

Prolonged GABA_A-mediated inhibition following single hair afferent input to single spinal dorsal horn neurones in cats

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1. To study the central processing mechanisms of sensory input from low threshold afferents to the spinal cord, we examined the excitatory response of single lumbar dorsal horn neurones to stimulation of hairs in the receptive field using a mechanically driven probe, and to activation of single hair follicle afferents using an intracellular current pulse to the cell bodies in the dorsal root ganglion. Experiments were done on anaesthetized, paralysed cats, spinalized at the L1 lumbar level.
2. Responses of spinal neurones to two types of hair afferent input were characteristically different. The excitatory response to input from a single group II hair afferent ($A\beta$; innervating guard hair follicle receptors) was multimodal, characterized by a small early depolarization followed by a sharp, large component with a slow, prolonged decay phase, whereas the response to input from a single group III hair afferent ($A\delta$; innervating down hair follicle receptors) was unimodal. The unitary EPSPs in response to activation of group III hair afferents had a slower rise time and longer decay time constant than those in response to activation of group II hair afferents.
3. When the receptive field of the afferent was located in the centre of the receptive field of the dorsal horn neurone, the gain of the central response was greater for the input from a single group II afferent (> 1) than that for the input from a single group III afferent (< 1).
4. In the case of single group II hair afferents, when pairs of single action potentials or pairs of trains of action potentials were generated at intervals of 20 ms to 3 s, the response in the dorsal horn neurone to the second volley was markedly depressed at intervals of less than 2 s, without any apparent inhibition of the on-going rate of firing. The response to the second volley in single group III afferents was less depressed.
5. This inhibition of the response to the second of a paired volley in single group II hair afferents was attenuated by administration of bicuculline, but not strychnine or naloxone. This indicates that the inhibition involves a GABA_A-receptor-mediated mechanism. Bicuculline did not affect the late component of the response to single group II hair afferent input, but unmasked a late component of the response to mechanical stimulation of hairs.
6. These results suggest that group II and group III hair follicle afferent inputs give rise to different types of excitatory responses in dorsal horn neurones and that these responses are regulated differentially by GABA_A receptor activation, possibly via a presynaptic mechanism.

Brown, Koerber & Noble (1987a) have described a prolonged (> 1 s) depression of the excitatory response in spinocervical tract neurones to the second of paired inputs from single group II hair (guard and tylotrich) follicle afferents. This inhibition appeared to be independent of postsynaptic potentials as it was not associated with any change in on-going firing activity in extracellular

recordings (Brown *et al.* 1987a) or postsynaptic hyperpolarization in intracellular recordings (Brown, Koerber & Noble, 1987b). More recently, Koerber & Mendell (1988) studied cord dorsum field potentials to determine the central responses to different frequencies of input from a variety of single afferents. They reported different central responses to different frequencies depending on the type of

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afferent studied; some responses increased in magnitude at higher frequencies while some were unchanged and others were inhibited. In particular the central response following an action potential in a single group II hair afferent was inhibited. These results suggest that central responses to different afferent inputs are subject to different regulatory controls.

The present study was undertaken to identify the chemical substrate underlying the inhibition of hair afferent input. We have combined the use of antagonists with the approach described by Brown, Koerber & Noble (1987*c*) to study inputs from single hair afferents to single dorsal horn neurones. Preliminary results of our study have been presented elsewhere (De Koninck & Henry, 1990).

METHODS

Animal preparation

Experiments were done on twenty-five cats anaesthetized with α -chloralose (60 mg kg⁻¹ i.v.) after induction with halothane (Fluothane; Ayerst, Saint-Laurent, Québec, Canada). The left common carotid artery and the left jugular vein were cannulated and a tracheal cannula was inserted. Spinal segments L5–S1 were exposed for recording. The L7 dorsal root ganglion was also exposed and an incision made in the connective tissue of the ganglion to expose the cell bodies for intracellular recording and stimulation of single dorsal root ganglion cells. The spinal cords were transected at the L1 vertebral level to eliminate the influence of supraspinal structures. Prior to the spinal transection, to minimize spinal shock, the L1 segment was injected with 0.1 ml lignocaine hydrochloride (1% Xylocaine; Astra, Sweden). The superficial peroneal and the tibial nerves were exposed at the level of the ankle. The nerves were kept in a pool of mineral oil and stimulated using bipolar stimulating electrodes. In some cases, no mineral oil was used and the skin was sewn back after placing the stimulating electrodes. These stimulating electrodes were surrounded by a curved thin plastic cover for isolation from the surrounding tissue.

The exposed spinal cord was covered with a pool of warm mineral oil to prevent cooling and drying. After bilateral pneumothorax the animals were paralysed with pancuronium bromide (Pavulon; Organon, West Hill, Ontario, Canada) at a level of 1 mg kg⁻¹ i.v. (repeated when required) and ventilated artificially. The level of anaesthesia was confirmed periodically throughout the experiment by examination of the arterial pressure, by checking the degree of pupillary constriction and by occasionally allowing the effects of the muscle relaxant to wear off transiently. An additional dose of α -chloralose (30 mg kg⁻¹) was given i.v. 7–9 h after the first injection and beyond that time when required. End-tidal CO₂ concentration was monitored continuously using a Beckman LB2 Medical Gas Analyzer and was maintained between 3.5 and 5.0%. Rectal temperature was maintained at 38 °C using a thermistor-controlled servo-mechanism and an infrared bulb. Arterial pressure was monitored continually throughout the experiment and, when necessary, diastolic pressure was maintained above 80 mmHg by intravenous infusion (5 ml over 2 min) of dextran (Macrodex; Pharmacia, NJ, USA; 6% in saline) or noradrenaline bitartrate by a continuous infusion (Levophed; Winthrop, Aurora, Ontario, Canada; 0.002% in saline).

Recording and stimulation

Four types of recording and stimulation protocol were used.

Extracellular recording from single dorsal horn neurones combined with natural stimulation of hairs. In these experiments, single or multibarrelled micropipettes were used to record extracellularly from functionally identified single dorsal horn neurones. Neurones were classified using a previously described scheme (Henry, 1976). The recording pipette contained 3 M NaCl (3–4 M Ω resistance). In some cases, glutamate (1 M; pH 7.4; Sigma, MO, USA) was applied by iontophoresis to raise the rate of firing of the dorsal horn neurone to enable observation of inhibition of glutamate-evoked activity. Hairs were moved using a feedback-controlled mechanical stimulator (Chubbuck, 1966; displacements ranged from 1 to 1000 μ m).

