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Enhanced excitability of dissociated primary sensory neurons after chronic compression of the dorsal root ganglion in the rat

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Abstract

A chronic compression of the dorsal root ganglion (CCD) produces ipsilateral cutaneous hyperalgesia and allodynia in rats. Intracellular electrophysiological recordings from formerly compressed neurons in the intact dorsal root ganglion (DRG) reveal lower than normal current thresholds (CTs) and abnormal spontaneous activity (SA) (Zhang JM, Song XJ, LaMotte RH. Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. J Neurophysiol 1999;82:3359–66). To determine if the neuronal hyperexcitability is intrinsic to the soma, L4 and L5 DRG neurons from rats that had prior CCD surgery or those that did not (controls) were dissociated, and intracellular recordings obtained 3–8 h (acute) or 24–30 h (1d) after culture. The CTs of large- (>45 μm diameter) and medium- ($30\sim45$ μm) sized neurons from control rats after acute or 1d culture were similar to those formerly recorded from the intact DRG and significantly lower for CCD than for control rats. However, the CTs of small- (≤30 μm) sized neurons from control rats were significantly lower in acute or 1d culture groups than they were in the intact DRG and not significantly different from those of dissociated small neurons from CCD rats. The overall incidence of SA was higher for CCD than for control neurons after 1d culture (10.3 vs. 1.8%) and similar to that obtained in the intact DRG. We conclude that the CCD-induced hyperexcitability of medium- and large-sized neurons remains after dissociation and is intrinsic to the soma. For small-sized neurons, the effects of CCD observed in the intact DRG are less apparent after dissociation possibly due to the hyperexcitability produced by the dissociation process itself.
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Keywords: Neuropathic pain; Dorsal root ganglion; Spontaneous activity; Hyperexcitability; Cell culture

1. Introduction

A chronic compression of the L4 and L5 dorsal root ganglia (CCD) produces cutaneous hyperalgesia and tactile allodynia on the ipsilateral foot in the rat (Hu and Xing, 1998; Song et al., 1999). The cell bodies (somata) of the dorsal root ganglia (DRGs) exhibit signs of hyperexcitability such as a lower current threshold (CT, or rheobase), a loss of accommodation and an increased incidence of abnormal spontaneous activity (SA) (Hu and Xing, 1998; Song et al., 1999; Zhang et al., 1999). Alterations in the intrinsic membrane properties of DRG somata might contribute to the cutaneous hyperalgesia and allodynia after CCD. Further studies of the underlying ionic mechanisms of the increased somal excitability after CCD would require the dissociation and culturing of DRG neurons (e.g. Yao et al., 2003). However, once removed from the animal and dissociated, the neurons are not only axotomized but also lack the close proximity to each other and to satellite glia and other non-neuronal cells that might affect neuronal excitability in the intact ganglion. The purpose of the present study was to determine whether the increased excitability of the DRG neurons (lower CT and more SA) produced by a CCD remains after dissociation and culture, i.e. is an intrinsic property of the soma. We investigated the electrophysiological properties of DRG neurons after dissociation and culture, and compared these properties for CCD and control (normal) animals. To compare the results from dissociated DRG neurons with

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those from intact DRG neurons under similar recording conditions, we used sharp-electrode intracellular recording in warmed bath solution. Some preliminary results of the present study have been published in abstract form (Ma et al., 2002).

2. Methods

2.1. Surgical procedure for rod implantation

Twenty adult female Sprague–Dawley rats weighing 150–180 g were housed in groups of three or four in a climate-controlled room under a 12 h light/dark cycle. The use and handling of animals were approved by the Institutional Animal Care and Use Committee of the Yale University School of Medicine and were in accordance with guidelines provided by the National Institutes of Health and the International Association for the Study of Pain.

