Postnatal Changes in Membrane Properties of Mice Trigeminal Ganglion Neurons

CARMEN CABANES, MIKEL LÓPEZ DE ARMENTIA, FÉLIX VIANA, AND CARLOS BELMONTE
Instituto de Neurociencias-Consejo Superior de Investigaciones Científicas, Universidad Miguel Hernández, San Juan de Alicante 03550, Spain

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Cabanes, Carmen, Mikel López de Armentia, Félix Viana, and Carlos Belmonte. Postnatal changes in membrane properties of mice trigeminal ganglion neurons. J Neurophysiol 87: 2398–2407, 2002; 10.1152/jn.00419.2001. Intracellular recordings from neurons in the mouse trigeminal ganglion (TG) in vitro were used to characterize changes in membrane properties that take place from early postnatal stages (P0–P7) to adulthood (>P21). All neonatal TG neurons had uniformly slow conduction velocities, whereas adult neurons could be separated according to their conduction velocity into Aδ and C neurons. Based on the presence or absence of a marked inflection or hump in the repolarization phase of the action potential (AP), neonatal neurons were divided into S- (slow) and F-type (fast) neurons. Their passive and subthreshold properties (resting membrane potential, input resistance, membrane capacitance, and inward rectification) were nearly identical, but they showed marked differences in AP amplitude, AP overshoot, AP duration, rate of AP depolarization, rate of AP repolarization, and afterhyperpolarization (AHP) duration. Adult TG neurons also segregated into S- and F-type groups. Differences in mean AP amplitude, AP overshoot, AP duration, rate of AP depolarization, rate of AP repolarization, and AHP durations were also prominent. In addition, axons of 90% of F-type neurons and 60% of S-type neurons became faster conducting in their central and peripheral branch, suggestive of axonal myelination. The proportion of S- and F-type neurons did not vary during postnatal development, suggesting that these phenotypes were established early in development. Membrane properties of both types of TG neurons evolved differently during postnatal development. The nature of many of these changes was linked to the process of myelination. Thus myelination was accompanied by a decrease in AP duration, input resistance (Rm), and increase in membrane capacitance (C). These properties remained constant in unmyleinated neurons (both F- and S-type). In adult TG, all F-type neurons with inward rectification were also fast-conducting Aδ, suggesting that those F-type neurons showing inward rectification at birth will evolve to F-type Aδ neurons with age. The percentage of F-type neurons showing inward rectification also increased with age. Both F- and S-type neurons displayed changes in the sensitivity of the AP to reductions in extracellular Ca2+ or substitution with Co2+ during the process of maturation.

INTRODUCTION

Neurons of dorsal root (DRG) and cranial sensory ganglia in adult mammals constitute a heterogeneous population in terms of transduction properties of their sensory endings and the quality of the sensation evoked by their activation. They also differ in a number of morphological and functional characteristics, such as size (Lawson 1979; Ramón-Cajal 1899), degree of myelination of the peripheral axon (Harper and Lawson 1985b), immunocytochemical properties of the soma (Alvarez et al. 1991; Dodd and Jessell 1985; Lawson and Waddell 1991), neuropeptide content (McCarthy and Lawson 1989; Quartu et al. 1992), and passive and active membrane properties of the cell body (Gallego and Eyzaquiere 1978; Harper and Lawson 1985a; Koerber et al. 1988; Liu and Simon 1994; López de Armentia et al. 2000; Ritter and Mendell 1992; Rose et al. 1986; Villiére and McLachlan 1996; Waddell and Lawson 1990).

Electrophysiological characteristics of primary sensory neurons are determined by the expression of various types of ion channels in their membrane (Gallego 1983; Roy and Narahashi 1992; Scroggs and Fox 1992; Scroggs et al. 1994) and are presumably associated with the encoding capacity and the transducing properties of the neuron’s peripheral nerve endings (Belmonte and Gallego 1983; Koerber et al. 1988).

Some specific characteristics of adult primary sensory neurons, such as somatic size and myelination, are different at the time of birth, experiencing further development during the early postnatal period (Coggeshall et al. 1994; Lawson 1979; Peters and Muir 1959). This also seems to be the case for some of the electrophysiological properties of the soma membrane (Fedulova et al. 1991; Fitzgerald and Fulton 1992; Spitzer 1979). Fulton (1987) suggested that at the time of birth, when myelination had not yet taken place, prospective C fibers already exhibited the long-duration action potential (AP) and a prolonged afterhyperpolarization (AHP) characteristic of mature C neurons. In contrast, putative Aδ neurons were more diverse in terms of their membrane properties: some of them were distinct electrophysiologically from C-fiber neurons, whereas others also displayed relatively long-lasting APs and AHPs, although they showed differences with putative C neurons in other active and passive membrane properties.

