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The lemniscal–cuneate recurrent excitation is suppressed by strychnine and enhanced by $GABA_A$ antagonists in the anaesthetized cat

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Abstract
In the somatosensory system, cuneolemniscal (CL) cells fire high frequency doublets of spikes facilitating the transmission of sensory information to diencephalic target cells. We studied how lemniscal feedback affects ascending transmission of cutaneous neurons of the middle cuneate nucleus. Electrical stimulation of the contralateral medial lemniscus and of the skin at sites evoking responses with minimal threshold induced recurrent activation of CL cells at a latency of 1–3.5 ms. The lemniscal feedback activation was suppressed by increasing the stimulating intensity at the same sites, suggesting recurrent-mediated lateral inhibition. The glycine antagonist strychnine blocked the recurrent excitatory responses while $GABA_A$ antagonists uncovered those obscured by stronger stimulation. CL cells sharing a common receptive field (RF) potentiate one another by recurrent activation and disinhibition, the disinhibition being produced by serial interactions between glycinergic and GABAergic interneurons. Conversely, CL cells with different RFs inhibit each other through recurrent GABA-mediated inhibition. The lemniscal feedback would thus enhance the surround antagonism of a centre response by increasing the spatial resolution and the transmission of weak signals.

Keywords: Cutaneous transmission, Disinhibition, Lateral inhibition, Medial lemniscus

Introduction

The cuneolemniscal (CL) neurons excited by displacing hairs or by touching the skin characteristically discharge many impulses in doublets (two spikes separated by ≈1 ms) (Amassian & De Vito, 1957; Galindo *et al*., 1968). The cuneolemniscal fibres emit recurrent collaterals before entering the medial lemniscus (ML) (Cajal, 1909; Fyffe *et al*., 1986) and ML stimulation induces on CL cells a repetitive synaptic response of one or two spikes following the antidromic action potential at a latency of 1.3–3.7 ms (Amassian & De Vito, 1957). These late spikes have been attributed to excitatory synaptic action produced by recurrent collaterals of CL neurons because they are induced by ML stimulation at the same threshold as, or even lower than, the antidromic response (Gordon & Jukes, 1964; Mariño *et al*., 1999; Canedo *et al*., 2000), thus ruling out current spread to the cerebral peduncle. The possibility remains that the excitatory responses might be due to the intrinsic properties of CL cells (Galindo *et al*., 1968; Canedo *et al*., 1998). However, ML stimulation induces recurrent excitatory and inhibitory postsynaptic potentials in CL and non-CL (nCL) neurons (Canedo *et al*., 2000), thus suggesting recurrent circuitry.

The nCL cells may release GABA and/or glycine as inhibitory neurotransmitters (Galindo *et al*., 1967). Both neurotransmitters appear to be implicated in feedback processes because ML stimulation increases their release (Roberts, 1974). The local GABAergic neurons comprise ≈25% of the neuronal population of the cat's middle cuneate nucleus (Rustioni *et al*., 1984; Roettger *et al*., 1989; Heino & Westman, 1991). The glycinergic cells make up ≈31% of the total cuneate neurons in the rat (Lue *et al*., 1997) where a substantial proportion of the nCL cells colocalize GABA and glycine (Popratiloff *et al*., 1996). Dorsal column nuclei cells stain for glycine and exhibit strychnine binding (Zarbib *et al*., 1981; Probst *et al*., 1986; Pourcho *et al*., 1992).

The lemniscal recurrent feedback may be functionally selective, activating, through recurrent collaterals, CL cells with similar receptive fields (RFs) while inhibiting CL neurons with different RFs (Canedo *et al*., 2000). If this assumption is correct, then widespread nuclear excitation via

high-intensity ML and cutaneous stimulation should mask the recurrent excitation. Then, blocking the inhibitory receptors of the recorded CL cells should uncover the short-latency recurrent excitation. To test this hypothesis, antidromically identified CL cells were extracellularly recorded while applying specific agonists and antagonists of GABA and glycine. The results have been reported partially in abstract form (Aguilar *et al*., 2002).

