Evidence That Long-Term Hyperexcitability of the Sensory Neuron Soma Induced by Nerve Injury in Aplysia Is Adaptive

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Gasull, Xavier, Xiaogang Liao, Michael F. Dulin, Cynthia Phelps, and Edgar T. Walters. Evidence that long-term hyperexcitability of the sensory neuron soma induced by nerve injury in Aplysia is adaptive. J Neurophysiol 94: 2218–2230, 2005. Peripheral axotomy induces long-term hyperexcitability (LTH) of centrally located sensory neuron (SN) somata in diverse species. In mammals this LTH can promote spontaneous activity of pain-related SNs, and such activity may contribute to neuropathic pain and hyperalgesia. However, few axotomized SN somata begin to fire spontaneously in any species, and why so many SNs display soma LTH after axotomy remains a mystery. Is soma LTH a side effect of injury with pathological but no adaptive consequences, or was this response selected during evolution for particular functions? A hypothesis for one function of soma LTH in nociceptive SNs in Aplysia californica is proposed: after peripheral injury that produces partial axotomy of some SNs, compensation for sensory deficits and protective sensitization are achieved by facilitating afterdischarge near the soma, which amplifies sensory input from injured peripheral fields. Four predictions of this hypothesis were confirmed in SNs that innervate the tail. First, LTH of SN somata was induced by a relatively natural axotomizing event—a small cut across part of the tail in the absence of anesthesia. Second, soma LTH was selectively expressed in SNs having axons in cut or crushed nerves rather than nearby, uninjured nerves. Third, after several weeks soma LTH began to reverse when functional recovery of the interrupted afferent pathway was shown by reestablishment of a centrally mediated siphon reflex. Fourth, axotomized SNs developed central afterdischarge that amplified sensory discharge coming from the periphery, and the afterdepolarization underlying this afterdischarge was enhanced by previous axotomy.

INTRODUCTION

The somata of many types of neurons display long-term hyperexcitability (LTH) after crush or transection of their peripheral axons, which is often expressed not only as a decrease in spike threshold or rheobase but also as an increase in repetitive firing during prolonged depolarizing pulses (for reviews, see Lai et al. 2004; Sung and Ambron 2004; Titmus and Faber 1990; Walters 1994). Hyperexcitability of mammalian sensory neuron (SN) somata in dorsal root ganglia (DRG) induced by peripheral axotomy can result in spontaneous firing in the DRG, which is thought to be a significant source of neuropathic pain after various forms of nerve injury (e.g., Nordin et al. 1984; Nystrom and Hagbarth 1981; Suzuki and Dickenson 2000; Wall and Devor 1983). An unanswered question is whether axotomy-induced LTH in or near a SN soma is merely a side effect of nerve injury or whether it has (or had in ancestral species) adaptive value that was selected for during evolution. One view is that soma LTH is just one facet of the dedifferentiation of a sensory or motor neuron that readies the neuron for regenerative growth, and this dedifferentiation is triggered by loss of trophic support from target cells and satellite cells distal to the site of axotomy (Gurttu and Smith 1988; Purves 1988; Titmus and Faber 1990; Wu et al. 1993). By this view, soma LTH is incidental to this growth state and, although it has pathological consequences such as the production of neuropathic pain, axotomy-induced soma hyperexcitability itself is not adaptive. Another possibility is that soma LTH is a side effect of altered synthesis of channels destined for regenerating peripheral terminals but incidentally expressed in the soma, with axotomy increasing expression of selected Na+ channels (e.g., Rizzo et al. 1995; Zhang et al. 1997) and decreasing expression of selected K+ channels (Ishikawa et al. 1999; Yang et al. 2004). To our knowledge, the only adaptive function that has been suggested is that axotomy-induced hyperexcitability might enhance Ca2+ influx into the soma, which may then trigger cellular responses that promote axon regeneration (Gordon et al. 1987; Titmus and Faber 1990). However, this hypothesis has not been tested.

On the basis of previous studies of injury-related plasticity in nociceptive SNs of the mollusc, Aplysia californica, we propose that soma LTH induced by peripheral injury functions to amplify subsequent sensory discharge coming from the injured region. Peripheral nerve crush in Aplysia induces long-lasting electrophysiological alterations in the centrally located SN soma that are similar to alterations described in mammalian DRG neurons (Sung and Ambron 2004; Ungless et al. 2002; Walters 1994; Walters et al. 1991) (see DISCUSSION). Interestingly, the axotomy-induced alterations in Aplysia SNs, including various aspects of soma LTH, are also similar to those produced in the same SNs by noxious cutaneous stimulation in the absence of obvious axotomy (Claworthy and Walters 1993; Cleary et al. 1998; Illich and Walters 1997; Lewin and Walters 1999; Scholz and Byrne 1987; Walters 1987b; Walters et al. 1983b). This similarity suggests that axotomy-induced soma LTH may be part of a larger adaptive response of nociceptive SNs to peripheral injury (Billy and Walters 1989; Claworthy and Walters 1993; Walters 1991, 1994; Weragoda et al. 2004).

Many peripheral injuries will 1) transect portions of peripheral sensory arbors, partially disconnecting some sensory neurons from their receptive fields, and 2) completely disconnect others at sites close enough to the receptive fields to permit rapid regeneration (see Fig. 1, A and B). We hypothesize that

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an adaptive response in surviving parts of injured SNs (and perhaps also nearby, uninjured SNs) then occurs to compensate for the partial loss of receptive fields, provide protective sensitization of the damaged region, and increase the sensitivity of regenerating sensory branches during regrowth into receptive fields. In *Aplysia* this involves 1) an increase in the sensitivity of still-functioning or regenerating peripheral portions of damaged SNs (Billy and Walters 1989; Dulin et al. 1995; Weragoda et al. 2004), 2) amplification of peripherally generated discharge when it reaches hyperexcitable central regions of the SNs (Walters 1987a; Walters et al. 1991), and 3) long-term facilitation of SN synapses (Walters 1987a; Walters et al. 1991). The early phase of SN soma LTH (1–2 days) induced by peripheral injury is triggered by activity-dependent signals (Lewin and Walters 1999; Lin et al. 2003; Walters 1987a, b). These signals may be identical to plasticity signals activated during aversive learning paradigms in *Aplysia* (e.g., Cleary et al. 1998; Manseau et al. 1998; Scholz and Byrne 1988, 1987). Later LTH (2 days to 3 wk) may require injuries severe enough to damage nerves and depends on molecular signals conveyed, at least in part, by retrograde transport to the soma from sites of peripheral axotomy (Ambron et al. 1995; Gunstream et al. 1995; Lin et al. 2003; Sung and Ambron 2004; Sung et al. 2001; Walters et al. 1991). Traumatic injuries in this soft-bodied animal are likely to damage nerve branches in deeper tissues and thus to produce both the rapid, activity-dependent and delayed, axotomy-dependent signals for LTH.

