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Brain Research 767 (1997) 214–219

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Research report

# Membrane properties of deep dorsal horn neurons from neonatal rat spinal cord in vitro

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Accepted 15 April 1997

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**Abstract**

Whole-cell patch-clamp recordings were undertaken to characterize and compare the membrane properties of deep dorsal horn neurons in transverse slices of rat lumbar spinal cord in two age groups, postnatal days (P) 3–6 and 9–16. In both age groups, significant correlations were observed between membrane time constant and cell resistance and between action potential height and its duration at half-maximal amplitude. Cell resistance and action potential half-width values were lower in the P9–16 age group. Neurons were divided into four categories based on their firing properties in response to intracellular current injection: single spike, phasic firing, repetitive firing, and delayed firing. The distribution of neurons within these categories was similar in both age groups which suggests that the firing properties of deep dorsal horn neurons are functionally differentiated at an early postnatal age. © 1997 Elsevier Science B.V.

*Keywords:* Spinal cord; Development; Slice

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**1. Introduction**

The integrative properties of deep dorsal horn (DDH) neurons (laminae IV–VI) represent a nodal point through which most primary afferent information must be processed. Hence the DDH is a critical site for segmental and descending neuromodulatory control. Some membrane properties of DDH neurons have been characterized intracellularly both in vivo [9,26] and in vitro [4,8,10,11,15–19]. Since the DDH comprises a heterogeneous population of neurons, it is not surprising that differences in measured intrinsic membrane properties were observed between the various neurons examined. Most DDH neurons discharge spontaneously. Fast and slow post-spike afterhyperpolarizations and an afterdepolarization were seen in cells in various combinations, both for unidentified DDH neurons [9–11,18,19] and spinothalamic tract cells [26]. Further, plateau potentials, bursting, and intrinsic membrane voltage oscillations have been observed in subpopulations of DDH neurons [11,12,15–17]. Recently, Lopez-Garcia and King [11] observed that dorsal horn neurons possess differences in their cellular membrane properties that relate to

their response properties to low and high intensity mechanical cutaneous stimuli. This observation suggests that the membrane properties of dorsal horn neurons are functionally differentiated.

A limitation of previous intracellular electrophysiological studies in DDH is their reliance on current clamp recordings with conventional sharp electrodes to determine intrinsic membrane properties. These recordings are affected by impalement-induced injury and its severe effect on estimates of passive membrane properties (for discussion see [21]). Further, in vitro studies relied on the use of high-impedance electrodes which limit current-passing capability and hence elucidation of voltage-dependent properties. Patch-clamp recordings offer some important advantages over conventional approaches. Due to low electrode impedance values, greater currents may be injected without fear of electrode rectification. Patch recordings also permit equally stable recordings from small cells while sharp electrodes may preferentially impale larger cell somas (see [27]). However, to date, few studies have published work using whole-cell recordings to study cell function in the mammalian spinal cord dorsal horn [28–30], none of which were undertaken in the DDH.

Because the integrative properties of DDH neurons are functionally divisible [11] and may profoundly alter their

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output properties in response to sensory input [15] or neuromodulators [1], a more complete and reliable understanding of their integrative mechanisms is required. The present study sought to characterize and divide neurons into subpopulations based on their intrinsic membrane properties, as well as to provide an analysis of the alterations in membrane properties with development. Experiments were undertaken in two age groups; postnatal (P) 3–6 and 9–16 days old. These age ranges may compare the cellular properties of DDH neurons before and after C fibre-evoked actions are observed in the DDH [3]. Results obtained from 9–16-day-old rats also permit comparison to other observations using similar-aged animals in vitro (e.g. [11,14,18–20,24]).