Materials and methods

All procedures conformed to the Spanish Physiological Society, the International Council for Laboratory Animal Science and the European Union (Statute N°86/809). Data were obtained from cats (2.5–5 kg) of either sex anaesthetized with α-chloralose (60 mg/kg i.v.; $n = 7$) or sodium pentobarbital (35 mg/kg i.v.; $n = 5$) after induction with ketamine (10–20 mg/kg i.m.) There was no evidence that choice of anaesthetic affected the results obtained. The animals were artificially ventilated at 20–30 strokes/min, with 25–40 mL/stroke according to body weight, and paralysed (pavulon 1 mg/kg/h, i.v.) Additional doses of anaesthesia (1/4 of a full dose) were regularly administered when necessary. The depth of anaesthesia was assessed by monitoring the heart rate, the electrocorticogram (ECoG; digitally filtered at a frequency band-pass of 1–100 Hz), and by observing the state of the pupil. Changes of heart rate (maintained in the range 90–140 beats/min) or in the pattern of the ECoG (high-amplitude and low-frequency waves were taken as sign of adequate anaesthesia), and dilated pupils, or pupils reacting rapidly to electrical stimuli, were considered to reflect inadequate anaesthesia in which case a supplementary quarter of a full dose of anaesthetic was immediately injected. The expired CO_2 (4–4.5%) and temperature (37.5 °C) were continuously monitored and adjusted when necessary throughout the experiment. A small hole was made over the lateral tip of the cruciate sulcus with a trephine to introduce a concentric bipolar electrode 1.5 mm deep to continuously monitor the ECoG. Glucose (5%) in saline was constantly infused (4 mL/h, i.v.)

The animals were suspended with clamps attached to the lumbar and thoracic vertebrae, and the dorsal medulla was exposed to insert electrodes in the middle cuneate nucleus (0–4 mm caudal to the obex). A bilateral pneumothorax was routinely performed to minimize pulsatile movements. Warm agar (4% in 0.9% saline) was added to all exposed tissues to increase stability and avoid desiccation. A craniotomy was performed at Horsley–Clark coordinates A2, L4.5 to lower a movable bipolar stimulating electrode to the ML (H-5) which served to antidromically identify CL cells according to standard criteria, in all cases including a collision test (Canedo & Towe, 1986; Canedo & Lamas, 1993) (Fig. 1A).

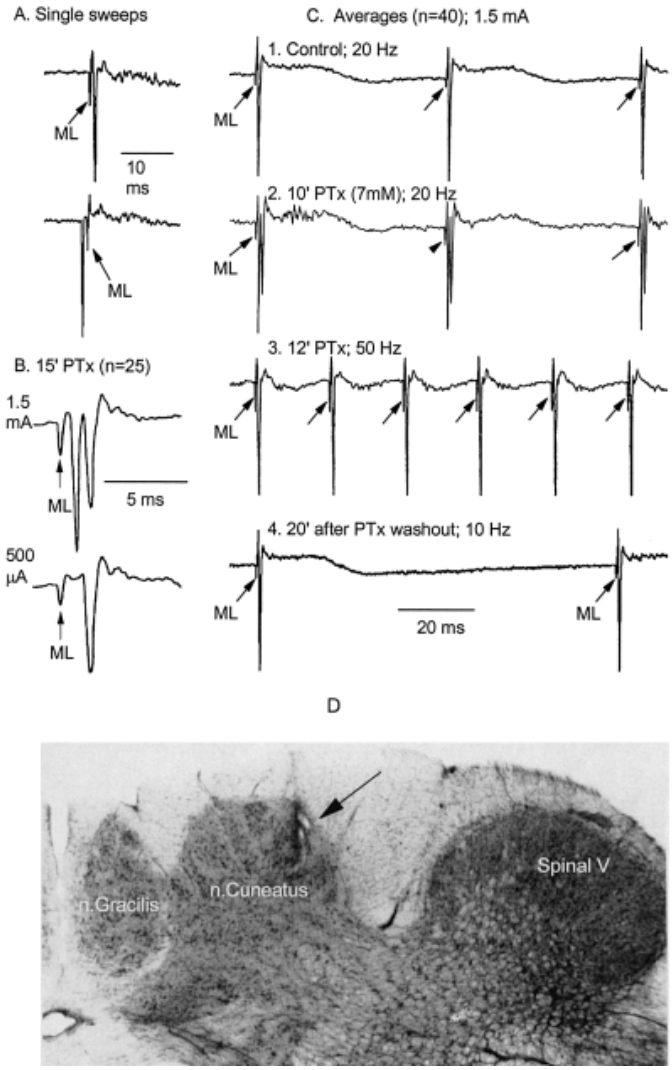

Figure 1. The recurrent activity is synaptically generated and it is modulated by $GABA_A$ receptors. (A) The record in the top single trace shows a short-latency, presumed antidromic, spike elicited by ML stimulation. Shocking the same ML site immediately after a spontaneous spike occurs (lower trace) generated no spike, confirming the antidromic nature. (B) ML stimulation (1.5 mA) after PTx application generated an antidromic spike followed by a recurrent response (upper). Decreasing the ML stimulating intensity below threshold for antidromic activation produced the recurrent response alone (lower). (C) The isolated antidromic spike (C1) was followed by a second recurrent spike after topical treatment with PTx (C2) that disappeared at a stimulating frequency of 50 Hz (C3) indicating that it was synaptically evoked. Chloralose anaesthesia. Arrows mark the ML stimulus artifacts. (D) Photomicrograph of a coronal histological section showing an electrode track within the cuneate nucleus, marked by an arrow.