In this report we test four predictions of this adaptive hypothesis, focusing on the later, axotomy-induced LTH of the SN soma. First, late SN soma LTH should be induced by relatively natural axotomizing events. Second, late LTH after axotomizing injury should be selectively expressed in axotomized SNs and perhaps also SNs with axons innervating the same fields as the axotomized SNs. Third, this LTH should reverse after functional recovery occurs, when it is no longer needed. Fourth, to have behavioral consequences, central SN LTH after axotomy should amplify sensory discharge coming from the periphery and enhance the afterdepolarization (ADP) that underlies central SN afterdischarge. The logic behind each of these predictions is given with the presentation of the results. Our findings support the hypothesis that LTH expressed in or near the soma of nociceptive SNs after peripheral injury contributes to sensory compensation and sensitization by promoting central afterdischarge that amplifies sensory input from injured peripheral fields.

**Methods**

**General**

*Aplysia californica* (70–250 g) were supplied by Alacrity Marine Biological Services (Redondo Beach, CA) and the National Institutes of Health *Aplysia* Resource Facility (Miami, FL). Animals were housed in aquaria containing artificial seawater (ASW; Instant Ocean, Burlington, NC) at 15–17°C for 1–10 days before surgery. Body weight was maintained on a diet of *Gracilaria* seaweed or dried seaweed laver.

**Axotomy**

Two methods were used to sever SN axons. In one, a small cut was made across part of the tail in the intact animal, producing relatively natural injury and both partial and complete axotomy of SNs known to innervate the ipsilateral tail region (Fig. 1, A and B). The cut, made without anesthesia or antiseptic conditions, went completely through the depth of the tissue across 30–40% of the width of the tail (never reaching the midline) about 1 cm posterior to the end of the ipsilateral parapodium. This cut should sever several branches of nerve p9 in the tail and completely axotomize 5–10 tail SNs with receptive fields along the posterior–lateral aspect of the tail, partially axotomize a similar number of SNs with large receptive fields reaching the midline of the tail or extending from the side of the tail into the posterior parapodial region, and fail to axotomize the remaining pleural SNs (about 10) having receptive fields in the tail region (Billy and Walters 1989; Walters et al. 1983a, 2004). Although the SN soma and major axon locations are not drawn realistically in Fig. 1B, the sizes and shapes of the SN receptive fields relative to the size of the tail are realistic (Billy and Walters 1989; Walters et al. 1983a, 2004).

A second axotomy method was used to produce complete transection of a much larger population of SNs. Most of the pedal nerves on one side of the body were crushed in vivo after anesthetizing the animal by冷却 to 5°C and injecting nearly 30% body volume of cold isotonic MgCl2 into the neck. An incision (about 1 cm) was made just above the pedal ganglia, and a pair of blunt forceps was used to...
crush all the pedal nerves except p1 (so that normal feeding behavior could occur) 1–2 cm from one pedal ganglion. The crush transected all the axons in each nerve while leaving the overlying nerve sheath intact (Steffensen et al. 1995). The injury site was too distant from the SN somata in the pleural ganglion to permit passive, electrotonically conducted effects on the somata (Ungless et al. 2002). The side crushed was alternated across animals. The incision was closed with stitches, and the animal was placed back into its home tank. One to 73 days after nerve crush, animals were injected with isotonic MgCl2 and ASW, which was then exchanged for buffered ASW composed of NaCl, 460 mM; CaCl2, 11 mM; KCl, 10 mM; MgCl2, 55 mM; and Tris buffer, 10 mM (pH 7.6). SNs were impaled with glass microelectrodes (8–25 MΩ) filled with 3 M K-acetate and 6 mM Fast Green dye (Sigma). In some cases, the SN soma was impaled with two beveled glass micro-electrodes, with one used for passing current and the other for measuring membrane potential. Two to ten SNs were sampled from similar locations in each VC cluster (Walters et al. 1983a) and were accepted if they had spike amplitudes >70 mV, resting potentials <−40 mV, and input resistance Rm >10 MΩ. Recordings were made at 19–21°C. Standard intracellular tests (Ungless et al. 2002; Walters et al. 1991) were performed in buffered ASW. Soma spike threshold was measured with an ascending series of 20-ms depolarizing pulses delivered at 2- to 15-s intervals until a spike was generated shortly after the termination of the pulse. Spike amplitude was measured from the resting potential to the maximal overshoot of the action potential. Spike duration was measured from the peak of the spike to the point at which membrane potential had returned to the resting potential. Repetitive firing was quantified by counting the spikes evoked by a 1-s intracellular depolarizing pulse using 2.5 × the threshold current and determined with the 20-ms pulses. In some cases repetitive firing was tested with 2- and 4-nA pulses. Input resistance was determined by measuring the change in potential at the end of a 1-s pulse of negative current (0.2 or 1.0 nA) injected into the soma. Afterdischarge was examined with brief, 2-ms pulses delivered to the SN soma or nerve p9, and was quantified by counting any spikes occurring after the last short-latency spike evoked by the final test pulse. Afterhyperpolarization (AHP) and afterdepolarization (ADP) amplitude were measured as the greatest hyperpolarization and depolarization, respectively, occurring within 2 s after the last spike. In some experiments action potentials in SN axons were evoked by extracellular stimuli to cut ends of nerve p9 delivered 3–4 cm from the pedal ganglion through polyethylene suction electrodes filled with ASW.

Behavioral tests

Recovery of function after nerve crush was monitored with the tail-evoked siphon reflex (Walters and Erickson 1986) (see Fig. 5A). The electrophysiological correlates reported here (Figs. 4 and 5, B and C) were conducted during the same period and often in the same animals used for behavioral tests by Dulin et al. (1995). Some of the behavioral data from that paper are replotted here (Fig. 5B) and referred to (Fig. 5C) to show the reciprocal relationship between reflex responses and SN soma excitability. To provide a better view of the siphon, part of each parapodium was excised (see Fig. 5A) (Carew et al. 1981) 1 day before pedal nerve crush, after anesthetizing the animal by cooling it to about 2°C. Siphon responses were tested in a blind procedure, at 2- to 3-day intervals, with brief, superficial pinch using fine forceps. Behavioral measures were restricted to noting whether an evoked siphon movement occurred.