The changes taking place in electrophysiological properties of mammalian primary sensory neurons during early postnatal maturation are largely unknown. To our knowledge, studies in the trigeminal ganglion have not been undertaken. This is the period when peripheral receptor organs become innervated (Belford and Killackey 1980) and the modality of peripheral
endings is being defined (Fitzgerald 1987). It also corresponds with the period of peripheral axonal myelination (Friede and Samorajski 1968). Knowledge on the development of their properties may contribute to explain the mechanisms underlying the establishment of functional heterogeneity of mature primary sensory neurons.

In the present work, passive and active membrane properties of neonatal mice trigeminal ganglion (TG) neurons were analyzed and compared with those of adult animals. We report marked changes in their membrane and firing properties, some linked to the process of myelination. Preliminary results have been reported in abstract form (Cabanes et al. 1999).

**METHODS**

**In vitro trigeminal ganglion preparation**

Mice (129/Sv × C57BL/6 strain) of different postnatal ages (P1–P50) were deeply anesthetized by an intraperitoneal injection of pentobarbitone sodium (90 mg/kg, Euta-Lender, Madrid) and perfused through the heart with cold, oxygenated physiological saline containing (in mM): 128 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgCl₂, 1 NaH₂PO₄, 16 NaHCO₃, and 5 glucose. The animals were killed by decapitation, and their heads placed inside a glass dish filled with ice-cold physiological saline where the TG was dissected free of surrounding tissues with its central and maxillary roots attached. After removal, the TG was transferred to a chamber filled with ice-cold physiological saline, oxygenated with a mixture of 95% O₂-5% CO₂. After careful cleaning under a dissecting microscope, the TG was pinned to the silicone elastomer (Sylgard)-coated bottom of a small (200 μl volume) recording chamber. The TG was perfused continuously (5–7 ml/min) with oxygenated physiological saline at room temperature (20–25°C).

The ganglion was illuminated tangentially with a fine (1 mm) fiber optic light source (F-O-Lite, WPI, Sarasota, FL) that produced a Nomarski-like image of the ganglion surface, viewed through the optics (×20 objective, 0.35 NA, and 19.9 mm working distance) of an Optiphot-2 upright microscope (Nikon, Tokyo, Japan). Single cells were impaled with a glass microelectrode. The cut ends of the central and peripheral (maxillary) roots of the TG nerve were inserted into tight suction electrodes for pulsed electrical stimulation, using a nerve stimulator (model S48) coupled to a photoelectric stimulus isolation unit that delivered constant current pulses (model PSIU6) both from Grass-Telefactor (West Warwick, RI). To calculate central and peripheral conduction velocities (CV), the distance between the tip of the suction electrode attached to the central or maxillary root and the microelectrode tip was measured, and this value was divided by the latency of the antidromic and orthodromic AP, respectively (Fig. 1). Distances were estimated with the help of an eyepiece micrometer scale in neonatal TG and with a calibrated ruler in adult ganglia.

**Electrical recording**

Cells were impaled with borosilicate glass microelectrodes (1.0 mm OD and 0.5 mm ID, Clark Electromedical Instruments) filled with 3 M KCl. Electrodes resistance ranged between 80 and 110 MΩ in adult

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**FIG. 1.** Active and passive membrane properties measured in trigeminal ganglion (TG) neurons. A: action potential (AP) parameters: a, resting membrane potential; b, AP overshoot; c, AP amplitude; d, AP duration at 50% of amplitude; e, repolarization time of AP at 90% amplitude; f, AP latency; g, AP maximum rate of rise; h, AP maximum rate of fall. B: afterhyperpolarization (AHP) parameters: i, AHP amplitude; k, AHP duration at 50% of amplitude. The trace corresponds to the same neuron as in A. C: voltage response to hyperpolarizing current pulses of 100-ms duration, showing the sag in potential, and voltage response to a depolarising current pulse used to determinate the threshold (rheobase) current. D: current-voltage (I-V) relationship at the peak (●) and at steady state (○) of the same neuron as in C. All examples in the figure correspond to neonatal animals.
FIG. 2. Histogram of conduction velocities in mouse trigeminal neurons. A: distribution of central (n = 29; mean conduction velocity (CV) = 1.2 ± 0.2 m/s) and peripheral (n = 18; mean CV = 1.1 ± 0.14 m/s) CVs in neonatal mice TG. B: distribution of central (n = 94; mean CV = 2.2 ± 0.2 m/s) and peripheral (n = 55; mean CV = 3.4 ± 0.3 m/s) CVs in adult mice TG.

ganglia and between 110 and 180 MΩ in neonatal ganglia. The higher-resistance electrodes in neonates were required to minimize impalement injury. Recordings were obtained using an Axoclamp-1A amplifier (Axon Instruments, Union City, CA). Voltage and current records were stored on videotape and/or digitized (sampling rate 5–28 kHz) with a 1401 A-D converter (Cambridge Electronic Design, Cambridge, UK) and stored in a PC. Data were accepted only if the evoked AP had an amplitude of ≥60 mV, measured from resting membrane potential to the peak overshoot.