Extracellular recording and application of drugs

In a first series of experiments ($n = 4$), the recording of single neurons was accomplished through tungsten electrodes (12 MΩ resistance), and the noncompetitive $GABA_A$ antagonist picrotoxin (PTx), the competitive $GABA_A$ antagonist bicuculline (BiCu) and the glycine antagonist strychnine were topically applied following the technique described by Davidson & Southwick (1971). A 3-mm-diameter ring was seated over the cuneate nucleus and the tungsten electrode introduced. The exposed tissue, except for the pial surface enclosed by the ring, was covered with agar. Polyethylene tubes introduced and removed fluid from the ring. The drugs were dissolved in cerebrospinal fluid (obtained from each animal upon opening of the cisterna magna)

at concentrations of 6–8 mm (PTx), 3–5 mm (BiCu) and 20–40 mm (strychnine). The extent of the diffusion of topically applied drugs is difficult to define. It is possible that they could reach neighbouring structures surrounding the cuneate nucleus (e.g. reticular formation). This diffusion might explain the fact that sensorimotor cortex responded to polysensory stimulation and generated paroxysmal activity after prolonged (> 1 h) application of PTx and BiCu. For this reason, the results with topical application of drugs were obtained during the first 45–50 min after application to avoid the influence of an overexcited cortex. In a second series of experiments (n = 8) the same drugs as well as GABA and glycine were iontophoretically ejected in an effort to ensure a local effect. To this end, 3–5-barreled pipettes were attached to a mechanical microdrive and used for extracellular recording and iontophoresis. The barrels were filled with one of: 3 m NaCl for recording, GABA (1 m, pH 4), BiCu methiodide (20 mm, pH 4), glycine (1 m, pH 3.5) or strychnine (10 mm, pH 5.5). Ejection currents were in the range 5–20 nA for glycine, 20–35 nA for BiCu and of 40–80 nA for GABA and strychnine. The ranges of currents used for GABA and glycine were established by observing their effect on responses to stimuli applied to the skin, and those for BiCu and strychnine were selected by measuring the current level necessary to reverse the effect of iontophoresed GABA (Fig. 2A) and glycine, respectively. When not in use, each drug barrel was subjected to a constant retention current of 15–25 nA of appropriate polarity.

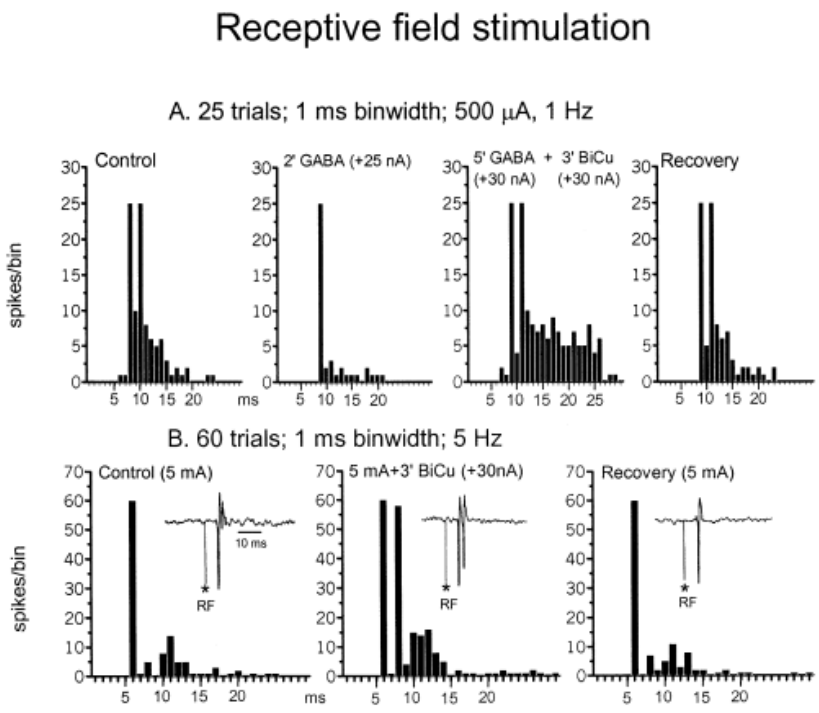

Figure 2. GABA masked the recurrent activation evoked by low-intensity skin stimulation. Bicuculline (BiCu) unmasked the recurrent activation obliterated by high-intensity skin stimulation. Histograms showing the response of two different CL cells (A and B) to RF stimulation. (A) Response to RF stimulation in control and during iontophoretic ejection of GABA, GABA + BiCu and recovery. Note that BiCu ejection not only reversed the effect of GABA on the recurrent spikes but also increased the later discharges (third histogram from the left). (B) Increasing the intensity of RF stimulation to 5 mA suppressed recurrent activation (left) that was uncovered upon BiCu ejection (middle); single specimen records are shown for each case in the insets, with the RF stimuli signaled by asterisks.