Statistical analysis

Data, presented as means ± SE, were analyzed using paired or unpaired t-test or by one- or two-way ANOVA followed by Newman–Keuls or Bonferroni post hoc tests, using Prism software (GraphPad, San Diego, CA). Except where indicated, two-tailed tests were used, with statistical significance set at P < 0.05. In some cases (Tables 1 and 2), one-tailed t-tests were used when planned, a priori comparisons were made on the basis of statistically significant findings from pilot studies. Frequencies were compared with Fisher’s exact test.

Results

Axotomizing injury induces late LTH of SN somata

An important prediction of the adaptive sensory compensation/sensitization hypothesis is that LTH of the SN soma should be induced by traumatic axotomy under natural conditions. This prediction has not been tested in mammalian preparations because procedures in use that axotomize defined populations of SNs are invasive and require sterile surgical procedures conducted under anesthesia. We took advantage of the well-defined somatotopic organization of nociceptive SNs in Aplysia (Walters et al. 1983a, 2004) to make a small cut through the body wall in the side of the tail (Fig. 1A) in the absence of anesthesia. This caused both complete and partial axotomy (Fig. 1B) of different SNs within a known sensory population (10–20 cells; see METHODS). Although the small cut caused transient defensive responses, including local withdrawal, inking, and escape locomotion (Walters and Erickson 1986), all cut animals survived and, indeed, all fed normally within 30 min after being injured, indicating that any effects on general motivational state were brief (Walters et al. 1981).

We waited 9–12 days after the tail cut to test tail SN soma excitability to reduce the contribution during testing of activity-dependent, nociceptive sensitization mechanisms that are independent of nerve injury (see DISCUSSION). Repetitive firing (Fig. 1C) was tested with a 1-s depolarizing pulse injected into the soma through the recording electrode, using a normalized current that was 2.5 × the 20-ms spike threshold current. Threshold currents for SNs innervating the cut side of the tail were not significantly different from those of contralateral controls (0.81 ± 0.06 nA vs. 0.89 ± 0.08 nA, respectively, n = 5 animals and 3–6 SNs tested per ganglion in each animal), or from naïve animals tested during the same period (0.86 ± 0.07 nA, n = 8 animals and 4–6 SNs tested per ganglion). However, significantly greater repetitive firing (Fig. 1D) was found in tail SNs ipsilateral to the tail cut than in SNs from naïve, uncut animals (P = 0.015, unpaired t-test) or SNs contralateral to the cut (P = 0.005, paired t-test). The contralateral SNs would not have been axotomized because pleural tail SNs do not send axons to the opposite side of the body (Billy and Walters 1989; Walters et al. 1983a, 2004).

To see whether LTH of SN somata produced by axotomizing tail cut requires either continuing release of neuromodulators during testing or other Ca2+-dependent expression mechanisms, we tested SNs in low-Ca saline (0.02% normal concentration) 8–11 days after the tail cut. This solution also allowed us to stimulate the nerve during testing to confirm that all included data were taken from neurons showing electrophysi-
ological evidence for an axon in the tail nerve (p9). The low-Ca saline produced a general increase in SN soma excitability compared with that seen in ASW, but did not alter the differences in excitability among the axotomized, contralateral control, and naïve SNs (Fig. 2A). Again, there was significantly greater repetitive firing in tail SNs ipsilateral to the tail cut than in contralateral SNs (Fig. 2B) (P = 0.007, paired t-test, n = 8 animals) or SNs from naïve animals (P = 0.007, unpaired t-test, n = 12 animals). Threshold currents for SNs innervating the cut side of the tail (0.72 ± 0.08 nA) were somewhat lower but not significantly different from those of contralateral controls (0.81 ± 0.08 nA) or from naïve animals (0.76 ± 0.05 nA). These data show that a population of SNs likely to have been axotomized by relatively natural trauma display LTH 8–11 days after the injury.

**Soma LTH is specific to SNs with axons in injured nerves**

The fact that late LTH of SNs likely to have been axotomized by unilateral tail cut was restricted to SNs ipsilateral to the injury is consistent with mechanisms that are specific to SNs with injured axons, as was previously suggested in nerve crush studies (Clatworthy and Walters 1994) and studies showing LTH after transection of SN neurites in dissociated cell culture (Ambron et al. 1996; Bedi and Glanzman 2001; Bedi et al. 1998). To investigate further the apparent specificity of cut-induced late LTH to SNs with injured axons, we compared the excitability of SNs likely to have been axotomized (cells with axons in nerve p9) to SNs tested in the same ganglion that would not have been axotomized by the tail cut (cells lacking axons in nerve p9). For each SN we were able to test for the presence of an axon in nerve p9 using electrical stimulation without any effects of the test shocks on soma excitability because all the tests were performed in low-Ca saline, which blocks sensitizing neuromodulatory effects in this preparation (Weragoda et al. 2004). These nerve tests were performed in the experiments just presented (Fig. 2), and the comparisons between SNs in the same clusters with and without apparent axons in nerve p9 (“p9 SNs” vs. “non-p9” SNs) are shown in Fig. 3A. In the low-Ca saline, the excitability of all groups was greater than typically observed in normal saline (compare Fig. 3A to Figs. 1C and 1D). No significant differences in soma excitability were found between p9 SNs and non-p9 SNs contralateral to the cut, or between p9 SNs and non-p9 SNs in naïve, uncut animals. However, in ganglia ipsilateral to the tail cut, the p9 SNs were more excitable than non-p9 SNs (P = 0.10, paired t-test), whereas the soma excitability of non-p9 SNs on the cut side was not significantly different from that of p9 or non-p9 SNs on the contralateral side. As with the data shown in Figs. 1 and 2, the sample of p9 SNs in the tail cut group probably includes some SNs that were not axotomized by the tail cut. These results support the hypothesis that mechanisms triggered directly by SN axotomy contribute to late LTH within injured SNs. Interestingly, we observed that both the p9 SNs and non-p9 SNs on the side contralateral to the tail cut were somewhat more excitable than the corresponding SNs in naïve animals (P < 0.05 in each case, unpaired t-test). This suggests that a tail cut can also induce a weak, widely expressed component of late soma LTH that is not specific to injured SNs.

