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Peripheral Inflammation Facilitates Aβ Fiber-Mediated Synaptic Input to the Substantia Gelatinosa of the Adult Rat Spinal Cord

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Whole-cell patch-clamp recordings were made from substantia gelatinosa (SG) neurons in thick adult rat transverse spinal cord slices with attached dorsal roots to study changes in fast synaptic transmission induced by peripheral inflammation. In slices from naive rats, primary afferent stimulation at Aβ fiber intensity elicited polysynaptic EPSCs in only 14 of 57 (25%) SG neurons. In contrast, Aβ fiber stimulation evoked polysynaptic EPSCs in 39 of 62 (63%) SG neurons recorded in slices from rats inflamed by an intraplantar injection of complete Freund's adjuvant (CFA) 48 hr earlier ($p < 0.001$). Although the peripheral inflammation had no significant effect on the threshold and conduction velocities of Aβ, Aδ, and C fibers recorded in dorsal roots, the mean threshold intensity for eliciting EPSCs was significantly lower in cells recorded from rats with inflammation (naive: 33.2 ± 15.1 μA, $n = 57$; inflamed: 22.8 ± 11.3 μA, $n = 62$, $p < 0.001$), and the mean latency of EPSCs elicited by Aβ fiber stimulation in CFA-treated rats was significantly shorter than that recorded from naive rats (3.3 ± 1.8 msec, $n = 36$ vs 6.0 ± 3.5 msec, $n = 12$; $p = 0.010$). Aβ fiber stimulation evoked polysynaptic IPSCs in 4 of 25 (16%) cells recorded from naive rat preparations and 14 of 26 (54%) SG neurons from CFA-treated rats ($p < 0.001$). The mean threshold intensity for IPSCs was also significantly lower in CFA-treated rats (naive: 32.5 ± 15.7 μA, $n = 25$; inflamed: 21.9 ± 9.9 μA, $n = 26$, $p = 0.013$). The facilitation of Aβ fiber-mediated input into the substantia gelatinosa after peripheral inflammation may contribute to altered sensory processing.

Key words: inflammation; pain; dorsal horn; synaptic transmission; neural plasticity; substantia gelatinosa

Peripheral tissue inflammation characteristically leads to increased pain sensitivity. This is the consequence both of a peripheral sensitization of high-threshold Aδ and C nociceptor terminals on exposure to inflammatory mediators (Levine and Taiwo, 1994) and to a central facilitation of synaptic input into the dorsal horn of the spinal cord; central sensitization (Woolf, 1983; Torebjork et al., 1992). Central sensitization is initiated in noninflamed animals by brief C-fiber inputs and manifests as a modification in the receptive field properties of dorsal horn neurons caused by the recruitment of subthreshold inputs (Woolf and King, 1990), and includes the transformation of nociceptive-specific cells into multireceptive cells with a low-threshold Aβ fiber input (Simone et al., 1989; Woolf et al., 1994). In human volunteers, central sensitization induced by activation of C-fibers with chemical irritants includes the generation of a tactile pain mediated by Aβ fibers (Torebjork et al., 1992; Koltzenburg et al., 1994). In *in vitro* neonatal spinal cord preparations, repetitive brief C-fiber stimulation produces an NMDA receptor-mediated heterosynaptic facilitation of Aβ fiber inputs to deep dorsal horn and ventral horn spinal neurons (Thompson et al., 1990, 1993).

Central sensitization is likely to contribute substantially to the hypersensitivity associated with experimental inflammation as a consequence of C-fiber input from spontaneously active C-fibers or augmented peripheral activation of sensitized C-fibers. Another mechanism may, however, participate in alterations in synaptic efficacy during inflammation, a change in the synaptic drive generated by Aβ sensory neurons innervating the inflamed area. In adjuvant-inflamed but not naive rats, for example, the hamstring flexor withdrawal reflex is progressively sensitized by repetitive light mechanical stimuli applied to the inflamed tissue (progressive tactile hypersensitivity), which can be mimicked by Aβ fiber electrical stimulation (Ma and Woolf, 1996a). Aβ fiber input in inflamed animals also generates an action potential afterdischarge in dorsal horn neurons, something only Aδ and C-fibers normally evoke (Neumann et al., 1996). Finally, Aβ fiber-mediated ventral root potentials recorded from an *in vitro* spinal cord preparation from inflamed neonatal rats, show windup, a phenomenon normally only associated with C-fibers (Thompson et al., 1994). One explanation for these changes in the central action of Aβ fibers after inflammation may be the novel expression of substance P and other synaptic modulators in some of these fibers (Neumann et al., 1996), which could result in synaptic events typical of C-fibers being generated by Aβ fibers.

The central changes involved in inflammation may result in the facilitation of Aβ fiber-mediated synaptic input to neurons in the superficial dorsal horn, especially lamina II (substantia gelatinosa, SG). The direct primary afferent input into the SG is predominantly Aδ and C fiber nociceptors (Willis and Coggeshall, 1991), and the novel recruitment of low-threshold Aβ-evoked synaptic potentials in these neurons might alter sensory processing sufficiently to contribute to the abnormal hypersensitivity typical of inflammation. We have now investigated, using an *in vitro* adult