## 2. Materials and methods

The lumbar spinal cords of 3–16-day-old neonatal rats were dissected free and sliced on a vibrating blade microtome in 400–700- $\mu\text{m}$  transverse sections. Older animals (P9–16) were first anaesthetized with 0.5–1.0 ml of 10%

Table 1  
Comparison of passive and active membrane properties in DDH neurons from two age groups

	3–7 Days old	9–16 Days old
Passive properties		
Membrane potential (mV)	$-59 \pm 7$ (39)	$-61 \pm 8$ (28)
Measured cell resistance ( $\text{M}\Omega$ )	$552 \pm 257$ (44)	$458 \pm 303$ (34) <sup>a</sup>
Calculated cell resistance ( $\text{M}\Omega$ )	$378 \pm 179$ (38)	$248 \pm 157$ (23) <sup>a</sup>
Membrane time constant (ms)	$45 \pm 17$ (44)	$40 \pm 17$ (34)
Cell capacitance (pF)	$16.7 \pm 19.2$ (29)	$22.9 \pm 33.2$ (18)
Active properties		
Action potential height (mV)	$85 \pm 16$ (42)	$83 \pm 17$ (30)
Action potential half-width duration (ms)	$4.6 \pm 2.8$ (42)	$3.2 \pm 1.6$ (30) <sup>a</sup>
Post-spike hyperpolarization amplitude (mV)	$10.2 \pm 3.9$ (31)	$11.6 \pm 3.8$ (25)
Rheobase (pA)	$62 \pm 27$ (38)	$64 \pm 36$ (29)
Threshold voltage (mV)	$22.0 \pm 6.5$ (40)	$20.7 \pm 5.5$ (29)

<sup>a</sup> Statistically significant differences at  $P < 0.05$ .

urethane i.p. (w/v) prior to decapitation. Slices were then incubated at 32°C for at least 1 h in oxygenated artificial cerebrospinal fluid (aCSF) containing (in mM); NaCl, 125;

