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Key role of the dorsal root ganglion in neuropathic tactile hypersensibility

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Abstract

Cutting spinal nerves just distal to the dorsal root ganglion (DRG) triggers, with rapid onset, massive spontaneous ectopic discharge in axotomized afferent A-neurons, and at the same time induces tactile allodynia in the partially denervated hindlimb. We show that secondary transection of the dorsal root (rhizotomy) of the axotomized DRG, or suppression of the ectopia with topically applied local anesthetics, eliminates or attenuates the allodynia. Dorsal rhizotomy alone does not trigger allodynia. These observations support the hypothesis that ectopic firing in DRG A-neurons induces central sensitization which leads to tactile allodynia. The question of how activity in afferent A-neurons, which are not normally nociceptive, might induce allodynia is discussed in light of the current literature.

Keywords: Dorsal root ganglion; Ectopic firing; Indwelling catheter; Neuropathic pain; Rhizotomy

1. Introduction

Nerve injury is frequently followed by tactile hypersensibility in the associated limb (tenderness to touch; ''tactile allodynia''). This neuropathic pain symptom is fundamentally paradoxical. If anything, partial denervation of the skin ought to blunt sensation, not amplify it. We and others (Devor et al., 1991; Devor and Seltzer, 1999; Gracely et al., 1992; Sheen and Chung, 1993) have proposed the explanation that ectopic afferent discharge originating at the nerve injury site and in axotomized primary sensory neurons in the dorsal root ganglion (DRG) triggers and maintains spinal ''central sensitization'' (Woolf, 1983). Signal amplification by central sensitization renders painful afferent input arriving in the cord along low threshold mechanoreceptive Aβ fibers (Campbell et al., 1988; Torebjork et al., 1992). The observed tactile allodynia is ''Aβ pain''.

In the Chung model of neuropathic pain (Kim and Chung, 1992) about half of the innervation of the hindlimb is eliminated by severing the L5 (or L5+6) spinal nerve just distal to the DRG. Under these circumstances massive ectopic afferent firing is observed in the corresponding dorsal roots beginning ca. 20 h after axotomy, precisely the time that tactile allodynia first appears (Liu et al., 2000b, 1999). This simultaneity is not just a coincidence, but appears to reflect a causal relation between the ectopia and the allodynia. For example, Chung and others showed that silencing the ectopia by applying tetrodotoxin, local anesthetics or related compounds to the neuroma or axotomized DRGs (Blenk et al., 1997; Lyu et al., 2000; Malan et al., 2000; Zhang et al., 2000), or by severing the connection between the DRGs and the spinal cord by dorsal rhizotomy (Na et al., 2000; Sheen and Chung, 1993; Yoon et al., 1996), reduces or eliminates the tactile allodynia. Likewise, evoking activity with irritants applied to the DRG induces tactile allodynia (Dobretsov et al., 2001; Homma et al., 2002; Hu and Xing, 1998; Zhang et al., 1999). Corresponding observations have been reported with respect to other animal models of neuropathy in

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which tactile allodynia is thought to be generated by the same mechanism (Devor and Seltzer, 1999).

Complicating this otherwise consistent picture, Li and collaborators (2000) and Eschenfelder et al. (2000) reported that, in their hands, dorsal rhizotomy does *not* reverse tactile allodynia in the Chung model. On the face of it, this result is a straightforward failure to replicate the results of the investigators noted above. However, this seemingly obvious conclusion may be misleading. The reason is that both groups, and others (Colburn et al., 1999), reported that L5 dorsal rhizotomy, alone, triggers tactile allodynia. If so, effects of rhizotomy cannot be used to rule out the hypothesis that tactile allodynia after spinal nerve section is caused by ectopic firing originating in the neuroma and axotomized DRG. The logic is simple. Allodynia originally caused by neuroma and DRG ectopia may simply be replaced by a new form of allodynia triggered by the rhizotomy. The new cause of allodynia obscures the old. The Chung group did not observe allodynia after rhizotomy alone (Sheen and Chung, 1993; Yoon et al., 1996).

So far only the Chung group and Na et al. (2000) have reported that blocking spinal access of peripheral ectopia eliminates tactile allodynia under conditions where rhizotomy does not, in itself, induce allodynia. Because of the importance of this result for understanding neuropathic pain mechanisms, we have repeated the experiment using a strain of rats in which massive DRG ectopia is known to occur following spinal nerve section (Liu et al., 2000a,b). Under our experimental conditions L5 dorsal rhizotomy did not induce allodynia. Moreover, it did eliminate allodynia triggered by spinal nerve section, as did application of anesthetics to the axotomized ganglion. Thus, our results are consistent with the original reports of the Chung and the Na groups, and support the conclusion that neuroma and DRG ectopia plays a key role in neuropathic tactile hypersensibility in the Chung model.