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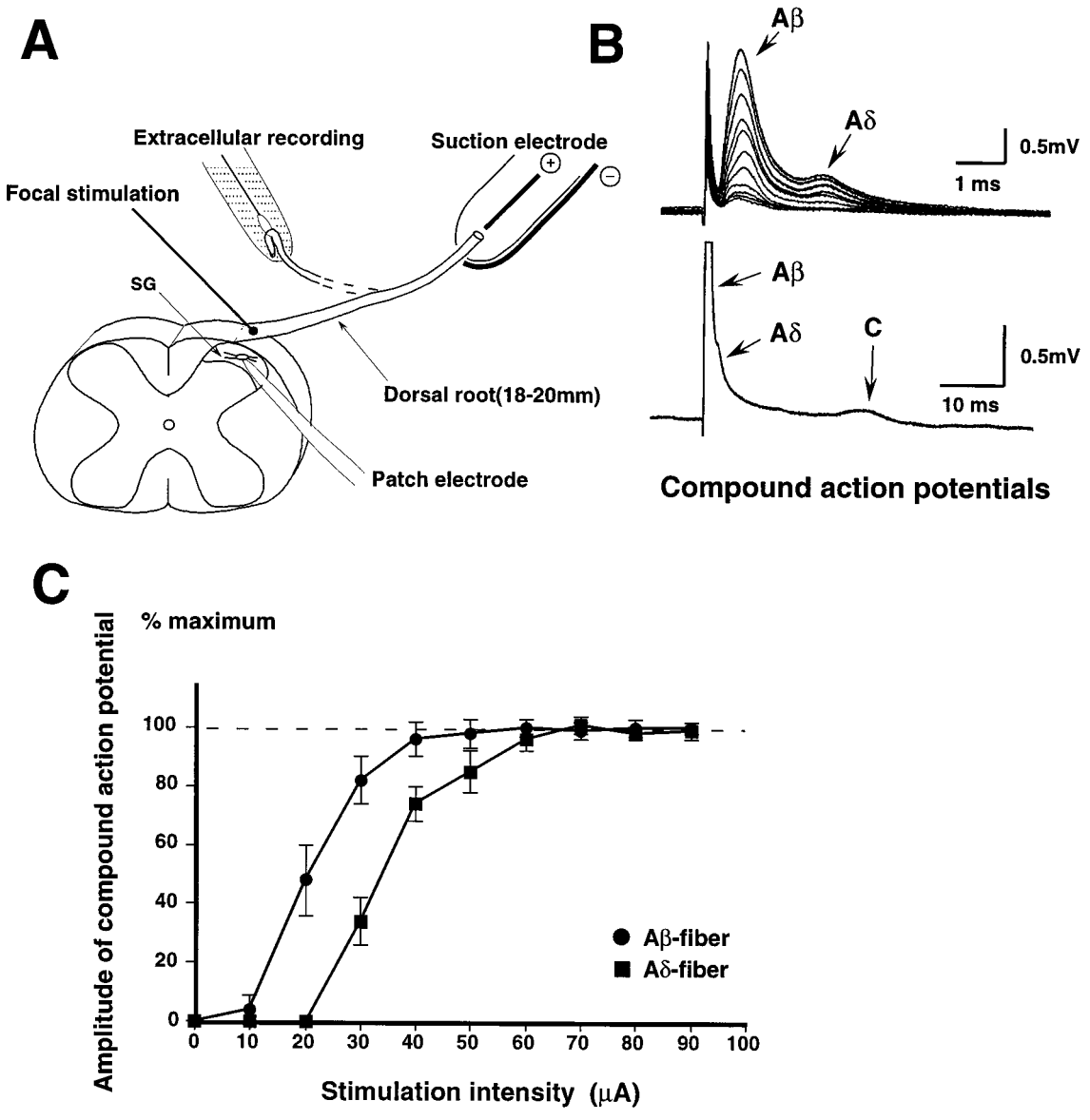

Figure 1. *A*, Schematic diagram of the experimental setup. Extracellular recordings were made from dorsal roots, and whole-cell patch-clamp recordings were made from SG neurons in adult rat spinal cord transverse slices with a long attached dorsal root. *B*, Representative extracellular recording of compound action potentials evoked at graded stimulus intensities (*top*, 12–50 μA; *bottom*, 300 μA). The threshold intensities for Aβ, Aδ, and C fibers were 12, 23, and 230 μA, respectively. The stimulus duration for Aβ and Aδ was 0.05 msec and for C fibers was 0.5 msec. Calculated conduction velocities for Aβ, Aδ, and C fibers were 27.3, 8.5, and 0.8 m/sec, respectively. *C*, The stimulus–response relationship of Aβ and Aδ compound action potentials ($n = 5$).

spinal cord preparation, the effect of inflammation on Aβ fiber-mediated fast synaptic responses in the SG.

MATERIALS AND METHODS

The methods for inducing inflammation, obtaining adult rat spinal cord slices, and blind whole-cell patch-clamp recordings from SG neurons have been described in detail previously (Yoshimura and Jessell, 1989; Yoshimura and Nishi, 1993; Ma and Woolf, 1996a). Briefly, inflammation was induced by an intraplantar injection of complete Freund's adjuvant (CFA; Sigma, St. Louis, MO; 100 μl) into the left hindpaw of adult male Sprague Dawley rats (10–11 weeks, 300–350 gm) under halothane (2.5%) anesthesia, producing an area of erythema, edema, and tenderness restricted to the hindpaw (Stein et al., 1988). Naive noninflamed animals or rats 48 hr after CFA injection were terminally anesthetized with urethane (1.5–2.0 gm/kg, i.p.), and the lumbosacral spinal cord was removed. The isolated spinal cord was then placed in preoxygenated cold Krebs' solution (2–4°C). After removal of the dura mater, all ventral and dorsal roots, except the L5 dorsal root on the left side, were cut, and the pia-arachnoid was removed. The spinal cord was placed in a shallow groove formed in an agar block and glued to the bottom of a microslicer stage with cyanoacrylate adhesive and held in place by the agar block. The spinal cord was immersed in cold Krebs' solution, and a 600-μm-thick transverse slice with attached dorsal root was cut on a vibrating microslicer (model DTK1500; Dosaka Co. Ltd., Kyoto, Japan). The spinal cord slice was then placed on a nylon mesh in the recording chamber and held in place by a titanium electron microscopy grid supported by a silver wire loop. The slice was perfused with Krebs' solution (15 ml/min) saturated with 95% O_2 and 5% CO_2 at 36 ± 1°C. The Krebs' solution contained (in mM): NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11. The length of preserved L5 dorsal root from the cathode of the suction electrode to the dorsal root entry zone was adjusted to 18–20 mm by cutting its distal end. Orthodromic stimulation of the dorsal root was performed with a suction electrode (Fig. 1*A*) using a constant-current stimulator (Neurolog). The

Table 1. Compound action potential

	Threshold (μA)			Conduction velocity (m/sec)		
	Aβ	Aδ	C	Aβ	Aδ	C
Naive (n = 12)	13.3 ± 2.7	24.7 ± 1.9	217.9 ± 76.9	27.0 ± 3.7	8.0 ± 1.2	0.8 ± 0.1
Inflamed (n = 14)	12.6 ± 2.7	25.4 ± 2.2	198.0 ± 77.3	26.0 ± 4.3	8.1 ± 1.1	0.9 ± 0.1