In a complementary approach we tested for the specificity of late LTH to injured SNs while testing in normal extracellular...
[Ca\(^{2+}\)]. Instead of a tail cut, we crushed pedal nerves under anesthetic conditions. On one side of the animal we crushed nerve p9 alone, whereas on the other side pedal nerves p6, p7, and p8 (which do not innervate the tail) were crushed and p9 was left intact. After 7–12 days, we compared the excitability of p9 SNs (identified presumptively by location in the VC cluster) that had been axotomized by p9 crush on one side to p9 cells on the opposite side that had not been axotomized but would have been exposed to ipsilateral neuromodulatory effects caused by crushing nerves p6, p7, and p8 on that side. SNs that were axotomized by crushing only nerve p9 were significantly more excitable than contralateral SNs that were not axotomized but had been exposed to any modulatory effects of ipsilateral pedal nerve crush (Fig. 3B) (P = 0.009, paired t-test, n = 5 animals). In this study repetitive firing was tested with 1-s, 2-nA pulses (Fig. 3B). Similar differences were found when these cells were tested with 4-nA pulses (not shown; p9 crush, 27.2 ± 4.1 spikes; non-p9 crush, 6.9 ± 1.3 spikes, P = 0.004, paired t-test). The 20-ms thresholds were not significantly different between the p9 SNs on the p9-crushed side and non-p9-crushed side. Naive animals were not tested in this study, although the amount of repetitive firing in SNs that were not axotomized was similar to that evoked by the 2-nA test pulses in SNs of naïve animals in other studies (Gasull and Walters, unpublished observations; see also Fig. 5C and text). These data support the conclusion that axotomy of SNs induces a late soma LTH having a prominent component that is specific to injured SNs.

**Crush-induced LTH decreases after behavioral recovery**

If soma LTH contributes to adaptive sensory compensation and sensitization during the period when a peripheral region has lost sensory innervation because of SN axotomy, the soma LTH might be expected to diminish after recovery of function, when the need for sensory compensation and protection of a wounded region has passed. To test this prediction we examined the time course of SN soma LTH after pedal nerve crush and then compared this time course to the time course of recovery of a reflex initiated by afferents in one of the crushed pedal nerves (see following text). Animals were dissected between 1 and 73 days after nerve crush and SNs in the tail region of the VC cluster were tested in excised ganglia. As previously observed (Walters et al. 1991), axotomized SNs in most animals displayed LTH (Fig. 4A). When we compared the mean repetitive firing response per sensory cluster across all animals tested between 1 and 73 days after nerve crush, the axotomized SNs displayed significantly more repetitive firing than contralateral SNs (12.1 ± 0.8 vs. 5.3 ± 0.5 spikes, P < 0.00001). There was also a significant decrease in the action potential threshold in these cells (Fig. 4B, 0.78 ± 0.04 vs. 1.01 ± 0.05 nA, P < 0.00001, n = 48 animals). In addition, we used within-animal t-test to assess differences in repetitive firing within individual animals. Significant within-animal LTH (comparing axotomized to contralateral SNs; n = 4 to 8 cells per sensory cluster) was not seen 1 day after crush, but was found in 15 of 23 animals tested between 3 and 73 days after crush. No animals were tested 2 days after nerve crush. Interestingly, statistically significant hyperexcitability (assessed with a paired t-test for each animal) was observed in 13 of 14 animals tested between 3 and 30 days after nerve crush, but in only two of nine animals tested 34 to 73 days after crush (P = 0.001, Fisher’s exact test). The latest significant within-animal hyperexcitability was observed 41 days after nerve crush. These results show that significant decay of crush-induced LTH has occurred by the second month after nerve crush.

Is the slow decline in soma LTH associated with gradual recovery of function in the pathways interrupted by nerve crush? To look for correlations between changes in SN excitability and different stages of functional recovery, we compared the time course of soma LTH to the time course of recovery of the tail-evoked siphon reflex. This reflex (Fig. 5A) requires nerve p9 for its initiation (Dulin et al. 1995) and is triggered solely by stimulation of the posterior region of the body innervated by nerve p9 (Walters and Erickson 1986; Walters et al. 1983a, 2004). It is mediated, at least in part, by activation of pleural SNs, which excite pleural interneurons that excite siphon motor neurons in the abdominal ganglion (Cleary and Byrne 1993; Walters et al. 1982). Crushing p9 axotomizes the tail SNs in the VC cluster, which then slowly regenerate toward their peripheral targets (Steffensen et al. 1995). Early phases of recovery might also involve functional reconnection of sprouting proximal axonal segments to segments distal to the transection (Bedi and Glanzman 2001; see also Bittner 1991; Dulin et al. 1995). We replotted all the excitability data from Fig. 4A and superimposed them on reflex recovery data collected at the same time and presented in a different format in an earlier paper (Dulin et al. 1995). After unilateral pedal nerve crush, the tail-evoked siphon reflex was abolished during the 1st wk on the nerve-crushed side of every animal (Fig. 5B, n = 22). By contrast, the reflex was always evoked by the same moderate-intensity test pinches applied to...
the contralateral side of the tail (not shown), or applied to either side of the tail before nerve crush (point C in Fig. 5B) (Dulin et al. 1995). Notice that SNs on the crushed side showed maximal hyperexcitability before reflex recovery was evident, and then displayed a progressive decline in hyperexcitability as the tail-evoked siphon reflex recovered in the 2nd and 3rd wk after nerve crush. The delay of 3–4 days before soma LTH was the observed in the study of Fig. 4 was tested for the presence of the tail-evoked siphon reflex before electrophysiological testing. Repetitive firing of SN soma tested on the crush site (Gunstream et al. 1995; Lin et al. 2003; Sung et al. 2004). A subset of the animals used in the studies presented in Figs. 4 and 5A received both the behavioral tests and the SN excitability tests, permitting a statistical evaluation of the prediction that LTH in SNs from animals showing no recovery of the tail-evoked siphon reflex is greater than LTH in SNs from animals showing at least partial recovery of the reflex. Within this subset, animals in the “No Recovery” group were tested 7, 15, 21, 36, or 41 days after crush, whereas animals in the “Reflex Recovery” group were tested 10, 12, 15, 20, 21, 23, 30, 34, 35, or 45 days after nerve crush. A one-way ANOVA followed by Newman–Keuls multiple comparison tests revealed that both groups of axotomized SNs were significantly more excitable than SNs in naïve, uninjured animals, and that axotomized SNs from animals in the No Recovery group were significantly more excitable than axotomized SNs from animals in the Reflex Recovery group (Fig. 5C). A separate comparison, using paired t-tests, showed that each group of axotomized SNs was significantly more excitable than the contralateral neurons in the same animals. In addition, an ANOVA on SNs in the two contralateral control groups and the naïve, uninjured control group revealed no differences in excitability among these control groups, suggesting that the parapodial excision and repeated behavioral testing (which were not performed on the naïve controls having intact pedal nerves) did not produce long-term effects on excitability of the tested SN somata. Taken together, these results suggest that functional reconnection of injured sensory axons to peripheral targets reduces LTH.

