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Activity-dependent changes in excitability of perirhinal cortex networks in vitro

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Abstract

Rat brain slices comprising the perirhinal cortex (PC) and a portion of the lateral nucleus of the amygdala (LA), in standard medium, can generate synchronous oscillatory activity that is associated with action potential discharge and reflects the activation of glutamatergic and GABAergic receptors. We report here that similar synchronous oscillatory events are recorded in the PC in response to single-shock, electrical stimuli delivered in LA. In addition, we found that the latency of these responses progressively increased when the stimulus interval was varied from 10 to 1 s; for example, the response latency during stimuli delivered at 1 Hz was more than twofold longer than that seen during stimulation at 0.1 Hz. This prolongation in latency occurred

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In memoriam of Toshiyuki Kano.

after approximately 5 stimuli, attained a steady value after 24–35 stimuli, and recovered to control values 30 s after stimulation arrest. These frequency-dependent changes in latency continued to occur during NMDA receptor antagonism but weakened following application of $GABA_A$ and/or $GABA_B$ receptor blockers. Our findings identify a new type of short-term plasticity that is mediated by GABA receptor function and may play a role in decreasing neuronal network synchronization during repeated activation. We propose that this frequency-dependent adaptive mechanism influences the excitability of limbic networks, thus potentially controlling epileptiform synchronization.

Keywords

Amygdala; $GABA_A$ receptors; $GABA_B$ receptors; NMDA receptors; Perirhinal cortex; Repetitive stimulation

Introduction

It is well established that amygdala and perirhinal cortex (PC) neuronal networks are interconnected both anatomically and functionally [23]. These limbic areas are characterized by synaptic plasticity and are intimately involved in learning and memory [9, 33, 42, 43, 49, 56, 57, 64]. Rhinal cortices, which include entorhinal and perirhinal structures, are critical components of the declarative memory system [55]. In particular, in patients affected by temporal lobe epilepsy refractory to antiepileptic drug treatment, surgical resection of rhinal cortices and amygdala causes a marked postoperative impairment in the ability to learn unrelated word pairs [63]. Clinical and experimental evidence also indicates that both amygdala and PC play pivotal roles in the generation of epileptiform discharges and in epileptogenesis [7, 23, 34, 41].

We have previously reported that spontaneous, network-driven events associated with action potential discharge can be recorded in rat brain slices comprising the PC along with a portion of the lateral nucleus of the amygdala (LA) during incubation with standard medium [32]. These network oscillations reflect the activation of ionotropic glutamatergic and GABAergic receptors and are influenced by gap junction conductance. Interestingly, similar in vitro spontaneous activity occurs in brain slices obtained from amygdala-kindled rats [39, 40] as well as in isolated rodent hippocampal slices obtained from “control” animals [37, 46, 47].

In this study, we employed focal electrical stimulation of the LA under similar experimental conditions (i.e., standard medium) to further establish the functional characteristics of these neuronal network interactions in the rat limbic system. As expected, single-shock electrical stimuli in LA induced field potential and intracellular responses in the perirhinal cortex that were similar to those that occur spontaneously. However, we discovered that the latency of these oscillatory, network-driven responses increased when stimuli were delivered at higher stimulation frequencies (e.g., the response latency during stimulation at 1 Hz was more than twofold longer than during 0.1-Hz stimulation). In addition, we found that these frequency-dependent changes in latency were unaffected by an NMDA receptor antagonist but became less marked during application of $GABA_A$ and/or $GABA_B$ receptor blockers.

Methods

Brain slice preparation and maintenance

Male, young adult Sprague-Dawley rats (130–180 g; Charles River, St-Constant, QC, Canada) were decapitated under halothane anesthesia according to the procedures established by the Canadian Council on Animal Care. All efforts were made to minimize animal suffering and the number of animals used. Horizontal brain slices (450 μm) were prepared, as reported in previous studies from our laboratory [6, 32], and were transferred into a tissue chamber, where they laid at the interface between artificial cerebrospinal fluid (ACSF) and humidified gas (95 % O_2, 5 % CO_2) at a temperature of 34 °C and a pH of 7.4. ACSF composition was (in mM) NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and D-glucose 10. 3-N[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid (CGP 55845, 4 μM), 3,3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP, 10 μM), and picrotoxin (PTX, 50 μM) were bath-applied in some experiments. Chemicals were acquired from Sigma (St. Louis, MO, USA) with the exception of CPP and CGP 55845, which were obtained from Tocris Cookson (Langford, UK). Surgical separation of the parahippocampal areas from the hippocampus proper was performed with a razor blade mounted on a micromanipulator at the beginning of the experiment to minimize the possible influence exerted by CA3-driven activity [6, 32] (Fig. 1A).

Electrophysiological recordings

Field potential recordings were made with ACSF-filled glass pipettes (resistance 1.2–10 MΩ) that were connected to high-impedance amplifiers. Sharp-electrode, intracellular recordings were performed in the PC with pipettes that were filled with 3 M K-acetate (tip resistance 70–120 MΩ). Intracellular signals were fed to a high-impedance amplifier with internal bridge circuit for intracellular current injection. The resistance compensation was monitored throughout the experiment and adjusted as required. Microelectrodes recording extracellular and intracellular signals were placed closely (~500 μm) in the deep layers of the PC (i.e., 600–800 μm from the pia) (Fig. 1A).

The fundamental electrophysiological parameters of the neurons included in this study were measured as follows: (i) resting membrane potential (RMP) after cell withdrawal, (ii) apparent input resistance (Ri) and membrane time constant (τ) from the voltage change in response to a hyperpolarizing current pulse (100–200 ms, <−0.5 nA), and (iii) action potential amplitude and duration from the baseline and at half-width, respectively. These characteristics were similar to those previously reported by D'Antuono et al. [16] and Kano et al. [32] (see Table 1). All PC cells could be identified as regularly firing neurons when injected with pulses of intracellular depolarizing current [16]. Field potential and intracellular signals were fed to a computer interface (Digidata 1200B, Axon Instruments, Union City, CA, USA) and were acquired and stored by using the pClamp 8 software (Axon Instruments). Subsequent data analysis was made with the Clampfit 8 software (Axon Instruments).