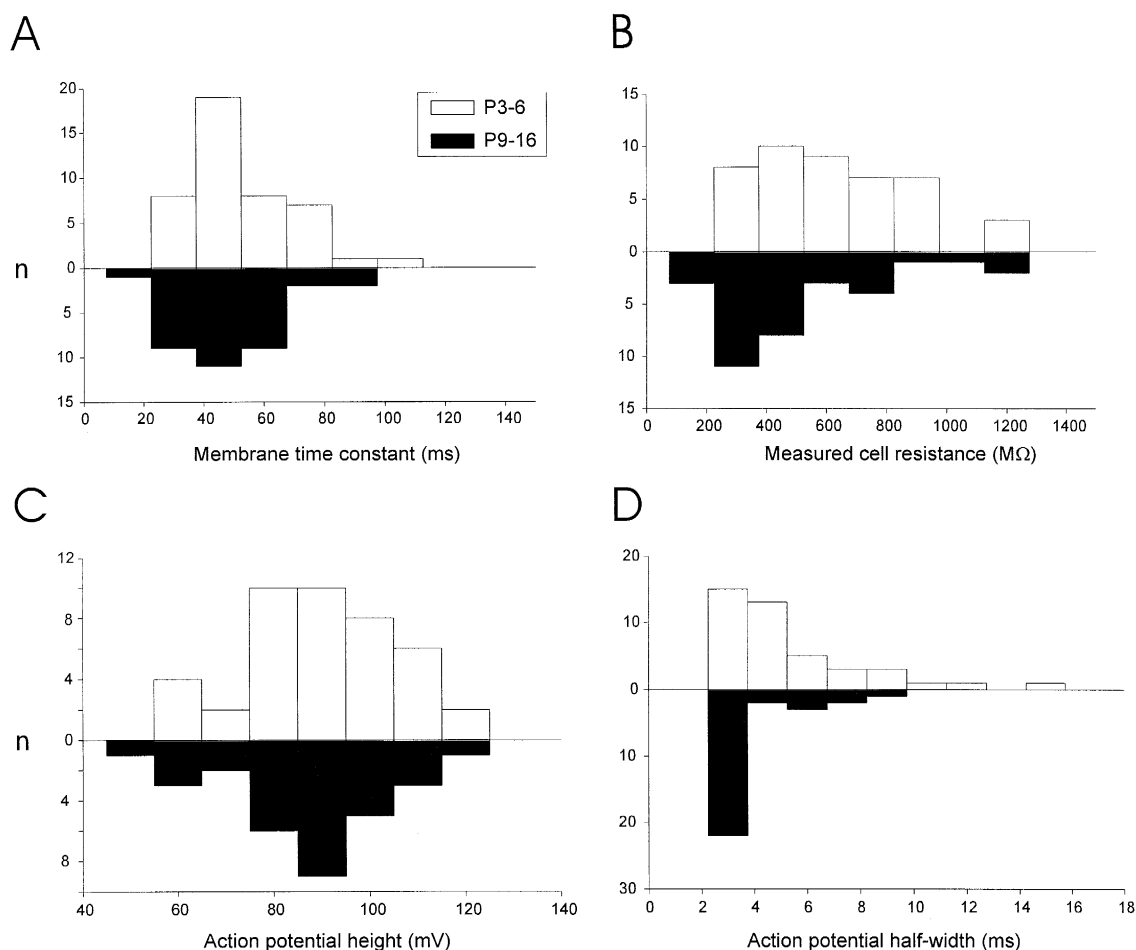


Fig. 1. Comparison of distribution of membrane properties in neurons from two age groups, P3–6 and P9–16. A: membrane time constant ( $\tau_m$ ); B: measured cell resistance ( $R_n$ ); C: action potential height; D: action potential duration at half-maximal amplitude (half-width).

KCl, 2.5; CaCl<sub>2</sub>, 2; MgCl<sub>2</sub>, 1; glucose, 25; NaH<sub>2</sub>PO<sub>4</sub>, 1.25; NaHCO<sub>3</sub>, 26. A spinal slice was fixed to the bottom of the experimental chamber. Patch pipettes had resistance values ranging from 4–7 M $\Omega$ . The whole-cell ‘blind’ patch-clamp recording technique was undertaken with recording electrodes containing (in mM): K-gluconate, 140; EGTA, 0.2; HEPES, 10; MgATP, 4; GTP, 1; pH 7.3 (see [6]). All recordings were made at room temperature. The recording chamber was continuously superfused with oxy-

genated aCSF at a rate of approx. 2 ml/min. Whole-cell recordings were undertaken with the Axopatch 1D amplifier (Axon Instruments) filtered at 5 kHz (4-pole lowpass Bessel). Voltage and current clamp data were acquired on a computer with the pCLAMP acquisition software (Axon Instruments). After rupturing the cell membrane to achieve the whole-cell configuration, the current clamp recording configuration was used to determine resting membrane potential. Then, at a standardized membrane potential of