Intracellular recording from dorsal horn neurones combined with natural stimulation of hairs. Single glass micropipettes filled with 3 M K(CH₃SO₄) were used (20–50 M Ω resistance). Further details are provided in De Koninck & Henry (1991) and De Koninck & Henry (1992). Hairs were moved using the feedback-controlled mechanical stimulator described above.

Extracellular recording from single dorsal horn neurones combined with intracellular recording and stimulation of single dorsal root ganglion cells. Single glass micropipettes (3 M KCl; 5–20 M Ω resistance) were used to record intracellularly from dorsal root ganglion (DRG) neurones. Each hair afferent was classified on the basis of its peripheral conduction velocity (Brown & Iggo, 1967), its responsiveness to down or guard hair movement, and on the character of its somal action potential (Rose, Koerber, Sedivec & Mendell, 1986; Koerber, Druzinsky & Mendell, 1988). When stable intracellular recording was obtained, intracellular depolarizing current pulses were used to generate an action potential in the afferent cell body. The intensity of each depolarizing pulse was adjusted to generate a single action potential in the afferent. This protocol has been shown to produce an action potential only in the afferent being recorded (Brown *et al.* 1987*c*). Simultaneous extracellular recording was obtained from single dorsal horn neurones using the extracellular multibarrelled micropipettes described above.

Dorsal root potential recording combined with natural stimulation on the ipsilateral hindfoot and electrical stimulation of sensory nerves. A dorsal root potential (DRP) was recorded using single patch-clamp-type glass micropipettes filled with 3 M NaCl (1 M Ω resistance) located on the surface of the L7 dorsal root close to its entry into the spinal cord. DRP was recorded in response to electrical stimulation of the exposed afferent nerves and natural stimulation on the ipsilateral hindfoot using the Chubbuck (1966) mechanical stimulator mentioned above.

Data acquisition and analysis

The intracellular signals were lowpass filtered at 10 kHz and the extracellular signals were bandpass filtered between 0.1 and 2 kHz, except for the cord dorsum recordings where no highpass filtering was used. The recordings were stored on magnetic tape and analysed off-line using locally designed software (Salter & Henry, 1987; De Koninck & Henry, 1992). Responses of dorsal horn neurones to a given input were quantified by counting the number of spikes in a fixed period at a fixed interval following a stimulus; baseline firing was

subtracted by determining the number of spikes in an identical period during on-going firing activity.

Administration of antagonists

Intravenous administration of antagonists was preferred to local administration to ensure that the antagonist would reach a wide number of receptor sites. Thus, antagonists were administered via the catheter in the external jugular vein. The drugs used and the doses selected were as follows: bicuculline hydrochloride (Research Biochemicals Inc., Natick, MA, USA), 0.3–1.0 mg kg⁻¹ (Duggan & Foong, 1985; Salter & Henry, 1988; De Koninck & Henry, 1990); strychnine sulphate (British Drug Houses, UK), 0.2–0.6 mg kg⁻¹ (Game & Lodge, 1975); naloxone hydrochloride (Narcan; Endo, Garden City, NY, USA), 0.1–0.5 mg kg⁻¹ (Calvillo, Henry & Neuman, 1974, 1979).

In these experiments, the cats were pretreated with hexamethonium bromide (10 mg kg⁻¹ i.v. every 5 h; Sigma, USA) to block the changes in blood pressure induced by bicuculline (Hong & Henry, 1990) and by strychnine (Hong, Yashpal & Henry, 1989).

RESULTS

In the present experiment, we focused on group II hair afferents (types G and T; innervating guard and tylotrich hair follicle receptors; Brown & Iggo, 1967) and group III hair afferents (type D; innervating down hair follicle receptors; Brown & Iggo, 1967) and on dorsal horn neurones which responded to movement of a single hair. Some of the

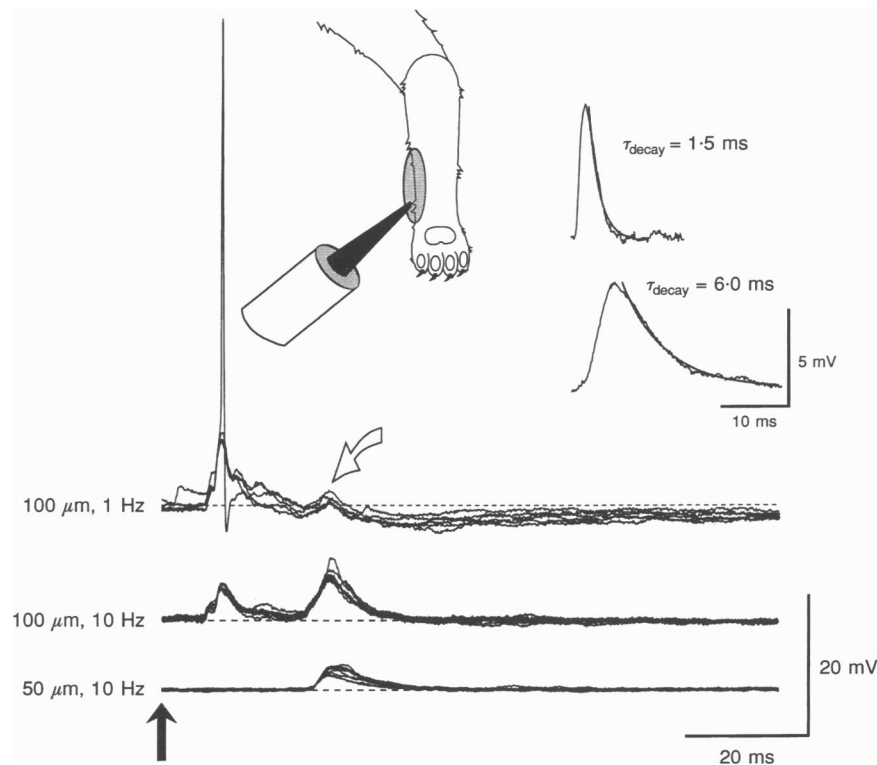


Figure 1. Response of a dorsal horn neurone to mechanical stimulation of hairs in the excitatory receptive field