Stimulation protocols and data analysis

Single-shock stimuli (0.1–0.3 mA, 50–100 μs) were delivered through a tungsten electrode that was positioned in the LA (Fig. 1A). These stimuli were delivered at 0.1, 0.2, 0.5, and 1 Hz for periods of up to 300 s while keeping the stimulus strength constant throughout the session. Threshold stimulation strength was established in each experiment as that capable of consistently eliciting an oscillatory network-driven response following stimuli delivered at 0.1 Hz; stimulation protocols were then performed at different frequencies in a random fashion by employing such stimulation strength. Each series of stimuli was followed by a period of "no stimulation" lasting at least 180 s.

The latency of these stimulus-induced responses was measured from the stimulus artifact to the rising phase of the first action potential associated with the stimulus-induced response (see Fig. 1C, inset b). Unless specified, values under each experimental condition were obtained by averaging the latency of the intracellular responses that were generated following the last 20 to 30 stimuli delivered during a series. As illustrated in Fig. 2B, these "late" stimulus-induced responses were characterized by latencies that displayed minimal variability (i.e., the latency appeared to have reached a nominal steady state). The duration of the stimulus-induced responses was obtained from the intracellular recording trace by measuring the time between the first action potential and the full membrane repolarization (Fig. 1D); duration values in each experimental session were also obtained by averaging the responses recorded following the last 20 to 30 stimuli delivered during each series.

Measurements in the text are expressed as mean ± SEM and n indicates the number of neurons studied under each specific protocol. Data were statistically compared with one-way or two-way analysis of variance (ANOVA) for repeated measures as appropriate, followed by the Fisher's least significant difference (LSD) post hoc test, and were considered significantly different if $p<0.05$.

Results

PC network responses to single-shock stimuli delivered in LA

Figure 1B shows spontaneous field potential activity recorded in PC and LA in two different brain slices incubated in standard medium. As reported by Kano et al. [32], these spontaneous events were characterized by a series of field oscillations at 5–11 Hz, with durations ranging between 0.2 and 1.4 s and intervals of occurrence between 7 and 22 s. Field events occurring more frequently (Fig. 1B, inset a) had shorter durations than events occurring at longer intervals (Fig. 1B, inset b).

Intracellular recordings from PC neurons demonstrated that in most cases (25 out of 31 neurons recorded from 16 slices) single-shock stimulation of the LA at 0.1 Hz evoked all-or-none sequences of action potential bursts that rode over rhythmic depolarizing oscillations of the membrane potential (Fig. 1C, inset a). In the remaining six neurons, low strength LA stimuli induced a postsynaptic response that could contain both excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP) components depending on the neuron resting membrane potential (not shown); in these experiments, only stronger electrical stimulation of the LA produced action potential discharges associated with

rhythmic membrane oscillations (Fig. 1C, inset b). As shown in Fig. 1D, simultaneous intracellular and extracellular recordings revealed that the stimulus-induced intracellular oscillatory responses were mirrored by field oscillations that were similar to those reported to occur spontaneously [32].

On average, the latency of the oscillatory network-driven responses generated by PC neurons following LA stimuli at 0.1 Hz was 28.1±1.1 ms (n=31 neurons). The distributions of the latencies and of the durations of the responses generated by these PC neurons following LA stimulation at 0.1 Hz are summarized in the frequency histograms shown in Fig. 1E. When stimuli were applied at 0.1 Hz, the mean latencies were fairly constant from one pulse to the next (Fig. 2B, top panel). Co-application of the NMDA receptor antagonist CPP and the non-NMDA glutamatergic receptor antagonist CNQX (10 μM; n=3 neurons) or CNQX only (n=3 neurons) abolished these stimulus-induced responses, revealing a hyperpolarizing IPSP when the membrane potential was depolarized to values less negative than −70 mV (not illustrated).

Stimulus-induced oscillatory network-driven responses increase in latency when stimuli are delivered at short intervals

When LA stimuli were delivered at 0.5 Hz, the latencies of the oscillatory network-driven responses were initially similar to those found at 0.1 Hz but then progressively increased after approximately 5 successive stimuli, peaking at 90 ms at around 10 stimuli, and attaining a relatively steady value of 55 ms after approximately 25 stimuli (Fig. 2A). This finding is quantified in the plots shown in Fig. 2B, which show the latency of the oscillatory network-driven responses induced by successive stimuli delivered at 0.1, 0.5, and 1 Hz in a PC neuron that was tested four times at these three different rates of stimulation. The "steady" latency of the responses induced by stimuli delivered at 0.5 Hz in this experiment was 1.5-fold longer than that observed during 0.1-Hz stimulation (Fig. 2B, middle panel). Moreover, an even larger latency prolongation emerged when stimuli were delivered at 1 Hz (Fig. 2B, bottom panel). These data indicate that the latency of the oscillatory network-driven responses measured during the steady state in the PC augments progressively as the rate of stimulation in LA is increased.

To further assess this phenomenon, we analyzed the latency of the responses generated by PC neurons (n=31) over the course of repetitive stimulation of the LA at 0.1, 0.2, 0.5, and 1 Hz. Figure 3C shows that the steady state latencies of these responses increased as a direct function of the stimulation rate. Thus, mean steady state latencies obtained during stimuli delivered at 0.5 Hz (47.3±2.8 ms) were significantly longer that those observed at 0.1 Hz (28.1±1.1 ms; p<0.01, Fisher's LSD test). Latencies further increased during 1-Hz stimulation trials (60.8±2.6 ms, p<0.01) (Fig. 3C). The intracellular recordings illustrated in Fig. 3A, B also show that PC responses were consistently induced by stimuli at threshold strength when delivered at 0.1 Hz but failed to be generated in response to every second stimulus when the rate was increased to 1 Hz. These failures were abolished by increasing the stimulus strength.

The increases in latency were paralleled by decreases in the duration of the oscillatory responses generated by PC neurons (Fig. 3A, inset b, and Fig. 3B, inset b). As summarized

in the histograms in Fig. 3D, the responses obtained at 0.5- and 1-Hz protocols were significantly (p<0.01) shorter than those recorded when stimulation was delivered at 0.1 Hz. A significant (p<0.05) difference was also found between 0.2- and 1-Hz protocols (Fig. 3D).

The latency increase elicited by repetitive stimulation of LA at frequencies higher than 0.2 Hz recovered within a few tens of seconds upon termination of the stimulation procedure. This feature was analyzed in detail in 11 PC cells that were tested for stimuli delivered at 0.1 Hz (control), then stimulated for 100 s at 1 Hz, and then further analyzed upon termination of the 1-Hz train. Representative samples of the stimulus-induced responses obtained in one of these experiments are illustrated in Fig. 4A. Data obtained from all experiments (n= 11 neurons) are summarized in the plot of Fig. 4B, which shows that the response latency gradually decreased and achieved a steady value similar to control (i.e., 0.1-Hz stimulation) approximately 30 s after termination of stimulation at 1 Hz.