Previous studies have shown that nerve crush in Aplysia significantly increases spike duration and decreases spike afterhyperpolarization (AHP) of SNs, as well as increasing repetitive firing and decreasing spike threshold (Clatworthy and Walters 1994; Gunstream et al. 1995; Walters et al. 1991). However, significant effects on additional electrophysiological properties of the SN soma were not seen. This pattern was somewhat different from that found when LTH was induced by induction of an inflammatory-like, foreign-body response around selected pedal nerves, which in addition to producing the above effects also caused significant increases in spike amplitude and input resistance (Clatworthy et al. 1994), or when LTH was produced by dissociation, which also increased spike amplitude (Sung et al. 2004). We wondered whether these additional effects may have been missed after nerve crush because they had reversed substantially during recovery of function because these earlier studies had not distinguished animals showing reflex recovery from animals that had not. To test this idea we compared seven different electrophysiological properties of SN soma in animals that showed at least partial recovery (the Reflex Recovery group, Table 1) to animals that showed no recovery of the tail-evoked siphon reflex (the No Recovery group, Table 2). These data came from the animals whose repetitive firing is plotted in Fig. 4, but in addition included animals tested later than 30 days after crush. As previously found after nerve crush (Clatworthy and Walters 1994; Gunstream et al. 1995; Walters et al. 1991), axotomized SNs in both groups showed significant alterations in repetitive firing, spike threshold, and spike duration, and these were the only properties altered in the Reflex Recovery group (Table 1). In contrast, the No Recovery group (Table 2) also displayed...
TABLE 1. Electrophysiological properties of sensory neurons in animals that showed recovery of the tail-evoked siphon reflex after nerve crush

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>Axotomized</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive firing, spikes</td>
<td>4.6 ± 0.4</td>
<td>8.3 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spike threshold, nA</td>
<td>0.87 ± 0.07</td>
<td>0.72 ± 0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>86.5 ± 1.8</td>
<td>85.6 ± 1.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spike duration, ms</td>
<td>3.6 ± 0.3</td>
<td>4.8 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>4.4 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>40.7 ± 4.4</td>
<td>42.3 ± 4.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>45.1 ± 0.9</td>
<td>44.3 ± 0.7</td>
<td>n.s.</td>
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</table>

Data are from 14 animals tested 10–73 days after nerve crush, and are expressed as means ± SE. Values of P were determined with one-tailed t-tests with n = 14 pairs of ganglia (2–8 SNs sampled per ganglion). One-tailed tests were used because the direction of each effect was predicted by previous studies. Repetitive firing was tested with a 1-s depolarizing pulse using 2.5× the current required to reach action potential threshold with a 20-ms pulse. Spike amplitude, duration, and AHP were measured with single-action potentials evoked by the 20-ms pulse. Input resistance was tested with a 1-s, 1-nA hyperpolarizing pulse. AHP, spike after hyperpolarization.

significant alterations of spike amplitude, AHP, and input resistance. These additional effects were found even though the smaller n in this group should have decreased the likelihood of detecting significant effects. Neither group showed any effect on resting potential. These results indicate that nerve crush induces long-term changes in many electrophysiological properties of the soma, and most or all of these alterations are at least partially reversible after behavioral recovery.

Peripheral generated sensory discharge is amplified by enhancement of afterdischarge and afterdepolarization generated near the soma during LTH

If LTH of SN somata induced by axotomizing injury contributes to adaptive sensory compensation and sensitization, this LTH must somehow amplify incoming bursts of action potentials. One previous study found that pedal nerve crush produced a long-term increase in the probability of generating afterdischarge following spikes elicited by brief test stimuli to the soma (Walters et al. 1991), but did not examine possible increases in central afterdischarge evoked by activity arriving from the periphery. Noxious tail shock also can produce a long-term increase in afterdischarge evoked by soma tests (Walters 1987a), but this tail shock did not cause axotomizing injury. Moreover, it is not known whether long-term enhancement of SN afterdischarge, like the immediate generation of SN afterdischarge following intense afferent activity (Clatworthy and Walters 1993), is associated with enhancement of the spike afterdepolarization (ADP) measured in the soma. To answer these questions, we crushed pedal nerves on one side in vivo and excised the ganglia 4–10 days later to examine SN afterpotentials and afterdischarge on each side. Test stimuli were single 2-ms pulses or brief trains of the same pulses delivered either directly to the soma or to nerve p9, ≥1 cm proximal to the crush site but still 2–3 cm away from the pedal ganglion. The nerve test stimuli were weaker than those used previously to produce immediate afterdischarge in naive, uninjured animals (Clatworthy and Walters 1993).

Representative responses are illustrated in Fig. 6, which shows the afterdischarge, ADP, and AHP evoked by each type of stimulus. Looking first at afterdischarge (Fig. 7A1), two-way ANOVA revealed significant effects of nerve crush, the type of test, and their interaction (P < 0.001 in each case). Multiple comparisons with Bonferroni post hoc tests showed that axotomized SNs exhibited more afterdischarge than did contralateral SNs when tested with the train of five pulses delivered to nerve p9 (P < 0.001) but not with the other test stimuli. In 10 of 11 animals at least one SN sampled on the nerve-crushed side displayed afterdischarge (16 of 18 SNs sampled), whereas in only three of 11 animals did control SNs display afterdischarge (three of 16 SNs sampled). Afterpotentials also were altered by nerve crush. Two-way ANOVA on ADP amplitude revealed significant overall effects of nerve crush, the type of test, and their interaction (P < 0.001 in each case). Multiple comparisons showed that axotomized SNs exhibited larger ADPs than did contralateral SNs when tested with each of the four test stimuli (Fig. 7A2, P < 0.05 to P < 0.001; see figure). Nearly identical results were found when the area under the curve of the ADP rather than its maximum amplitude was analyzed (not shown). Finally, examination of AHP amplitude revealed significant overall effects of nerve crush and the type of test (P < 0.001 in each case), but not their interaction. Multiple comparisons showed that axotomized SNs exhibited smaller AHPs than did contralateral SNs when tested with each

FIG. 6. Examples of afterpotentials observed in SN somata after discharge evoked by electrical test stimuli applied to either the soma or nerve p9. Each column of responses is from a single SN on the side contralateral to the nerve crush (A) or the nerve-crushed side (B). Soma and nerve test pulses were 2 ms long. ADP, afterdepolarization; AHP, afterhyperpolarization.