Parameters measured and data analysis

The following electrophysiological parameters were measured: resting membrane potential (V_m), input resistance (R_in), and membrane time constant (τ). R_in was calculated from the slope of the peak current-voltage (I-V) relationship, following injection of hyperpolarizing current pulses (duration, 100–150 ms; Fig. 1, C and D). The value of τ was obtained from the single exponential fit to the onset phase of a small hyperpolarizing voltage response. The membrane capacitance (C) was estimated using the relation: τ = R_in × C. A rectification index in the voltage response to hyperpolarizing pulses was calculated according to the relationship: [(R_in at peak) – (R_in at steady state)]/R_in at peak] × 100. Neurons showing a rectification index higher that 5% for current pulses that reached a peak voltage between −100 and −120 mV were considered as “rectifying” neurons. The following parameters of the AP were measured (Fig. 1, A and B): AP amplitude, AP overshoot, AP duration at 50% amplitude, AP repolarization time at 90% of amplitude, AP maximum rate of rise (dV/dr max), AP maximum rate of fall (dV/dr min), amplitude of the afterhyperpolarization (AHP), and duration of the AHP at 50% of maximal amplitude. The pattern of AP discharge to long (100–150 ms) depolarizing current pulses was also analyzed (Fig. 1C). Neurons were tested at a constant intensity value twice AP threshold.

Records were analyzed using a commercial software package (Cambridge Electronic Design) and custom built in-house software. Data are presented as means ± SE. Statistical comparisons were made using Student’s t-test for means when the distribution was normal and Mann-Whitney Rank Sum Test (MWR) when the distribution was not normal. We used the Z test for comparison of proportions (Sigmastat 2.0; Jandel Scientific Software, Erkrath, Germany). One-way ANOVA was used to analyze possible differences between subpopulations of adult neurons.

RESULTS

Intracellular recordings were obtained from 172 TG neurons of 33 mice. According to the age of the animal, neurons were separated in two groups: neonatal neurons (ages P0–P7; n = 37) and adult neurons (ages P21–P50; n = 135).

Functional types of neurons

NEONATAL NEURONS. The conduction velocity (CV) of neonatal TG neurons was low with a mean of 1.1 ± 0.14 m/s (range from 0.27 to 3 m/s, n = 18) in the peripheral axon and 1.2 ± 0.16 m/s in the central CV (n = 29), possibly reflecting a lack of myelination at this stage. The distributions of axonal CVs were unimodal (Fig. 2A). It should be pointed out that peripheral and central branches of excised TG were very short in neonatal animals (<3 mm and 2 mm, respectively), hampering a reliable estimate of the CV.

Nevertheless, two separate populations of TG neurons were clearly discernible in neonatal animals based on the shape of the AP (Gallego and Eyzaguirre 1978; Yoshida and Matsuda 1979): neurons with a hump or inflexion in the repolarizing phase of the AP (S-type neurons, Fig. 3A) and neurons without a hump in the repolarizing phase of the AP (F-type neurons, Fig. 3B). The differentiated records of the AP (Fig. 3, A and B, A: top: TG neuron exhibiting a long-duration action potential with a strong inflexion on the repolarization phase (S-type neuron); bottom: differentiated record of the action potential showing a 2nd negative peak. B: top: neuron with short-duration action potential with no inflexion (F-type neuron); bottom: differentiated record of the spike without a second, delayed negative peak. Action potentials in A and B were evoked by electrical stimulation from the peripheral branch (maxillary) of the trigeminal nerve. Both neurons had high R_in (A, 225 MΩ; B, 215 MΩ). - - - , 0 potential level.

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Passive membrane properties and rectification of adult and neonatal mice trigeminal neurons

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<th>TABLE 1. AP and AHP properties of adult and neonatal mice trigeminal neurons</th>
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Means ± SE are given for each parameter. In the case of percent comparisons, the Z test was used. Other parameters as in Table 1. **AHP**: amplitude of the afterhyperpolarization (AHP). The AP duration was measured at half amplitude. To compare the means of different parameters in the 4 subgroups of adult neurons (Aδ F type, C F type, Aδ S type, and C S type) the Kruskall-Wallis one-way analysis of variance on ranks test was used. In this case, differences between subgroups are indicated on the bottom row, labeled overall P. The absence of symbols indicates the lack of significant differences (P > 0.05). Differences between adult and neonatal F- and S-type neurons are indicated in the text and Figs. 6 and 7. **AP duration**, maximal rate of rise; **AP overshoot**, maximal rate of fall. ‡ and ‡‡, P < 0.05 and P < 0.001, respectively, for differences (t-test) between F- and S-type neurons in adults and neonates.

**bottom** were used to evidence the presence or absence of the hump, which appeared as an additional negative component in the time course of the AP derivative. The chance of encountering either type of AP was about equal in neonatal TG, 51% (19/37) of the neurons were of the F-type, whereas 49% (18/37) were S-type neurons.