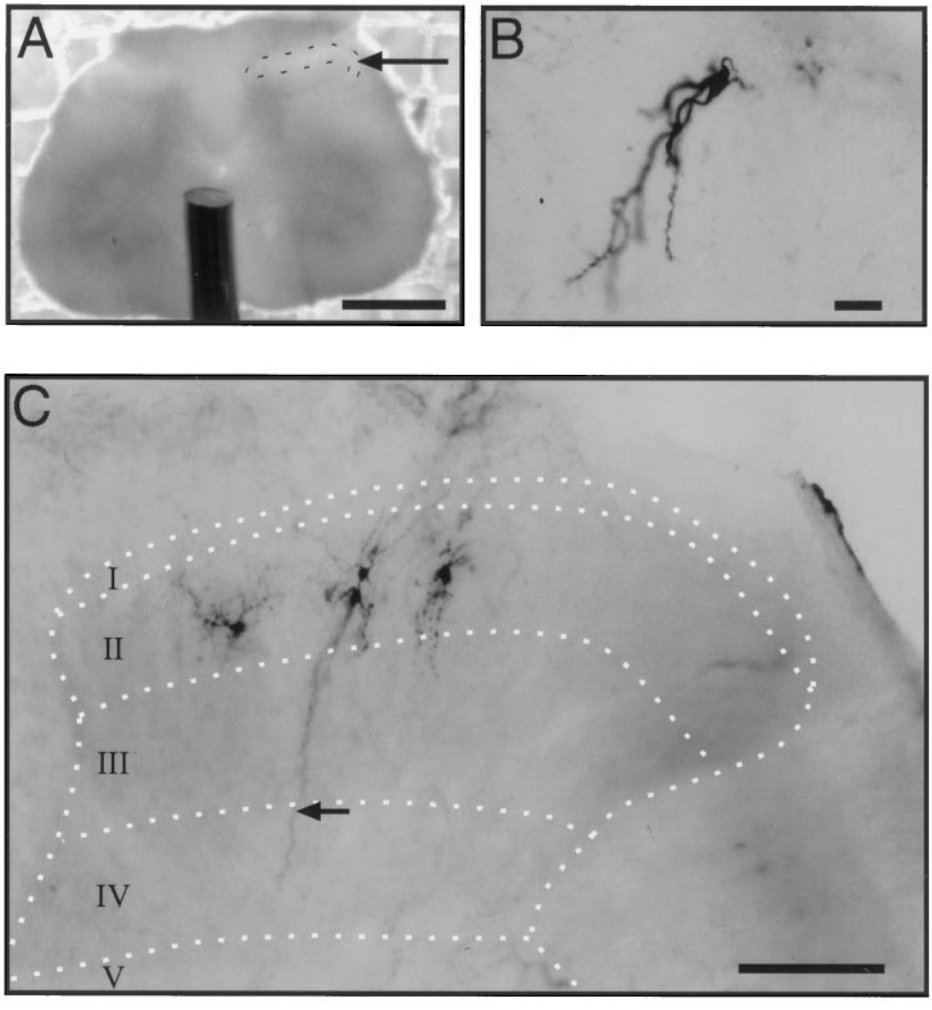

Figure 2. Identification of SG and SG neurons in the transverse spinal cord slices. *A*, Photomicrograph of the slice preparation from naive rat showing that the SG can be identified as a translucent pale area in the superficial dorsal horn (*dotted area*) enabling targeting of the recording electrode to the this region. Scale bar, 600 μm. *B*, A representative SG neuron injected with Neurobiotin. Scale bar, 20 μm. *C*, A low-power photomicrograph of a slice from naive rat showing SG neurons filled with Neurobiotin. Note all neurons lie within the middle third of the dorsoventral plane of SG and have the features typical of stalk cells. The dendrites of some cells extend ventrally into deeper laminae as indicated by the *arrow*. Scale bar, 150 μm.

stimulus intensity necessary to activate Aα/β, Aδ, and C fibers and the afferent fiber conduction velocity was determined by extracellular recording of compound action potentials from the dorsal root near the dorsal root entry zone in each experiment. The minimum stimulus intensities and duration to activate Aα/β, Aδ, and C fibers were ~10 μA (0.05 msec), 25 μA (0.05 msec), and 200 μA (0.5 msec), respectively (Fig. 1*A*,*B*; Table 1). In some experiments, focal stimulation was performed with a monopolar silver wire electrode (50 μm diameter), insulated except at the tip, positioned just distal to the dorsal root entry zone to estimate conduction velocity of the fibers responsible for particular synaptic responses.

Blind whole-cell patch-clamp recordings were made from neurons located in SG (Figs. 1*A*, 2*A*). With a light source directed under the slice, the SG, because of its relative lack of myelin is readily identifiable as a distinct translucent region in the superficial dorsal horn (Fig. 2*A*) (Yoshimura and Nishi, 1993). The recording electrodes were positioned, in all cases, under direct visual control into the middle third of SG, identified as above, in the dorsoventral plane and within its medial half in the mediolateral plane. The location of recorded neurons was confirmed in selected instances by the intrasomatic injection of Neurobiotin (0.3%; Vector Laboratories, Burlingame, CA).

Two pipette solutions were used in this study, the first, which was used in most cases with TEA and Cs, contained (in mM): Cs-sulfate 110, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, TEA 5, and ATP-Mg salt 5, and the second, without Cs and TEA, contained (in mM): potassium gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, ATP-Mg salt 5, and Na-GTP 0.5. The resistance of a typical patch pipette was 5–10 MΩ. Voltage-clamped neurons were held at a membrane potential of −70 mV for recording EPSCs and at 0 mV for recording IPSCs. At 0 mV, only IPSCs produce upward deflections (Baba et al., 1998), because the reversal potentials of EPSCs are ~0 mV (Yoshimura and Jessell, 1990).

Membrane currents were amplified with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) in voltage-clamp mode. Signals were filtered at 2 kHz and digitized at 5 kHz. Data were analyzed using pClamp 6 (Axon Instruments). Membrane potential and input resistance were measured shortly after establishing whole-cell clamp.

In preliminary experiments in 15 SG cells recorded in the absence of TEA/Cs in the pipette solution, no indication of an augmentation of K^+ channel-associated slow synaptic currents after inflammation was detected. Because the Cs/TEA-containing pipette solution, although obscuring such K^+ currents, improved space clamp and the capacity to

Table 2. Criteria for the classification of synaptic responses

Classification	Threshold	Monosynaptic (Fixed latency)	Polysynaptic (Variable latency)	Latency
Aβ mono	<50 μA	+		<2.2 msec
Aβ poly	<25 μA		+	
Aδ mono	>25 μA	+		2.2–10 msec
Aβ/Aδ poly	25–50 μA		+	
Aδ poly	>50 μA		+	
C mono	>200 μA	+		20–40 msec

record IPSCs, we used it to record the fast A fiber-mediated synaptic responses that were under investigation in this study.