TABLE 2. Electrophysiological properties of sensory neurons in animals that displayed no reflex recovery after nerve crush

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>Axotomized</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive firing, spikes</td>
<td>4.7 ± 0.4</td>
<td>14.5 ± 1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spike threshold, nA</td>
<td>0.88 ± 0.08</td>
<td>0.61 ± 0.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>89.0 ± 1.9</td>
<td>93.1 ± 2.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Spike duration, ms</td>
<td>2.7 ± 0.2</td>
<td>4.7 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>AHP, mV</td>
<td>5.9 ± 0.4</td>
<td>4.4 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>39.4 ± 3.3</td>
<td>47.1 ± 2.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>44.6 ± 1.6</td>
<td>44.1 ± 1.2</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Data are from 9 animals tested 4–41 days after nerve crush, and are expressed as means ± SE. Values of P were determined with one-tailed t-tests with n = 9 pairs of ganglia (2–4 SNs sampled per ganglion).
of the test stimuli, except for the single pulse to the nerve (Fig. 7A3, \( P < 0.05 \) to \( P < 0.01 \); see figure). These data show that when afferent discharge is evoked by peripheral nerve stimulation, prior axotomy causes significant enhancement of afterdischarge and the ADP, as well as significant depression of the AHP, as monitored in the SN soma.

Central afterdischarge and ADP evoked immediately by intense peripheral stimulation in uninjured animals requires Ca\(^{2+}\) influx into central neurons (Clatworthy and Walters 1993). We asked whether elimination of significant Ca\(^{2+}\) influx would also block the enhancement of afterdischarge and ADP produced by prior nerve crush. Afterdischarge and the ADP, but not the AHP, were largely eliminated by conducting the soma and nerve tests in low-Ca saline (0.02% normal concentration) (Fig. 7B). Two-way ANOVA revealed no significant overall effects on afterdischarge or ADP during any of the four tests in low-Ca saline. However, similar analysis of the AHP results showed significant overall effects in low-Ca saline. Significant effects of axotomy were still observed on the AHP, but not on afterdischarge or the ADP.

The dependency of the ADP and afterdischarge on extracellular Ca\(^{2+}\) might reflect a role for Ca\(^{2+}\)-dependent exocytosis of neuromodulators onto central regions of the SN during afferent activity (Clatworthy and Walters 1993). Although some synapses are made directly onto SN cell bodies in Aplysia (Zhang et al. 1991), most synapses in molluscan nervous systems occur in the neuropil, below the cell body layer (Chase 2002). If the ADP responsible for afterdischarge is generated in the neuropil, or depends on neuromodulators released in the neuropil, then excising the SN somata above the level of the neuropil should greatly reduce the ADP and afterdischarge. In testing this prediction, we first confirmed our previous finding (Ungless et al. 2002) that LTH of the soma is observed in the excised sensory cluster 5–7 days after pedal nerve crush, and found similar effects of axotomy on repetitive firing evoked by 1-s, 2-nA pulses in SNs in excised clusters (crushed vs. control, 13.5 ± 1.7 vs. 2.7 ± 0.6 spikes; \( P < 0.0001, n = 15 \) and 11, respectively) and in ganglia (14.1 ± 1.2 vs. 3.7 ± 0.9 spikes, \( P < 0.0001, n = 25 \) and 10, respectively). Like axotomized SNs tested in ganglia preparations (see Tables 1 and 2), previously axotomized SNs (\( n = 15 \)) tested in excised clusters also exhibited significantly lower soma spike thresholds (\( P < 0.05 \)), smaller AHPs (\( P < 0.01 \)), and longer spike durations (\( P < 0.05 \)) than contralateral SNs (\( n = 11 \)). No significant differences were found in input resistance tested in excised clusters and in ganglia. In preliminary experiments we found no evidence of ADPs after single spikes or five-pulse trains evoked by test pulses to excised somata. Even after increasing the train length to ten pulses to increase the chances of generating ADPs during soma tests, the ADPs were quite...
small. No significant differences in ADP amplitude were found in excised SNs that had previously been axotomized compared with contralateral control SNs (0.9 ± 0.2 vs. 0.7 ± 0.2 mV, n = 15 and 11, respectively). No afterdischarge was evoked in any of the excised neurons.

Taken together, these data suggest that most of the enhancement of the central ADP observed after SN axotomy depends on mechanisms or factors outside of the soma, yet close enough to cause rapid, measurable effects on soma afterpotentials during afferent activity.

**DISCUSSION**

This paper considers an important physiological question that has not been addressed explicitly in any system: Can injury-induced LTH expressed in SN somata be adaptive? Although some of our findings have mechanistic implications, an analysis of the mechanisms of LTH and afterdischarge in *Aplysia* SNs will be presented elsewhere.

**Questions about the functional and pathological significance of SN soma LTH**

Dramatic and prolonged LTH of the soma after peripheral axotomy has been described in many types of neurons in diverse species (for reviews, see Lai et al. 2004; Sung and Ambron 2004; Titmus and Faber 1990; Walters 1994). Pain physiologists have been particularly interested in axotomy-induced LTH of somatic SNs because soma LTH seems likely to contribute to neuropathic pain, most straightforwardly by promoting spontaneous activity in what would otherwise be silent or near-silent nociceptive afferents (Nordin et al. 1984; Nystrom and Hagbarth 1981; Suzuki and Dickenson 2000). However, the contributions to neuropathic pain of LTH in a SN soma are not straightforward. For example, after nerve injury far more DRG neurons exhibit soma LTH than develop spontaneous activity in what would otherwise be silent or near-silent nociceptive afferents (Abdulla and Smith 2001; Gallego et al. 1987; Gurtu and Smith 1988; Kim et al. 1998; Ma et al. 2003; Song et al. 2003; Stebbing et al. 1999; Study and Kral 1996; Zhang et al. 1997), many DRG neurons that develop spontaneous activity after nerve injury are not nociceptive (e.g., Ma et al. 2003; Michaelis et al. 2000), and ectopic spike generators at sites of axonal injury probably contribute more to spontaneous activity after nerve injury than do ectopic spike generators in or near the soma (Gorodetskaya et al. 2003). Thus the significance of axotomy-induced LTH of the SN soma for neuropathic pain is still poorly understood and the possibility that such LTH has natural functions (as opposed to neuropathic consequences) in at least some species has not been explored. Evidence for specific adaptive functions of central LTH of SNs could point to selection pressures that contributed to the early evolution of these mechanisms, and perhaps provide insight into why axotomy-induced hyperexcitability mechanisms exist in the centrally located SN soma of so many species, including humans.