Effects induced by concomitant application of $GABA_A$ and $GABA_B$ antagonists

Next, we tested the hypothesis that GABA receptor signaling contributed to the latency prolongation of oscillatory network-driven responses in PC neurons (n=17), using the $GABA_A$ and $GABA_B$ receptor blockers PTX (50 μM) and CGP 55845 (4 μM), respectively. Co-application of these drugs increased the excitability of PC networks as indicated by the consistent ability of threshold stimuli to induce oscillatory network-driven responses during any of the frequencies tested (Fig. 5A–C). In addition, the latencies of the responses obtained during 1-Hz stimulation were significantly shorter (p<0.05) with PTX+CGP 55845 than in control conditions (Fig. 5D and Table 2; note that differences among frequencies of stimulations are shown in Fig. 5, whereas differences between groups of treatment are indicated in Table 2), suggesting that activation of GABA receptors contributed to the increase in latency observed with increasing stimulus frequency.

GABA receptor blockers also caused alterations in the duration of neuronal responses as a function of stimulus frequency (Fig. 5E). At 0.1-Hz stimulation, the duration was significantly (p<0.05) higher in the PTX+CGP 55845 group compared with control values (Table 2). In addition, a frequency-dependent decrease (p<0.05; two-way ANOVA for repeated measures) in average values of duration was observed during application of medium containing PTX+ CGP 55845 (Fig. 5E). Specifically, the response duration was significantly (p<0.05) shorter at 0.5 Hz (442.5± 59.9 ms) and at 1 Hz (312.4±28.1 ms) than at 0.1 Hz (766.5 ±187.1 ms), so that duration values were not significantly different from control values at frequencies higher than 0.1 Hz (Table 2).

Effects induced by pharmacological blockade of $GABA_A$ receptors

Experiments with just the $GABA_A$ blocker PTX demonstrated that frequency-dependent changes in the latency of the stimulus-induced responses were partially dependent on $GABA_A$ receptors. Specifically, the increase in latency observed under PTX treatment at 1 Hz was significantly (p<0.05) less pronounced than that under control conditions (Fig. 6A, B and Table 2). No differences were found at the other frequencies of stimulation.

PTX also prolonged the effect exerted by stimulus frequency on the duration of the responses. Thus, durations were significantly (p<0.01) shorter in control conditions than

under PTX at 0.1 and 0.5 Hz (Fig. 6B and Table 2). No statistical difference was observed at 1-Hz stimulation (Fig. 6B and Table 2).

Effects induced by pharmacological blockade of $GABA_B$ receptors

Similar effects were also seen during the sole application of the $GABA_B$ receptor blocker CGP 55845. When treatment groups were compared at the same frequency of stimulation, latencies were significantly longer in control conditions than in the presence of the $GABA_B$ blocker at 0.5 Hz ($p<0.05$) and 1 Hz ($p<0.01$), whereas no difference was found at 0.1 Hz ($p=0.27$) (Fig. 6D and Table 2). At all the different frequencies of stimulation that were tested, mean values for the durations of the responses were similar in control slices and under CGP 55845 exposure (Fig. 6D and Table 2).

NMDA receptors do not contribute to the stimulus-rate-dependent changes in latency

Finally, we examined whether frequency-dependent effects on latency and/or duration were dependent on NMDA receptor function, using the NMDA receptor antagonist CCP. Blocking NMDA receptors did not significantly affect response latency ($p=0.29$) (Fig. 7 and Table 3); however, it did significantly ($p<0.05$) reduce response duration compared to control at 0.1 Hz, but not at 1 Hz (Fig. 7C and Table 3).

Discussion

The main novel finding obtained in this study is that the latency of the responses generated by PC networks following single-shock stimuli delivered in the LA progressively increases when the stimulus frequency is varied from 0.1 to 1 Hz. We have also found that the frequency-dependent changes in latency recover to control values shortly after stimulation arrest and are not dependent on NMDA receptor signaling, suggesting that they do not reflect long-term depression (LTD) of synaptic transmission. Finally, we have found specific changes in duration and latency of these responses during pharmacological blockade of $GABA_A$ or $GABA_B$ receptors and changes in response duration with inhibition of NMDA receptors.

PC networks generate oscillatory network-driven events in response to LA inputs

Reciprocal innervation between principal cells of LA and PC [49, 54, 61] constitutes the basis for the highly correlated neuronal activity observed in these two limbic areas. This evidence is in line with the reported ability of LA and PC networks maintained in vitro under control conditions to generate spontaneous and synchronous oscillatory network-driven events [32] as well as with the data obtained here by recording the responses generated by PC neurons following single-shock electrical stimuli delivered in LA. We have found that the activation of inputs originating from LA in these brain slices causes robust discharges of action potential firing in the PC; these responses are characterized by a sustained depolarization that is mirrored by field potential activity similar to that observed spontaneously.

These stimulus-induced responses, like those occurring spontaneously [32], are mediated by ionotropic glutamatergic receptors as indicated by the ability of an AMPA receptor

antagonist to abolish them, whereas NMDA receptor antagonism did not eliminate the responses but reduced their duration. Several studies have identified a primary role played by non-NMDA receptors in sustaining synchronous activity, including epileptiform discharges in cortical structures such as the PC [6, 10, 15, 19, 29, 58].

Mechanisms involved in the frequency-dependent prolongation in latency

The principle new finding of these experiments is that when the stimulation rate in LA is increased stepwise from 0.1 to 1 Hz, the latency of the responses generated by PC networks increases. Although we did not systematically analyzed the effects induced by different stimulus strengths on such latency prolongation, similar frequency-dependent increases could be observed with both low-intensity (i.e., threshold stimuli that were capable of consistently inducing responses at 0.1 Hz) and high-intensity stimuli. Several mechanisms can contribute to this phenomenon. One obvious candidate is LTD of glutamatergic synaptic transmission, which is known to be induced by 1-Hz stimulation [5, 12, 28, 38, 48, 62]. However, our data demonstrate that the latency prolongation recovered to prestimulation, “control” values with a few tens of seconds, suggesting that mechanisms underlying LTD are not contributing to these changes. Furthermore, NMDA receptors, which are known to play a role in synaptic plasticity, including LTD [5, 12, 45], are not participating to the latency prolongation of these stimulus-induced responses since these dynamic changes persisted during application of an NMDA receptor antagonist.