Statistical analysis on differences in threshold and latencies of neurons recorded in control and inflamed tissue was performed using a nested ANOVA and on the proportions of cells with particular synaptic response by logistic regression with GEE techniques. Results presented are mean ± SD.

RESULTS

Identification of SG neurons

The neurons recovered after intrasomatic injection of Neurobiotin showed that targeting the electrode into the SG resulted in recordings from neurons with cell bodies in lamina II in all cases ($n = 21$) (Fig. 2*C*). These cells had, moreover, morphological features and cell body diameters similar to those described previously in the rat SG using Golgi (Beal and Bicknell, 1985) and intracellular HRP (Woolf and Fitzgerald, 1983)-labeling techniques and included stalked and islet cells, the most common cell types of the region. A distinctive feature in several cells was dendrites extending ventrally into the deeper laminae of the dorsal horn (Fig. 2*C*).

Membrane properties and spontaneous synaptic responses of SG neurons

The average membrane potentials of SG neurons recorded from naive preparations were −64.5 ± 6.2 mV ($n = 21$) and, in animals with inflammation they were −65.7 ± 7.4 mV ($n = 25$). Mean input resistance was 746 ± 357 MΩ ($n = 11$) in naive and 834 ± 453 MΩ ($n = 14$) in cells from inflamed animals, suggesting that similar sized cells were recorded in both cases. The frequency of spontaneous EPSCs was 35.9 ± 17.6 Hz ($n = 12$) from naive and 32.1 ± 24.1 Hz ($n = 15$) from cells recorded in preparations from inflamed animals. The frequency of spontaneous IPSCs was 23.2 ± 13.0 Hz ($n = 5$) in naive and 17.9 ± 10.7 Hz ($n = 6$) in preparations from inflamed animals. No significant differences in these passive and active membrane characteristics were detected between neurons recorded from slices prepared from naive and CFA-treated rats.

Primary afferent threshold and conduction velocity

Primary afferents could be divided into three distinct groups, corresponding to Aα/β, Aδ, and C fibers, on the basis of the threshold and conduction velocity of compound action potentials recorded extracellularly on the dorsal root (Fig. 1*B*). Figure 1*C* illustrates the stimulus response functions of Aα/β fiber and Aδ fiber volleys in the dorsal root at a pulse width of 50 μsec and shows that at <25 μA, only an Aβ wave is detectable with a maximum amplitude at 50 μA. Any new response elicited above 50 μA is likely to be, therefore, Aδ-mediated. It is possible that at thresholds below that necessary to detect an Aδ wave, a few single Aδ fibers may be activated. Table 1 shows that the stimulation thresholds and conduction velocities for the Aα/β, Aδ, and C fibers recorded in preparations from naive and rats with an inflamed hindpaw did not differ significantly. The values obtained for threshold and conduction velocity are in agreement with those found in earlier studies *in vivo* (Lynn and Carpenter, 1982; Harper and Lawson, 1985; Villiere and McLachlan, 1996).

Synaptic responses in SG neurons

Whole-cell patch-clamp recordings were made from 57 SG neurons in slices prepared from naive rats ($n = 12$) and 62 neurons in slices from rats inflamed 48 hr before with CFA ($n = 14$). All SG neurons recorded responded to orthodromic dorsal root stimulation. Table 2 shows the criteria for the classification of synaptic responses into Aβ or Aδ monosynaptic or polysynaptic in terms of threshold, response to repetitive inputs, and latency. Identification of EPSCs as monosynaptic was based on a constant latency and absence of failures with repetitive stimulation at a frequency of 20 Hz (Fig. 3*A*, *middle*, *bottom*) (Yoshimura and Jessell, 1989). Polysynaptic EPSCs, in contrast, had variable latencies and showed failures at 20 Hz (Fig. 3*A*, *top*; see Fig. 6*B*). At stimulus thresholds between 25 and 50 μA, it was not possible because of the stimulus response profile of the afferent volleys (Fig. 1*C*) to differentiate unambiguously any polysynaptic responses elicited into Aβ or Aδ, and we have classified these, therefore, as Aβ/Aδ (Table 2).

Most SG neurons recorded from naive rat slices exhibited either monosynaptic or polysynaptic Aδ fiber-mediated EPSCs. A small proportion of cells (25%) from the control preparations had Aβ fiber-mediated polysynaptic input, but none had a monosynaptic Aβ fiber input (Table 3), in agreement with earlier findings (Yoshimura and Nishi, 1993). No cells with an input exclusively from C-fibers were found. Polysynaptic IPSCs were recorded in some neurons at a holding membrane potential of 0 mV (Fig. 3*B*) and were mediated by $GABA_A$ and/or glycine receptors, as evidenced by the antagonism with bicuculline and strychnine (Fig. 3*C*). As for the EPSCs, the IPSCs in most SG neurons in naive rats were mediated by Aδ fibers, confirming the previous study (Yoshimura and Nishi, 1995), and only a small proportion of cells had Aβ fiber-mediated polysynaptic IPSCs (16%) (Table 3).

Synaptic responses in SG neurons recorded from rats with an inflamed hindpaw

In contrast to the naive situation, SG neurons recorded from slices obtained from rats with an inflamed hindpaw exhibited Aβ fiber-evoked polysynaptic EPSCs in the majority of cases (39 of 62; 63%; Table 3) ($p < 0.001$). No Aβ fiber-mediated monosynaptic EPSCs could be detected in these rats. Figure 4*A* shows the distribution of the minimum stimulus intensity threshold for eliciting EPSCs in slices from naive and rats with