When a cell was well isolated from the background, it was tested with stimulation of the ML, and only antidromically responsive CL neurons were considered for further study. The excitatory RF was determined and a bipolar needle electrode (9 mm intertip separation) placed into the skin area which activated the CL neuron under study with minimal threshold and latency. Rectangular pulses of 0.05–1 ms duration and up to 5 mA intensity were applied to the peripheral RF. Shorter duration (0.05–0.1 ms) and lower intensity (up to 2 mA) pulses were used to stimulate the ML. The effect of the stimulation was averaged for 25–75 trials in control conditions, again during

ejection of drugs, and finally after recovery. Stability of the recording situation was ascertained by comparing responses collected during the first and the last set of trials. Data were retained when there was no significant difference in the response. Histograms were constructed from the accumulated number of spikes for the total number of trials.

Recordings were stored on magnetic tape and analysed offline. The recording electrode was considered to be in the cuneate nucleus when gentle tapping and brushing of the ipsilateral forearm produced short-latency cellular responses.

The animals were killed with an overdose of anaesthetic, and histological procedures confirmed that all the recordings reported in this study were obtained from the middle cuneate nucleus (Fig. 1D).

Results

The data set includes 60 CL cells, of which 21 were studied with topical application of drugs and 39 were studied with iontophoresis. Because both techniques gave similar results they were grouped together for statistical purposes.

A total of 42 CL neurons (70%) showed a short-latency spike following the antidromic response (1–3.5 ms latency from the peak of the antidromic spike to the peak of the subsequent response), which is presumably produced through CL recurrent collaterals. Most of these recurrent excitations (30/42; 70%) were observed only after being uncovered using one of two different strategies: (i) placing the lemniscal electrode at the site of minimum threshold for antidromic activation, and (ii) applying $GABA_A$antagonists.

Effect of $GABA_A$ antagonists on recurrent responses

Topical (Fig. 1C) and iontophoretical (Figs 2 and 3) application of $GABA_A$ antagonists unmasked recurrent responses not seen in control conditions. In the control condition the CL neuron illustrated in Fig. 1 responded to lemniscal stimulation (1.5 mA) generating only an antidromic spike (Fig. 1A and C1). Upon topical application of PTx, the cell produced a second, recurrent, spike (Fig. 1C2) that failed at a stimulating frequency of 50 Hz (Fig. 1C3). ML stimulation at subthreshold intensity for antidromic activation produced solely the recurrent response (Fig. 1B, lower trace), indicating that it was not linked to the antidromic response but rather induced through collaterals of other CL neurons.