We have, however, obtained evidence for a partial role of both $GABA_A$ and $GABA_B$ receptors in latency prolongation. Specifically, either $GABA_A$ or $GABA_B$ antagonism reduced significantly the frequency-dependent differences in latency prolongation. GABAergic transmission plays an important role in modulating oscillatory activity of neuronal networks [8, 14, 35, 44, 50, 52, 53], which depends on the interplay between postsynaptic inhibition, mediated by $GABA_A$ and $GABA_B$ heteroreceptors, and presynaptic inhibition due for instance to the activation of $GABA_B$ autoreceptors [51]. Interestingly, a differential modulation of $GABA_B$-dependent versus $GABA_A$-mediated contribution to oscillatory activity has been proposed in the case of γ oscillations in the auditory cortex [44]. Possible explanations for the different contributions of GABA receptors could include (i) agonist-induced co-internalization of $GABA_B$ receptors associated with $GABA_A$ receptors at hyperactive synapses [3]; (ii) phosphorylation or dephosphorylation of $GABA_B$ receptors caused by postsynaptic activation [22]; (iii) decreased expression of $GABA_B$ receptors due to glutamate spillover [60]; and (iv) altered GABA transporter activity, affecting the availability of GABA to perisynaptic $GABA_B$ receptors [24]. These phenomena may be involved in the slightly different effects produced by $GABA_A$ or $GABA_B$ agonist exposure during network stimulation, as found on latency (more affected by CGP 55845) and duration (affected only by PTX, whose power was decreased in presence of CGP 55845) of neuronal responses.

Our data suggest that stimulus-induced oscillations generated by PC neuronal networks are associated with GABA release that in turn inhibits neuronal activity and thus attenuates the ability of the network to generate synchronous firing. In line with this view, a powerful feed-forward inhibitory network could be activated in the PC following electrical stimuli

delivered in the LA, and this mechanism might become more and more pronounced as the stimulation frequency is increased, thus delaying the responses generated by PC neurons; the presence of such potent feed-forward inhibition has been indeed demonstrated in the PC following stimulation of both entorhinal cortex and neocortex [18].

These effects were however partial, indicating that additional mechanisms contribute to the frequency-dependent increase in response latency. In addition to neuromodulators such as adenosine, 5-hydroxytriptamine, or acetylcholine—which are presumably released during stimulation and are known to reduce neurotransmitter release [21, 26, 30]—an important role in augmenting the latency during repetitive stimulation at rates higher than 0.5 Hz may also be played by the transient elevations in extracellular [K^+] that occur during intense neuronal activity and result from synaptic and non-synaptic mechanisms [1, 25, 27, 31, 36]. Several studies suggest that an increase in extracellular [K^+] can paradoxically decrease the propagation of neural activity in axonal pathways along with the presynaptic release of neurotransmitters [20]. Finally, extracellular alkalinization—which occurs during synchronous neuronal activity [11, 17]—is known to reduce gap junction coupling [13, 59], thus decreasing excitability and prolonging the time required for the neuronal recruitment associated with the stimulus-induced response.

Relevance of the frequency-dependent increase in latency

We have presented here evidence for a new type of frequency-dependent adaptive feature that is characterized by latency prolongation of the oscillatory responses generated by PC neurons during repetitive stimulation of the LA. For example, the latency more than doubled when the stimulus frequency was increased from 0.1 to 1 Hz. The increases in latencies were reduced, but not eliminated, by antagonism of $GABA_A$ and $GABA_B$ receptors, indicating that GABAergic transmission is partly, but not solely, responsible for this phenomenon. The increase in latency demonstrated in our study may be relevant for explaining the ability of low-frequency stimulation to reduce the duration of seizure-like events as previously reported in both 4-aminopyridine and low Mg^{2+} in vitro models of epileptiform synchronization [2, 4, 6], in which stimulation of parahippocampal cortices at 1 Hz appeared to be an effective procedure.

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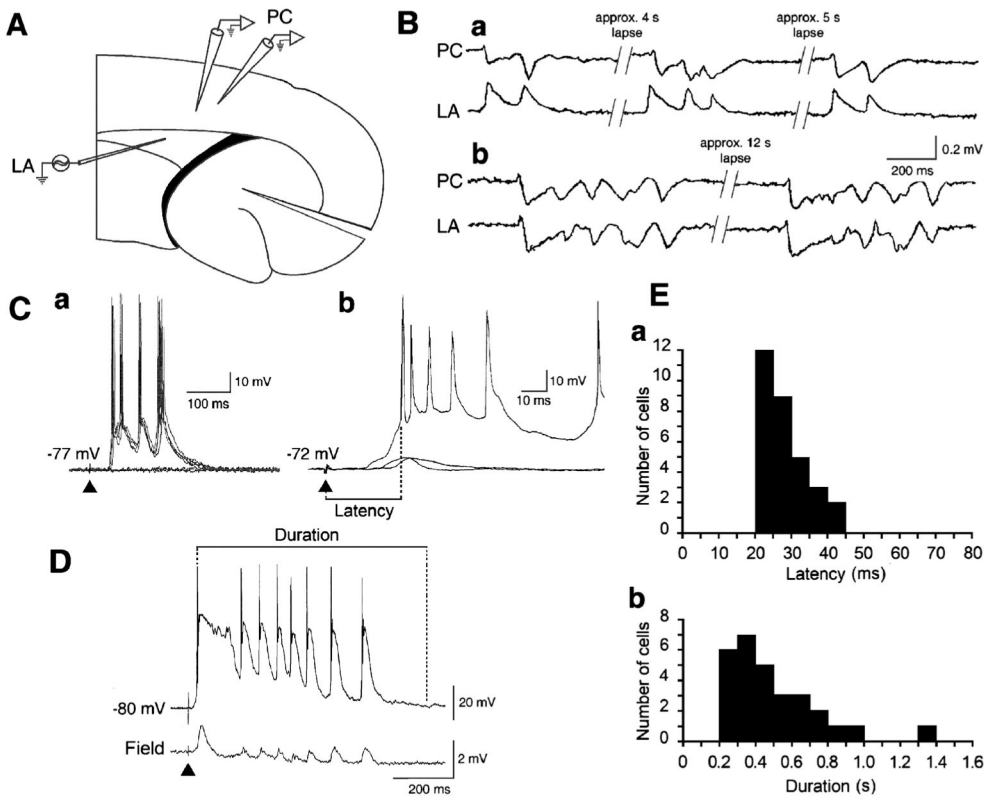

Fig. 1.