In CCD rats ($n=11$), under pentobarbital sodium anesthesia (50 mg/kg i.p.) and using aseptic precautions, the transverse process and intervertebral foramina of L4 and L5 were exposed unilaterally as previously described (Song et al., 1999). A stainless steel L shaped rod (0.63 mm in diameter and 4 mm in length) was inserted into each foramen, one at L4 and the other at the L5 ganglion. The incision was closed in layers. Nine unoperated rats were used as controls.

2.2. Neuronal dissociation and culture

Five to 7 days after surgery, the rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and the L4 and L5 DRGs were exposed. In CCD rats, the correct placement of each implanted rod was confirmed (Yao et al., 2003). All the rods were in the right position (Song et al., 1999) so that all 22 DRGs from CCD animals were accepted for the experiments. The cell dissociation and culture procedures were as previously described (Caffery et al., 1992; Honmou et al., 1994; Yao et al., 2003). Briefly, DRGs were removed from control or CCD rats and placed in complete saline solution (CSS) for cleaning and mincing. The CSS contained (mM): NaCl 137, KCl 5.3, $MgCl_2$ 1, $CaCl_2$ 3, Sorbitol 25, and Hepes 10, adjusted to pH 7.2 with NaOH. The DRGs were then digested for 20 min with collagenase A (1 mg/ml; Boehringer Mannheim, Gmbh, Germany) and, for another 20 min, with collagenase D (1 mg/ml; Boehringer Mannheim, Gmbh, Germany) and papain (30 units/ml, Worthington Biochemical, Lakewood, NJ) in CSS containing 0.5 mM EDTA and 2 μg cysteine at 37 °C. The cells were dissociated by trituration in culture medium containing 1 mg/ml bovine serum albumin and 1 mg/ml trypsin inhibitor (Boehringer Mannheim, Gmbh, Germany) and plated on glass coverslips coated with 0.1 mg/ml polyornithine and 1 mg/ml laminin (Boehringer Mannheim, Gmbh, Germany). The culture medium contained equal amounts of Dulbecco's Modified Eagle Medium and F12 (Gibco, Grand Island, NY) with 10% FCS (HyClone Laboratories, Logan, UT) and 1% Penicillin (100 U/ml)/ Streptomycin (0.1 mg/ml) (Life Technologies, Rockville, MD). The cells were incubated at 37 °C (5% CO_2 balanced air) for 1 h after which culture medium without the trypsin inhibitor was added. All chemicals were from Sigma–Aldrich (St Louis, MO) unless otherwise stated.

2.3. Sharp-electrode intracellular recording

Intracellular recordings were obtained 3∼8 h (acute) or 24∼30 h (1d or 1 day) after the dissociation. Coverslips were transferred to a recording chamber that was mounted on the stage of an upright microscope (BX50-WI, Olympus Optical, Tokyo, Japan). The chamber was perfused with a bath solution containing (mM): NaCl 130, KCl 3, $CaCl_2$ 1, $MgCl_2$ 1, Hepes 10, Glucose 10, adjusted to pH 7.4 and osmolarity 300 mosM. The solution was pre-heated to 36 °C by means of an in-line heater with a controller (TC-344A, Warner Instruments Inc., Hamden, CT). The bottom of the recording chamber was made of aluminum and was electrically warmed to assist in maintaining the bath temperature in the recording chamber at 36 ± 0.5 °C. The intracellular recording electrodes were fabricated from borosilicate glass (World Precision Instruments, Sarasota, FL) and pulled on a Flaming/Brown micropipette puller (P-97, Sutter Instrument, Novato, CA). Electrodes were filled with 1.0 M KCl (impedance: 40∼80 MΩ) and positioned by a micromanipulator (MIS-5000, Burleigh Instruments, Inc., Fisher, NY). Prior to electrode insertion, the size of a soma to be studied was classified according to its mean diameter (i.e. average of the longest and shortest diameters) as small (≤30 μm), medium (31∼45 μm) or large (>45 μm) (Ma et al., 2003). We have found that for intact DRG neurons, a classification of neurons by the diameters of their cell bodies (somata) produced virtually the same groups of neurons as a classification by their axonal conduction velocities. In addition, CCD does not change the validity of the classification. First, most neurons with large- and medium-sized somata had A-fiber conduction velocities, whereas most neurons with small-sized somata had C-fiber conduction velocities (Ma et al., 2003; Zhang et al., 1999). Second, in the intact ganglion, the relationship between somal size and conduction velocity of intact DRG neurons was not significantly different between CCD and control neurons, indicating that the size of somata did not change after CCD (Zhang et al., 1999). Thus, we used somal diameter as a means of classifying cells both in the intact DRG and after cell dissociation. The presence of an inflection on the falling phase of the action potential was characteristic for nociceptive DRG neurons in vivo (Koerber et al., 1988; Ma et al., 2003; Ritter and Mendell, 1992). We also classified the dissociated DRG neurons according to the presence or absence of an inflection on the falling phase of action potential evoked by current injection. Although the shape of action potential might be distorted by the current, we have found that, in the intact DRG, in most of the cases, the presence or absence of inflection was not affected by current injection in the intact DRG neurons (Ma et al., 2003).

