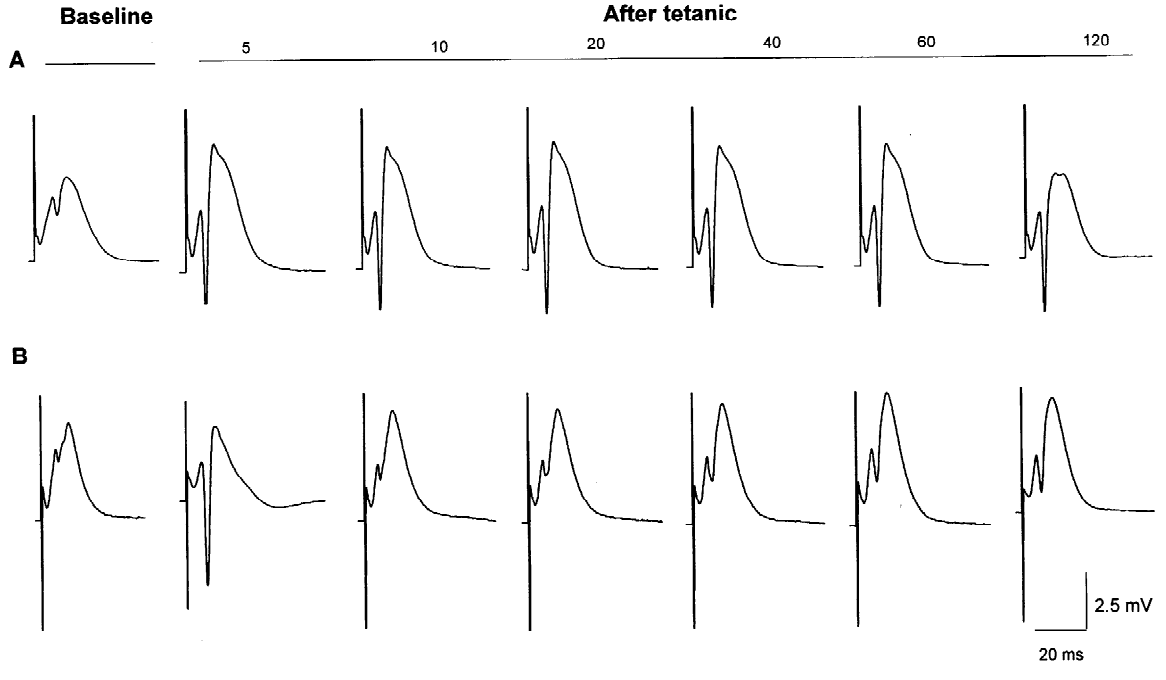

Fig. 4. A typical example of the effects of intra-MRN injection of saline (A) or lidocaine (B) on LTP maintenance in the dentate gyrus of the hippocampus. 5–120 min, time after the tetanic stimulation. Baseline, baseline recording for 60 min before PP tetanization. Saline or lidocaine were injected 5 min after the tetanic stimulation.