2. Methods

2.1. Animals and surgery

Experiments were carried out using adult (250–500 g) male rats of the Wistar-derived Sabra strain (Lutzky et al., 1984; Chung model and rhizotomy experiments), or Sabra rats of the HA selection line (Devor and Raber, 1990; indwelling catheter experiments). All work adhered to national legislation on humane care and use of laboratory animals, and corresponding guidelines of the International Association for the Study of Pain (Zimmermann, 1983). In light of the prior published work on this topic, we used the minimal number of animals sufficient to establish correspondence to one of the two previously published conclusions.

Three surgical procedures were used, all performed under general anesthesia (sodium pentobarbital, Nembutal, 50 mg/kg, i.p.) with aseptic precautions.

2.2. Spinal nerve section (Chung model; Kim and Chung, 1992)

The L5 spinal nerve was exposed unilaterally 5–10 mm distal to the ganglion, tightly ligated with 5-0 silk, and cut just distal to the ligature. Care was taken not to touch the L4 spinal nerve. The L6DRG contributes minimally if at all to hind-paw innervation in Sabra rats (Devor et al., 1985), and in our hands including the L6 segment enhances tactile allodynia only marginally. Nonetheless, in some of the animals we also cut the L6 spinal nerve (i.e., L5+6) because this was done in the study of Li et al. (2000), and in the original description of this model by Kim and Chung (1992). Results using these alternative surgeries were equivalent, and have been combined.

2.3. Dorsal rhizotomy

After carrying out a minimal laminectomy, the L5 (or L5+6) dorsal root (DR) was identified on one side, withdrawn with a fine glass hook through a small nick made in the dura, and cut with microscissors. The distal DR end was ligated with 6-0 silk to facilitate later identification at autopsy, and both ends were reinserted intradurally. A piece of gelfoam was placed on the dural defect. Rhizotomy was carried out in intact rats, and in rats with tactile allodynia 3–7 days following ipsilateral spinal nerve injury.

2.4. Indwelling catheter

Immediately following spinal nerve section, a fine sterilized polyethylene catheter (PE10, o.d. = 600 μm), filled with sterile saline, was implanted with its tip on the L5DRG (experimental), or on the L4DRG or the L4 spinal nerve 2–4 mm distal to the L4DRG (control). The catheter tip, which was thinned to about 200 μm (o.d.) by pulling over a heat source, was either inserted into the root foramen in parallel with the spinal nerve until it lay on the dorsal surface of the DRG, or was rested on the exposed surface of the L4 spinal nerve. In a few rats the tip of the catheter was inserted past the DRG and 3–5 mm into the spinal intrathecal space. Just distal to the foramen the catheter was anchored to underlying tissue using methacrylate tissue glue (Histoacryl, B. Braun, Melsungen, Germany) as well as several ties of 6-0 silk secured to protuberances formed on the catheter wall. Further proximally, the catheter had a pre-formed U-turn which permitted its proximal end to be tunneled under the skin to the back of the neck where it was anchored to muscle using silk sutures, and then

exteriorized through a small opening in the skin. The exteriorized proximal end of the catheter was sealed with a removable stainless steel plug. Catheters were sized so that their internal volume was 10.0 μl.

Following surgery, incisions were closed in layers using silk sutures and skin wound clips, a topical bacteriostatic powder was applied, and penicillin (50 kU/kg, i.m.) was given prophylactically. Rats were then returned to the institutional animal colony where they were maintained individually in transparent plastic shoebox cages bedded with wood shavings. The day:-night cycle was 12 h:12 h (lights on 06:00), and rat food pellets (Kofholk, Petah Tikva, Israel, product #19510) and water were available ad libitum. Following the period of experimental observations animals were killed with an overdose of Nembutal (100 mg/kg i.p.) and a broad postmortem dissection was performed. Methylene blue (1%) was injected into the indwelling catheter in order to mark the position of the catheter tip and assess the extent of fluid spread. At the time of testing the investigator knew which segment was targeted, L4 or L5, but was blind as to whether the catheter tip rested on the DRG, the spinal nerve or in the intrathecal space.