The classification of neonatal TG neurons into S and F type had some predictive value in the sense that electro-physiological properties co-segregated with the shape of the AP. All active properties measured, except the amplitude of the AHP, were different between S and F neurons (Table 1). APs of S-type neurons had a significantly longer duration (P < 0.001), a larger amplitude, and overshoot (P < 0.05). The maximal rate of AP depolarization (P < 0.05) and repolarization (P < 0.001) were also slower than in F-type neurons. The duration of the AHP that follows an AP was almost twice as long in S-compared with F-type neurons (P < 0.001, MWR test). In contrast to the marked differences in AP shape and AHP duration, a distinction between S and F neurons of neonatal mice based on passive membrane properties was less clear. As summarized in Table 2, S neurons had slower membrane time constant (P ≤ 0.05) than F neurons, but the estimates of average input resistance and membrane capacitance were similar in the two groups. These data suggest that size differences are not marked between both classes of neonatal neurons. The rectification index was also similar (Table 2).

**ADULT NEURONS.** In contrast to neonatal TG neurons, peripheral and central CV values of adult TG neurons showed a broader distribution (ranging from 0.24 to 8 m/s) that extended into the range typical of myelinated axons (Fig. 2B). For individual neurons, a good correlation (R² = 0.69) was found between their central and peripheral CVs. The slope of this relationship was close to 0.53, the peripheral CV being always faster (data not shown). Neurons with peripheral CV <1.4 m/s or central CV <1 m/s were classified as unmyelinated (C neurons), whereas those with peripheral CV >1.4 m/s or central CV >1 m/s were considered thin myelinated (Aδ neurons). Seventy percent (95/135) of adult TG neurons were classified as Aδ and 30% (40/135) as C neurons.

In adult TG, it was also possible to record neurons with and without a hump in the AP and thus classify them as S- and F-type neurons. The percentages of F- and S-type neurons were 39% (52/135) and 61% (83/135), respectively. As was the case for neonatal neurons, adult F- and S-type neurons were clearly distinct electrophysiologically. Thus all active properties measured...
sured, except the amplitude of the AHP, were different between the two groups (Table 1). APs of S-type neurons had a significantly longer duration ($P < 0.001$), a larger amplitude and overshoot ($P < 0.001$). The maximal rate of AP depolarization ($P < 0.001$) and repolarization ($P < 0.001$) was also slower than in F-type neurons. The duration of the AHP that follows an AP was fivefold longer in S- compared with F-type neurons ($P < 0.001$, MWR test). As was the case for neonates, there were no marked differences between passive properties of F- and S-type neurons (Table 2).

In adult ganglia, S- and F-type neurons could be further subdivided according to their conduction velocity into slow conducting (C type) and faster conducting (Aδ) neurons. Figure 4 summarizes graphically the changes in peripheral CV of F- and S-type neurons during postnatal development. The mean peripheral CV of all F-type neonatal neurons was $1.3 \pm 0.24$ m/s ($n = 10$). During postnatal development most F-type neurons became faster conducting. Thus 90% of adult F-type neurons had CVs in the Aδ range (mean: $4.6 \pm 0.38$ m/s, $n = 21$ peripheral CV and $3.5 \pm 0.24$ m/s, $n = 31$ central CV). The remaining 10% had CVs in the C range ($1.2 \pm 0.11$ m/s, $n = 2$ peripheral CV and $0.6 \pm 0.11$, $n = 4$ central CV; Fig. 7A). In contrast, adult S-type neurons belong in nearly equal proportion to the Aδ (58%, 48/83) or the C neuron (42%, 35/83) group (Fig. 6A).

A plot of AP duration against peripheral CV showed a distinct clustering of neurons (Fig. 5). Neurons with CV < 1 m/s were all S type. All C S-type neurons had long-duration APs (> 1.9 ms). Ninety-two percent of neurons with AP < 0.75 ms had CV > 2 m/s, and of those neurons fulfilling these two criteria, 82% (18/22) were F type. Between these two groups spread most S-type neurons conducting within the Aδ range. There was a weak negative linear correlation between AP duration and axonal CV ($r^2 = 0.23$) when considering all subtypes of cells but this correlation disappeared within each of the individual subgroups of Fig. 5.

Changes in electrophysiological properties with age

The percentage of S- and F-type neurons was compared between neonatal and adult mice. Neither the percentage of S-type neurons (49% in neonatal, 61% in adult) nor of F-type neurons (51% in neonatal, 39% in adult) changed significantly during postnatal development ($P = 0.365$, Z test), suggesting that these phenotypes are already established and invariant at the early postnatal period. However, some properties of the AP changed in S- and F-type neurons during development (see following text).