Electrophysiological recordings were performed with continuous current-clamp in bridge mode using an AxoClamp-2B amplifier, stored digitally via Digidata 1322A interface, and analyzed offline with pClamp 8 software (Axon Instruments, Union City, CA). Efforts were undertaken to minimize the errors induced by the non-linearity in electrode characteristics. The bridge balance and capacity compensation were carefully adjusted to minimize the transient voltage change at the start of current injection. The *I*/*V* relationship for our electrodes over the range of currents used in the study showed that the measured voltage drops across out electrodes were within 3.5 mV of those expected based on linear characteristics.

A neuron was accepted for study only when it exhibited a resting membrane potential (RMP) more negative than −45 mV. Action potentials (APs) were evoked by injecting current steps of 100 ms duration through the intracellular recording electrode from −0.5 nA in increments of 0.05 nA (or 0.1 nA for large-sized neurons) until evoking one or more APs, or reaching 4 nA. The current threshold (CT, nA) was defined as the minimal current required to evoke an AP (Fig. 1). The input resistance (R_{in}, MΩ) was calculated from a hyperpolarization current step of −0.2 nA, the same current level used to bridge-balance the electrode resistance.

For each neuron isolated for study, a continuous recording was obtained for 3 min without the delivery of any external stimulus. If spontaneous discharge persisted during this period, the neuron was classified as spontaneously active (SA) (Fig. 3).

2.4. Statistical analyses

Values are presented as means ± SE. Two-way analyses of variance (ANOVAs) followed by posthoc pairwise comparisons (Student–Newman–Keuls method) (SigmaStat version 2.03, SPSS Inc., San Rafael, CA) were used to determine the statistical significance of differences between the electrophysiological parameters obtained from DRG neurons under different experimental conditions (CCD vs. Control, and acute vs. 1d culture). One-way analyses of variance followed by the same posthoc pairwise comparisons were used to compare the electrophysiological parameters of dissociated neurons with those of neurons previously recorded in our laboratory from the intact DRG (Zhang et al., 1999). Chi-Square (χ^2) tests were used to assess differences between experimental groups in the incidence of SA. A probability of 0.05 was chosen as the criterion for significance.