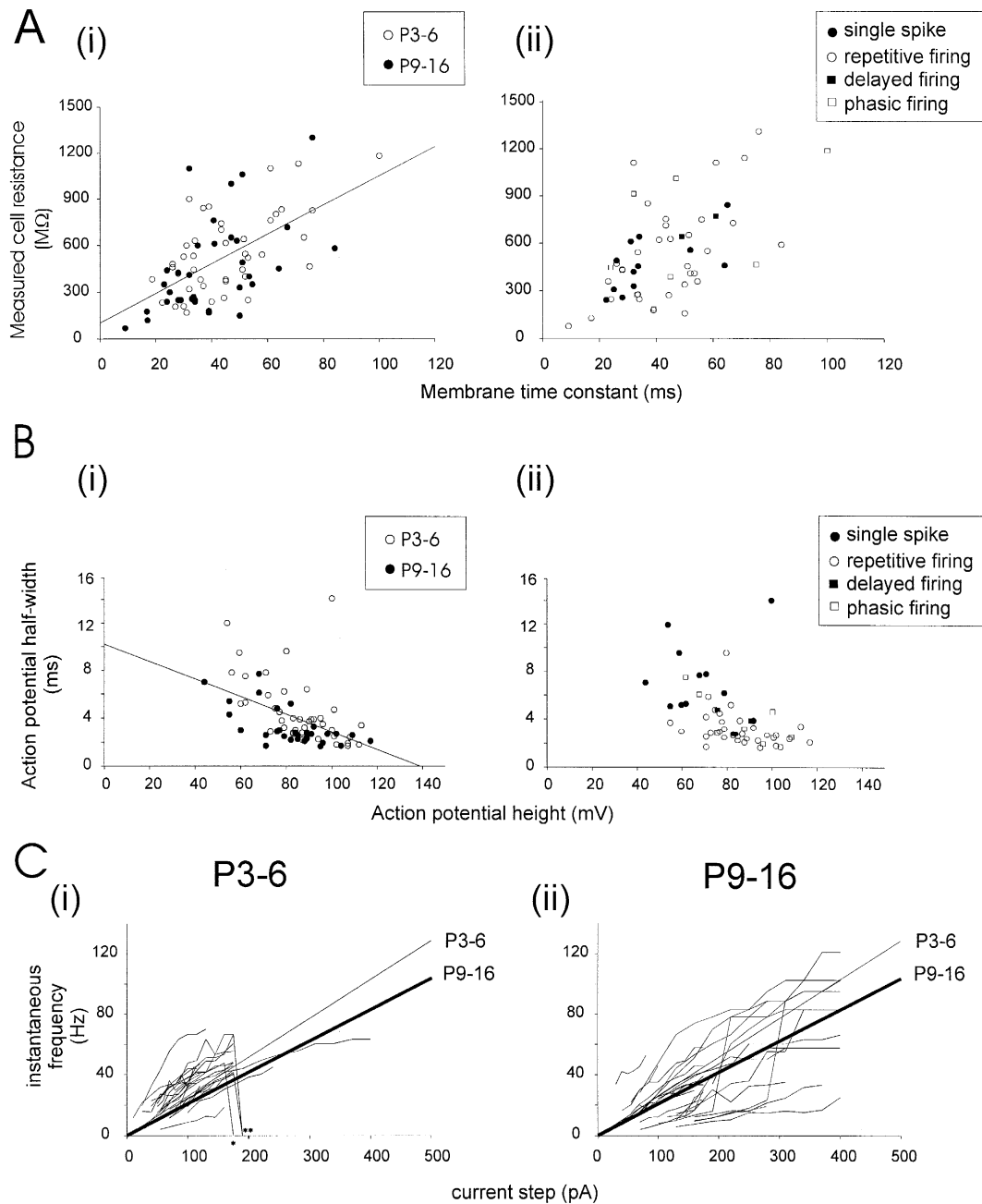


Fig. 2. Scatterplots of passive (A) and active (B) membrane properties. A: relation between input resistance and membrane time constant divided into two age groups (i) or based on their firing properties (ii). B: relation between action potential amplitude and duration at half-maximal amplitude divided into two age groups (i) or based on their firing properties (ii). C:  $f-I$  relation of instantaneous frequency and current injection. Instantaneous frequency was calculated from the interval between the first two spikes following current injection. Line emanating from origin reflects linear regression fit of all  $f-I$  curves for P3–6- (thin line) and P9–16-day-old animals (thick line).

–75 mV, a series of hyperpolarizing and depolarizing current steps were undertaken to obtain estimates of membrane time constant, cell resistance, spike height, rheobase, threshold voltage, and firing properties. Mean access resistance was measured in current clamp mode as  $59 \pm 24$  M $\Omega$  and compensated. Following switching to the voltage clamp configuration, a series of hyperpolarizing and depolarizing voltage steps were applied to obtain peak and steady-state current–voltage relations as well as values for a determination of cell resistance and whole cell capacitance (for methodological details see [7]). The membrane was voltage clamped at –95 mV. The 40 mV hyperpolarizing voltage step was used to fit the fast exponential decay of the current transient at step onset. The data points were fit using the equation  $y = I_{ss} + (I_0 - I_{ss})e^{(-t/\tau)}$ , and employing the least squares minimization algorithm (pCLAMP).  $I_{ss}$  is the steady-state current produced by the voltage step,  $I_0$  is the amplitude of the current response at time zero, and  $\tau$  is the time constant. Neuron resistance and cell capacitance were calculated from these values (see [13]). All values are reported as mean  $\pm$  S.D.