ACTION POTENTIAL AND PASSIVE MEMBRANE PROPERTIES. S-type neurons. Figure 6A shows the changes taking place in central and peripheral CV of S-type neurons during postnatal development. The mean peripheral CV of type S neonatal neurons was $0.9 \pm 0.15$ m/s ($n = 8$). In adults, this population segregated into two subgroups with different CVs: a group in the Aδ

![Diagram showing typical changes in TG AP duration and CV with age.](http://jn.physiology.org/)

FIG. 4. Postnatal changes in AP shape of S and F-type neurons vary according to mature CV. Diagram showing typical changes in TG AP duration and CV with age. In brackets is shown the mean CV ± SE for each subgroup. Neonatal mice TG neurons (left) were classified according to AP shape. Adult mice TG neurons (right) were classified according to AP shape and CV. The majority of F-type neurons in adult mice are fast conducting (Aδ, top right), only few F-type neurons conducted in the C range (2nd from top right). The CVs of adult S-type neurons were more heterogeneous than F-type neurons.

FIG. 5. Segregation of TG neurons from adult animals into subgroups according to their peripheral CV and AP duration. Plot of AP duration against CV velocity in adult TG neurons. Neurons with AP-duration > 2 ms and peripheral CV < 1 m/s form a group (C S type, ▲) clearly different from neurons with AP < 0.75 ms and CV > 2 m/s (Aδ F type, ○). Between these 2 groups, one finds preferentially S-type neurons conducting in the Aδ range (●). These group had APs with a broad range of duration (> 0.75 ms and < 3.5 ms). The AP duration was measured at half amplitude.
Moreover, conducting in the A range of neurons conducting in the C range; as shown in Table 2, changes were more pronounced in Table 1. During postnatal development, the changes in the AP and AHP of F-type neurons during maturation are summarized in Fig. 7. The AP duration in the majority (those in the Aδ range) of F-type neurons decreased with age (P < 0.001; Fig. 7B; Table 1). This reduction was explained by a faster rate of AP depolarization and repolarization (P < 0.001; Fig. 7B; Table 1). In contrast to S-type neurons, the AHP duration in F-type neurons did not change significantly during postnatal development, particularly in Aδ neurons, which represent a large majority of this population of TG cells (Fig. 7D; Table 1).

Passive properties changed markedly in type F neurons but only in those neurons that became myelinated. Thus mean R_m decreased (P < 0.05) in adult Aδ neurons but remained similar in adult C neurons (Table 2). However, the average τ and membrane capacitance of Aδ F-type neurons was higher (P < 0.05) than in neonatal F-type neurons.

INWARD RECTIFICATION. About one-third (11/35) of neonatal neurons displayed time-dependent inward rectification in response to the injection of a negative current pulse. This rectification is presumably due to activation of the I_h current (Mayer and Westbrook 1983; Scroggs et al. 1994). Inward rectification was abolished by 1 mM extracellular Cs⁺ (data not shown). The incidence of inward rectification among F- and S-type neonatal neurons was similar (26% of F neurons, 37% of S neurons, P = 0.714, Z test). The size of this rectification, computed as a rectification index (see METHODS), was also similar (22 ± 4.6% for F neurons, 29 ± 9.5% for S neurons, P = 0.898, Z test; Table 2).

We also examined changes in passive electrical properties. As shown in Table 2, changes were more pronounced in neurons that became myelinated. Thus mean R_m in S neurons did not change significantly in the subgroup of adult C neurons but was markedly reduced in the Aδ group (P < 0.001; Table 2). Moreover, τ was slower in both, Aδ and C. The mean capacitance (C) was significantly higher in adult Aδ neurons (P < 0.05; Table 2) but did not change significantly with age in C neurons.

F-type neurons. Figure 7A shows the changes taking place in central and peripheral CV of F-type neurons during maturation. As already mentioned, almost all F-type increased their CV postnatally. The changes in the AP and AHP of F-type neurons during maturation are summarized in Fig. 7. The AP duration in the majority (those in the Aδ range) of F-type neurons decreased with age (P < 0.001; Fig. 7B; Table 1). This reduction was explained by a faster rate of AP depolarization and repolarization (P < 0.001; Fig. 7B; Table 1). In contrast to S-type neurons, the AHP duration in F-type neurons did not change significantly during postnatal development, particularly in Aδ neurons, which represent a large majority of this population of TG cells (Fig. 7D; Table 1).

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In adult animals, time-dependent inward rectification (IR) was present in about half of the neurons (62/134), with no significant differences between F- (56%, 29/52) and S-type neurons (40%, 33/83; \( P = 0.10, Z \) test). IR was more frequent in F-type neurons of adult animals (56%) than in F-type neurons of neonates (26%; Table 2, \( P < 0.05, Z \) test). Moreover, in adult neurons the incidence of IR was tightly linked to the process of myelination. Thus IR was present in 62% of adult Aδ F-type neurons while none of the C F-type neurons had this property (Table 2). In the case of S-type neurons, these percentages were 56% for Aδ neurons and only 18% for C neurons (\( P = 0.001, Z \) test).