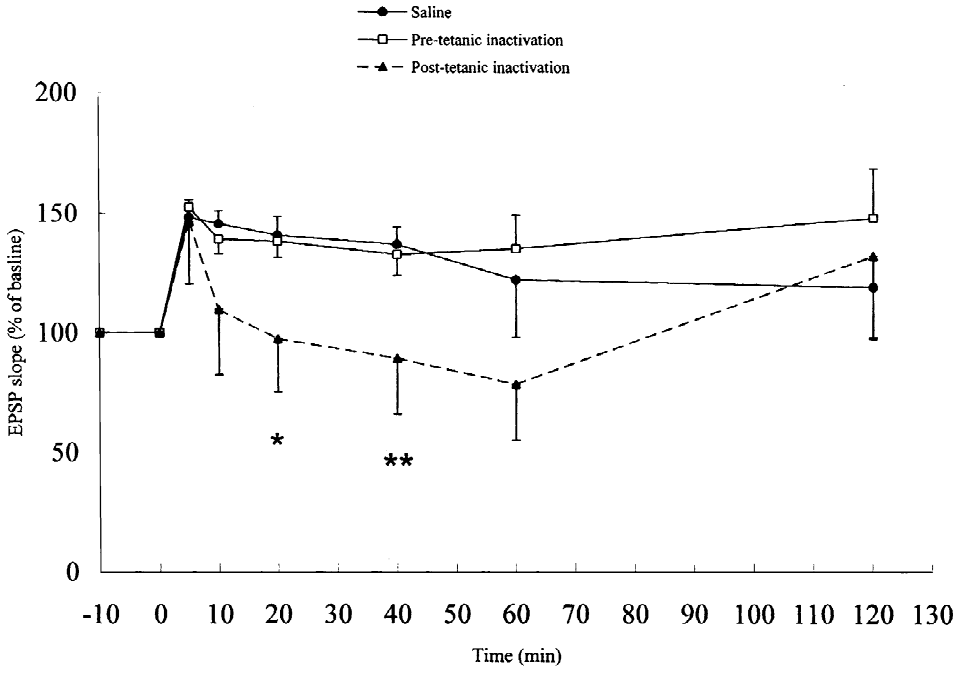

Fig. 5. The effects of intra-MRN injections of lidocaine before and after tetanic stimulation on induction and maintenance of LTP of the population EPSP in the dentate gyrus of the hippocampus. Data are plotted as an average of the percentage change from baseline responses. Values are mean+S.E.M. Control group received saline instead of lidocaine. $^{**}P<0.001$, $^{*}P<0.01$. $N=7$ for control group and $N=8$ for each test group.

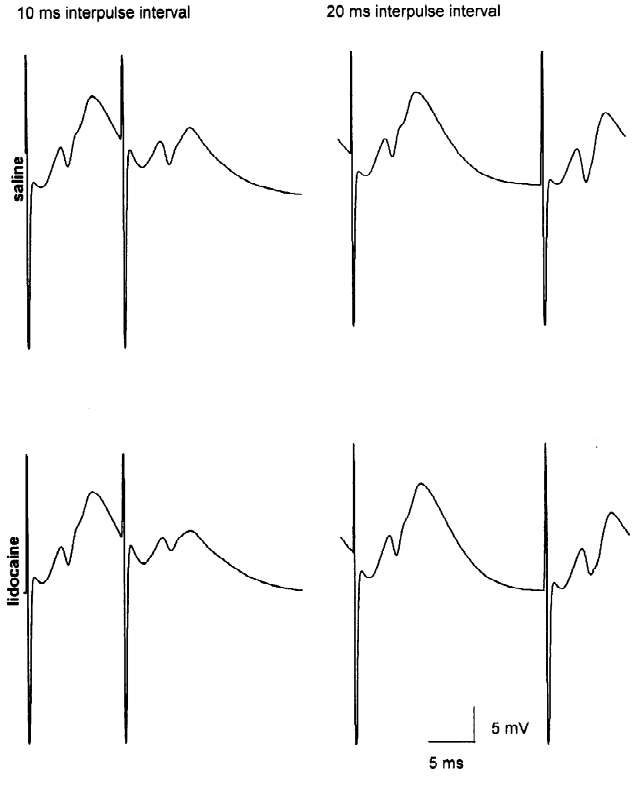

Fig. 6. An example of averaged extracellular evoked responses elicited from the dentate gyrus with paired-pulses (10 ms and 20 ms interstimulus intervals) using intra-MRN injection of saline or lidocaine 5 min after tetanic stimulation. Calibration: vertical 5 mV, horizontal 5 ms.

10-ms interval. The results indicate that reversible inactivation of MRN enhances recurrent inhibition in the dentate gyrus of the rat hippocampus.

4. Discussion

The main findings of the present study are: (1) MRN inactivation has no effect on baseline activity of granular cells of the dentate gyrus. (2) MRN ablation before tetanus has no effect on LTP induction in the dentate gyrus but it enhances PS amplitude when this area returns to its normal activity. (3) Post-tetanus inactivation of MRN enhances LTP decay but when neuronal activity of MRN returns to the normal level, this effect will be eliminated. (4) Reversible inactivation of MRN enhances recurrent inhibition in the dentate gyrus of the rat hippocampus.