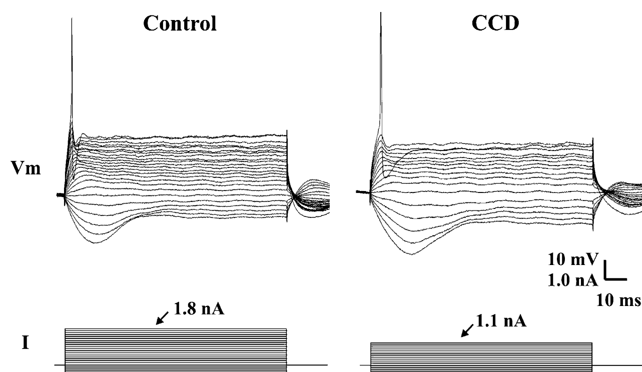

Fig. 1. Examples of action potentials (AP) evoked by current injection in control and CCD neurons. APs were evoked by injecting current steps of 100 ms duration from −0.5 nA in increments of 0.1 nA until evoking one or more APs. The current threshold was defined as the minimum current required to evoke an AP. Typical recordings of membrane potential (upper traces) and current (lower traces) are given for two large-sized DRG neurons (diameters of 47 and 48 μm), one from a control and the other from a CCD rat. The current threshold was 1.8 nA for the control neuron and 1.1 nA for the CCD neuron. V_m, membrane potential; *I*, current.

3. Results

Sharp-electrode intracellular recordings were obtained from 459 DRG neurons, including 237 CCD neurons from 22 compressed ganglia (11 rats) and 222 control neurons from 18 control ganglia (nine rats). Of the CCD neurons, 120 were obtained after acute dissociation including 37 large (L), 46 medium (M), and 37 small-sized (S) neurons. An additional 117 CCD neurons were recorded after 1d culture: 44 L, 43 M, and 30 S. Of the control neurons, 113 were obtained after acute dissociation (37 L, 42 M and 34 S) and 109 after 1d culture (37 L, 40 M, and 32 S). All S neurons except two from CCD and one from control animals showed an inflection on the falling phase of action potential. In acutely dissociated DRG neurons, 13.5% L and 58.7% M CCD neurons and 13.5% L and 45.2% M control neurons exhibited an inflection. After 1d culture, 13.6% L and 51.2% M CCD neurons and 16.2% L and 47.5% M control neurons showed an inflection. There was no significant difference in the proportion of inflected neurons between CCD and control, or between acute and 1d culture within each size category (χ^2 tests, $P<0.05$).

3.1. Effects of CCD on the membrane properties of dissociated DRG neurons

In comparison with control neurons of the same size, large- and medium-sized CCD neurons exhibited significantly lower mean CTs after both acute and 1d culture (Fig. 1, * in Table 1). In contrast, for small-sized neurons, no significant differences in the mean CT were found between CCD and control neurons after either acute or 1d culture. With one exception, the CCD and control neurons of a given size category did not differ significantly in mean RMP or mean R_{in}. The exception was the large-sized CCD neurons whose mean RMP after acute dissociation was significantly more depolarized than that of control neurons.

Comparisons between acute and 1d culture of means obtained for a given size category and treatment condition revealed differences only for large-sized neurons (§ in Table 1). First, the RMP of large-sized control neurons became significantly more depolarized after 1 day in culture. Second, for both CCD and control large neurons, the mean R_{in} decreased significantly after 1 day in culture.

3.2. Comparison of membrane properties of intact and dissociated DRG neurons

The electrophysiological properties of these dissociated neurons were compared in Table 1 with those previously obtained in our laboratory from the intact ganglion recorded in vitro under similar conditions, i.e. with sharp electrodes and a warm bath temperature (Zhang et al., 1999). Small neurons exhibited more changes in electrophysiological parameters after dissociation than large- and medium-sized neurons († in Table 1). Control neurons of small size

Table 1
Effects of CCD on electrophysiological properties of intact and dissociated DRG neurons