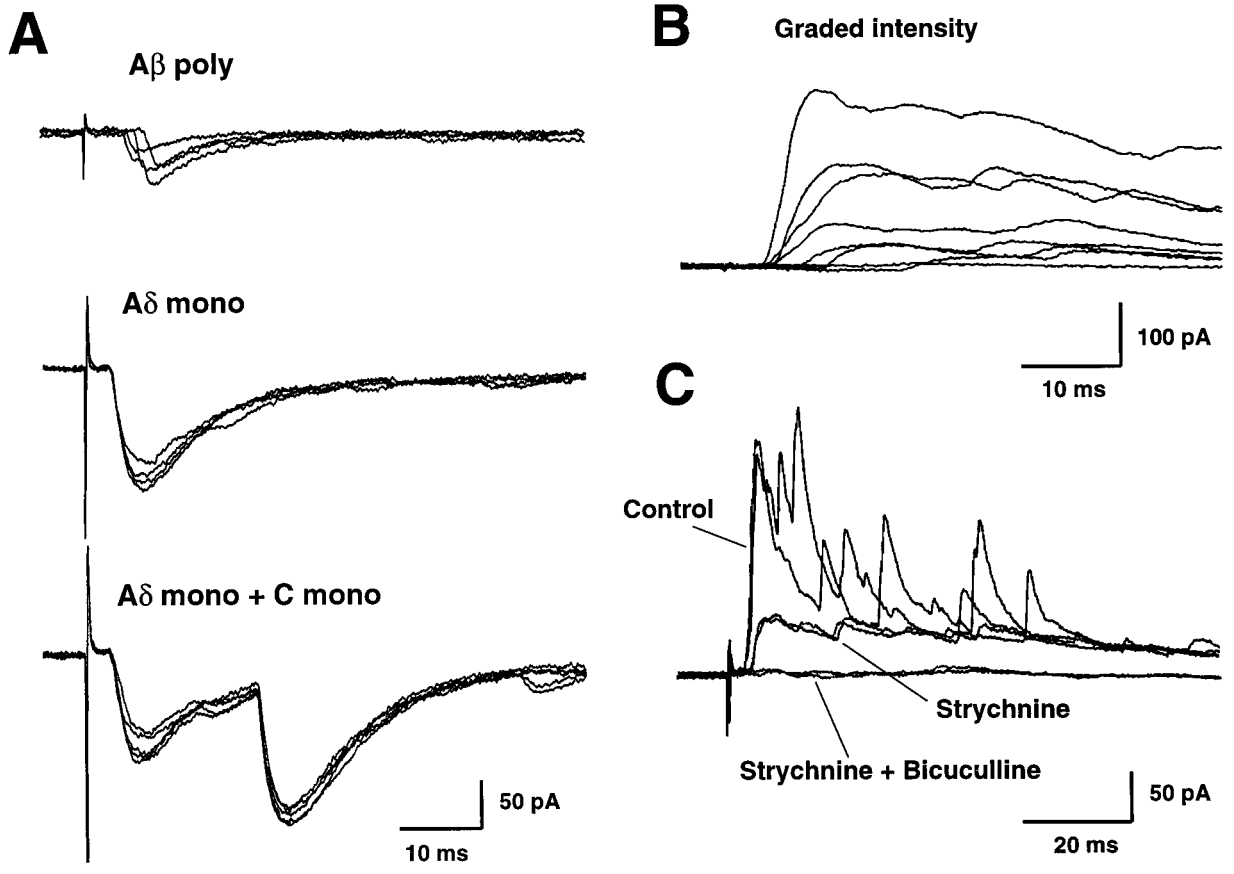

Figure 3. *A*, The *top*, *middle*, and *bottom* show respectively, EPSCs evoked by Aβ (14–20 μA, 0.05 msec), Aδ (32–50 μA, 0.05 msec), and C (200–500 μA, 0.5 msec) fiber intensities. Four to five traces are superimposed in each panel. *Top*, Aβ fiber-evoked polysynaptic EPSCs. *Middle*, Aδ fiber-evoked monosynaptic EPSCs. *Bottom*, Aδ and C fibers evoked monosynaptic EPSCs. Note that the latencies were constant for the monosynaptic EPSCs and variable in the polysynaptic responses. The above records were obtained from a single neuron. *B*, Polysynaptic IPSCs evoked by graded stimulation. As the intensity was increased from 15 to 40 μA, 0.05 msec, the latency of the IPSC shortened. *C*, The effects of strychnine (2 μM) and bicuculline (20 μM) on IPSCs. Strychnine eliminated the short-latency component of the IPSC, whereas bicuculline reduced the longer latency component.

Table 3. Classification of synaptic responses

	Aβ-mono	Aβ-poly	Aδ-mono	Aβ/Aδ poly	Aδ-poly	C-mono
EPSCs						
Naive (n = 57)	0 (0%)	14 (25%)	15 (26%)	25 (44%)	8 (14%)	8 (14%)
Inflamed (n = 62)	0 (0%)	39 (63%)	12 (19%)	19 (30%)	1 (2%)	6 (10%)
IPSCs						
Naive (n = 25)	0 (0%)	4 (16%)	0 (0%)	19 (76%)	2 (8%)	0 (0%)
Inflamed (n = 26)	0 (0%)	14 (54%)	0 (0%)	12 (46%)	0 (0%)	0 (0%)

inflammation. In slices from CFA-treated rats, the mean threshold intensity was 22.8 ± 11.3 μA, which was significantly lower than that in the naive preparations (33.2 ± 15.1 μA; $p < 0.001$; n = 57 for naive rat and 62 for CFA-treated rats). The threshold in the inflamed preparations is well below that for eliciting Aδ volleys (Fig. 1*C*). This difference cannot be caused by changes in afferent fiber excitability because peripheral inflammation had no significant effect on either the thresholds or the conduction velocities of Aβ, Aδ, and C fibers (Table 1). Figure 5*A* shows the distribution of the latencies of EPSCs evoked at a stimulus intensity of 20 μA, 0.05 msec (above the threshold for Aβ but below the threshold of Aδ fibers). Mean latencies of EPSCs in naive and CFA-treated rats at this stimulus intensity were 6.0 ± 3.5 msec (n = 12) and 3.3 ± 1.8 msec (n = 36), respectively (p = 0.010). In the CFA-treated rats, Aβ fiber-mediated polysynaptic EPSCs with a very short latency (<2.0 msec), which is much shorter than that of Aδ fiber-mediated monosynaptic EPSCs (latency, 2.2–3.5 msec), could be detected (Fig. 5*A*, Fig. 6). These short-latency EPSCs were never recorded in cells from naive animals at this stimulus strength. The conduction velocity calculated by two point stimulation along the length of the dorsal root was in Aβ fiber range (>15 m/sec) (Fig. 6*C*). Stimulation at an intensity of 100 μA 0.05 msec, which is supramaximal for Aβ fibers and above the Aδ threshold (Fig. 5*B*), also resulted in a shorter mean latency of EPSC in inflamed rats (2.6 ± 1.0 msec; n = 60) than