The MRN is one of the subcortical structures known to exert powerful modulatory effects on hippocampal synaptic transmission. Field potential studies conducted in anesthetized [2,17,21] and freely moving [9,33,39] animals indicate that electrical stimulation of the MRN can markedly facilitate subsequent PP-evoked dentate granule cell discharge. While the facilitatory effects of activation of MRN have been well described, the mechanisms underlying the phenomenon have not been definitively established. There is a hypothesis suggesting the possibility that MRN activation produces an initial inhibition of granule cells

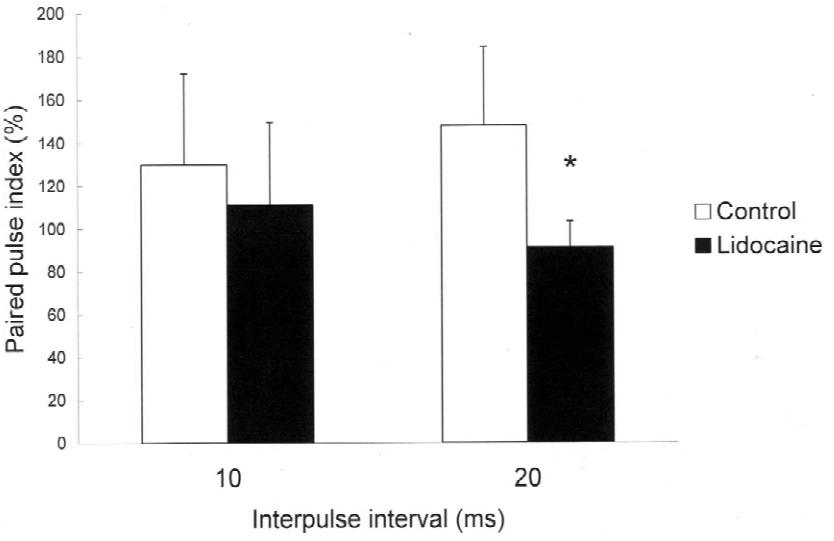

Fig. 7. The effects of intra-MRN injection of lidocaine on recurrent inhibition in the dentate gyrus of the hippocampus. Values are mean+S.E.M. PS2/PS1 (PPI) under intra-MRN injection of saline or lidocaine. Note, PPI in lidocaine condition in 20 ms interval is <100 indicating an increase in synaptic inhibition. $N=7$ for each condition. $^{*}P<0.05$.

which leads to a greater synchronization of granule cell responses to subsequent PP stimulation [2]. It has also been suggested that MRN stimulation could activate interneurons which excite granule cell excitability by inhibition of inhibitory interneurons (i.e. disinhibition) [14,26,39]. Freund in his anatomical studies indicated that the majority of afferents from MRN to the hippocampus terminate on GABAergic interneurons [13]. This finding confirms the disinhibition hypothesis and indicates that MRN neurons exert their role by disinhibition of hippocampal main cells.

Several possible underlying mechanisms may subserve the effect of MRN inactivation on LTP. The most direct suggestion would be a decrease in the release of 5-HT onto the dentate granule cells following MRN ablation. This decreased level of extracellular 5-HT may interact with the normally underlying mechanisms of LTP maintenance. Bliss et al. [4] found that depletion of 5-HT by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT) or *para*-chlorophenylalanine (PCPA) reduced the amount of LTP which could be induced in the DG of rats anaesthetized with pentobarbitone. Stanton and Sarvey [34] reported that depletion of 5-HT by 5,7-DHT had no effect on the amount of LTP which could be induced in the DG of in vitro hippocampal slices. The difference between these two depletion experiments may be due to pre-operatory method such as elimination of any tonic serotonergic influence during slice preparation or methods used to quantify LTP (i.e. changes in population EPSP slope [4] vs. changes in PS amplitude [34]).

Our findings indicated that even in the absence of MRN, synapses in the DG can be potentiated after PP tetanic stimulation. If MRN inactivation is applied before tetanus, during MRN functional ablation (i.e. up to 60 min after tetanus), there is no decay in LTP. However, 2 h after tetanic stimulation not only LTP decreased, but also increased significantly. It seems that dentate gyrus granule cells may have been in a hypersensitivity state after a course of MRN inactivation. This finding suggests that MRN neurons probably have no role in the events that happen during LTP induction but have an important role in the maintenance of LTP.

MRN afferents terminate on distinctive hippocampal GABAergic interneurons that terminate on main hippocampal cells. These connections are the basis of MRN activity and disinhibition of hippocampal main cells. Also it has been shown that axon terminal autoreceptors (5-HT_{1B} and 5-HT_{1D}) in hippocampal formation regulate serotonin release [22].