Size category	Large		Medium		Small	
Treatment	CCD	Control	CCD	Control	CCD	Control
CT (nA)						
Intact	0.95±0.11**	1.89±0.21	0.72±0.08**	1.68±0.14	0.58±0.05**	0.90±0.08
n	46	24	26	27	34	26
Acute	1.00±0.12**	1.88±0.14	0.79±0.08*	1.12±0.10††	0.31±0.04††	0.31±0.03††
n	37	37	46	42	37	34
1d	1.27±0.13**†	2.07±0.19	0.63±0.07*	0.90±0.09††	0.29±0.03††	0.40±0.05††
n	44	37	43	40	30	32
RMP (mV)						
Intact	−66.16±1.29	−66.67±1.43	−64.00±1.30	−63.91±1.43	−61.24±1.66	−61.94±1.69
n	47	24	26	27	36	26
Acute	−61.78±1.07*†	−65.81±1.12	−59.83±1.15†	−62.69±1.20	−54.22±1.43††	−56.97±1.38†
n	37	37	46	42	37	34
1d	−63.50±0.84	−62.49±1.09†§	−58.44±1.37†	−60.43±1.18	−52.87±1.14††	−56.00±1.40††
n	44	37	43	40	30	32
R_{in} *(MΩ)*						
Intact	32.90±2.37	34.46±6.01	56.72±6.58	37.08±3.28	104.66±11.04	99.31±14.31
n	46	17	26	17	34	20
Acute	34.87±3.24	30.59±2.22	61.31±8.56	54.78±5.67	147.21±17.42†	204.28±31.39†
n	37	37	46	42	37	34
1d	25.57±2.13†§	22.16±1.75†§§	45.98±4.82	54.70±6.17	124.50±18.99	157.96±27.96
n	44	37	43	39	30	32

Values are means±SE; *n*, sample size; CT, current threshold; RMP, resting membrane potential; R_{in}, input resistance. Intact, results from intact ganglion (Zhang et al., 1999); acute, acutely dissociated DRG neurons; 1d, 1-day cultured DRG neurons; *, $P<0.05$; **, $P<0.01$, CCD vs. control; †, $P<0.05$; ††, $P<0.01$; acute or 1d culture vs. intact DRG; §, $P<0.05$; §§, $P<0.01$, acute vs. 1d culture.

showed a significantly lower CT, a more depolarized RMP after either acute or 1d culture, and a higher R_{in} after acute (but not 1d) culture than they did in the intact ganglion. As described, CCD produced no additional changes in these parameters in dissociated small neurons.

Each of the changes produced by the dissociation of the small control neurons could act to increase their excitability. In contrast, dissociation produced no such effects in large or medium cells save for a decrease in the mean CT for medium-sized cells († in Table 1). Thus, while for the intact DRG the effect of CCD was to lower the mean CT in all three size categories (Zhang et al., 1999), for dissociated neurons, CCD lowered the CT only for the large- and medium-sized cells. For small-sized dissociated neurons, CCD produced no further lowering of the CT beyond that produced by the dissociation process itself.

3.3. Effects of CCD on the incidence of SA

The overall incidence of SA in CCD neurons, while not significantly different from that of control neurons after acute dissociation, became significantly higher after 1d culture (χ^2 tests, $P<0.05$) (Fig. 2). After acute dissociation, SA was recorded in 5 (L 3, S 2) of the 120 CCD neurons (4.2%) and 2 (M 1, S 1) of the 113 control neurons (1.8%). After 1d culture, 12 (L 5, M 5, S 2) of the 117 CCD neurons and 2 (L 1, S 1) of the 109 control neurons exhibited SA (10.3 and 1.8%, respectively). However, no significant difference was found between CCD and control neurons within each size group, either after acute or 1d culture (χ^2 tests), possibly because of the small sample sizes. In the intact DRG, 12 of 109 CCD neurons (11.0%) and 2 of 77 control neurons (2.6%) exhibited SA (Zhang et al., 1999). Thus, the incidence of SA after CCD was similar for intact- and 1d cultured DRG neurons but was lower after acute dissociation (Fig. 2).