**An adaptive sensory compensation/sensitization hypothesis for SN soma LTH**

One potential explanation for axotomy-induced LTH in central SN somata is that positive selection pressures shaped the evolution of this response in species that were ancestral to those expressing it today. In some contemporary animals this LTH might be expressed in the absence of continued selection pressures, but in other species the selection pressures might still operate and be identifiable. Several lines of evidence suggest that injury-related selection pressures have had major influences on the evolution of mechanisms of long-term neural plasticity (Cohen-Armon et al. 2004; Walters 1987b, 1991, 1994; Walters et al. 1991; Weragoda et al. 2004). Because much of this evidence has come from SN plasticity in *Aplysia*, we were curious to see whether evidence could be found that would implicate the continued operation of these pressures on axotomy-induced LTH of *Aplysia* SN somata; i.e., that this form of LTH is adaptive in this species.

Specifically, we have looked for evidence to support the hypothesis that axotomy-induced LTH of *Aplysia* somata helps to compensate for partial loss of receptive fields (Fig. 1B) and to provide protective sensitization of a damaged peripheral region by amplifying peripherally generated discharge when it reaches hyperexcitable central components of SNs that innervate the injured region. Although we are not in a position to measure adaptiveness directly (e.g., by looking for effects on organismic survival or reproductive success), we have tested several logical predictions of this model and in each case found evidence consistent with the hypothesis that axotomy-induced LTH of SN somata in *Aplysia* is an adaptive response to peripheral injury rather than a nonadaptive or maladaptive consequence of axotomy.

**SN soma LTH is induced by a natural axotomizing event**

The prediction that LTH of central SN somata can be induced by natural axotomy has not been tested before because deliberate axotomizing procedures in experimental use have resulted in minimal or nonreproductive injury. Furthermore, a natural injury that would axotomize most SNs in the L4 or L5 DRGs (which are commonly used in mammalian nerve injury models), or that would axotomize all the SNs in a pleural ganglion in *Aplysia*, would be so deep or extensive that the animal would be likely to die from blood loss or infection before any adaptive benefit could be realized. If axotomy-induced central SN LTH is adaptive, it should be induced by axotomizing events that are survivable and that occur in the absence of anesthesia. The well-defined somatotopic organization of nociceptive SNs in *Aplysia* allowed us to make a small cut in the body wall at a site that ensured at least partial axotomy of a small population of approximately 20 known SNs (Walters et al. 1983b, 2004). This unilateral tail cut in the absence of anesthesia produced tissue damage and brief defensive behavior similar to that produced by lacerations during attacks on young *Aplysia* by crabs (*Callinectes sapidus*) in the laboratory (Walters, unpublished observations; Walters et al. 1993).

The observations that all the animals 1) survived the tail cut and 2) fed normally within 30 min after injury suggest that the injury was not severe and (in contrast to the effects of repeated electrical shock used in many conditioning paradigms; see Walters et al. 1981) that the effects on general motivational state were brief. Nevertheless, the tail cut produced selective, very long lasting hyperexcitability of SN somata in the p9 region of the VC cluster, expressed as enhancement of repetitive firing evoked by a test pulse to the soma (Fig. 1). We do...
not think this LTH was caused by intense activation of nociceptors during the tail cut because unpublished observations in our laboratory, as well as other studies (Cleary et al. 1998; Walters 1987a,b) indicate that brief, superficial noxious stimuli, even when very intense, produce central SN alterations lasting only a few days, whereas the tests in the present study were delivered 9–12 days after tail cut. Instead the LTH was probably caused by effects of deeper injury: axotomy (Ambron et al. 1996; Bedi et al. 1998; Walters et al. 1991) and/or inflammatory-like responses around axons at the site of injury (see Clatworthy et al. 1994; Farr et al. 1999, 2001). The difference in LTH between SNs on the cut and uncut sides was not as dramatic as is often observed after nerve crush (compare Fig. 1D to Figs. 3B, 4A, and 5C). This can be explained by the location of the tail cut, which would have spared axons in nerve p9 that travel rostral or medial to the cut site (schematized in Fig. 1B), and these nonaxotomized SNs (which do not exhibit late LTH; see Fig. 3) would have been included in our sampling of SNs in the p9 region of the VC cluster. In contrast, our crushing procedure axotomizes all of the axons in each crushed nerve (Steffensen et al. 1995). Interestingly, the tail-cut–induced LTH, like LTH induced by axotomizing nerve crush (Ungless et al. 2002), was observed under test conditions in which release of extrinsic neuromodulators was blocked (Fig. 2), indicating that this LTH is intrinsic to SN membrane in or near the soma. These results strongly suggest that natural axotomy, or events closely associated with axotomy such as inflammatory-like reactions at a site of nerve injury, induce central SN LTH.

SN soma LTH is specific to SNs innervating an injured region

To minimize interference from long-lasting nociceptive sensitization on vital activities of an animal such as feeding and reproduction, injury-activated sensitization mechanisms should be targeted selectively to wounded regions that need sensory compensation and extra protection (Walters 1994). Partial evidence for such specificity in SN soma LTH came from a previous study showing that ipsilateral crush of selected pedal nerves does not produce LTH of ipsilateral cerebral SNs that lack axons in pedal nerves, that ipsilateral cerebral nerve crush does not produce LTH of ipsilateral pleural SNs that lack axons in cerebral nerves, and that crush of the nearby pleural–abdominal connective, which lacks axons of pleural SNs, fail to induce LTH in pleural SNs (Clatworthy and Walters 1994; Walters et al. 1991). Furthermore, axotomy by itself may be sufficient to induce SN soma LTH because it is also produced by transection of SN neurites in dissociated cell culture (Ambron et al. 1996; Bedi and Glanzman 2001; Bedi et al. 1998). In the present study we applied a more stringent test of nerve specificity, asking whether axotomy-induced LTH generalizes to SN somata within the same pleural sensory cluster that have axons in ipsilateral pedal nerves other than the one undergoing axotomy. Both tail cut (Fig. 3A) and selective crush of nerve p9 (Fig. 3B) produced significant LTH of SN somata with axons in nerve p9. No LTH was found in somata of nonaxotomized SNs when neighboring SN somata with axons in other pedal nerves were axotomized (Fig. 3B). In addition, a very weak, general component of LTH was suggested by the slightly greater excitability of SNs contralateral to the tail cut than of SNs from naïve animals that was sometimes seen (Fig. 2B; but also see Fig. 1D). Nevertheless, the much greater hyperexcitability of SNs on the tail-cut side shows that axotomizing injury produces central SN LTH that is highly specific to either axotomized SNs or SNs that have receptive fields within the region innervated by SNs that had been axotomized (see also Ma et al. 2003). In either case, SN LTH is selectively targeted to SNs that innervate an injured region.