**DISCHARGE PATTERN.** TG neurons responded to the intracellular injection of a long (100–150 ms) depolarizing current pulse either with a single AP (phasic response) or with a repetitive discharge of APs (tonic response). Phasic responses were present in 75% (18/24) of neonatal neurons tested, whereas the rest responded tonically. The proportion of neonatal F- and S-type neurons exhibiting a tonic response was similar (4/14 F neurons; 2/10 S neurons; \( P = 0.981, Z \) test). Tonic neurons were more excitable than phasic neurons because they required lower current values to fire APs. In tonic neurons, the rheobase values were 0.3 ± 0.18 nA (\( n = 4, R_\text{in} = 194 \pm 45 \text{ MΩ} \)) for F-type neurons and 0.17 ± 0.17 nA (\( n = 2, R_\text{in} = 265 \pm 195 \text{ MΩ} \)) for S-type neurons. In phasic neurons, the thresholds were 0.9 ± 0.58 nA (\( n = 10, R_\text{in} = 124 \pm 27 \text{ MΩ} \)) and 0.9 ± 0.19 nA (\( n = 8, R_\text{in} = 170 \pm 45 \text{ MΩ} \)) for F- and S-type neurons, respectively (\( P < 0.05, t\)-test). These differences in threshold were very clearly linked to the discharge pattern and not to the shape of the action potential because mean threshold currents were not different (\( P = 0.78 \)) in F-type neurons (0.7 ± 0.15 nA, \( n = 14 \)) compared with S-type neurons (0.8 ± 0.12 nA, \( n = 10 \)).

In adult mice, TG neurons tonic discharges were very infrequent, corroborating the findings of Puil and Spigelman (1988) in guinea pig. Only 8% (5/63) of adult neurons tested fired repetitively in response to long depolarizing pulses. All neurons firing tonically were C neurons of the S type that lacked IR in response to hyperpolarizing pulses. In marked contrast to the neonate, all adult F-type neurons discharged phasically. As was the case for neonatal neurons, threshold currents required to evoke an AP were lower in tonic compared with phasic cells (0.4 ± 0.49 nA, \( R_\text{in} = 206 \pm 36 \text{ MΩ} \), \( n = 5 \) vs. 1.0 ± 0.63 nA, \( R_\text{in} = 101 \pm 10 \text{ MΩ} \), \( n = 67; P < 0.05 \)). Although the proportion of neurons discharging tonically was higher in neonatal than in adult neurons (6/24 vs. 5/63, respectively) differences among both groups did not reach the significance level (\( P = 0.074, Z \) test).

**EFFECTS OF LOW Ca\(^{2+}\) ON THE AP.** During development, a marked change in the sensitivity of the AP to reductions in external Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_{\text{in}}\)) and to application of Co\(^{2+}\) were noticed. These results are summarized in Fig. 8 for the different types of neurons. In neonatal TG neurons, somatic APs of both S- (\( n = 2 \)) and F-type (\( n = 6 \)) neurons were highly sensitive to reductions in [Ca\(^{2+}\)]\(_{\text{in}}\). In all neurons tested (\( n = 8 \)), the AP amplitude decreased significantly on reduction in [Ca\(^{2+}\)]\(_{\text{in}}\) (82 ± 1.7 mV in control vs. 40 ± 11.3 mV in 250 μM Ca\(^{2+}\); \( P < 0.05, t\)-test; Fig. 8, A and C). The sensitivity of the AP to low external Ca\(^{2+}\) appears to be restricted to the first postnatal week because no effect was observed in neurons

**FIG. 8.** Effect of low external calcium (250 μM) or cobalt (2.5 mM) on the AP of trigeminal neurons. For each panel, the axonally evoked AP recorded in control solution is shown in black, whereas the AP recorded in low Ca\(^{2+}\) or cobalt solution is shown in gray. The AP amplitude of S- and F-type neonatal neurons was strongly reduced by low-Ca\(^{2+}\) solution (A and C) or Ca\(^{2+}\) substitution with Co\(^{2+}\) (E and G). In contrast, APs of S- and F-type neurons from adult mice were not reduced in amplitude by extracellular calcium reduction (B and D) or Ca\(^{2+}\) substitution with Co\(^{2+}\) (F and H). The only effect of low Ca\(^{2+}\) or Co\(^{2+}\) substitution in adult TG neurons was a reduction in the hump of S-type neurons.
tested at age P10 (n = 3, data not shown). In contrast to results in immature neurons, low [Ca\textsuperscript{2+}]\textsubscript{i} had no effect on the AP (and AHP) of adult type-F neurons (n = 7; Fig. 8B).

In adult S-type neurons (n = 3), the mean duration of the AP was shortened slightly (1.3 ± 0.4 vs. 1.0 ± 0.1 ms, P = 0.484) due to a reduction of the hump but the mean amplitude (97 ± 2.0 vs. 100 ± 2.2 mV, P = 0.400, MWR test) was also unaffected (Fig. 8D).