2.5. Experimental procedure

Tactile sensitivity of the hindpaws was measured before surgery and at intervals beginning 24 h after surgery. We used an ascending staircase of calibrated nylon von Frey monofilaments with initial bending forces (in mN) of: 8.4, 19.0, 35.1, 53.9, 104.2, 157.5, 217.8 and 286.6 (equivalent to mass of: 0.8, 1.9, 3.4, 5.3, 10.2, 15.4, 21.4 and 28.1 g). These calibrations were verified, and if necessary adjusted through trimming, by mounting the monofilament in a microdrive and lowering it onto the pan of an electronic balance. When there was no response to the stiffest monofilament, we sometimes tested using a stiff "pinprick" stimulus.

Rats were placed on a raised wire mesh screen and the mid-plantar skin, just proximal to the foot pads and midway between medial and lateral edges of the paw, was briefly probed five times at 1-s intervals, with a force just sufficient to bend the monofilament. Trials began with the 0.8-g monofilament. If the animal failed to respond with at least a momentary twitch/withdrawal of the foot on at least three of the five probes, the next stiffest monofilament was tried, and so forth. "Threshold" was the force of the first monofilament in the series that evoked $\geqslant 3/5$ responses. On a given test day this ascending staircase threshold-seeking procedure was repeated at least three times on each foot, usually alternating from side to side, with $\geqslant 3$ min allowed between trials on each foot. The average of values obtained on each foot was used to represent the rat's response threshold for that test day. An exception was experiments involving injections through an indwelling catheter where threshold at each time point postinjection was based on a single set of ascending staircase probes. Preoperative (preop) data are the average of all tests carried out prior to surgery, usually three in number.

Experiments involving drug injection through indwelling catheters were carried out 1–18 days postoperatively (dpo) using rats that demonstrated tactile allodynia. We waited at least 24 h between subsequent injections in any given rat. The criterion used for allodynia was paw withdrawal threshold less than half of the preoperative baseline on at least 2 test days. This criterion was met in 70% of the rats originally operated; rats that did not meet this criterion were excluded from the study. Injections were accomplished in two stages. First, 10 μl of lidocaine (2%, Teva, Petah Tikva), or in some cases 5 μl lidocaine followed by 5 μl saline, were loaded in the catheter. This flushed the 10 μl of saline that previously filled the catheter onto the surface of the ganglion or the spinal nerve, or into the intrathecal space, and loaded the internal volume of the catheter with lidocaine (5 or 10 μl). Sensory testing (baseline) was carried out following this control saline flush. At time 0 (Figs. 2 and 3) the lidocaine that was pre-loaded in the catheter was ejected with a push of 5 or 10 μl of saline. This refilled the catheter with saline. In some experiments the volume of lidocaine applied was increased to as much as 25 μl as noted in Section 3, and in two rats 1% lidocaine was tried.

In all of the rats that met the stated criteria for inclusion in the study we confirmed at autopsy that the intended spinal nerve(s) or DR(s) had indeed been cut, and in all but one with an indwelling catheters we found the catheter tip on the targeted ganglion, the spinal nerve or intrathecally. Dye injection at postmortem dissection revealed that 5–10 μl volumes covered the DRG but spread only 1–2 mm within the foramen, did not reach the spinal cord, and certainly did not get anywhere near neighboring L4 spinal roots or ganglia which lie at a distance of 8–12 mm.

Statistical comparisons are based on non-parametric χ^2 or Fisher exact probabilities tests with a significance criterion of $p = 0.05$.

3. Results

3.1. Dorsal rhizotomy

Prior to spinal nerve cut rats rarely responded even to the stiffest von Frey filament (28.1 g) although they consistently showed paw withdrawal to pinprick. Such responses were plotted as 28.1 g. As illustrated in Fig. 1 (upper panel) tactile allodynia develops on the hindpaw ipsilateral to the surgery within 24 h of spinal nerve transection. There was no obvious change on the