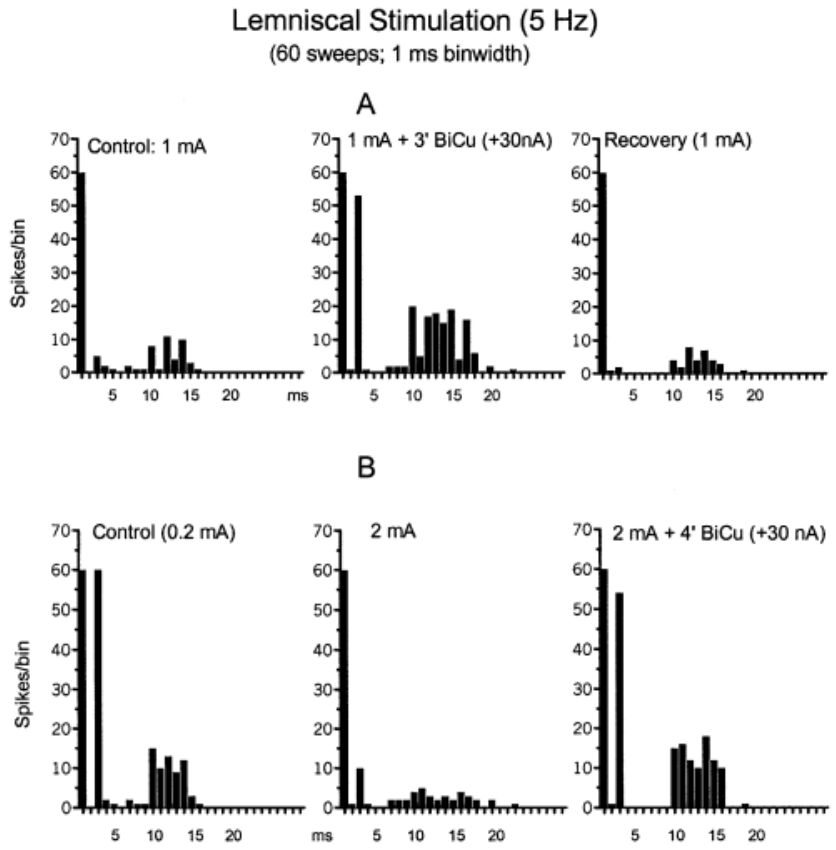

Figure 3. Increasing the intensity of medial lemniscus (ML) stimulation blocked the recurrent activation. Response from a CL cell to ML stimulation in different experimental conditions. (A) ML stimulation (1 mA) did not induce recurrent excitation (left), which was unmasked by BiCu ejection (middle). (B) The recurrent excitation was present when stimulating the ML at the site of minimum threshold for antidromic activation (left). Increasing the ML stimulating intensity to 2 mA at the same site blocked the recurrent activation (middle) that was subsequently uncovered by BiCu ejection (right).

The recurrent responses obtained when stimulating at low intensity (< 1 mA) in the excitatory centre of the peripheral RF were blocked by iontophoretically ejecting GABA (Fig. 2A). Concurrent ejection of BiCu reversed the effect of GABA and also increased the long-latency responses (see also the examples for other different CL neurons in Figs 2B and 3). On the other hand, high-intensity (3–5 mA) stimulation of the skin masked the recurrent effects (Fig. 2B, left).

The ML stimulating electrode was moved to test whether ML stimulation produced different recurrent effects when applied near the axon of the recorded CL neuron and beyond. The data shown in Fig. 3 illustrate a CL cell responding antidromically to ML stimulation that also generated a recurrent response upon BiCu ejection (Fig. 3A, middle) and when stimulating at the site of minimal threshold (Fig. 3B, left). Increasing the ML stimulating intensity at the site of minimal threshold abolished the recurrent response (Fig. 3B, middle). The recurrent activation again reappeared after ejecting BiCu (Fig. 3B, right), thus indicating that it might have been induced through recurrent-induced lateral inhibition. A similar behaviour was observed in 36 CL cells. The remaining CL neurons presenting recurrent responses (6/42 or 14.3%; all silent at rest) did not show any evidence of lemniscal feedback inhibition able to suppress the recurrent spikes when stimulating the RF (Fig. 4) or the ML at increasing intensities. These cells continued to generate doublet firing at 2 mA lemniscal stimulating intensity even when the stimulating electrode was placed all the way through the ML, and after iontophoretic ejection of strychnine and GABA.