Each set of traces represents five superimposed records of the response to a brief mechanical pulse from a mechanical stimulator apposed to hairs (time of stimulus indicated by the filled vertical arrow). The displacement of the mechanical stimulator was restricted to hairs and did not make contact with the skin. At a frequency of stimulation of 1 Hz (uppermost traces), a large, fast-rising and slowly decaying multimodal response was recorded. The latency to onset was 6.8 ms, consistent with an afferent conduction velocity in the region of 60 m s⁻¹ (A β , group II hair afferents; type G hair; Brown & Iggo, 1967). At a higher frequency of stimulation (10 Hz; middle traces), the early component of the response was markedly depressed, unmasking a second response (open curved arrow) with a latency to onset of 22.8 ms consistent with an afferent conduction velocity in the region of 15 m s⁻¹ (A δ , group III hair; type D hair; Brown & Iggo, 1967). At a lower intensity of stimulation (50 μ m, also at 10 Hz; lowermost traces), only the late component was observed, suggesting that the threshold for this late component was lower than that of the early response and that these two groups of responses result from activation of different types of afferent. The dashed line through each trace is a projection of the resting membrane potential to emphasize the hyperpolarization at low frequency of stimulation. The EPSPs shown as insets represent averages of five early (upper) and late (lower) EPSPs at 15 Hz stimulation to emphasize the difference in the rise time and the decay time constant (τ_{decay}) of these two types of EPSP. Recording was with a K(CH₃SO₄)-filled electrode.

neurons also responded to noxious stimulation of the skin and were thus classified as wide dynamic range neurons. Others responded only to innocuous mechanical stimulation and were classified as non-nociceptive neurons. Based on the depth of the recordings and on previous experience with extracellular labelling of recording sites (De Koninck & Henry, 1989) and with intracellular labelling (De Koninck, Ribeiro-da-Silva, Henry & Cuello, 1992; De Koninck, Ribeiro-da-Silva, Henry & Cuello, 1993), the dorsal horn neurons included in this study were estimated to be located in laminae III–IV of Rexed.

Two types of EPSP in response to hair stimulation

Two types of response to hair stimulation were observed during intracellular recording from dorsal horn neurons. At low frequencies of stimulation (≤ 1 Hz), there was an early response (latency, 7–15 ms), consisting of the summation of *fast* EPSPs (rise time of individual EPSP, 0.8–1.5 ms between 10 and 90% of the peak; decay time constant, 1.4–2.8 ms). This early response peaked at 2–3.5 ms and slowly decayed with a time constant of 15–42 ms (Fig. 1). In some cases, a late response (latency, 19–25 ms) was also observed, consisting of slower rising and slower decaying EPSPs (rise time of individual EPSP, 2.7–3.5 ms; decay time constant, 4.6–6.3 ms; Fig. 1). Figure 1 shows the response of one neurone, chosen because it illustrates a mixed response to hair stimulation. However, such a mixed response was not observed with all neurons. The early response occurred at a latency consistent with conduction in $A\beta$ afferent fibres (group II; Brown & Iggo, 1967) and the later response occurred at a latency consistent with conduction in $A\delta$ afferent fibres (group III; Brown & Iggo, 1967).

At higher frequencies of stimulation (10–20 Hz) the later ($A\delta$ latency) response was more prominent, while the early response ($A\beta$ latency) was depressed. This phenomenon is shown in Fig. 1 (see also Fig. 6 for the extracellular equivalent). Even at a frequency of 1 Hz, there was some depression of the early response (not shown).

Figure 1 also illustrates the characteristic difference in shape of the early (presumed group II hair) response with that of the later (presumed group III hair) response. The multimodal appearance of the early response to stimulation at ≤ 1 Hz suggests that it had a large polysynaptic component, which is consistent with its degradation at increased frequencies of stimulation. At a frequency of stimulation of 10 Hz, mono- and disynaptic EPSPs could be resolved.

In Fig. 1 there is a late, slow IPSP observable after the end of the excitatory response (peaking around 50 ms). This IPSP disappeared at higher frequencies of stimulation (5–10 Hz), which may account for the increase in the size of the late (group III hair) response at these frequencies, as shown in Fig. 1 (see also Fig. 6 for the unmasking of the late component).

Responses to activation of single afferents

To investigate in more detail each of the components of the responses to stimulation of hairs described above, we used the approach developed by Brown *et al.* (1987c), to examine the responses of dorsal horn neurons to input from single hair afferents by applying intracellular current pulses to dorsal root ganglion afferent neurons. Eight coupled pairs of a single group II hair afferent to a single dorsal horn neurone and three coupled pairs of a single group III hair afferent to a single dorsal horn neurone were studied. Figures 2 and 3 illustrate responses in dorsal horn neurons from single group II and group III hair afferents, respectively. Note the similarity in shape and time course between the extracellularly recorded response to the single group II afferent in Fig. 2 and the early response recorded at 1 Hz in Fig. 1 (upper trace) and between the response to the single group III afferent in Fig. 3 and the late response in Fig. 1 (middle trace).

Similar findings to those reported by Brown *et al.* (1987c) were obtained. Namely, the size of the response was larger when the receptive field of the group II hair afferent was closer to the centre of the receptive field of the dorsal horn neurone. The gain of the central response to group II hair afferent input was greater than 1 (> 1 spike in the dorsal horn neurone per spike in the DRG cell body). However, for group III hair afferents, the gain was smaller. In fact, several action potentials generated in the cell body of the afferent (3–6 action potentials at 20 Hz) were necessary to elicit a spiking response in the dorsal horn neurone. The rise and decay time of the response to input from group III hair afferents was characteristically longer than the rise and decay time of the response to group II hair afferents (Figs 1–3).

Responses to paired activation of single afferents

Brown *et al.* (1987a) have described a prolonged (> 1 s) depression of the excitatory response in dorsal horn neurons to the second of paired *activation* of a single hair follicle afferent; in the following description, each *activation* refers to either a single action potential or a short train of action potentials (3–6 action potentials at 20 Hz). To study the mechanism underlying this inhibition that follows the input from hair afferents, responses to paired activation of single hair afferents were examined at varying interstimulus intervals.