Field and intracellular responses recorded from the PC following LA stimulation at 0.1 Hz. **A** Schematic drawing of the combined slice used in our study and position of the recording electrodes; abbreviations in this and the following figures are as follows: *LA*, lateral amygdala; *PC*, perirhinal cortex. **B** Spontaneous field events recorded simultaneously from PC and LA in two different experiments are shown in insets *a* and *b* [32]; note that these oscillations, when occurring more frequently, have shorter duration than those occurring at longer intervals. **C, D** Oscillatory responses recorded with intracellular electrodes from PC neurons in three different brain slices following single-shock stimulation of the LA at 0.1 Hz. Note in **C** inset *a* the all-or-none action potential bursts riding over rhythmic depolarizations while in **C** inset *b* the oscillatory response emerges only when strong electrical stimuli are delivered. Note also in **D** that stimulus-induced bursting discharges are mirrored by field oscillations similar to those reported to occur spontaneously in **B. E** Plot histograms of the latency and of the duration of the responses generated by PC neurons following LA stimulation at 0.1 Hz. Measurements were obtained from intracellular recordings as shown in **C** inset *b* and **D**

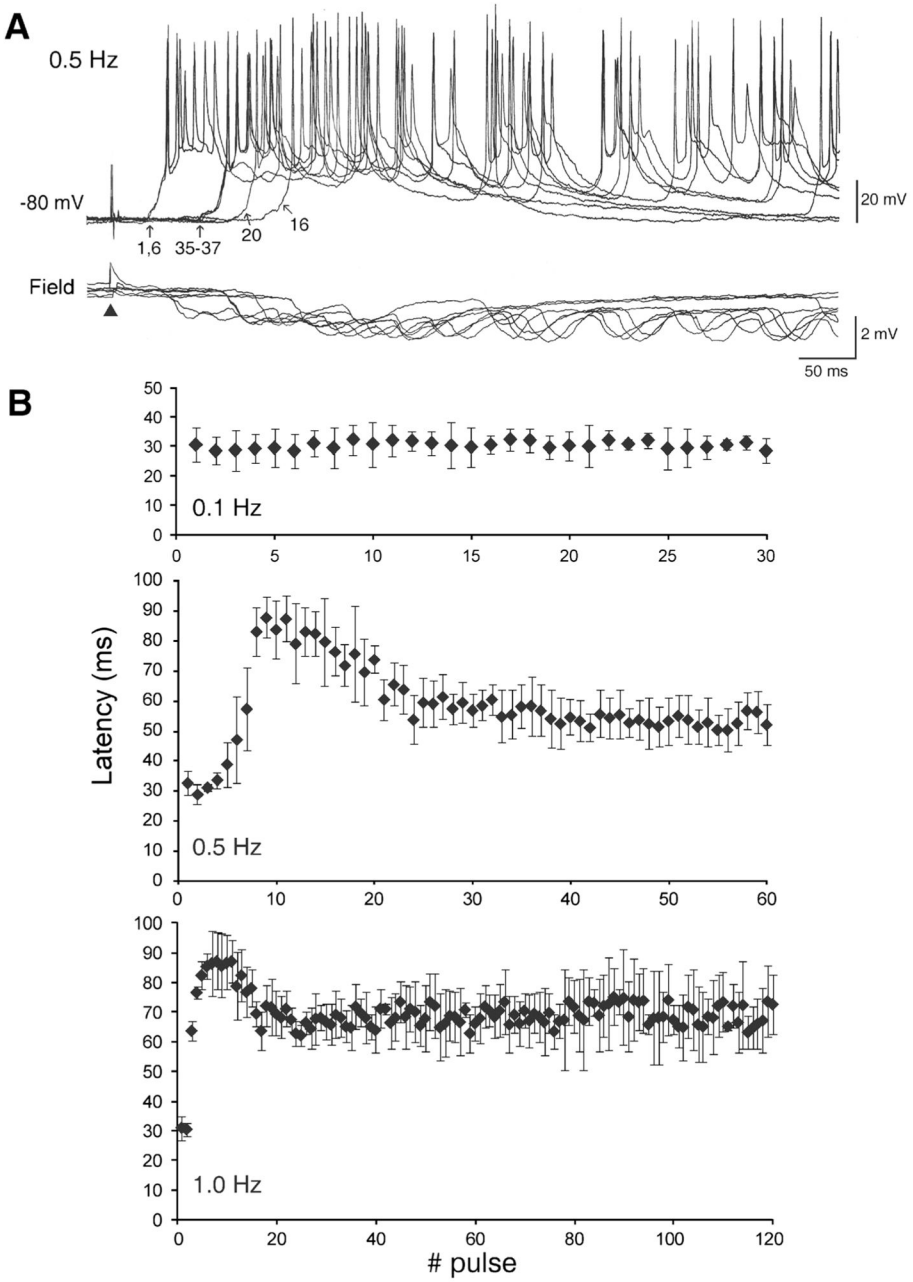

Fig. 2.

PC oscillatory network-driven responses increase in latency during continuous LA stimulation at 0.5 and 1 Hz. **A** Intracellular and field recordings obtained from the PC during repetitive stimuli delivered at 0.5 Hz; responses are superimposed and some of them are numbered according to the stimulation sequence order. Note that the latency progressively increases approximately after the first 5 successive stimuli and attains a relatively steady value after approximately 20 stimuli. **B** Plots of the latency of the oscillatory network-driven responses induced by stimuli delivered in LA at 0.1, 0.5, and 1 Hz in the experiment shown in **A**; these data are the average of the results observed during four successive trials. Note

that the “steady” latency of the responses induced by stimuli at 0.5 Hz is longer than that observed during 0.1-Hz stimulation, that the latency further increases when stimuli are delivered at 1 Hz, and that, in the latter case, the latency prolongation develops over time