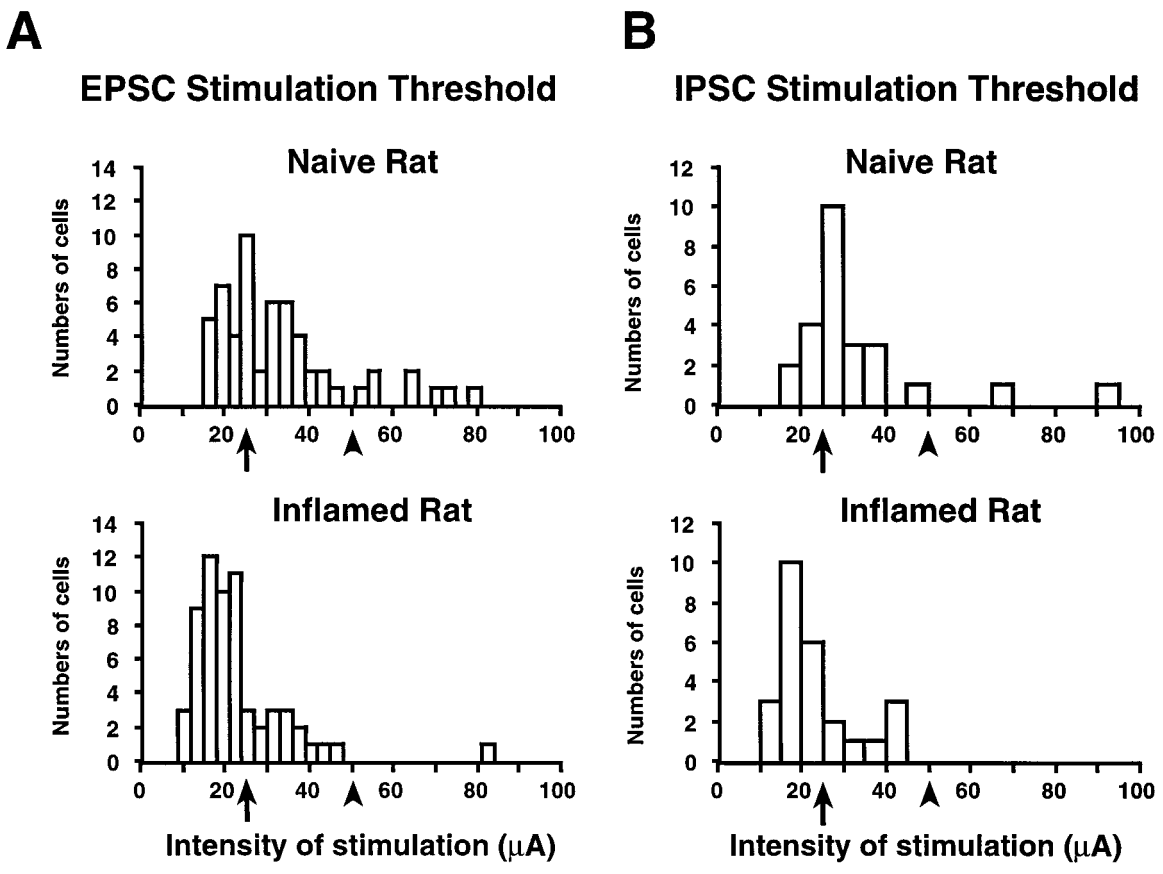

Figure 4. Distribution of the minimum stimulus threshold intensities necessary for eliciting EPSCs and IPSCs in cells recorded in the SG in slices from naive and CFA-treated rats. The mean stimulus threshold intensity required to evoke EPSCs in naive and CFA-treated rats was 33.2 ± 15.1 μA (n = 57) for naive and 22.8 ± 11.3 μA (n = 62) for CFA-treated rats ($p < 0.001$; nested ANOVA). The mean stimulus threshold intensity required to evoke IPSCs in naive and rats with an inflamed hindpaw was 32.5 ± 15.7 μA (n = 25) for naive and 21.9 ± 9.9 μA (n = 26) for the CFA-treated rats (p = 0.013; nested ANOVA). The *arrow* indicates the stimulus intensity at which an Aδ volley begins to be detected, all responses below this value are exclusively Aβ. The *arrowhead* represents the stimulus value at which a maximal Aβ volley is elicited. All responses elicited above this intensity are exclusively Aδ-evoked. For values between the *arrow* and the *arrowhead*, the responses evoked may be Aβ- and/or Aδ-evoked.

in naive rats (3.1 ± 1.1 msec; n = 53; $p < 0.05$). The suprathreshold stimulus also shortened the EPSC latency compared with the submaximal stimulus.

In SG neurons recorded from rats with inflammation, polysynaptic IPSCs were evoked by Aβ fiber intensity stimulation in about half of cells (14 of 26; 54%; Table 3), which is significantly greater than naives ($p < 0.001$). Figure 4*B* shows the distribution of the minimum stimulus intensity threshold for eliciting IPSCs in slices from naive and CFA-treated rats. In slices from rats with an inflamed hindpaw, the mean threshold intensity was 21.9 ± 9.9 μA, which was significantly lower than that in the naive preparations (32.5 ± 15.7 μA; p = 0.013; n = 25 for naive rat and 26 for inflamed group).

DISCUSSION

We have found that a localized peripheral inflammation for 48 hr results in a facilitation of short-latency fast Aβ fiber-mediated polysynaptic EPSCs and IPSCs in SG neurons that receive sensory input from sensory fibers innervating the inflamed area.

Aβ fiber-mediated synaptic input to the SG

There is substantial evidence that the primary function of neurons in the SG is to integrate noxious afferent information carried by the high-threshold Aδ and C fibers that terminate in this region of the superficial dorsal horn (Willis and Coggeshall, 1991). The SG cells, acting as inhibitory and excitatory interneurons, modify the output of projection neurons in both lamina I and the deeper layers of the dorsal horn (Willis and Coggeshall, 1991). The vast majority of SG neurons have high-threshold receptive fields, but an excitation of SG neurons by innocuous mechanical stimuli and Aβ fiber electrical stimulation has been reported in a small number of cells in *in vivo* studies (Kumazawa and Perl, 1978; Bennett et al., 1980; Woolf and Fitzgerald, 1983). Studies in adult spinal cord slices with an attached dorsal root show a similar picture. Although short-latency fast excitatory synaptic responses in SG cells in these preparations have been found to be predominantly mediated by Aδ fibers, Aβ fiber-mediated EPSCs are also detected, but only in a small proportion of SG neurons. These Aβ fiber-mediated EPSCs always have a variable and longer latency than the more common monosynaptic Aδ EPSCs (Yoshimura and Jessell, 1989; Yoshimura and Nishi, 1993). *In vitro* studies with immature young rat spinal cord preparations have not reported Aβ fiber-mediated fast EPSCs, which may reflect developmental changes or technical issues relating to the thickness of the slice and the length of dorsal root available (Bleazard et al., 1994; Randic et al., 1995; Sandkuhler et al., 1997).