Four neurone pairs, each pair consisting of a single group II afferent and a single dorsal horn neurone, were studied. When each activation of the single afferent consisted of one action potential, the response of the dorsal horn neurone to the second activation of the single afferent was increasingly depressed as the interstimulus interval was decreased. Figure 4 shows an example of the response of a single dorsal horn neurone to paired activation of a single group II hair afferent. This inhibition of the dorsal

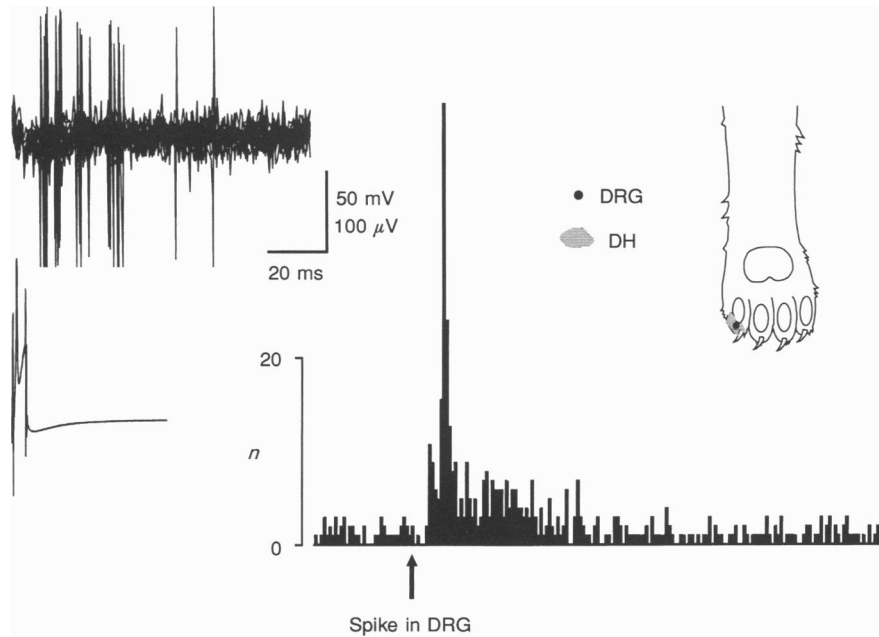


Figure 2. Response of a single dorsal horn neurone to activation of a single group II hair follicle afferent

The records on the left are 15 superimposed traces of the extracellular response of the dorsal horn neurone (upper) to single action potentials in a single group II hair afferent produced by 15 single intracellular depolarizing current pulses in the dorsal root ganglion cell body (lower). The schematic diagram on the right represents the receptive fields of the dorsal horn neurone (DH) and of the dorsal root ganglion afferent (DRG). The peristimulus time histogram (PSTH) represents the cumulative response of the dorsal horn neurone (in spikes per bin; bin width, 1 ms) to 250 single action potentials generated in the single afferent delivered at 3 s intervals (at arrow). The time scale is the same for the records on the left and the histogram.

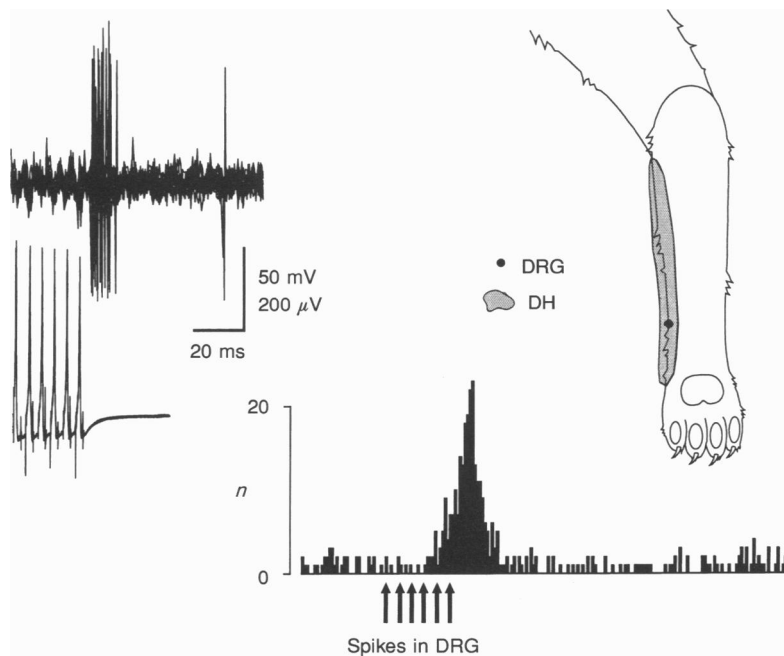


Figure 3. Response of a single dorsal horn neurone to activation of a single group III hair follicle afferent

Same protocol as that described for Fig. 2, with the exception that trains of action potentials (lower trace on the left and arrows below the PSTH) have to be used to evoke an observable spiking response in the dorsal horn neurone.

horn neurone response was seen at interstimulus intervals of up to 2 s (see also Brown *et al.* 1987a). The same inhibition was observed when each activation of the single afferent consisted of trains of three to six action potentials at 20 Hz. Comparing the inhibition of the peak response with that of the slow decay phase of the response of the dorsal horn neurone revealed, in every case, that the firing during the slow decay phase was proportionately more inhibited than the firing at the peak of the response for a given interstimulus interval (Fig. 4; see arrows).

Two neurone pairs, each consisting of a single group III afferent and a single dorsal horn neurone, were also studied. The response of the dorsal horn neurone to the second of paired activation (train) in a single group III afferent was also depressed at short interstimulus intervals. However, this depression was smaller than that

seen with group II afferents, suggesting that the input from group III hair afferents is not as sensitive to frequency as that from group II hair afferents (Fig. 5).

The on-going firing activity of the dorsal horn neurone was not inhibited in any case following the excitatory response to single hair afferent activation (see Figs 2–5; see also Brown *et al.* 1987a).