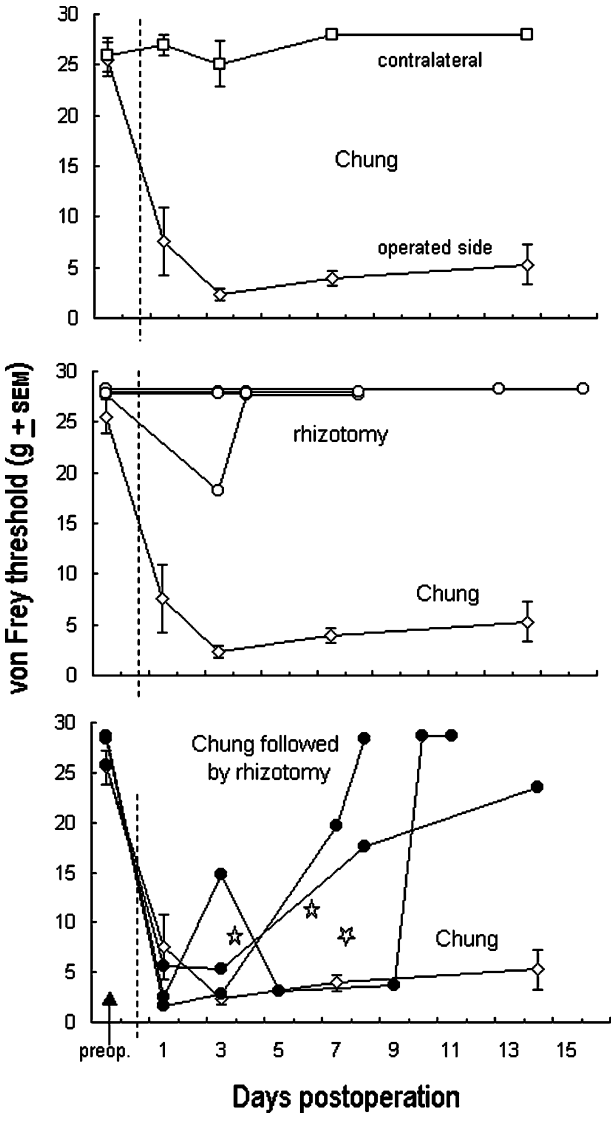

Fig. 1. Tactile allodynia in the Chung model of neuropathic pain requires axonal continuity between the axotomized DRG and the spinal cord. Upper panel, spinal nerve transection (vertical dashed line,11 rats) rapidly induced tactile allodynia on the ipsilateral hindpaw (operated side) as measured by reduced threshold for paw withdrawal upon stimulation with von Frey monofilaments. Middle panel, L5 ($n = 3$) or L5+6 ($n = 1$) dorsal rhizotomy failed to induce tactile allodynia (rhizotomy, 4 rats, open circles). The rat that showed a small but insignificant decrease in response threshold 3 dpo is the one in which the L5+6 DRs were cut. Lower panel, once tactile allodynia had been established in the Chung model (criterion drop in response threshold on at least 2 test days) the L5 dorsal root was cut (time postoperation indicated by stars). This rhizotomy eliminated the allodynia, returning withdrawal threshold toward the preoperative baseline (3 rats, filled circles). In one rat allodynia was still observed on the first test day after rhizotomy, suggesting that central sensitization might persist following rhizotomy by as much as 24 h. Open diamonds in the middle and lower panels are copied from the upper panel (Chung operated side) to facilitate comparison.

contralateral side. Dorsal rhizotomy in rats without spinal nerve injury failed to induce criterion tactile allodynia (4 rats, Fig. 1 middle panel). However, dorsal rhizotomy, performed after the emergence of tactile allodynia induced by spinal nerve section eliminated the allodynia (3 rats, Fig. 1 lower panel). Effects of rhizotomy were significant in comparison with Chung operated animals that did not undergo follow-up rhizotomy (Fisher test, $p < 0.01$).

3.2. Lidocaine infusion on DRG or spinal nerve

In four rats that had developed tactile allodynia following spinal nerve section we applied 5 or 10 μl of 2% lidocaine onto the surface of the L5DRG. This dose range was selected as being insufficient to block axonal conduction (see below), but adequate to reduce ectopic spike initiation in the ganglion; these two processes have very different sensitivities to lidocaine (Devor et al., 1992). Relief from allodynia began 2–16 min after lidocaine injection, and the effect lasted for 17 to >280 min (Fig. 2). So long as tactile allodynia was present, the effect of lidocaine did not vary consistently with time following spinal nerve injury. There was also no obvious relation between response latency and duration.

Variability from experiment to experiment precluded a clear determination as to whether 10 μl lidocaine was more effective than 5 μl. However, dose surely must play a role. For example, in the rat in which 10 μl lidocaine had only a brief effect (circles in Fig. 2, tactile allodynia returned within 17 min) an injection of 5 μl failed to relieve allodynia at all (not plotted). Likewise, in one rat that responded to 5 μl of 2% lidocaine there was a lesser response to 1% lidocaine (inverted triangles, Fig. 2). Since two other trials in this rat using 1% lidocaine had no effect at all, 2% lidocaine was used in all subsequent animals.

In a fifth rat 10 μl lidocaine 2% did not relieve allodynia. In this rat postmortem examination revealed that the catheter tip lay just lateral to the L5DRG rather than on its dorsal surface as in the other rats. Perhaps tissue barriers in this animal prevented sufficient lidocaine from penetrating the DRG capsule (data not plotted in Fig. 2).