### 3. Results

The overall sample contained 78 neurons, 44 from animals aged 3–6 days and 34 from animals aged 9–16 days. Table 1 provides a summary of measured passive and active membrane properties separated into these two age groups. Membrane time constant, a measure of cell membrane resistivity, is similar in both age groups. An unchanged membrane resistivity coupled to a reduction in cell input resistance is consistent with a predicted increase in cell size with development (e.g. [2,23]; see also [5]). A comparison of the distribution of membrane time constant and measured resistance values for the two age groups is provided in Fig. 1A and B. Note that while the distribution of membrane time constant values were similar in both age groups, there was a leftward shift in the distribution of measured cell resistance in the older animals. In the present sample there was a significant positive correlation between membrane time constant and cell resistance when each age group was examined separately and when the sample was combined together ( $P < 0.001$ , Pearson Product Moment) with a correlation coefficient of 0.58 (Fig. 2A(i)). Hence part of the variation in cell resistance observed between neurons is due to variations in membrane resistivity.

Table 2  
Comparison of firing properties of DDH neurons in two age groups

Firing properties	P3–6 ( $n = 29$ )	P9–16 ( $n = 26$ )
Single spike	8	4
Phasic	4	4
Repetitive	16	17
Delayed	1	1

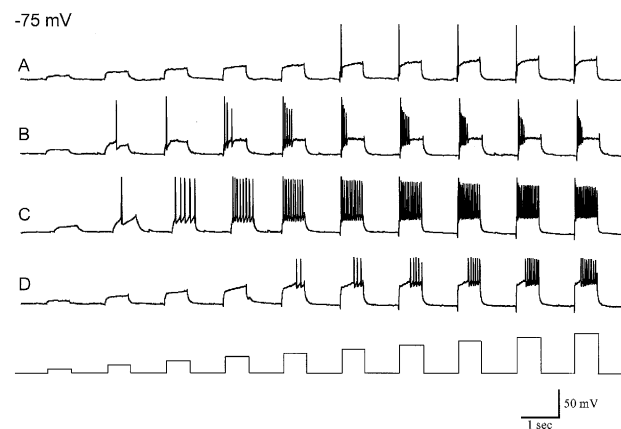


Fig. 3. Neurons can be subdivided based on their firing properties in response to current step injections. A: single spike; B: phasic firing; C: repetitive firing; D: delayed firing.

Measures of active membrane properties are also provided in Table 1. Action potential height was identical in both age groups but action potential duration at half-maximal amplitude (half-width) was observed to undergo a significant reduction in duration in the P9–16 age group. This reduction is due to a decrease in the number of longer duration half-width values (Fig. 1D). Post-spike hyperpolarization amplitude, rheobase and voltage threshold values did not differ statistically between the two age groups. There was a significant inverse correlation between action potential height and half-width when each age group was examined separately or combined (Fig. 2B;  $P < 0.001$ , Pearson Product Moment) with an overall correlation coefficient of 0.49.

The population of neurons were divided into four categories based on their firing properties in response to 700-ms pulses of intracellular current injection; repetitive firing, phasic firing, single spike and delayed firing (see [11]). The distribution of neurons within these categories are provided in Table 2 and examples are shown in Fig. 3. The majority of neurons in both age groups were capable of repetitive firing (Table 2) and there was no difference in the distribution of firing properties between the two age groups ( $\chi^2$ ). Neuron firing properties are compared to measured passive and active membrane properties in Fig. 2A(ii) and B(ii). Neurons which were only capable of firing a single spike had significantly lower action potential heights ( $69 \pm 15$  mV) and longer half-width values ( $6.0 \pm 2.9$  ms) than neurons capable of repetitive firing ( $86 \pm 15$  mV and  $3.0 \pm 1.0$  ms, respectively,  $P < 0.001$ ). In comparison, the mean values for neurons with phasic firing properties were similar to those neurons which underwent repetitive firing. There were no differences in the passive membrane properties between neurons subdivided based on their firing properties.