Response to paired stimulation of hairs

As described earlier (see Fig. 1), the response to stimulation of hairs could be divided into two components: an early, fast-rising large response with a slow decay and a late, smaller and slower-rising component (see curved arrows in Fig. 6A pointing to late component). At short interstimulus intervals, while the early component of the response to the second stimulus was reduced, the late component increased

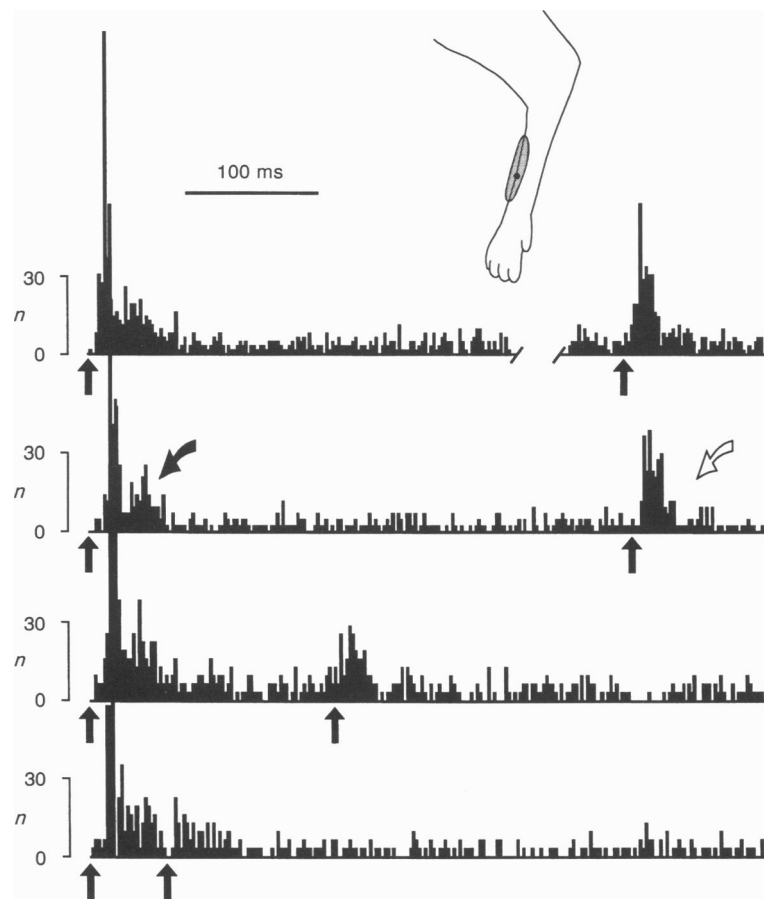


Figure 4. Response of a single dorsal horn neurone to paired action potentials generated in a single group II hair afferent at different interstimulus intervals

Each peristimulus time histogram (bin width, 2 ms) is the cumulative response of the dorsal horn neurone to, from top to bottom, 100, 75, 50 and 50 pairs of stimuli (normalized to 100). The interstimulus intervals are, from top to bottom, 750, 500, 175 and 50 ms. Even at interstimulus intervals of 1.5 s, an inhibition of the second response was observed (not shown). Note that the late phase of the excitatory response to the conditioning stimulus (filled curved arrow) is absent in the response to the second activation (open curved arrow). Vertical straight arrows below each PSTH indicate the times of occurrence of the action potential in the hair afferent cell body. The schematic diagram represents the locations of the receptive fields of the group II hair afferent (black dot) and the excitatory receptive field of the dorsal horn neurone (grey area).

(see second curved arrow in Fig. 6A), similar to the observation upon repetitive stimulation (Fig. 1). Such unmasking of this late component is presumably due to disinhibition (i.e. attenuation of the IPSP occurring at that latency; see Fig. 1) and fading of the decay phase of the early component (Figs 1, 2 and 4).

Effect of bicuculline on the inhibition following input from single group II hair follicle afferents

Systemic administration of antagonists to known inhibitory agents in the dorsal horn were tested against the depression of the response to the second stimulus of paired activation of single group II hair afferents. A first dose of bicuculline (0.6 mg kg⁻¹ i.v.) attenuated by 45% the inhibition of the response to the second stimulus to a single group II hair afferent at an interstimulus interval of 300 ms ($n=3$; not shown). A second dose of bicuculline (cumulative dose, 1.2 mg kg⁻¹) further attenuated the

inhibition by more than 80%. In two cases, partial recovery from the response to bicuculline was observed 30 min after the injection, at which point the experiment was terminated.

Effect of bicuculline on the inhibition following stimulation of hairs

As observed with input from single afferents, bicuculline attenuated the inhibition of the response to the second of paired mechanical stimuli to the hair receptive field ($n=5$; Fig. 6). Strychnine (0.6 mg kg⁻¹; $n=2$) and naloxone (0.5 mg kg⁻¹; $n=3$) had no effect on either type of inhibition.

In Fig. 6B, while administration of bicuculline (0.6 mg kg⁻¹, i.v.) did not affect the size of the early component of the first response, it enhanced the early component of the second response. Administration of bicuculline also enhanced or unmasked the late component of the response to both the first and the second stimuli, suggesting that the IPSP masking this late component (see above and Fig. 1) was mediated via GABA_A receptors. Such

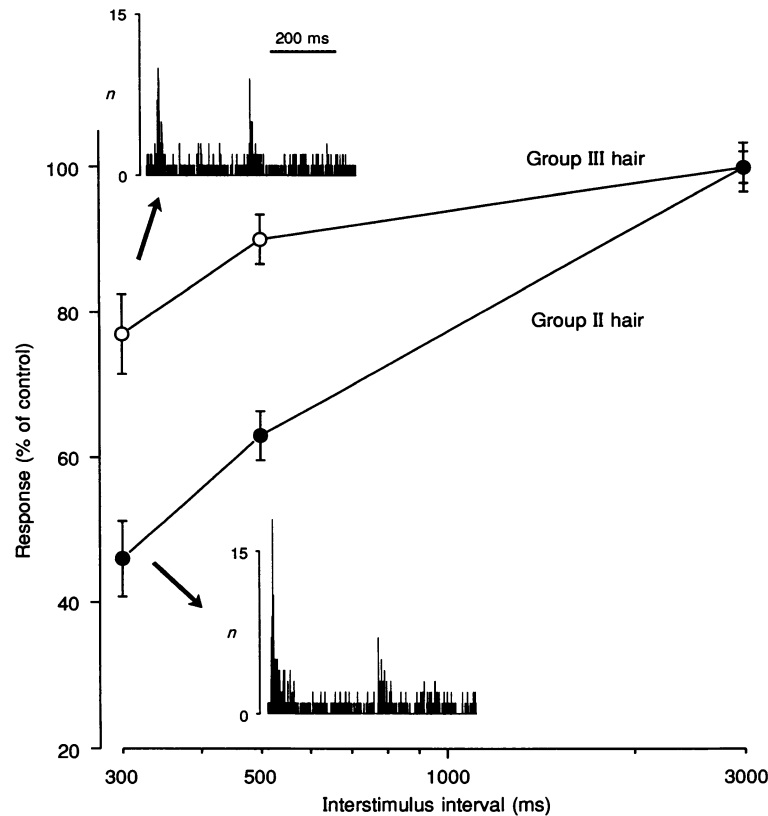


Figure 5. Comparison of the inhibition of the response of a dorsal horn neurone to the second of paired activation of a single group II hair afferent (●) to similar inhibition in a different dorsal horn neurone to stimulation of a single group III hair afferent (○) in the same cat