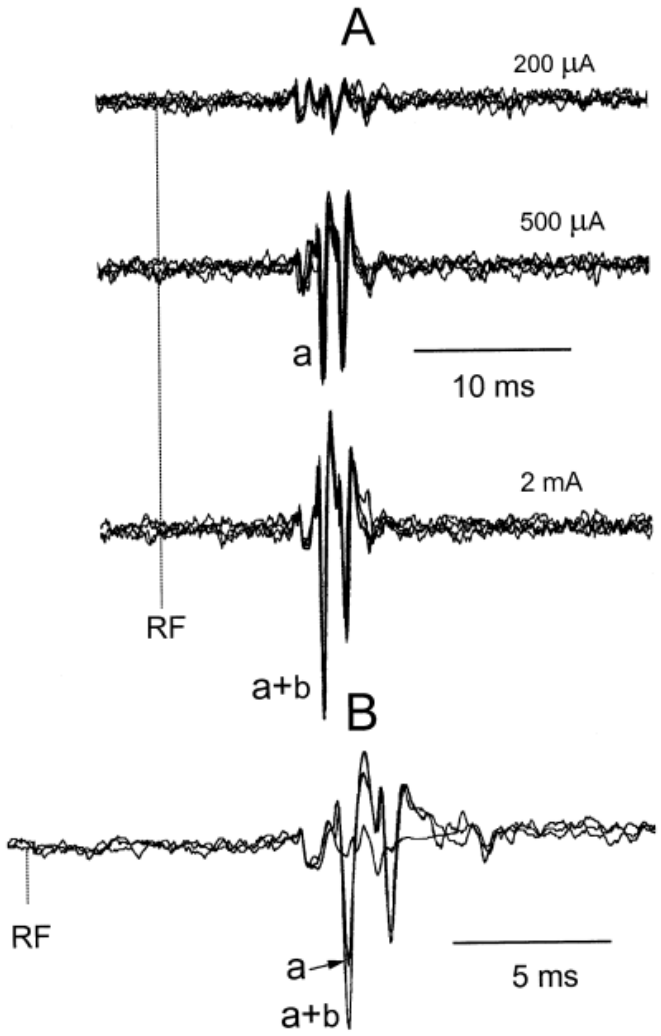

Figure 4. Fourteen per cent of the double-spiking CL cells did not show evidence of lateral inhibition. (A) Traces of five superimposed sweeps each showing that RF stimulation induced a doublet of spikes on a low-threshold CL cell (spikes a, middle trace). Increasing the RF stimulating intensity did not abolish the second spike of the doublet, and induced a single spike on a different higher-threshold CL cell (spike b) superimposed on the first spike of the lower-threshold doublet (third trace). (B) Three superimposed sweeps showing the response to RF stimulation from subthreshold to suprathreshold for both spikes a and b. The vertical dotted lines signal the RF stimuli.

In summary, these data show that the ML recurrent collaterals not only serve to increase the activity of CL cells with axons in close proximity but also that most collaterals contribute to decrease the propagated activity of CL cells with a nonadjacent RF. This recurrent contribution to lateral inhibition was blocked by $GABA_A$ antagonists.

Effect of strychnine

The most conspicuous effect produced by topical and iontophoresed strychnine were depression of the spontaneous activity of CL cells and block of the evoked recurrent excitation (Fig. 5). Strychnine completely suppressed the spontaneous activity of all the CL cells tested (n = 10, Fig. 5A) and the recurrent activation elicited by ML (Fig. 5B) and RF (not shown) stimulation for the great majority of tested cells (20/26 or 77%).

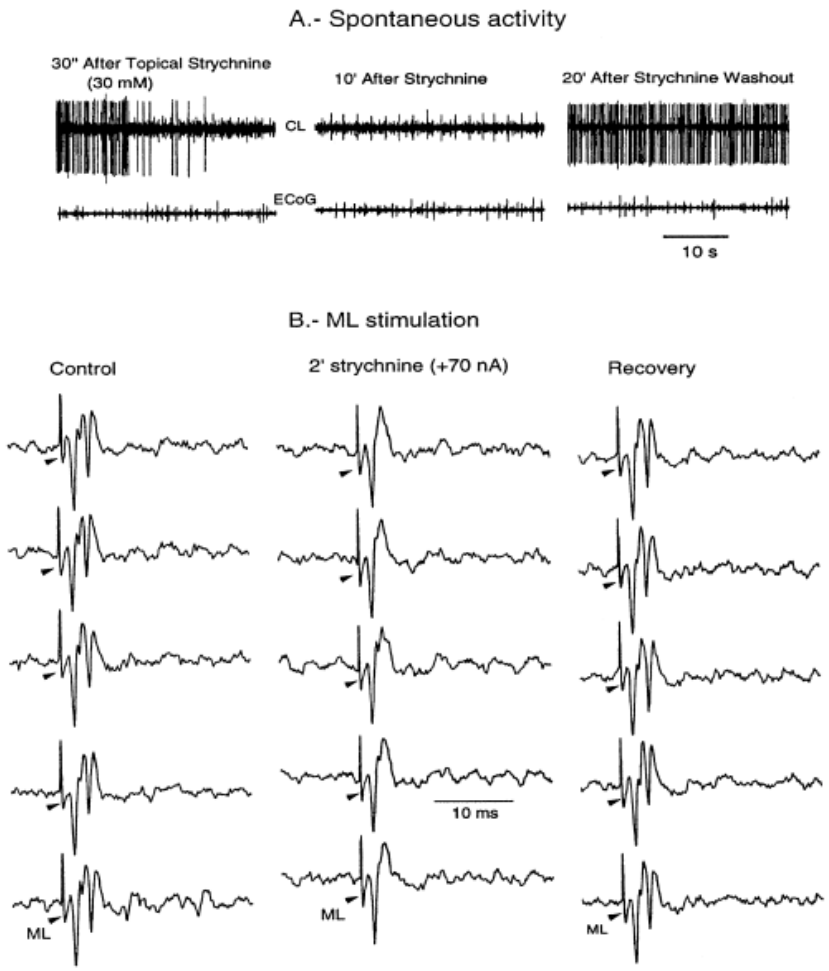

Figure 5. Strychnine blocked the spontaneous activity and the recurrent activation of CL cells. (A) Topical application of strychnine blocked the spontaneous firing of a CL cell without affecting the electrocorticographic activity (ECoG); nembutal anaesthesia. (B) Single sweeps. Iontophoretical ejection of strychnine in a different experiment blocked the second, recurrent, spike induced by ML stimulation (middle column). ML stimulation (250 µA) throughout. The black arrowheads signal the ML stimuli.