Frequency–current ( $f$ – $I$ ) relations were constructed for the neurons in the two age groups as presented in Fig. 2C. Several differences are notable. First, in response to an increasing magnitude of current injection, several neurons

in the P3–6 age group lost their ability to fire repetitively (asterisks on abscissa in Fig. 2C(i)). Further, neurons from older animals were capable of higher firing frequencies. A comparison of the regression lines shows that the older animals have a reduced slope in their  $f-I$  relationship (Fig. 2C).

#### 4. Discussion

The present data documents and compares the intrinsic membrane properties of neurons from P3–6 and P9–16 neonates. It suggests that membrane resistivity is relatively constant throughout this period in development. Hence the observed reduction in cell resistance is probably largely due to an increase in cell size; though our present estimate of cell size, cell capacitance, underwent a non-significant 37% increase. A more interesting observation is the significant reduction in action potential duration with age. This was correlated with differences in the membrane firing properties observed and may also account for the increased firing frequencies seen in the older animals. We divided neurons into four categories based on their firing in response to injection of depolarizing current steps. Lopez-Garcia and King [11] suggested that a similar subdivision was correlated with functional differences in primary afferent synaptic input. The present observation that the distribution of different types of neuronal firing properties does not change significantly between P3–6 and P9–16 supports that notion that DDH neurons may be well differentiated functionally even at early postnatal ages.

Mean resting potential and action potential values in DDH neurons in the present study were similar to those observed using sharp electrodes in the transverse slice and hemisected preparations of similar-age animals [10,11]. However, our cell resistance values using similar measurement procedures were about 5–9-times greater than those reports using sharp electrodes [10,11], and membrane time constant was about 4-times greater [11]. This difference is best explained by a leak conductance at the soma due to conventional microelectrode impalement [21]. These differences are important because synaptic integration, especially of slow synaptic events, is significantly influenced by the electrotonic structure of neurons [21]. The values for action potential duration at half-maximal amplitude reported here in the P9–16 age group are longer than previous reports [10,11] and may be due to the fourfold greater membrane resistivity observed with patch recordings.

An advantage of the transverse slice preparation is its reliable visual targeting of neurons within specific laminae [10]. However, a problem with the present study is the lack of cellular identification in the functionally heterogeneous deep dorsal horn. Previous *in vitro* approaches have commonly relied on the use of differences in neuron response properties to nociceptive and non-nociceptive primary af-

ferent inputs to subdivide neurons into three general categories, nociceptive-specific (NS), low-threshold (LT), and wide dynamic range (WDR) (for review see [25]). While in older neonates (e.g. P10–14), electrical stimulation of nerve roots in slice may be sufficient to allow for such a discrimination [22], the synaptic input in younger neonates (P3–6) is more difficult to characterize due to the immature state of primary afferent myelination [3]. In rats 10–14 days old, Lopez-Garcia and King [11] recorded from dorsal horn neurons in the hindlimb-attached hemisected spinal cord *in vitro*. Differences between low- and high-intensity mechanoreceptor stimulation-induced synaptic responses were observed to be well correlated with variations in intrinsic membrane properties. For example, the majority of WDR neurons fired repetitively in response to intracellular depolarizing current injection while NS neurons fired bursts of action potentials at the onset of the depolarizing current step. It is possible that a similar correlation between DDH neuronal firing properties and physiological identity exists in the P3–P6 neonates presently examined.

We conclude that, even at early postnatal ages, DDH neurons express a diversity in cellular membrane properties that allow for a range of encoding patterns, from the strongly phasic single spike to high-frequency repetitive firing. The heterogeneity in membrane properties of DDH neurons is maintained in adulthood [9] presumably to support the unique signalling requirements of the numerous functional subpopulations of DDH neurons (for review see [25]). Future studies in the neonate should employ whole-cell recordings from a more intact *in vitro* preparation (e.g. the isolated intact spinal cord; see [7]) to allow for a detailed comparison of intrinsic membrane properties to neuron identity based on afferent input and axonal projections.

#### Acknowledgements

Supported by the Thorlakson Foundation Fund and the Manitoba Medical Services Foundation.

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