Points on the graph represent means \pm S.E.M. ($n=100$) of the number of spikes in a 30 ms window around the response to the second activation of the single afferent. Values are normalized to 100 according to the size of the conditioning response. The peristimulus time histograms (bin width, 1 ms) illustrate the phenomenon represented by the points on the graph (arrows; each represents the average of responses to 100 sets of stimuli in the respective single afferent). Note that the group III hair input is weaker than that of the group II hair, but is much less inhibited at lower interstimulus intervals than the latter.

unmasking of a late component was not seen with responses to input from single group II hair afferents (Fig. 2) following bicuculline administration (not shown), suggesting that these two components are due to activation of different classes of afferent. Indeed, the latencies of the early and the late components of the responses to stimulation of hairs (Figs 1 and 6) are consistent with the early one being mediated via activation of group II afferents and the late one via group III afferents.

Interestingly, following an initial dose of bicuculline, the inhibition of the early component of the second response of

the dorsal horn neurone was attenuated, but remained partially depressed (38 % of the control response; Fig. 6*B*). On the other hand, the amplitude of the unmasked late component of the second response was 85 % of its control counterpart (see arrows in Fig. 6*B*). This result emphasizes again the differential sensitivity of the early and the late components of the response upon repetitive stimulation, which is similar to the result of activation of single group II *vs.* group III afferents (Fig. 5). This further strengthens the hypothesis that each component is mediated via activation of separate classes of afferent.

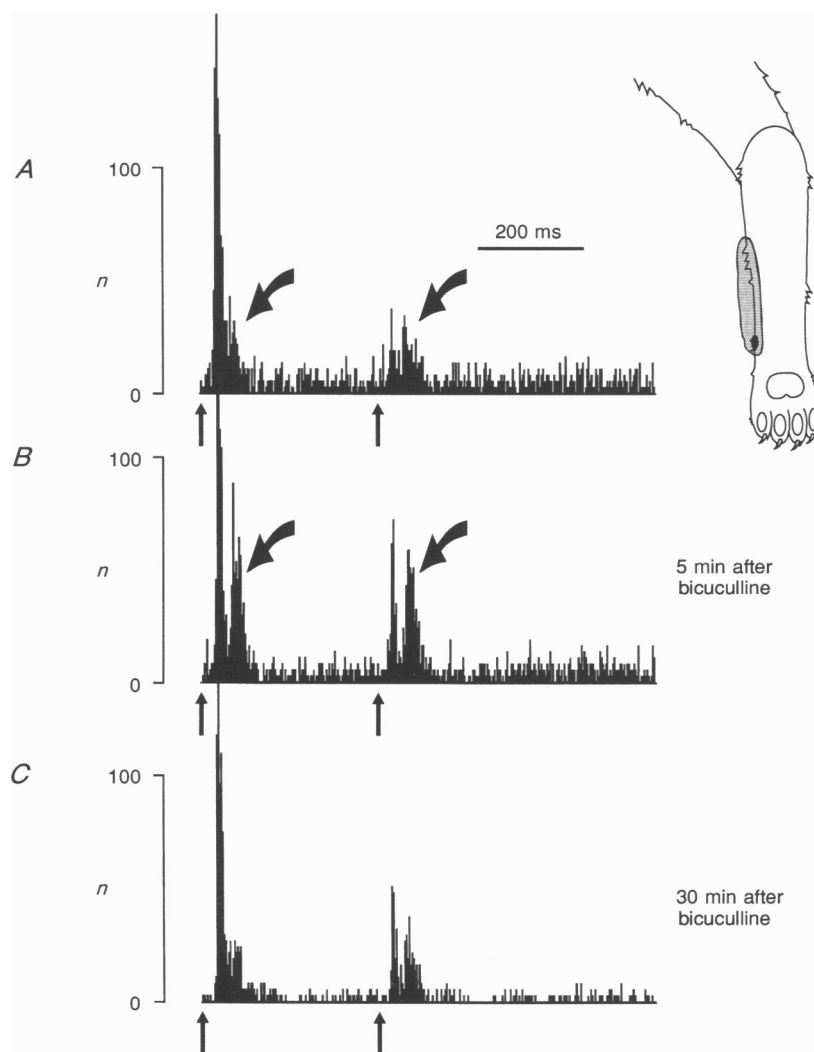


Figure 6. Bicuculline reversibly attenuates the inhibition of the response of a single dorsal horn neurone to the second of paired mechanical stimuli to the excitatory hair receptive field

The peristimulus time histograms are cumulative responses of the dorsal horn neurone to 150 pairs of mechanical pulses to hairs (bin width, 2 ms; interstimulus interval, 300 ms; the 100 μm displacement of the probe did not touch the skin). Data were taken before (A), 5 min after (B), and 30 min after (C) a single dose of bicuculline (0.6 mg kg^{-1} i.v.). Note in A that whereas the early component of the response to hair stimulation is markedly depressed after the second stimulus (14 % of control response), the late component (curved arrows) is much less depressed (83 % of control; see also Fig. 1). After the administration of bicuculline (B), the depression of the early component to the second stimulus is attenuated and a large late component of the response is unmasked in both responses to the first and second stimuli (vertical, straight arrows). See text for further discussion.