The patterns of SA in dissociated neurons were classified as bursting, regular and continuous or irregular (Fig. 3). The incidence of each pattern, 52.4% for bursting, 33.3% for regular and 14.3% for irregular, was similar to that recorded from the intact DRG after CCD (Hu and Xing, 1998; Song

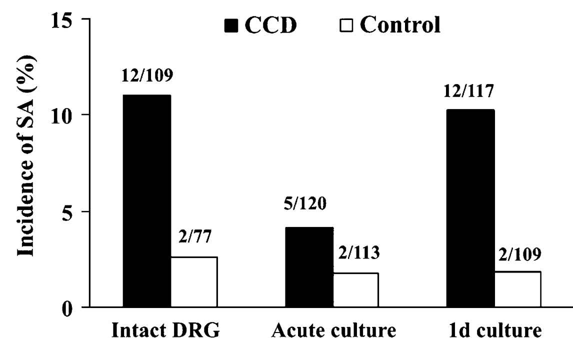

Fig. 2. The incidence of abnormal spontaneous activity (SA) in DRG neurons. The incidence of SA is defined as the number of SA neurons divided by the total number sampled for each group (×100). The numbers upon which the percentages are based are given for CCD and control neurons (solid and open bars, respectively) from three groups (left to right): intact DRG (Zhang et al., 1999), acute culture, and 1d culture.

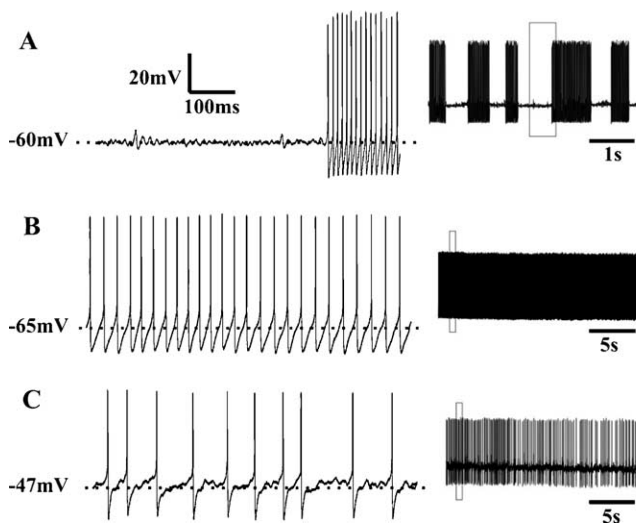

Fig. 3. Typical patterns of spontaneous activity obtained from dissociated CCD neurons. Examples are given for large- (A), medium- (B) and small-sized (C) DRG neurons after 1d culture. These patterns were categorized as bursting (A), regular/continuous (B) and irregular (C). Each record on the left is an enlargement of the response within the boxed area indicated in the corresponding panel on the right.

et al., 1999; Zhang et al., 1999). Most neurons with bursting or irregular SA exhibited subthreshold oscillations of the membrane potential as described for SA neurons in the intact DRG after CCD (Xing et al., 2001; Zhang et al., 1999) and in intact and dissociated DRG neurons after axotomy (Amir et al., 1999; Liu et al., 2002). In some instances, spontaneous discharges were immediately preceded by an increase in the amplitude of oscillation, whereas in other cases it was not (Fig. 3A).

4. Discussion

4.1. Injury-induced hyperexcitability in the somata of DRG neurons

The hyperexcitability of DRG neurons after CCD, as indicated by a lower than normal CT and an increased incidence of SA, has been observed in previous electrophysiological studies of the intact DRG (Hu and Xing, 1998; Song et al., 1999, 2003; Zhang et al., 1999). Similar changes in excitability were found in DRG neurons after injuries of the peripheral nerve or inflammation of peripheral tissues (Abdulla and Smith, 2001; Bennet and Xie 1988; Djouhri et al., 2001; Kim et al., 1998; Liu et al., 2000; Ma et al., 2003; Stebbing et al., 1999; Wall and Devor, 1983; Xu et al., 2000). In the intact DRG, the somata of sensory neurons are surrounded by satellite glial cells and other non-neuronal cells. Injurious and/or inflammatory stimuli may trigger the release of chemical substances from non-neuronal cells and/or neighboring neurons that could conceivably alter neuronal excitability (e.g. for review, Amir and Devor, 1996; Amir and Devor, 2000; Devor and Wall, 1990; Watkins and Maier, 2002; Xu and Zhao, 2003).