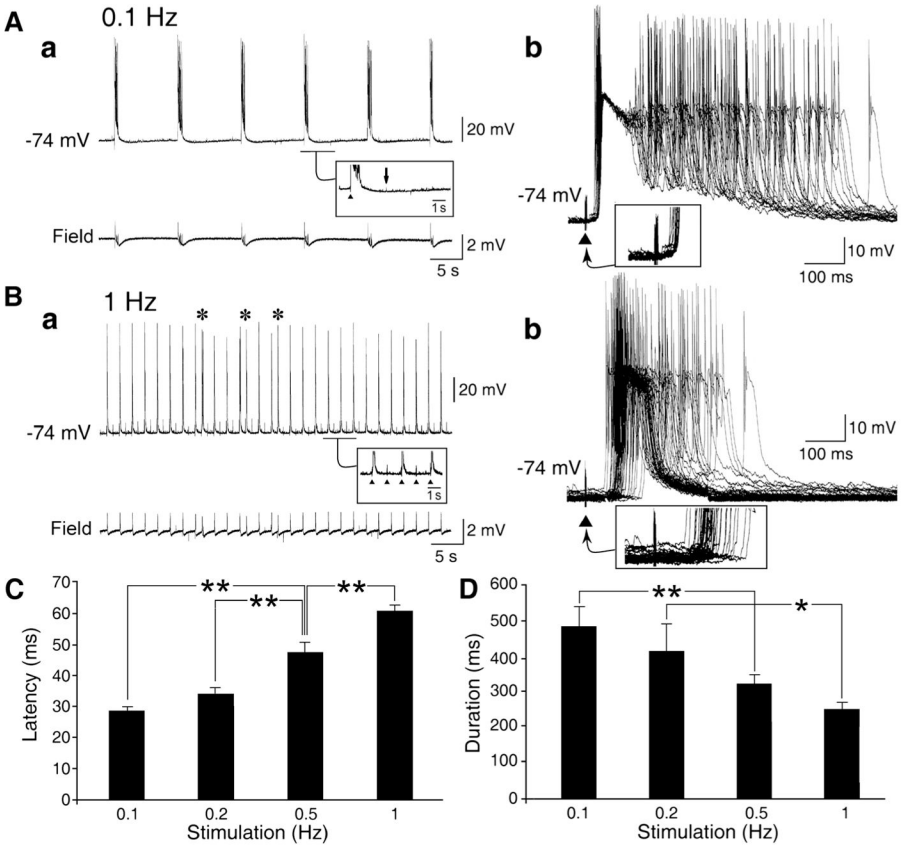

Fig. 3.

Latency and duration of the oscillatory network-driven responses recorded in the PC during continuous LA stimulation at different frequencies**. A**, **B** Intracellular (*upper trace*, −74 mV) and field (*lower trace*, Field) recordings obtained in the PC during continuous stimulation at 0.1 and 1 Hz. Samples were obtained once the responses had attained a "steady" latency. Note that in both inset *a*'s of **A** and **B**, at threshold strength, PC responses were consistently induced by stimuli delivered at 0.1 Hz, but not when the stimulus rate was increased to 1 Hz. Inset *b*'s of **A** and **B** illustrate in detail the prolongation of the response latencies. **C**, **D** Plots of the latency and of the duration of the responses induced by LA stimuli delivered at 0.1, 0.2, 0.5, and 1 Hz during the "steady" state in 31 neurons. Note in **C** that the latency values progressively increase when the stimulation rate is brought to values larger than 0.2 Hz while there is no significant difference between 0.1- and 0.2-Hz protocols. Note also in **D** that the duration of the oscillatory responses decreases by increasing the stimulation rate and that statistical differences (one-way ANOVA for repeated measures followed by post hoc Fisher's LSD test; *single asterisk* denotes significance at $p<0.05$; *double asterisks* denote significance at $p<0.01$) similar to those seen for the changes in latency characterize these measures

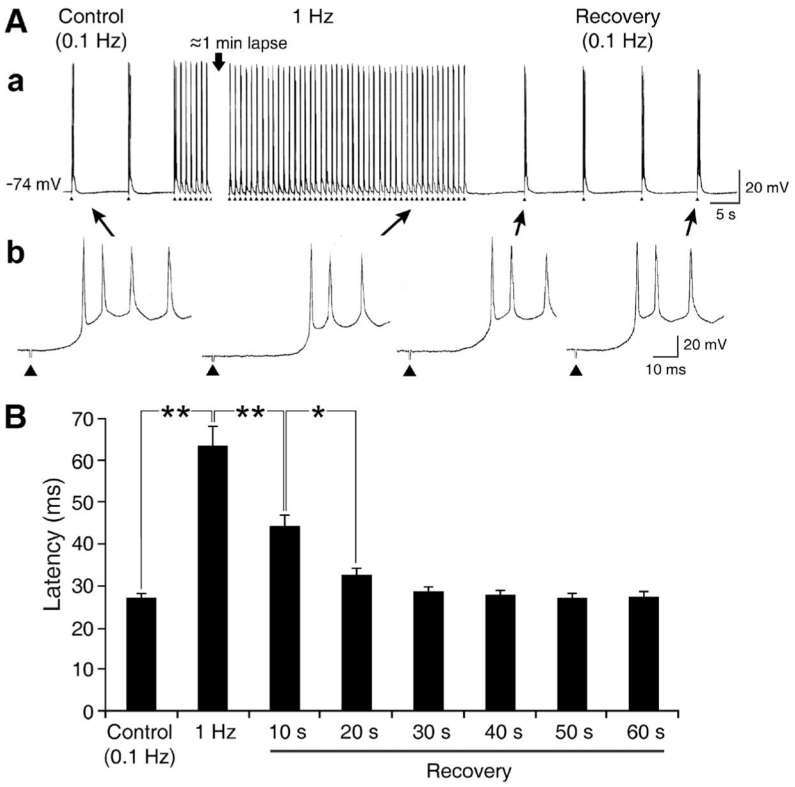

Fig. 4.