Dorsal root potential in response to natural stimulation of the receptive field and to electrical stimulation of a sensory nerve

The long time course of the depression of the response to the second of paired activation of hair afferents is of the same order as that of the dorsal root potential (DRP) following an afferent volley (for reviews see Levy, 1977; Nicoll & Alger, 1979; Davidoff & Hackman, 1985). In six cats, the DRP was recorded following both mechanical stimulation of the hindpaw and electrical stimulation of the tibial and superficial peroneal nerves (Fig. 7A). The

amplitude of the DRP varied with the intensity of the stimulus (Fig. 7A). Note that, even at small intensities, the time course of the mechanism reflected by the DRP is long (> 1 s; Fig. 7A). Bicuculline (0.3–1.0 mg kg⁻¹ i.v.) reversibly blocked the DRP ($n = 5$ cats; Fig. 7B), suggesting that it reflects the presence of a prolonged GABA_A-mediated mechanism following input from afferent activation. The graph in Fig. 7C emphasizes the similarity between the time course of the DRP and that of the depression of the response to the second of paired activation of a single hair afferent. The time to peak of the DRP was 182 ± 35 ms and decayed with a time constant of 955 ± 122 ms. The smooth

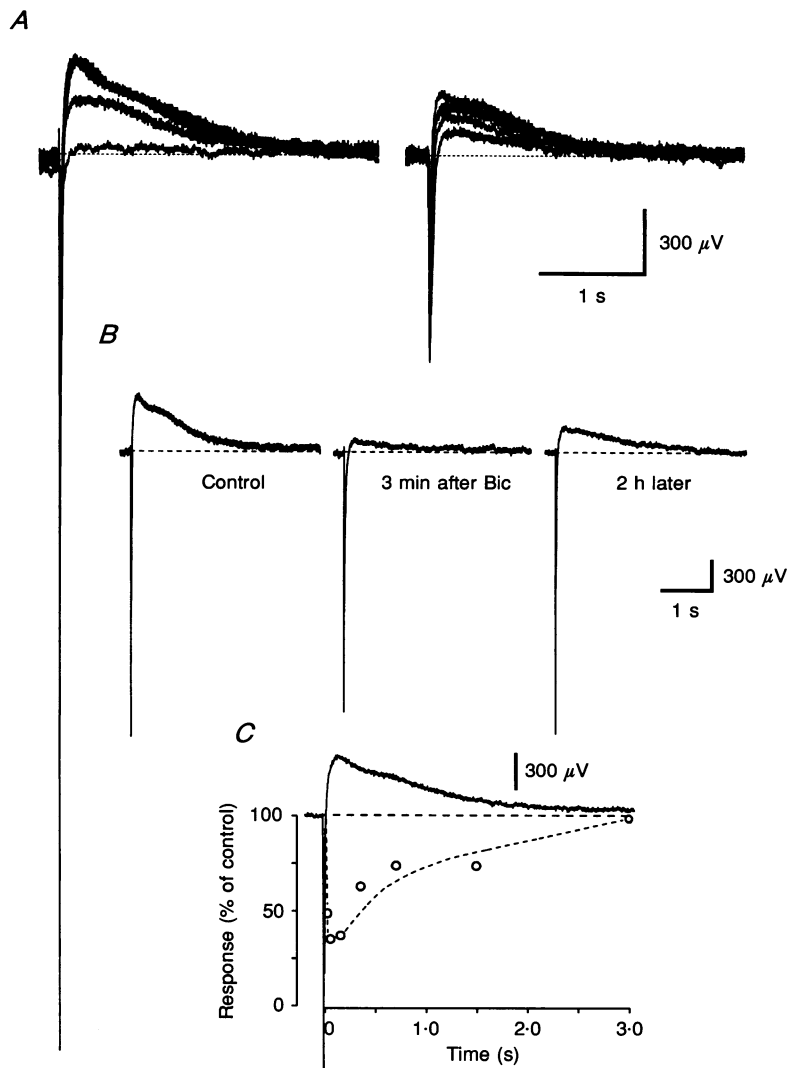


Figure 7. Time course and bicuculline sensitivity of the L7 dorsal root potential (DRP)

A, DRP recorded following electrical stimulation of the sciatic nerve (left) or mechanical stimulation of the ipsilateral hindfoot (right). Each trace is an average of responses from 35 stimuli (50, 60, 80, 100 and 300 μA respectively for electrical stimulation; 5, 10, 30, 50 and 100 μm for mechanical stimulation; stimulation rate, 0.1 Hz). Note the prolonged time course of the DRP (> 1 s in duration) even at small intensities. B, bicuculline (Bic; 0.6 mg kg⁻¹ i.v.) reversibly blocked the DRP. Each trace is an average of 35 responses from electrical stimulation of the sciatic nerve (75 μA at 0.1 Hz). C, comparison of the time course of the DRP with that of the inhibition of the response of a dorsal horn neurone to the second of paired inputs from a single group II hair afferent in the same cat. The trace at the top of the graph is an average DRP from 35 stimuli (75 μA at 0.1 Hz). See text for further discussion.

curve fitted through the points, representing the inhibition of the second of paired responses, peaks at 190 ms and has a decay time constant of 1115 ms.

DISCUSSION

In the present study, we have characterized physiologically the excitatory and inhibitory responses of single dorsal horn neurones to activation of single group II and group III hair follicle afferents and characterized pharmacologically the prolonged inhibition that follows input from single group II hair afferents.

Excitatory responses

At low frequencies of impulses in the afferent, the central response to each type of afferent was characteristically different. The response to group II hair follicle afferent input was multimodal whereas that to group III hair afferent input was unimodal. The central gain of the response to group II hair afferent input was also greater than that to group III hair afferent input (Brown *et al.* 1987c; Koerber & Mendell, 1988). One possible explanation for this difference may be that the response to group II input is due to the addition of a small early monosynaptic component and larger di- and polysynaptic components, as suggested by Hongo & Koike (1975) and Brown *et al.* (1987c); conversely, the response to group III input may be mediated more directly via simpler circuitry, for example mainly monosynaptic (and/or disynaptic) components.

This possible difference in the organization of the input of the two types of afferent and the dorsal horn neurones studied here may account for the fact that, at short interstimulus intervals and at higher frequencies of stimulation, the response to group III hair afferent input is markedly less inhibited than that to the group II hair input. Indeed, the prolonged decay phase, presumably the polysynaptic component, of the response to group II hair input appears to be most sensitive to repetitive stimulation (see also Brown *et al.* 1987b).