Our findings indicate that the CCD-induced increase in excitability of medium- and large-sized neurons persists in the absence of signals from neighboring cells. First, the dissociated DRG neurons were immediately surrounded by few or no satellite cells. Second, superfusion would likely wash away any substances released by neighboring cells in culture. Thus, the higher incidence of SA and the lower CTs of larger and medium-sized neurons in CCD neurons are indicative of a hyperexcitability that is intrinsic to the soma.

4.2. Effects of dissociation on the membrane properties of DRG neurons

The electrophysiological parameters we obtained from dissociated small neurons from control rats are similar to those previously reported for acutely dissociated and short-term cultured DRG neurons using sharp-electrode intracellular recording (Caffery et al., 1992), or whole-cell patch-clamp recording (Abdulla and Smith, 2001; Gold and Traub, 2004; Study and Kral, 1996). A similarity in recording conditions used in our laboratory (intracellular electrodes, warm bath temperature) allows us to make, for the first time, a direct comparison between the electrophysiological parameters presently obtained from dissociated neurons and those we previously recorded from the intact DRG (Zhang et al., 1999). In comparison with neurons in the intact DRG, small neurons from both control and CCD rats exhibited more changes in electrophysiological parameters after dissociation than large- and medium-sized neurons. Perhaps small-sized neurons are more vulnerable than larger sized neurons, due to the loss of their axons and/or their satellite glia after dissociation. Acutely dissociated small-, but not medium- and large-sized neurons exhibited significantly higher R_{in} than those in the intact ganglions. An implication may be that the axon of a small neuron contributes more to the R_{in} than the axon of a larger neuron. Another effect of dissociation on the membrane properties of small neurons was a more depolarized RMP. Both the higher R_{in} and depolarized RMP could contribute to the lower CTs of dissociated small neurons.

The depolarized RMP in dissociated small neurons could raise a concern about the effects of possible 'damage' caused by the sharp electrode. Although electrode penetration is inevitably accompanied by some membrane damage and leakage, such effects are believed to be minimal and not likely to affect our results for the following reasons. First, efforts were undertaken to minimize damage by the use of higher impedance (70∼80 MΩ) electrodes. Second, the higher

R_{in} of dissociated, small-diameter neurons indicated a minimal effect of leaking caused by the electrode. Third, a neuron was accepted for study only if it showed a stable resting membrane potential (RMP) more negative than −45 mV during the 3 min period of observation after electrode insertion. Lastly, although the RMPs of small neurons were more depolarized and the CTs lower after dissociation than they were in the intact ganglion, the values are within range of those published by other laboratories using similar procedures of culture and patch-clamp recording (Abdulla and Smith, 2001; Gold and Traub, 2004). Therefore, we believe that the depolarized RMP and lowered CT in dissociated small neurons were more a result of the dissociation procedure rather than the damage and leakage caused by the electrode.

One can only speculate as to why the decrease in CT for small neurons after dissociation was not further decreased after CCD. One possibility is that the CCD-induced changes in small neurons were not intrinsic to the somata, but rather due to the changes in the satellite glia and/or other non-neuronal cells which were removed after dissociation. Another possibility is that the ion channels affected most by CCD were distributed closer to the axon hillock in smaller than in larger neurons and that this region of the cell membrane was removed during the dissociation. The 1d cultured small neurons exhibited a trend toward lower CTs in CCD vs. control despite a decreased R_{in} in both groups (Table 1), indicating a possible correlation between neurite outgrowth and neuronal hyperexcitability. In studies of nerve injury, dissociated, cultured DRG neurons whose axons formerly projected into an injured, as opposed to a normal, sciatic nerve exhibited an enhanced rate of neurite outgrowth (Hu-Tsai et al., 1994; Lankford et al., 1998). This raises the possibility that previously compressed, though not axotomized, CCD neurons may also be more responsive to the culture environment. Preliminary observations during the course of the present experiment are in accordance with this hypothesis. That is, we typically found that neurite outgrowth in culture was more rapid for CCD than for control neurons (Fig. 4).