Five control rats that developed criterion tactile allodynia were used to confirm that the anti-allodynic effect of L5DRG superfusion did not result from conduction block in the intact L4 segment or from redistribution of lidocaine elsewhere. In these rats we applied to the L4 segment the full lidocaine dose that proved sufficient to eliminate allodynia when applied to the L5DRG. Moreover, just to be sure, we also tried doubling this dose. Applying lidocaine 2% to the L4DRG in volumes of 5, 10 or even 20 μl did not eliminate tactile allodynia (2 rats). Likewise, 15, 20 or even 25 μl of 1% or 2% lidocaine applied to the L4 spinal nerve failed to reverse allodynia (Fig. 3). This proves that the elimination of allodynia following li-

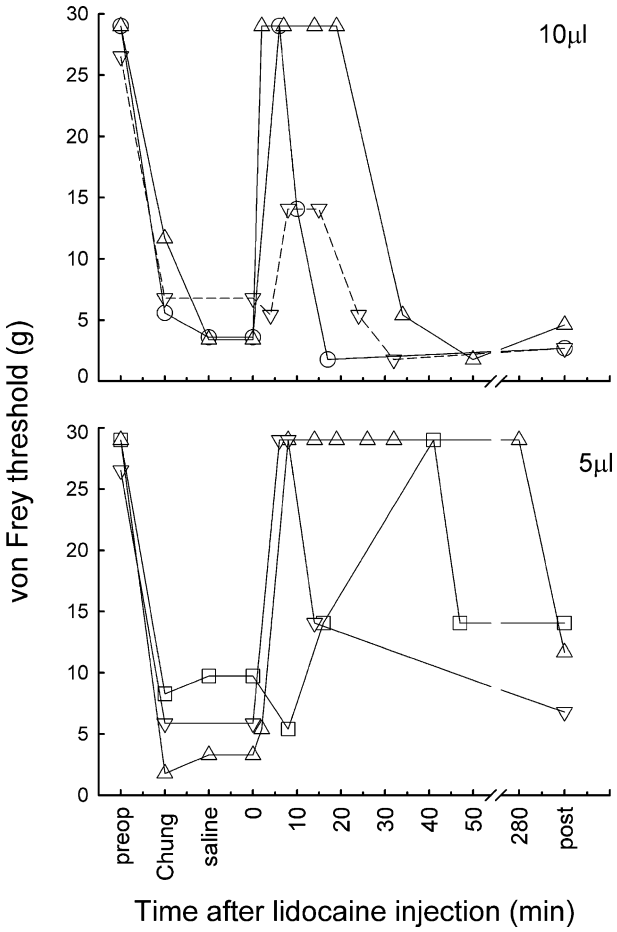

Fig. 2. Silencing ectopia by superfusing the axotomized DRG with a local anesthetic transiently eliminates tactile allodynia. The L5 ($n = 1$, inverted triangles) or L5+6 ($n = 3$, remaining symbols) spinal nerves were severed, inducing tactile allodynia (Chung). Superfusing the L5DRG with saline had no effect, but superfusion with 2% lidocaine (solid lines, 5 μl (lower panel) or 10 μl (upper panel)) transiently eliminated the allodynia. Use of 1% lidocaine attenuated allodynia but did not eliminate it (dashed line, upper panel). In one experiment (triangles) tactile allodynia had not returned 280 min after the injection, but it did by the next test day (post), consistent with the observation that lidocaine effects may persist. Each symbol type represents a different rat ($n = 4$). Data plotted at $t = 0$ are the last measurements made before the lidocaine injection.