Similar effects were observed after replacing Ca\textsuperscript{2+} (2.5 mM) with the inorganic Ca\textsuperscript{2+}-channel blocker Co\textsuperscript{2+}. Changes in the AP evoked by axonal stimulation in four neonatal S-type and three neonatal F-type neurons were pronounced (Fig. 8, E and G). The AP amplitude decreased from 84 ± 3.3 mV in control to 58 ± 9.1 mV in Co\textsuperscript{2+} (P < 0.05, n = 7). It is unlikely that these effects are due to nonspecific membrane actions: in neonatal mice, Co\textsuperscript{2+} replacement caused only a modest depolarization (1.8 ± 1.1 mV, n = 7) and had no significant effect on R\textsubscript{in} and τ (n = 3). In adult mice, the effects of Co\textsuperscript{2+} substitution on the AP were different depending on the type of neuron. In F-type neurons (n = 20, all A6 neurons), 2.5 mM Co\textsuperscript{2+} did not produce changes in V\textsubscript{m}, AP, or AHP properties (Fig. 8F; AP amplitude: 80 ± 2.9 vs. 77 ± 4.7 mV in cobalt, P = 0.451; AP duration: 0.6 ± 0.1 vs. 0.7 ± 0.1, P = 0365, MWR test). In S-type neurons (2 C and 21 Aδ), the AP duration and the AHP duration were significantly reduced with Co\textsuperscript{2+} (1.5 ± 0.2 vs. 1.1 ± 0.2 ms and 26.8 ± 8.8 vs. 7.1 ± 1.8 ms, respectively, P < 0.05), but the AP amplitude was unaffected (94 ± 1.9 vs. 92 ± 2.9 mV; Fig. 8H). In these neurons, R\textsubscript{in} and τ were not modified by Co\textsuperscript{2+} (n = 15).

**TTX Sensitivity of the AP.** APs evoked by nerve stimulation (peripheral or central branch) were always abolished by bath application of TTX (100 nM) in neonatal (n = 11) and adult (n = 12) TG neurons (data not shown). Additionally, in 9 of 11 neonatal neurons (type S or F), TTX blocked the AP evoked by depolarizing current pulses injected into the soma (Fig. 9A).

The AP of the two remaining neurons was resistant to TTX. One of these neurons exhibited tonic activity, which persisted in TTX (Fig. 9B). In adult TG neurons, some APs were totally blocked by bath application of TTX in 8 of 12 neurons, all of them Aδ (4 F-type and 4 S-type neurons; Fig. 9C). In three additional Aδ neurons (all F type), the somatic AP was blocked only partially (not shown). Finally, in one neuron (C type), the AP was slightly reduced by 100 nM TTX (Fig. 9D).

**DISCUSSION**

This report describes in detail changes in the electrophysiological properties of cranial primary sensory neurons of mice during postnatal development. To prevent alterations in cellular phenotype that may take place in sensory neurons when maturation is studied under culture conditions, a preparation consisting of acutely excised TG of mice superfused in vitro was employed.

The results show that at the moment of birth, TG neurons were already electrophysiologically diverse. During the first 3 wk of postnatal development, further changes in conduction velocity and passive and active membrane properties take place, leading to an heterogeneous population of unmyelinated and myelinated primary sensory neurons characteristic of adult animals (Djouhri et al. 1998). Furthermore, some of the electrophysiological changes appear to be strongly linked to the process of myelination because they developed only in those neurons that increased their axonal conduction velocity. The functional variety of sensory neurons at birth suggests that the specification of some properties takes place early, before myelination but after TG neurons have reached their peripheral targets.

Previous morphological studies have shown that, at birth, most sensory axons are unmyelinated but become myelinated during the first three postnatal weeks (Peters and Muir 1959). In agreement with this and with previous electrophysiological studies in maturing DRG neurons (Fitzgerald 1987; Fulton 1987), conduction velocity of TG neonatal neurons was low and increased with age to reach maximum values of ~8 m/s in the adult animal. In vivo investigations in adult cat pulpal afferents have reported the presence of fast-conducting Aβ fibers (Cadden et al. 1983). We did not find trigeminal fibers in this conduction range. This may be caused by species differences or to an experimental bias: due to the small size of the peripheral root in the mouse trigeminal nerve in vitro preparation (~10 mm), fast conducting fibers could be easily lost, buried in the stimulus artifact.

The existence of different functional types of primary sen-
sory neurons in newborn rodents was suggested by the morphological studies of Lawson (1979) and Coggeshall et al. (1994). They described two subpopulations of neonatal DRG neurons in the mouse and rat with different diameter and growing rates: small-dark cells that arrested their soma growth already at P10 and larger-lighter cells that continued their development until P20. Moreover, recordings from rat cutaneous primary afferents have shown that at P0, primary sensory neurons already differ in the transduction properties of their peripheral endings in spite of the absence of differences in conduction velocity (Fitzgerald 1987). The shape of the AP has been used to distinguish type S and F neurons as two functional types of adult primary sensory neurons (Belmonte and Gallego 1983; Gallego and Eyzaguirre 1978; Koerber et al. 1988; López de Armentia et al. 2000). In the present work, S- and F-type neurons were found in the trigeminal ganglion at birth in about equal numbers. This proportion did not vary during postnatal development, indicating that the developmental mechanism underlying AP shape specification are established early in development and do not change appreciably throughout the maturation process. However, the present work also shows that some membrane properties of F and S neurons evolve differently postnatally, presumably due to a variable expression of ion channels in the membrane (Fedulova et al. 1991; Gallego and Eyzaguirre 1978).