Latency prolongation returns to prestimulus conditions shortly after termination of repetitive stimulation. **A** Intracellular recording from a PC neuron during stimulation delivered at 0.1 Hz (*Control*), during continuous stimulation at 1 Hz (*1 Hz*) for approximately 100 s, and after termination (*Recovery*). Expanded recordings shown *below* are representative samples of the responses shown in on *top*. **B** Plots of the latency values obtained from 11 neurons in which the protocol shown in **A** was employed. Note that the response latency gradually decreased upon termination of the 1-Hz stimulation and returned to values similar to those seen under control conditions within 30 s (one-way ANOVA for repeated measures followed by post hoc Fisher's LSD test; *single asterisk* denotes significance at $p<0.05$; *double asterisks* denote significance at $p<0.01$)

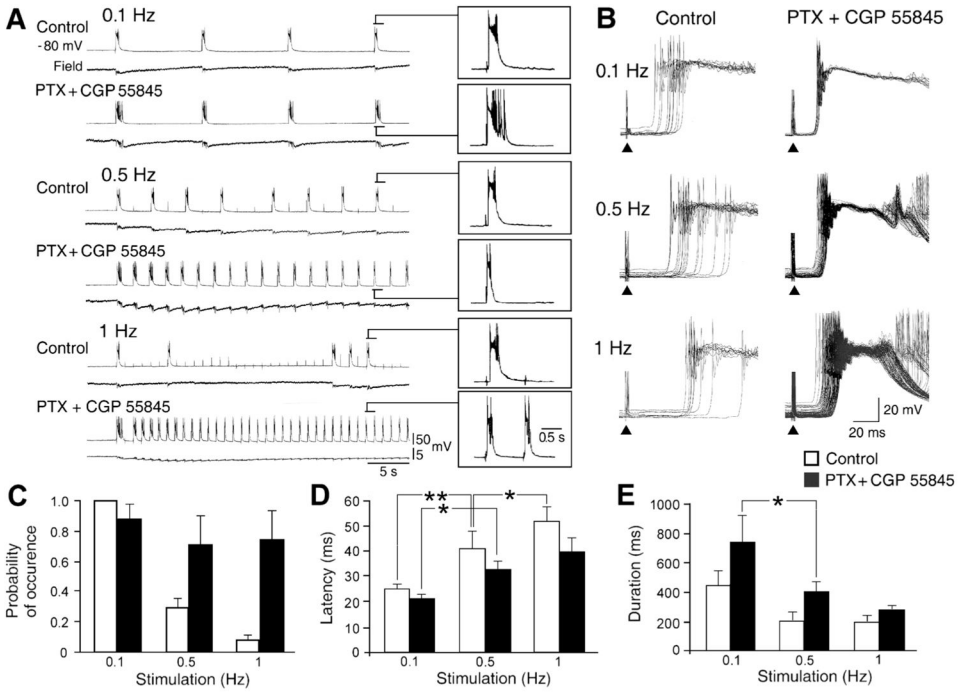

Fig. 5.

Effects induced by $GABA_A$ and $GABA_B$ receptor antagonists on latency prolongation and duration decrease occurring during LA stimulation at different frequencies. **A** Intracellular (*upper trace*, −80 mV) and field (*lower trace*, Field) recordings obtained in the PC during continuous stimulation at 0.1, 0.5, and 1 Hz; samples were obtained once the responses had attained a "steady" latency. Note the inset *a*'s of **A** and **B**, at threshold strength, PC responses were consistently induced only by stimuli delivered at 0.1 Hz, but not when the stimulus rate was increased to 0.5 or 1 Hz; in contrast, responses are always seen during application of picrotoxin (PTX, 50 μM)+CGP 55845 (4 μM). **B** illustrates in detail the prolongation of the response latencies under control conditions and during application of these GABA receptor antagonists. **C** Histogram illustrating the probability of occurrence of responses to stimuli delivered at 0.1, 0.,5 and 1.0 Hz under control conditions and in the presence of PTX+CGP 55845. **D**, **E** Plots of the latency and of the duration of the responses induced by LA stimuli delivered at 0.1, 0.5, and 1 Hz during the "steady" state condition in 17 neurons. Note that GABA receptor antagonists did not prevent but lessened the changes of latency in response to increased frequency of stimulation. Less marked changes were instead observed for the duration of neuronal response. *Single asterisks* denote significance at $p<0.05$; *double asterisks* denote significance at $p<0.01$; two-way ANOVA for repeated measures followed by post hoc Fisher's LSD test. Only differences among the different frequencies of stimulation are shown; refer to Table 2 for differences between groups of treatment

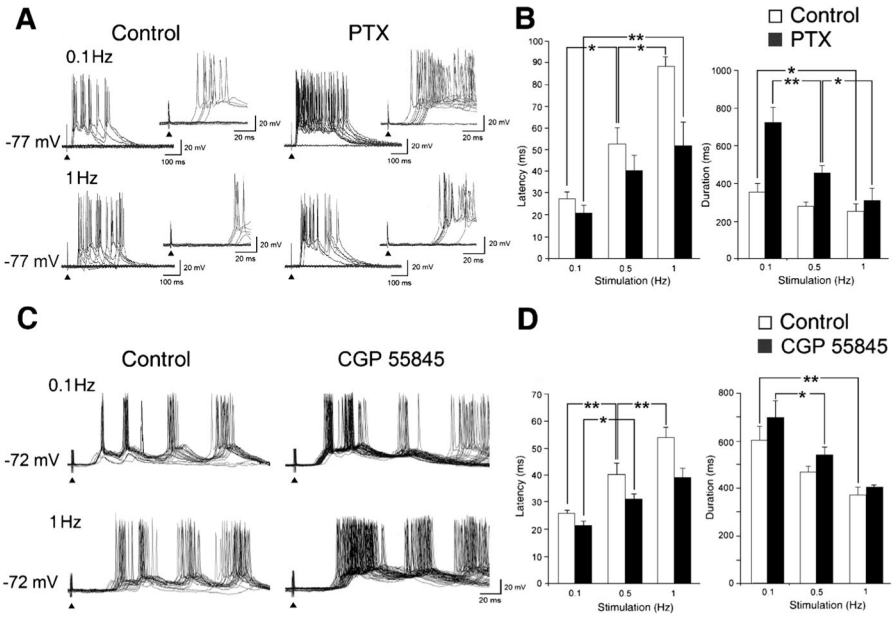

Fig. 6.