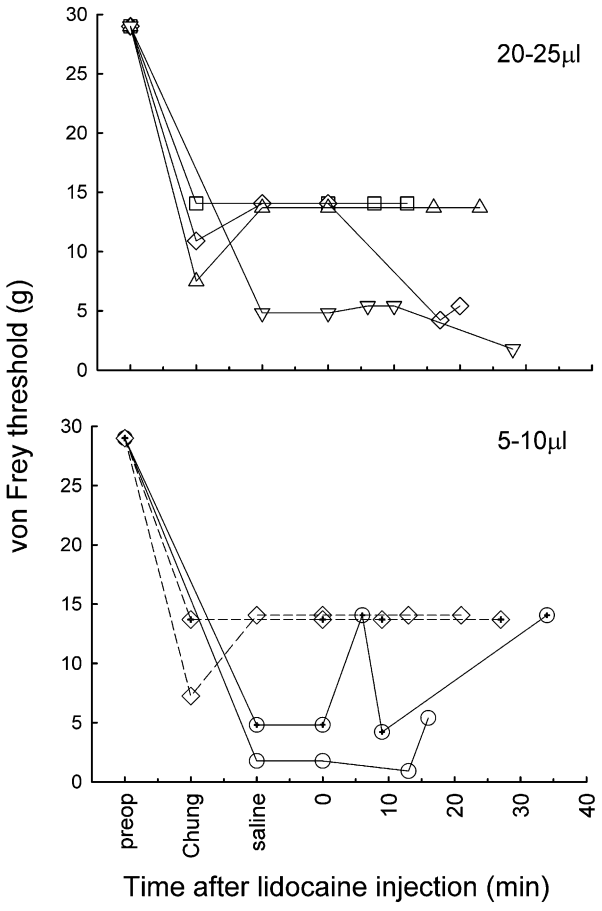

Fig. 3. Control experiments. Lidocaine applied to the L4DRG or L4 spinal nerve did not eliminate tactile allodynia, even transiently. Each symbol type represents a different rat ($n = 5$). Repeat trials in two of the rats are marked with a plus sign in the symbols (circle, diamond). The L5+6 ($n = 1$, circle) or L5 spinal nerves ($n = 4$, remaining symbols) were severed, inducing tactile allodynia. In two rats the L4DRG was superfused with 5 μl (circle), 10 μl (circle plus) or 20 μl 2% lidocaine (inverted triangle). In three rats 20 or 25 μl 2% lidocaine was applied to the L4 spinal nerve (square, diamond and triangle). One of these rats also received trials of 15 (diamond) or 20 μl (diamond plus) 1% lidocaine (dashed lines, lower panel). Data plotted at $t = 0$ are the last measurements made before the lidocaine injection.

docaine application to the L5DRG could not have been due to drug spread to the L4 segment, or to drug redistribution to the spinal cord or to the systemic circulation. Moreover, it proves that these doses of lidocaine are insufficient to block nerve conduction. With only the L4 segment still innervating the foot (L5 or L5+6 had been cut), L4 nerve block would be expected to render the foot completely anesthetic. In fact, lidocaine injection at L4 did not even appear reduce the original degree of tactile allodynia (Fig. 3). Lidocaine was significantly more effective at reversing allodynia when applied to the L5 than the L4 segment (Fisher's test, $p = 0.02$).

3.3. Intrathecal lidocaine

In six rats the catheter tip was inserted past the DRG and slightly into the intrathecal space as confirmed postmortem by dye injection. In three of these rats injection of lidocaine (2%, 5–15 μl, L5 segment) eliminated allodynia on the side of the injection as indicated by transient return of von Frey threshold to >28.1 g, but with preserved motor function and response to pinprick. In another three lidocaine (2%, 10–25 μl, L4 segment) produced anesthesia (no response to von Frey filaments or noxious stimuli including pinprick) and flaccid paralysis. These effects lasted 10–25 min. Anti-allodynia and anesthesia/paralysis prove that the drug was active, and that in the intrathecal space (in the absence of a

perineurial diffusion barrier), it was capable of blocking axonal conduction with expected sensory and motor consequences.

4. Discussion

Transection of L5 or L5+6 spinal nerves (Chung model of neuropathic pain) induced tactile allodynia of the hindpaw with rapid onset (Kim and Chung, 1992). Transection of the corresponding DRs did not do so. Transection of the L5 DR eliminated tactile allodynia previously triggered by spinal nerve section. Finally, applying a small quantity lidocaine to the L5DRG in animals with tactile allodynia in the Chung model transiently reversed the allodynia. This did not occur when lidocaine was applied to the L4 segment. These results are consistent with the observations of the Chung and the Na groups (Na et al., 2000 Sheen and Chung, 1993; Yoon et al., 1996), and inconsistent with the results of Li et al. (2000) and Eschenfelder et al. (2000). As such, they support the hypothesis that tactile allodynia in the Chung model is a consequence of central sensitization triggered by ectopic afferent discharge largely originating in the axotomized DRG neurons and the spinal nerve neuroma (Devor and Seltzer, 1999; Liu et al., 2000b).

4.1. The validity of conclusions based on dorsal rhizotomy

We do not know why dorsal rhizotomy alone triggers tactile allodynia in some hands (Colburn et al., 1999; Eschenfelder et al., 2000; Li et al., 2000), but not others (Sheen and Chung, 1993; Yoon et al., 1996; the present study). A large body of clinical experience indicates that dorsal rhizotomy and ganglionectomy reliably yield immediate relief from neuropathic limb pain, without inducing tactile allodynia (e.g., Gybels and Sweet, 1990; Hosobuchi, 1980; Loeser, 1972; White and Sweet, 1969). Pain due to deafferentation (anesthesia doulourosa) frequently emerges weeks or months afterwards, presumably due to late central reorganization (Albe-Fessard and Lombard, 1983; Levitt, 1985; Loeser and Ward, 1967; Loeser et al., 1968). Perhaps under as yet undefined circumstances this occurs rapidly in some rats. Variations in animal strain, surgical procedure and environment can have dramatic effects on pain phenotype (Defrin et al., 1996; Devor et al., 1982; Mogil et al., 1999; Raber and Devor, 2002; Shir et al., 1998, 2001; Wiesenfeld and Hallin, 1981; Zeltser et al., 1996).