SN soma LTH reverses slowly during recovery of function

Because nociceptive sensitization can interfere with normal behavior (Walters 1994), it should be adaptive to turn off long-term sensitizing mechanisms, such as central SN LTH, once recovery of function and/or regeneration have occurred. We found that crush-induced enhancement of repetitive firing peaked nearly 1 wk after pedal nerve crush and then slowly declined over the next few weeks (Fig. 4). Significant within-animal LTH was found as late as 41 days after the crush. However, a previous study had shown large variation in the time before any recovery of function could be detected in the central reflex (tail-evoked siphon withdrawal) used to monitor functional reconnection of nerve p9 to the tail after p9 crush (Dulin et al. 1995). We found the degree of LTH to be inversely related to the incidence of animals exhibiting the tail-evoked siphon reflex during the month after p9 crush (Fig. 5B), and SNs sampled in animals showing some behavioral recovery showed significantly less LTH than those sampled in animals showing no recovery (Fig. 5C). Furthermore, when nerve-crushed animals showing reflex recovery were excluded, LTH was expressed not only as enhanced repetitive firing, decreased spike threshold, and increased spike duration, but also as increased spike amplitude, decreased AHP, and increased input resistance (Tables 1 and 2). Interestingly, increased spike amplitude also develops in isolated pleural SNs 2 days after dissociation (Sung et al. 2004), a procedure that also involves axotomy and a lack of functional reconnection to peripheral targets. The gradual reversal of various manifestations of central LTH (see also Bedi and Glanzman 2001) during reflex recovery supports the adaptive prediction that central LTH is eventually turned off when it is no longer useful.

Central SN LTH amplifies afferent discharge by increasing the ADP above spike threshold

The sensory compensation/sensitization hypothesis for linking central SN LTH to adaptive behavioral consequences predicts that surviving portions of partially axotomized SN receptive fields (Fig. 1B) can still respond to peripheral stimuli and will send sensory discharge to the central soma where central LTH amplifies the peripheral sensory discharge. Thus we predicted that modest sensory discharge evoked by peripheral nerve stimulation (for reliable experimental control) would be amplified by afterdischarge in or near the soma long after peripheral axotomy, as had already been found to occur shortly after strong noxious stimulation of the body surface or a peripheral nerve (Clatworthy and Walters 1993; Walters et al. 1983b). Using nerve crush to produce peripheral axon injury without the complicating effects of peripheral receptive field sensitization (Billy and Walters 1989; Walters 1987a), we...
found that a moderate intensity nerve test stimulus, which failed to evoke afterdischarge in control SNs, evoked afterdischarge in nearly all of the sampled SNs having axons in crushed nerves (Fig. 7A). This afterdischarge was unlikely to have been generated at the site of nerve crush, where local hyperexcitability extends for <2 mm (Wерагода et al. 2004), because the test site was distant (>1 cm) from the crush site. Additional evidence for a central locus for generation of the afterdischarge was its correlation with ADP amplitude. No significant afterdischarge was found in axotomized (or control) SNs in response to the 2-ms soma pulses, in contrast to a previous study in which 33% of the axotomized SNs displayed afterdischarge to a 20-ms soma pulse (Walters et al. 1991). Thus whereas the present study showed that a brief, simultaneous burst ofafferent activity in a set of previously axotomized SNs is able to evoke afterdischarge in the activated SNs, the earlier observations suggest that under some conditions this central sensitizing effect can be potent enough for a single spike in a single pleural SN to evoke central afterdischarge in that cell.

If ADP amplitude increases above spike threshold, afterdischarge should be produced. We found a close relationship between the probability of generating afterdischarge and the amplitude of the ADP (Fig. 7A) (see also Clatworthy and Walters 1993; Walters et al. 1983b) and, most important, prior axotomy increased ADP amplitude (Fig. 7A2). Our observation that low-Ca saline nearly eliminated both the ADP and afterdischarge while causing only a small reduction in the AHP (Fig. 7) supports a mechanistic link between afterdischarge and the ADP (see also Clatworthy and Walters 1993). Although at least part of the mechanisms necessary for generating enhanced ADPs after axotomy may be located outside of the soma, the site of ADP generation is close enough to cause marked depolarization of the soma. Proximity to the soma is also suggested by the finding that artificial hyperpolarization of the soma during intense nerve stimulation in naïve animals blocks afterdischarge and reveals a large underlying ADP (Clatworthy and Walters 1993). This proximity suggests that LTH mechanisms monitored in the soma are expressed close enough to the site of ADP generation to promote afterdischarge after peripheral injury. LTH mechanisms involved in decreasing spike threshold and increasing input resistance should enhance the likelihood of generating afterdischarge, whereas mechanisms involved in increasing repetitive firing and decreasing the AHP should enhance the number of spikes generated (Table 2).

The ADP evoked by soma stimulation in axotomized SNs was dramatically reduced when the test stimulus was delivered to excised somata largely separated from underlying neuropil, even though the same excised SN somata showed effects of prior axotomy on repetitive firing, spike threshold, spike duration, and AHP. The latter effects also occur after axotomy induced by cluster excision or soma dissociation, but neither the presence nor the enhancement of an ADP has been reported when SN somata are excised or dissociated (Ambron et al. 1996; Bedi et al. 1998; Gunstream et al. 1995; Sung et al. 2004). This suggests that a factor critical for generating the ADP exists in the neuropil, close to the soma. Given the dependency of the ADP and afterdischarge on extracellular Ca\(^{2+}\), an intriguing possibility is that generation of the ADP requires Ca\(^{2+}\)-dependent release of a sensitizing neuromodulator onto SN branches within neuropil near the soma during nociceptive discharge. Of course, other Ca\(^{2+}\)-dependent mechanisms may also be involved in ADP generation (e.g., Martinez-Pinna et al. 2000).

Our results suggest that LTH expressed in or near the SN soma after peripheral axotomy contributes to sensory compensation and sensitization by facilitating afterdischarge near the soma, which functions to amplify sensory input from injured receptive fields. Although soma LTH may have additional functions, our findings argue against the possibility that axotomy-induced soma LTH is merely a side effect of injury that has no adaptive significance. They also add to growing evidence for contributions of long-lasting alterations of neuronal excitability to memory-like functions in diverse systems (Dаoudal and Dебанне 2003; Wаlters 1994; Zhang and Linden 2003). Gastropod molluscs provide excellent opportunities to address evolutionary questions about mechanisms of neuronal plasticity (e.g., Erixon et al. 1999; Marinesco et al. 2003). Comparisons of SN soma LTH across a variety of animal groups should provide insight into the uses, evolution, and mechanisms of this widespread form of neural plasticity.

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