A vast majority of F neurons in the adult trigeminal ganglion belong to the Aδ type, suggesting that most neonatal F neurons, which had relatively slow conduction velocity values, became myelinated during maturation. Their AP duration decreased during this process in parallel to augmented rates of AP depolarization and repolarization perhaps due to an enhanced expression of Na+(Ogata and Tatebayashi 1992) and K+ channels (Seifert et al. 1999), respectively. Also, with postnatal development Rm became comparatively lower and membrane capacitance larger in F Aδ neurons, probably reflecting an increase in cell soma surface with maturation (Lawson et al. 1974). In contrast, AHP amplitude and duration was similar in neonatal and adult F neurons, which suggests that in this type of neurons the ionic mechanisms supporting the AHP were already established at the moment of birth. Less than 10% of adult F neurons remained unmyelinated. These neurons seem to represent a truly different population within F neurons and have been observed in the guinea pig trigeminal ganglion also (Cabanes, unpublished data). In adult animals, all F neurons showing inward rectification were myelinated, which strongly suggests that F neurons displaying IR in early stages of postnatal development will become F myelinated neurons in the adulthood.

The present observations also indicate that the evolution of membrane properties of S-type neurons with age is more heterogeneous than in F neurons, and these differences were apparently associated with myelination. Compared with neonatal neurons, mature S neurons conducting in the Aδ range displayed a shorter duration AP attributable to faster depolarization and repolarization rates. A longer AHP was also noticeable in the group of adult S neurons. The changes in AP could be associated to increased expression of K+ currents during postnatal development in the TG neurons that became myelinated. Also, the comparatively reduced Rm together with the larger C observed in adult S Aδ neurons suggests that in the population of S neurons that develop a myelin sheath, the soma membrane surface increased during postnatal development as was the case for F Aδ neurons. Contrarily, S neurons that remained unmyelinated showed similar Rm C, and v values with age, suggesting that their cell body size stayed constant. In this group of adult S neurons of the C type, the duration of the AP was longer and with a more prominent hump than in neonatal cells. The AHP was also more prolonged. Increases in AP duration have been reported in DRG neurons of adult rats exposed to high nerve growth factor levels (Ritter and Mendell 1992). It could be speculated that variations during development of AP duration in Aδ and C neurons of the S type may be due to variable expression of trkA receptors in each subpopulation of cells (Bennet et al. 1996; Mu et al. 1993).

The marked changes in Ca2+ sensitivity of TTX-sensitive APs in F- and S-type neurons during maturation suggests that neither express their full complement of mature properties at birth. At present, we are unable to assign a functional value to this observation, but the new findings partially contradict the proposal of Fitzgerald and Fulton (1992). According to these authors, C fibers would not undergo any further postnatal maturation. In unpublished experiments, we have found that changes in Ca2+ sensitivity of the AP are regulated by the expression of the neurokinin 1 receptor (Cabanes, unpublished results).

In our experiments, a high percentage of neonatal TG neurons had their AP abolished by TTX. These data agree with those obtained by Fedulova et al. (1991) in rat DRG, showing that at birth most neurons already express TTX-sensitive sodium current. In contrast, others authors found TTX-resistant (TTXr) Na+ currents in ~40% of neonatal DRG neurons (Nobukuni and Hideharu 1992), and Roy and Narashis (1992) reported that TTXr Na+ current expression was strongly age dependent: it was 100% at ages P5 and only 10% at ages >P11. In this last study, the high percentage of TTXr Na+ currents in young animals may be due to the use of cultured neurons. In contrast, we and Fedulova et al. (1991) performed experiments on acutely excised ganglia.

Altogether, the present data show that maturational processes in mouse primary sensory TG neurons were not completed at the moment of birth. The evolution with age of TG neurons electrophysiological properties indicates that myelination, occurring in most if not all F neurons and in a part of S neurons, tend to reduce AP duration in both types of cells. The acquisition of a myelin sheath was also accompanied by changes in those passive membrane properties associated with a parallel increase in the surface area of the soma. Changes in other membrane properties like AHP duration or discharge pattern did not appear to be directly correlated with the myelination process and may be linked to the development of a functional specialization of adult neurons. The variable stage of maturation of primary sensory neurons at birth should be taken into consideration when comparing results obtained in intact and cultured neonatal neurons with those of adult animals.

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Present address of M. López de Armentia: Div. of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia.

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