Whatever the reasons for the immediate induction of allodynia by rhizotomy in the hands of some investigators, the supposed ''persistence'' of this allodynia following rhizotomy cannot be used as evidence to dismiss a role for DRG and neuroma ectopia in the allodynia observed before the rhizotomy (Eschenfelder et al., 2000; Li et al., 2000). Allodynia following rhizotomy in the Chung model may simply reflect the *replacement* of one mechanism of tactile allodynia, related to ectopia, with another mechanism, related to spinal deafferentation. This flawed inference does not confound conclusions under experimental conditions, like ours, in which L5 dorsal rhizotomy alone does *not* trigger tactile allodynia. Nonetheless, to gain added confidence, we carried out the experiments using indwelling catheters. As predicted, silencing of ectopia originating in the L5 segment using topically applied local anesthetic temporarily reversed the allodynia.

4.2. Role of L4 afferents in tactile allodynia in the Chung model

Following L5 or L5+6 spinal nerve transection sensory responsiveness of the hindpaw, including allodynia, is due to innervation by cutaneous L4 afferents. L4+5 rhizotomy or spinal nerve section renders the foot anesthetic (non-responsive even to pinprick) and hence a target for autotomy. The L6 segment contributes to cutaneous innervation of the hindpaw only in rats with a postfixed lumbosacral plexus, and even in these L6 innervation is limited to a dorsolateral part of the foot which was not tested here (Devor et al., 1985; Sheen and Chung, 1993).

In light of the critical role of L4 afferents in hindpaw sensation in the Chung model, one might predict that tactile allodynia is due to the sensitization of cutaneous L4 nociceptor endings in the skin of the partially denervated paw. This hypothesis is untenable, however, both because direct evidence of such sensitization is lacking, and because neither L5 rhizotomy nor application of lidocaine to the L5DRG should have any effect on supposed exaggerated responsiveness of L4 nociceptor endings in the hindpaw.

In could be argued that the anti-allodynic effect of lidocaine applied to the L5 segment is due to spread of the blocking agent to the L4 segment. Given that sensory responsiveness of the hindlimb is totally dependent of conduction in L4 afferents, partial block of L4 might be expected to reduce hypersensibility of the paw. If this had been the case, however, application of an equal dose of lidocaine directly to the L4 segment should have had at least the same anti-allodynic effect as application to L5. In fact, lidocaine applied to L4 had virtually no effect on tactile allodynia.

4.3. Role of L5 ectopia in tactile allodynia in the Chung model

Why, then, was tactile allodynia reversed when lidocaine was applied to the L5DRG? Conduction block of sensitized cutaneous nociceptors in the L5 segment could not be the answer for two reasons. First, L5

afferents had been disconnected from the skin by the original spinal nerve transection. Second, the amount of lidocaine we applied to the L5DRG was titrated to be well below that required to block impulse conduction. If the amount used had been enough to produce conduction block, then application of the same dose to the L4 segment (DRG or nerve) would have caused the foot to become anesthetic and paralyzed, just as occurred with intrathecal injection at L4. In fact, this dose, and even higher doses, did not produce anesthesia or even significantly reduce allodynia.

Rather than blocking impulse propagation in sensitized nociceptors, we propose that lidocaine acts by reducing ectopic discharge originating in the L5DRG. We have shown previously that ectopic firing in neuromas and DRGs is suppressed by much lower concentrations of lidocaine than required for nerve block (Amir et al., 1999; Devor et al., 1992; Matzner and Devor, 1993). The rapid reversal of tactile allodynia with sub-blocking concentrations of lidocaine is consistent with the hypothesis that ectopic firing originating in axotomized L5 afferents is essential for triggering and maintaining spinal central sensitization (Devor and Seltzer, 1999). In the presence of central sensitization, input along intact low threshold cutaneous afferents in the L4 segment give rise to a sensation of pain in response to light tactile stimulation of the hindpaw.