With respect to our results in pretetanic inactivation of MRN (Fig. 3), based on concurrent stimulation of MRN and PP that indicate increase in the amount of LTP [2,17,39], we expected that pretetanic inactivation of MRN by lidocaine injection might decrease the amount of LTP in DG, but in our result a significant difference in LTP induction was not observed. This may have occurred for different reasons. Firstly, MRN may act in a different manner during stimulation and inactivation (especially during stimulation with changing stimulation parameter). Secondly, MRN inactivation causes the removal of both direct and indirect MRN modulatory inputs to the hippocampus which may cancel the effects of each other. Finally, nonserotonergic neurons that also inactivate during MRN ablation may act differentially in these two conditions (i.e. stimulation and inactivation) as some investigators have shown in their role on hippocampal transmission [9]. In addition, there is some evidence indicating that serotonin depletion in the hippocampus up to 96% has

little to no effect on facilitation caused by MRN stimulation [9,29]. Our results are in agreement with these findings.

Concerning the significant increase in the extent of LTP of PS 120 min after tetanic stimulation, there is no direct evidence to explain this phenomenon but it had been shown that the maximum effect of lidocaine lasts for 60 min [25,30] so, 120 min after tetanic, MRN neurons have reached their normal activity. But in the presence of lidocaine and during MRN inactivation some events may have happened in the hippocampus that resulted in an increase in the excitability of dentate gyrus granular cells. Based on disinhibition mechanism [15,16], probably MRN ablation enhances the GABAergic interneurons inhibition. This inhibition in turn causes synchronization of hippocampal neurons during MRN inactivation, so that when they return to their normal activity they respond to PP stimulation and the PS amplitude will increase. However, the exact mechanism of increased LTP after pretetanic inactivation of MRN remains to be documented.

Our results in post-tetanic inactivation of MRN are in agreement with the disinhibition mechanism of principal hippocampal cells by MRN neurons [10,11,15,20]. Removal of MRN inputs to DG by lidocaine can increase GABAergic interneurons inhibition on hippocampal principal cells and therefore decreases the excitability of these cells and the amount of LTP. When the lidocaine effect is removed and MRN neuronal activity returns to the normal level, the PS amplitude will reach the control level.

In the interpretation of the difference between the results of experiments 2 and 3 it seems that the major factor is the pairing of MRN inactivation and PP tetanic stimulation. When inactivation is induced before tetanic stimulation (experiment 2) it has no effect on LTP induction nor on its maintenance. But if the inactivation is applied after LTP induction (experiment 3), the LTP maintenance is suppressed. It seems that the mechanism of action of MRN on LTP maintenance is different in the pre- and post-tetanic conditions which remains to be elucidated.

Recurrent inhibition is one inhibitory circuit in the hippocampus. In this kind of inhibition, the inhibitory interneurons excite via the axon collateral branch of principal cells. This inhibitory circuit is a feedback mechanism for controlling the activity of hippocampal principal cells. Paired pulse stimulation of the PP provides a measure of recurrent inhibition of dentate granule cell field potentials that is reflected in the depression of the second PS relative to the first PS. Previous studies have shown that GABAergic interneurons play a major role in recurrent inhibition in the hippocampus [1,6,35]. Since MRN serotonergic neurons inhibit these inhibitory interneurons [12,13,15,20], one can speculate that MRN inactivation may increase the efficacy of recurrent inhibition. Our results are in agreement with this hypothesis. Ablation of MRN inputs to the hippocampus by intra-MRN injection of lidocaine and PP stimulation by paired pulse at 20 ms intervals increased recurrent inhibition. Our results indicate that MRN has a negative role in recurrent inhibition in the DG of the hippocampus.

In our previous studies we have found that reversible inactivation of the median raphe enhances consolidation and retrieval but not acquisition of passive avoidance learning in rats [31]. In addition, inactivation of the MRN has no effect on various aspects of reference memory but enhances working memory performance in the Morris water maze [32]. From our behavioural and electrophysiological experiments, we can conclude that MRN has a negative role on memory consolidation and retrieval in classical conditioning, spatial memory and hippocampal neuronal circuit, but the role of the direct and indirect pathways of this nucleus to the hippocampus and the importance of serotonergic and nonserotonergic neurons remains to be elucidated.

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