Because Aβ fibers do not project directly to SG but to lamina III-VI (Brown, 1981; Woolf, 1987) and because the dendrites of many SG neurons do not leave SG (Light et al., 1979; Bennett et

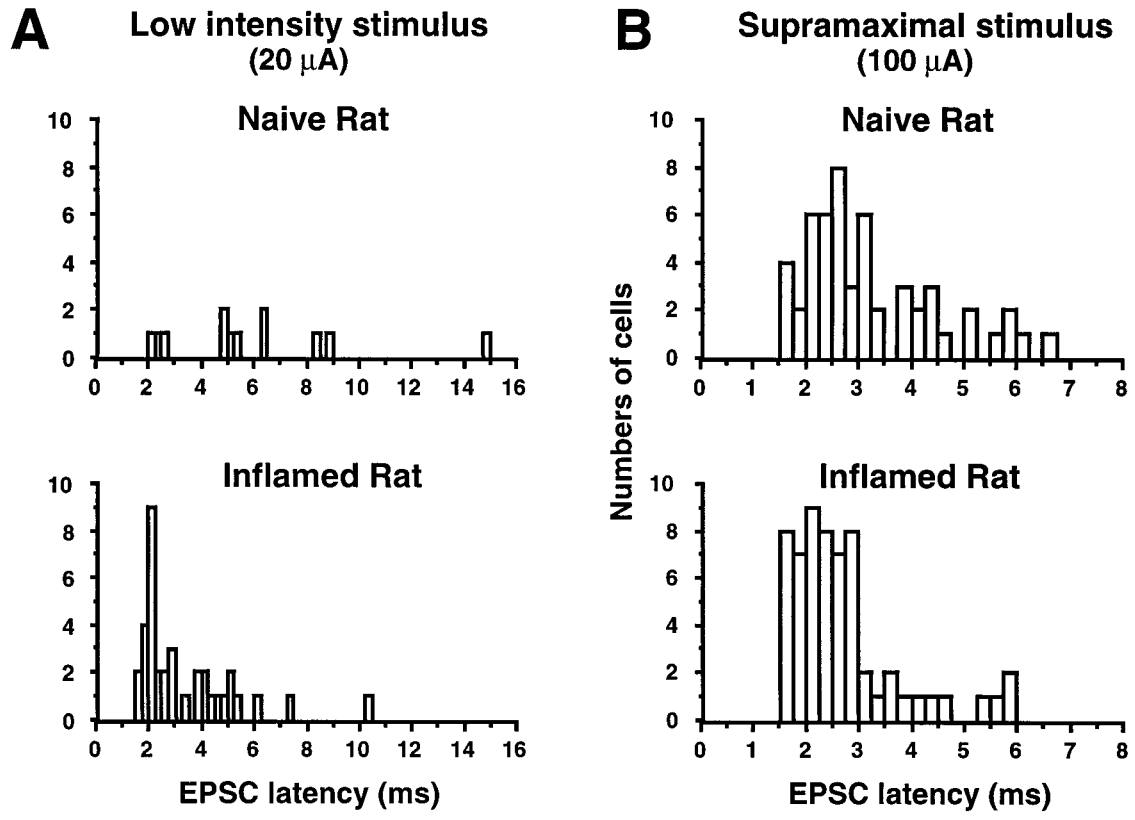

Figure 5. Distribution of the latencies of EPSCs in cells recorded in the SG in slices from naive and CFA-treated rats. *A*, The EPSC latencies evoked by Aβ fiber intensity (20 μA, 0.05 msec, below Aδ fiber threshold) were significantly shortened in the rats with an inflamed hindpaw; 3.3 ± 1.8 msec versus 6.0 ± 3.5 msec ($p = 0.010$; nested ANOVA; $n = 12$ in naive and $n = 36$ in inflamed rats). Aβ fiber-mediated EPSCs with short latencies (<2.0 msec) were only observed in the preparations from rats with an inflamed hindpaw. *B*, Distribution of the latencies of EPSCs evoked by supramaximal Aβ fiber stimulation intensity. Mean latency of EPSCs in CFA-treated rats was 2.6 ± 1.0 msec ($n = 60$), which was significantly shorter than that recorded in naive rats (3.1 ± 1.1 msec; $n = 53$; nested ANOVA; $p < 0.05$).

al., 1980), it has been commonly assumed that all responses to Aβ fiber stimulation must depend on polysynaptic pathways. In support of this is our failure ever to detect an Aβ fiber-evoked monosynaptic EPSC in the SG. However, there are some cells in the SG with dendrites that extend into the deep dorsal horn (Woolf and Fitzgerald, 1983; Fig. 2*C*). The question should be therefore, why, given this potential anatomical substrate for a direct Aβ input to some SG cells, has no such input ever been seen in naive animals or even after inflammation?

Potential mechanisms responsible for the facilitation of Aβ fiber mediated-input into SG after inflammation

There are two possible general mechanisms that could result in the recruitment of fast Aβ-evoked synaptic responses in the SG; a strengthening of pre-existing ineffective or silent synapses or the establishment of novel synapses by a structural alteration in synaptic connectivity. The former is likely to operate after inflammation, and the latter may well contribute to changes after nerve injury (Woolf et al., 1992). A functional change in synaptic connectivity could be caused by presynaptic or postsynaptic alterations, either increasing excitability or reducing inhibition and may operate at the first synapse between the afferent and dorsal horn neurons or on subsequent neurons in the polysynaptic chain that carries the Aβ fiber input to the SG from deep laminae. One example of a presynaptic change in primary afferents that could increase synaptic strength is a shift in transmitter content in A fibers. After inflammation, for example, some Aβ fibers, which are normally not substance P-immunoreactive, begin to express this peptide (Neumann et al., 1996). Aβ fibers also acquire the novel capacity to induce an NK1-mediated windup-like phenomenon (Thompson et al., 1994; Herrero and Cervero, 1996a,b). Inflammation also changes the nature of those peripheral stimuli that can evoke activity-dependent c-fos expression in the dorsal horn from predominantly nociceptors in the naive state (Hunt et al., 1987; Presley et al., 1990) to one that includes Aβ fibers (Ma and Woolf, 1996b). Other mechanisms that may potentially increase synaptic strength include increased transmitter release, increased postsynaptic receptors, reduced uptake or breakdown of transmitters, post-translational changes in receptor function, or alterations in postsynaptic membrane excitability. We found no change in the membrane potential of the SG neurons from CFA-pretreated preparations, but because the change in synaptic responsiveness was polysynaptic and not monosynaptic it is not possible to dissect out easily what is responsible and where it is acting. Nevertheless, inflammation has been shown to result in changes in the phenotype of dorsal horn neurons, including the upregulation of NK1 receptors and alterations in dynorphin expression so that postsynaptic mechanisms may be important (Ruda et al., 1988; Noguchi et al., 1991; Schafer et al., 1993; McCarson and Krause, 1994).