Another conclusion can be drawn from the rapid elimination of allodynia following lidocaine superfusion of the L5DRG, and its rapid return when the effect of the lidocaine fades. Specifically, central sensitization induced by the L5 ectopia tends to dissipate rapidly (within minutes) following elimination of the afferent activity that drives and maintains it, and is restored rapidly when the ectopia resumes. The dynamic nature of central sensitization is consistent with observations of many other authors who have reported that tactile allodynia comes and goes in close association with noxious afferent drive (Gracely et al., 1992; Koltzenburg et al., 1994; Torebjork et al., 1992).

Recent evidence indicates that following L5 spinal nerve section spontaneous activity at very low firing frequencies arises in L4 afferent C-fibers. It has been proposed that this activity, rather than ectopic activity from L5, is responsible for the maintenance of central sensitization and hence tactile allodynia in the Chung model (Ali et al., 1999; Boucher et al., 2000; Wu et al., 2001). This L4 activity is thought to be generated at mid-nerve, within the sciatic nerve trunk, rather than in the L4DRG or at L4 sensory endings in the skin. In our animals superfusion on the L4DRG and spinal nerve with lidocaine is unlikely to have blocked the L4 ectopia, as it did not produce sensory block. Thus, the L4 ectopia hypothesis is not necessarily undermined by the failure of this treatment to eliminate tactile allodynia. On the other hand, had ectopic L4 activity in our animals been sufficient to sustain central sensitization without a contribution from L5, lidocaine applied to the L5 segment would not have suppressed tactile allodynia. We conclude that while spontaneous ectopic activity in adjacent uninjured L4 nociceptive afferents might make a small contribution to central sensitization in the Chung model, it is not sufficient to explain tactile allodynia. Likewise, in animals in which the L5+6 spinal nerve was cut tactile allodynia was reversed by lidocaine superfusion of the L5DRG alone. This indicates that ectopia contributed by axotomized L6DRG neurons, alone and in conjunction with L4 ectopia, is not sufficient to maintain central sensitization and tactile allodynia.

4.4. Can A-fiber activity trigger central sensitization in the Chung model?

Our evidence that ectopic firing originating in axotomized L5 afferents plays a key role in the etiology of neuropathic tactile hypersensibility raises a potential problem. Electrophysiological recordings show that ectopic activity during the early stages of tactile allodynia in the Chung model occurs almost exclusively in afferent A-fibers (Boucher et al., 2000; Han et al., 2000; Liu et al., 2000a,b,c). The primacy of A-afferents is confirmed by the observation that depletion of capsaicin-sensitive C-fibers does not prevent the development of tactile allodynia in the Chung model (Chung, J.M., personal communication; Kinnman and Levine, 1995; Okuse et al., 1997; Ossipov et al., 1999; Shir and Seltzer, 1990). On the other hand, it is widely believed that central sensitization can only be triggered and/or maintained by (substantial levels of) afferent activity in afferent C-fibers (Woolf and Thompson, 1991; Woolf and Wall, 1986). Can this apparent inconsistency be resolved?

In fact, although activity in afferent A-fibers does not normally induce central sensitization, a good deal of independent evidence indicates that cellular changes induced by axotomy can render A-afferents capable of inducing central sensitization. Following axotomy Aβ afferents begin to express substance P (SP), one of the peptides present in intact C-fibers that are thought to mediate C-fiber induced central sensitization (Neumann et al., 1996; Noguchi et al., 1995). A-fiber selective activity induced by spinal nerve section causes release of SP in the spinal cord (Malcangio et al., 2000; Meyer-Tuve et al, 2001). Correspondingly Aβ activity in injured, but not intact, nerve fibers triggers Fos expression in postsynaptic neurons in superficial and deep laminae of the dorsal horn (Day et al., 2001; Molander et al., 1994; Shortland and Molander, 1998; Tokunaga et al., 1999). Normally, activity in C-fibers is required to drive Fos expression. Interestingly, behavioral observations suggest that afferent Aβ activity might enhance central sensitization in the presence of inflammation, even without frank nerve injury (Kim et al., 2001; Ma and Woolf,

1996). Repetitive light brushing on an inflamed paw evokes ''progressive tactile allodynia''. Furthermore, such brushing also works when it is applied outside of the area of direct inflammation, in the area of secondary hyperalgesia. This effect is most likely mediated by SP released in the spinal cord from Aβ afferents (Neumann et al., 1996); even when inflamed, cutaneous C-afferent endings do not readily respond to light brushing.

5. Conclusion

Taken together, the data presented suggest that spinal nerve injury both causes massive spontaneous ectopic firing in axotomized DRG A-neurons, and renders them capable of triggering and maintaining central sensitization due to a neurochemical switch in neuronal phenotype. Tactile allodynia is a consequence of these pathophysiological changes in the axotomized DRG neurons.

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