Effects induced by antagonizing the $GABA_A$ or the $GABA_B$ receptor on the latency prolongation and duration decrease occurring during LA stimulation at different frequencies**.** **A** Samples of superimposed intracellular recordings obtained during LA stimuli delivered at 0.1 and 1 Hz under control conditions and during application of the $GABA_A$ receptor antagonist picrotoxin (PTX). **B** Histograms illustrating the characteristics of the responses to stimuli delivered at 0.1, 0.5, and 1.0 Hz under control conditions and in the presence of PTX: note that changes in latency are less marked in presence of PTX. The duration of the responses induced by LA stimuli was also affected by changing the frequency of stimulation from 0.1 to 0.5 and 1 Hz, as shown by histograms of average values measured during the "steady" state condition. **C** Samples of superimposed intracellular recordings under treatment with the $GABA_B$ receptor antagonist CGP 55845 are shown. **d** Histograms of latency and duration of responses to stimuli delivered at 0.1, 0.5, and 1.0 Hz under control conditions and in the presence of CGP 55845 illustrate major effects on latencies. Changes in the duration of the responses induced by LA stimuli were similar both in control and in drug treatment conditions. *Single asterisk* denotes significance at $p<0.05$; *double asterisks* denote significance at $p<0.01$; two-way ANOVA for repeated measures followed by post hoc Fisher's LSD test. Only differences among the different frequencies of stimulation are shown; refer to Table 2 for differences between groups of treatment

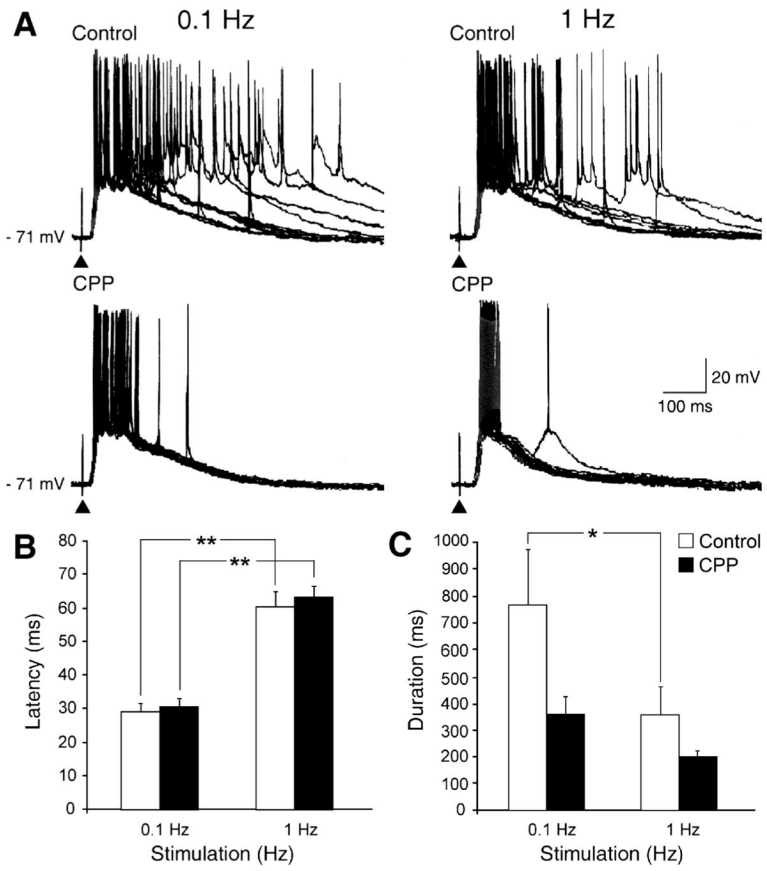

Fig. 7.

Effects induced by the NMDA receptor antagonist CPP on latency prolongation and duration decrease occurring during LA stimulation at 0.1 and 1 Hz. **A** Samples of superimposed intracellular recordings obtained during LA stimuli delivered at 0.1 and 1 Hz under control conditions and during application of the NMDA receptor antagonist CPP. **B** Histograms illustrating the characteristics of the responses to stimuli delivered at 0.1 and 1.0 Hz under control conditions and in the presence of CPP: note that no effects of drug treatment are present on latency. The duration of the responses induced by LA stimuli varied in control medium by changing the frequency of stimulation from 0.1 to 1 Hz. *Single asterisk* denotes significance at $p<0.05$; *double asterisks* denote significance at $p<0.01$; two-way ANOVA for repeated measures followed by post hoc Fisher's LSD test. Only differences between the two frequencies of stimulation are shown; refer to Table 3 for comparisons within groups of treatment

Table 1

Fundamental electrophysiological properties of the PC neurons recorded intracellularly in the deep layers

RMP	−70±1.0 mV	(*n*=25)
R_i	38.7±1.6 MΩ	(*n*=20)
τ	15.2±0.8 ms	(*n*=10)
APA	86.1±1.5 mV	(*n*=22)
APD	1.4±0.1 ms	(*n*=21)

Values are expressed as mean±SEM.

RMP resting membrane potential, R_i input resistance, τ time constant, *APA* action potential amplitude, *APD* action potential duration

Table 2

Changes in latency and duration with GABA blockers

	Latency			Duration		
Treatments	0.1 Hz	0.5 Hz	1 Hz	0.1 Hz	0.5 Hz	1 Hz
ACSF	25.6±1.6	40.8±6.3	54.1±5.1	461.6±103.3	277.3±48.9	219.5±45.1
	p=0.38	p=0.18	p<**0.05**	p<**0.05**	p=0.36	p=0.51
PTX+CGP 55845	20.9±1.9	33.1±3.1	40.0±4.2	766.5±187.1	422.5±59.9	312.4±28.1
ACSF	29.4±3.7	54.0±7.1	86.2±1.9	381.1±65.0	289.9±18.5	262.8±27.6
	p=0.51	p=0.19	p<**0.05**	p<**0.01**	p<**0.01**	p=0.07
PTX	21.7±4.3	44.8±8.7	55.9±13.5	742.4±90.6	465.9±47.2	330.8±53.9
ACSF	25.3±1.0	40.4±4.8	55.3±2.5	604.2±63.9	467.5±19.8	371.1±31.2
	p=0.27	p<**0.05**	p<**0.01**	p=0.15	p=0.27	p=0.55
CGP 55845	22.9±1.4	31.3±1.8	39.5±2.9	698.6±66.1	548.8±31.2	408.3±6.8

Values are expressed as mean± SEM. Significant p values are indicated in bold. Fisher's LSD test

Table 3

Changes in latency and duration with CPP

	Latency		**Duration**	
Treatments	0.1 Hz	1 Hz	0.1 Hz	1 Hz
ACSF	29.4±3.1	63.7±4.2	755.1±219.5	365.3±99.2
	p=0.82	p=0.55	p<**0.05**	p=0.28
CPP	30.4±3.0	66.5±2.5	366.0±67.7	208.9±14.2

Values are expressed as mean±SEM. Significant p values are indicated in bold. Fisher's LSD test