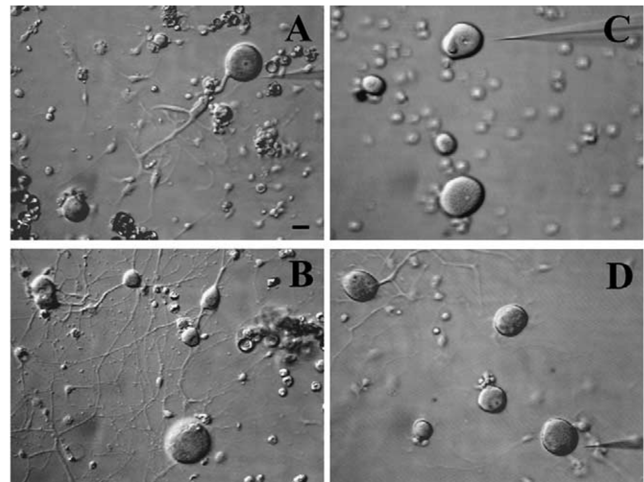

Fig. 4. Representative pictures of neurite outgrowth in cultured neurons. (A) and (C) CCD and control neurons after approximately 5 h in culture, respectively. (B) and (D) CCD and control neurons after 24 h in culture, respectively. Some CCD neurons (A) exhibited neurite outgrowth as early as 4–5 h in culture. This was rarely observed for control neurons (C). After 24 h of culture, control neurons began to develop some neurites (D), and the neurite outgrowth in CCD neurons was more significant (B). The photographs were obtained under infrared, differential interference contrast at 200× magnification. Scale bar (in A): 20 μm.

4.3. CCD-induced SA in dissociated and intact DRG neurons

The 1d cultured, but not the acutely dissociated, CCD neurons exhibited a similar incidence of SA to that of CCD neurons in the intact ganglion (Fig. 2), although the two groups of cultured neurons had little differences in CT or other membrane properties. Possibly some CCD neurons that were SA in the intact DRG ceased firing after acute dissociation only to recover after 1d in culture. In contrast, after a chronic constriction injury (CCI) of the sciatic nerve in the rats, the increased incidence of SA in DRG neurons in the intact ganglion (Kajander and Bennett, 1992) was also observed in neurons of each size category after acute dissociation (Peterson et al., 1996; Study and Kral, 1996). Similarly, large-sized DRG neurons from ganglia that had received a spinal nerve ligation (SNL) exhibited a higher incidence of SA than those from control ganglia both in the intact DRG and after acute dissociation (Liu et al., 2000, 2002). The fact that abnormal SA is present in acutely dissociated neurons after the latter two models of neuropathic pain and not after CCD may reflect differences in the cellular mechanisms triggered by prior axotomy (CCI and SNL) as opposed to DRG compression in the absence of axotomy (CCD).

It is not clear whether the SA neurons in 1d culture were the same ones that were SA in the intact DRG or whether additional neurons became SA due to changes brought about by the culture. The fact that the control neurons remained largely ‘silent’ after 1d culture might suggest that the 1d cultured SA neurons were from the same group of SA neurons in the intact ganglion. During the enzymatic and mechanical dissociation process, DRG neurons were deprived of axons and satellite glial cells. It is possible that some ion channels responsible for the spontaneous activity, but not for the lowered current threshold were removed temporarily after acute dissociation but recovered in the 1d-cultured group. This hypothesis could be tested in a future study.

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