Discussion

General

This study provides evidence that most CL cells receive excitatory afferents from lemniscal recurrent axons and that this recurrent excitation is blocked by GABA and facilitated by glycine. The recurrent responses are usually masked by high-intensity stimulation of the ML or the RF and can be uncovered by blocking $GABA_A$ receptors. These findings suggest that the lateral inhibition produced on CL cells by lemniscal feedback is mostly, if not solely, due to the activation of local GABAergic neurons.

The incidence of recurrent responses increased when low-intensity electrical stimuli were applied to ML or peripheral sites evoking responses at minimal thresholds, presumably by a stronger activation of central excitatory regions relative to inhibitory surrounds (Brown *et al*., 1974; Canedo & Aguilar, 2000).

GABA and glycine influences on recurrent responses

GABA, strychnine and high-intensity stimulation selectively blocked the second, recurrent, spike in the doublets of CL cells. The suppression of the recurrent excitation by strychnine

reinforces the suggestion that CL cells do not possess glycine receptors (Kelly & Renaud, 1973). Alternatively, while the GABAergic receptors might be mostly located in the soma and proximal dendrites (Lue *et al*., 1994), the glycine receptors could be located in the distal dendrites (Popratiloff *et al*., 1996; Lue *et al*., 2000), unlikely to be affected by antagonist iontophoresed near the soma or, possibly, the modulatory effect of strychnine might not be detectable on spiking activity. A parsimonious view of our results suggests that glycinergic cells disinhibit CL neurons through interposed GABAergic cells (Fig. 6). Blocking the glycine receptors would allow the GABAergic cells to inhibit the cuneolemniscal transmission.

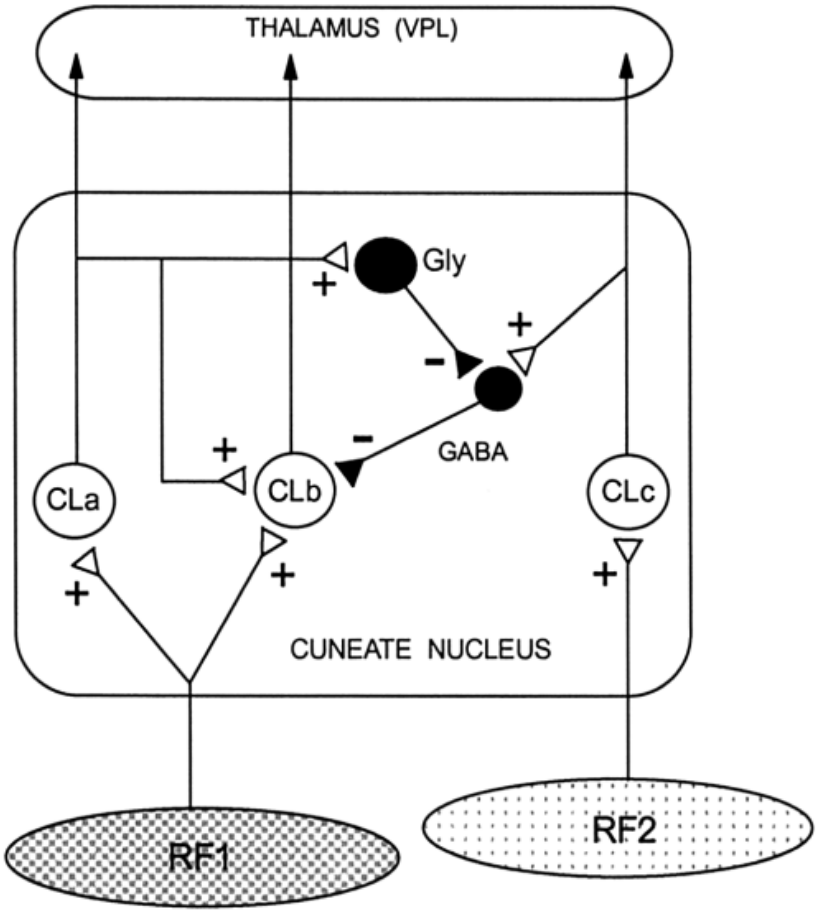

Figure 6. Proposed lemniscal feedback intracuneate mechanisms. Stimulation of receptive field 1 (RF1) will activate CL cells a and b (CLa, CLb) that recurrently activate and disinhibit each other. Stimulation of the neighbouring RF2 will activate the CL cell c (CLc) that will induce lateral inhibition on CLa and CLb through GABAergic local neurons. Inhibitory inputs from CLa to CLc and facilitatory ones from CLb to CLa have been omitted for simplicity. VPL, ventroposterolateral nucleus of the contralateral thalamus; Gly, glycine; +, excitation; –, inhibition.