When the response stimulation of hairs was reduced to simpler components (possibly mono- and disynaptic) by increasing the frequency of stimulation, the evoked responses from the two types of afferents remained different (slower rise and decay of the response to group III hair afferent activation), suggesting that perhaps the coupling between the two classes of afferent and dorsal horn neurones studied here may differ. This difference may be due to the chemical nature of the synapses between the afferent and the dorsal horn neurone, for example, NMDA *vs.* non-NMDA-receptor-mediated EPSPs. In their extracellular studies, Salt & Hill (Salt & Hill, 1981; Hill & Salt, 1982) have reported that while responses to low threshold mechanical stimulation are sensitive to non-specific glutamate receptor antagonists, they are insensitive to NMDA receptor antagonists. However our results emphasize the fact that, using mechanical stimulation of hairs, the group III hair response is easily

hidden by the decay phase of the response to group II input and by an IPSP following the group II ($A\beta$ latency) response. Hence, it is possible that the group III ($A\delta$ latency) response (Figs 1 and 7) cannot be resolved in conventional extracellular iontophoretic studies using hair stimulation. Further detailed pharmacological intracellular studies of isolated $A\beta$ - and $A\delta$ -latency EPSPs are needed to resolve this issue.

Inhibitory mechanisms

The results of the present study confirm those of Brown *et al.* (1987a) showing that a prolonged (> 1 s) period of inhibition follows excitatory input from hair afferents to dorsal horn neurones. Our results further demonstrate that this inhibition is mediated largely via a GABA_A receptor mechanism. On the other hand, glycine- or opiate-mediated mechanisms do not seem to be involved in this inhibition. Even though bicuculline attenuated the inhibition by more than 85 %, suggesting a major GABA_A-receptor-mediated component, the possibility remains that another inhibitory mechanism may also be involved.

Early postsynaptic inhibition

Stimulation of a group of hairs in the excitatory receptive field gives rise to IPSPs (Fig. 1; Hongo & Koike, 1975; Brown *et al.* 1987b; Noble & Short, 1989; De Koninck & Henry, 1990; Short, Brown & Maxwell, 1990) of short duration (~ 100 ms) which appear to be mediated in part via activation of GABA_A receptors (Fig. 6; De Koninck & Henry, 1990; Salter, De Koninck & Henry, 1993). The time course of these IPSPs (as well as other IPSPs from low threshold stimulation: Hongo, Jankowska & Lundberg, 1966, 1968; De Koninck & Henry, 1992; Salter *et al.* 1993) is much shorter than that of the prolonged inhibition (> 1 s) described here following input from a single hair afferent. Activation of single hair afferents does not appear to give rise to IPSPs (Brown *et al.* 1987b). In addition inhibition of the on-going firing activity of a dorsal horn neurone was never seen following input from a single hair afferent, suggesting that it was not due to a postsynaptic hyperpolarization (see also Brown *et al.* 1987a). As bicuculline did not change the decay phase of the excitatory responses from single afferents, it is unlikely that an IPSP would be hidden in the decay phase of this excitatory response. It is possible, however, that the mechanism giving rise to IPSPs triggered from activity in a single afferent is too small to be observed under the present conditions. Another possibility is that the IPSP arises from activation of a different class of afferent. Brown *et al.* (1987b) suggested that activation of some group III afferents may be responsible for these IPSPs. However, the latency of the IPSPs recorded here was shorter than that of EPSPs to group III afferents (Figs 1 and 6). Tapper, Brown & Ritz (1987) recorded postsynaptic potentials of dorsal horn neurones evoked by activation of single slowly adapting type 1 (SA1) afferents and found no evidence of

prolonged IPSPs. Another type of rapidly conducting afferent may be responsible for these IPSPs.

Late presynaptic inhibition

The prolonged inhibition (> 1 s) described here outlasts the duration of previously described GABA_A-mediated IPSPs (Hongo *et al.* 1966, 1968; Brown *et al.* 1987*b*; De Koninck & Henry, 1990, 1992; Salter *et al.* 1993) in dorsal horn neurones, suggesting that it is not due to a postsynaptic mechanism. Hence, the majority of this prolonged inhibition appears to involve GABA_A-receptor-mediated mechanisms presynaptic to the dorsal horn neurones from which recordings were made.

The fact that the DRP is blocked by bicuculline (see also Gmelin & Zimmermann, 1983) indicates that a pronounced and prolonged GABA_A-receptor-mediated mechanism distributes widely throughout the spinal cord following input from a single peripheral stimulus. This DRP has been suggested to reflect primary afferent depolarization (see Willis & Coggeshall, 1978). The similarity in time course and sensitivity to bicuculline of the DRP and the prolonged inhibition of the second response to paired group II input suggests that the latter may reflect largely primary afferent depolarization.

Tapper, Wiesenfeld & Craig (1983) also reported a prolonged period of inhibition following input from single SA1 afferents to single lamina IV dorsal horn neurones. They suggested it was possibly due to a postsynaptic inhibition. However, Tapper *et al.* (1987) did not find evidence of IPSPs following input from single SA1 afferents which could account for this prolonged inhibition and suggested that it was mainly due to a presynaptic mechanism. Tapper *et al.* (1983) also suggested that PAD was playing a role in controlling the decay phase of the excitatory response to a single impulse in an SA1 afferent. In our study, however, administration of bicuculline did not change the decay phase of the excitatory responses from single hair afferents, suggesting that PAD did not play a major role in controlling this decay phase.

Functional significance

Koerber, Seymour & Mendell (1991) have suggested that the behaviour of the central response upon different patterns of repetitive stimulation in primary afferents may reflect and match the properties of their peripheral receptors. In the present case, whereas the effectiveness of a single group II hair afferent input seems much stronger in eliciting a central response than does a single group III hair afferent input, the effectiveness of the group II hair input drops dramatically with repetitive stimulation, which is not the case with the group III hair input (see also Brown *et al.* 1987*c*; Koerber & Mendell, 1988). One consequence of such characteristics may be that, upon stimulation of the hair receptive field, as the response to group III input occurs at a later latency (A δ conduction velocity) than does the group II input (A β conduction velocity), at low

frequencies of stimulation, the group III hair response is masked by a brief period of postsynaptic inhibition (which will affect any late input) following the early group II response. As the frequency of stimulation is increased and the response to group II hair input is attenuated by a specific presynaptic mechanism, the group III response becomes a more significant component of the overall response.

Conclusion

The results of our study have extended those of Brown *et al.* (1987*a, b, c*). We suggest that the depression of hair afferent input upon repetitive activity is mainly mediated through a presynaptic GABA_A-receptor-mediated mechanism. In addition, we have shown that the two different hair inputs discussed in the present study lead to different central excitatory responses and that these central responses are differentially inhibited upon repetitive activation. This may reflect a different central encoding of the information mediated by each of these inputs.

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