Although a decrease of GABAergic and glycinergic inhibition could result in an augmentation of Aβ fiber-mediated responses in the SG, this is unlikely because we found a facilitation of Aβ

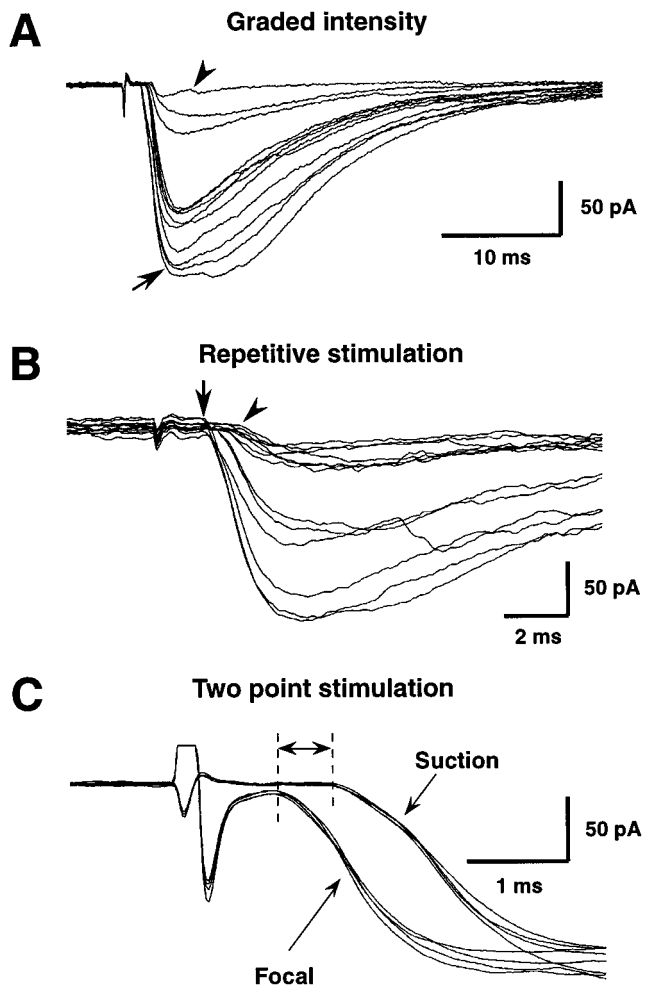

Figure 6. Aβ fiber-evoked polysynaptic EPSCs with short and variable latencies recorded in an SG cell from a slice from a rat with an inflamed hindpaw. *A*, The effect of stimulus intensity. As the intensity was increased (12–25 μA; 0.05 msec), the latency of the Aβ fiber-evoked EPSC shortened. The shortest latency was 1.6 msec at an intensity of 22–25 μA. The *arrowhead* identifies the EPSC evoked by the lowest, and the *arrow* identifies the EPSC evoked by the highest stimulus strengths. *B*, A shift in latency of the Aβ-evoked EPSCs was observed with 20 Hz repetitive stimulation at 25 μA, indicating a polysynaptic synaptic response. The *arrow* identifies the first EPSC, and the *arrowhead* identifies the last EPSC in the train. *C*, Stimulation of the dorsal root by a peripheral suction electrode and the entry zone with a focal electrode were performed to calculate the conduction velocity of fibers responsible for the evoked EPSC. Conduction velocity calculated by the difference of latencies was 32.5 m/sec (length of dorsal root, 19.5 mm).

fiber-mediated IPSCs as well as EPSCs after inflammation. We cannot exclude the possibility, however, that disinhibition occurs in laminae III or IV. This too seems unlikely, though, because both GABA and the $GABA_A$ receptor are upregulated in the dorsal horn after peripheral inflammation (Castro-Lopes et al., 1994).

After peripheral nerve injury, Aβ fibers sprout from lamina III into lamina II (Woolf et al., 1992), and Aβ fiber-mediated monosynaptic EPSCs, which are never normally observed in SG, can be detected (Okamoto et al., 1996). We have been unable, however, to detect any evidence of Aβ fiber sprouting after CFA inflammation at 48 hr (Q-P. Ma and C. Woolf, unpublished observations), which is in keeping with the lack of any monosynaptic input after this treatment.

Functional consequences of augmented Aβ input to the SG

Several studies recording from large cells in the deep dorsal horn have shown that inflammation alters receptive field size and properties (Ren et al., 1992a,b; Ren and Dubner, 1993). Synaptic input to lamina II cells is, as we show here, also modified. A recruitment of low-threshold mechanoreceptive input to nociceptive-specific neurons, including those in the superficial dorsal horn, occurs after central sensitization induced by capsaicin or mustard oil (Simone et al., 1989; Woolf et al., 1994). Central sensitization may contribute to the change in SG responsiveness to Aβ input after inflammation caused by an ongoing activity in C-fibers generated by the presence of inflammatory mediators in the inflamed tissue. Such a mechanism is unlikely, though, to be a major contributor in the present experiments, in which the sensory fibers are disconnected from the periphery, unless the inflammation-induced activity generates very long-lasting changes in membrane excitability.

The processing of sensory information in the spinal cord is dynamic, and it is this modifiability that is a major contributor to alterations in sensation after inflammation or nerve injury. The fact that an area of the spinal cord normally devoted almost exclusively to nociceptive input begins after inflammation to receive low-threshold synaptic input is a further indication of the plasticity of the system. What causes the changes and whether they contribute to inflammatory pain hypersensitivity needs now to be established.

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