The present results can be explained notwithstanding the interneurons colocalizing GABA and glycine (Popratiloff *et al*., 1996). The colocalizing interneurons may serve other roles, for example, modulation of primary afferent input through an independent circuit, or via the different distribution of receptors (GABA proximal, glycine distal), may subserve other functions. Furthermore, a species difference may exist because colocalizing interneurons have not been described in the cat.

Intrinsic vs. recurrent doublet firing

It might be argued that doublet firing is produced intrinsically and not a circuit property. However, the axons of CL neurons do emit recurrent collaterals reentering the nucleus (Cajal, 1909; Fyffe *et al*., 1986). It is also true that the CL cells have a propensity to generate bursting discharges when hyperpolarized but tend to present tonic discharges when depolarized (Canedo *et al*., 1998). Because the lemniscal axons release excitatory amino acids (De Biasi *et al*., 1994), their monosynaptic recurrent action is likely to be excitatory. It can hardly be produced through inhibitory interneurons leading to subsequent postinhibitory rebounds because of its short latency. These rebounds have been observed at a latency of 5 ms or longer following the antidromic spike (Canedo *et al*., 2000). Furthermore, recurrent excitation can be generated by ML stimulation with currents below threshold for antidromic activation, corroborating previous intracellular results showing ML-induced recurrent excitatory postsynaptic potentials at a similar short latency to that described here (Canedo *et al*., 2000). Further support for a role of the intrinsic circuitry in the generation of doublet firing is that iterative ML stimulation at 50 Hz abolished the recurrent excitation without affecting the antidromic response (Fig. 1C).

Relationship to previous work

When using a fixed electrode to shock the ML at subthreshold intensity for antidromic activation, the incidence of lemniscal recurrent excitatory effects was lower (Gordon & Jukes, 1964) than that described here. Obviously, using a fixed stimulating electrode increases the probability of stimulating lemniscal axons with different RFs of the recorded neuron, thus producing feedback inhibition. The same argument would also explain the scarce lemniscal–cuneate recurrent observations reported when using high-intensity ML stimulation to increase the probability of identifying CL cells (Andersen *et al*., 1964).

The assumption that high-intensity peripheral and ML stimulation activates CL cells with different RFs that will tend to cancel each others' activity is also substantiated by prior work. Amassian & De Vito (1957) reported that increasing the ML stimulating intensity often resulted in the inhibitory suppression of the short-latency recurrent responses that followed the antidromic spikes. Gordon & Seed (1961) and Andersen *et al*. (1964) claimed that most of the excitatory lemniscal recurrent effects are exerted by axon collaterals of CL cells on nCL cells, and Andersen *et al*. (1964) reported that stimulation of an afferent nerve blocked the responses induced by stimulating a different afferent nerve.

Functional significance

The probability that a doublet generates a propagated response on thalamic cells is greatly increased and the probability that a prethalamic cell may synchronize several target thalamic neurons increases more than 12 times for the second spike compared to the first (Usrey *et al*., 1998). The recurrent excitation and disinhibition produced by CL cells with shared RFs (CLa and CLb in Fig. 6) would serve not only to increase the spatial resolution but also to potentiate weak signals at prethalamic level. Blocking the glycinergic receptors releases the GABAergic cells from inhibitory input. These GABAergic neurons subsequently hyperpolarize the CL cells thus obliterating the recurrent excitation. Blocking of $GABA_A$ receptors precludes inhibition of CL cells allowing the focused recurrent excitation (Canedo & Aguilar, 2000; Canedo *et al*., 2000) to reach firing threshold.

The present results together with data related to the influence of the sensorimotor cerebral cortex on the cuneolemniscal transmission (Aguilar *et al*., 2001) suggest that the circuit design diagrammed in Fig. 6 may be shared by recurrent and cortico-cuneate fibres. In this way the cortex could select its own input from a particular region of the skin, as occurs during voluntary discrimination and active touch, by potentiating a centre of activation surrounded by an inhibited periphery at the first stage of prethalamic processing.

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Abbreviations
BiCu, bicuculline; CL, cuneolemniscal neuron; ECoG, electrocorticogram; GABA, gamma-aminobutyric acid; ML, medial lemniscus; nCL, non-cuneolemniscal neuron; PTx, picrotoxin; RF, receptive field.

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