TEMPORAL SEQUENCE OF CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF OCULOMOTOR MOTONEURONS DURING POSTNATAL DEVELOPMENT

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Abstract—The temporal sequence of changes in electrophysiological properties during postnatal development in different neuronal populations has been the subject of previous studies. Those studies demonstrated major physiological modifications with age, and postnatal periods in which such changes are more pronounced. Until now, no similar systematic study has been performed in motoneurons of the oculomotor nucleus. This work has two main aims: first, to determine whether the physiological changes in oculomotor nucleus motoneurons follow a similar time course for different parameters; and second, to compare the temporal sequence with that in other neuronal populations. We recorded the electrophysiological properties of 134 identified oculomotor nucleus motoneurons from 1 to 40 days postnatal in brain slices of rats. The resting membrane potential did not significantly change with postnatal development, and it had a mean value of −61.8 mV. The input resistance and time constant diminished from 82.9–53.1 MΩ and from 9.4–4.9 ms respectively with age. These decrements occurred drastically in a short time after birth (1–5 days postnatally). The motoneurons’ rheobase gradually decayed from 0.29–0.11 nA along postnatal development. From birth until postnatal day 15 and postnatal day 20 respectively, the action potential shortened from 2.3–1.2 ms, and the medium afterhyperpolarization from 184.8–54.4 ms. The firing gain and the maximum discharge increased with age. The former rose continuously, while the increase in maximum discharge was most pronounced between postnatal day 16 and postnatal day 20. We conclude that the developmental sequence was not similar for all electrophysiological properties, and was unique for each neuronal population. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: postnatal development, oculomotor system, motoneurons, slice preparation, rat.

Studies on the temporal sequence of postnatal changes in the electrophysiological properties of motoneurons (Mns) using the in vitro brain slice preparation are rather scarce. Physiological studies of Mns have been carried out using this procedure within hypoglossal (Berger et al., 1996; Greer and Funk, 2005; Nunez-Abades et al., 1993; Viana et al., 1994, 1995), facial (Aghajanian and Rasmussen, 1989; Magarinos-Ascone et al., 1999), oculomotor (Guertaud, 1988; Tsuzuki et al., 1995), and abducens (Russier et al., 2003) brainstem nuclei, and in some spinal motoneuronal populations (Fulton and Walton, 1986; Ziskind-Conhaim, 1988). With the exception of the Mns within the hypoglossal nucleus, the reported data come from neonatal or adult Mns, and lack a comparison of the physiological data over the course of postnatal development. In the hypoglossal nucleus, the physiological properties of the neonatal and adult Mns have been compared (Viana et al., 1994, 1995), while in genioglossal Mns, the changes in such properties along postnatal development have been systematically studied (Nunez-Abades et al., 1993). The comparison between neonatal and adult Mns has demonstrated remarkable differences in input resistance, time constant, rheobase, action potential, medium afterhyperpolarization (mAHP), and firing rate (Viana et al., 1994, 1995). Moreover, in genioglossal Mns, postnatal periods have been reported during which the changes in the abovementioned electrophysiological parameters are statistically significant (Nunez-Abades et al., 1993). Consistent with these findings, marked physiological differences along postnatal development have also been reported in other neuronal populations belonging to the tractus solitarius (Vincent and Tell, 1999), vestibular (Murphy and du Lac, 2001), trigeminal (Cabanes et al., 2002; Guido et al., 1998), pre-Botzinger (Elsen and Ramirez, 2005), lateral lemniscus (Ahuja and Wu, 2000), and accumbens (Belleau and Warren, 2000) nuclei, and in those of thalamic (Perez-Velazquez and Carlen, 1996; Metherate and Aramakis, 1999) and cortical areas (Zhou and Hablitz, 1996).

The oculomotor system has been extensively studied (Scudder et al., 2002; Sparks, 2002) to elucidate how eye movements are coded, and for a better understanding of synergies with other skeletal motor systems (Grantyn et al., 2004; Isa and Sasaki, 2002). In particular, the physiological properties of Mns within the motor nuclei that generate eye movements have been characterized in both chronic and acute preparations (Grantyn and Grantyn, 1978; Delgado-Garcia et al., 1986; Fuchs et al., 1988; de la Cruz et al., 1989; Durand, 1989; Stahl and Simpson, 1995; Sylvestre and Cullen, 1999). One of the most notable characteristics of these Mns is that they reach a high firing rate to lead saccadic eye movement. Current knowledge of the Mns within the oculomotor nucleus (OCM) in the slice preparation is restricted to neonatal (Tsuzuki et al., 1995) or adult (Guertaud, 1988) periods. Furthermore, comparison of neonatal and adult electrophysiological data in
OCM Mns demonstrates marked modifications with postnatal development (Carrascal et al., 2005). However, until now there has been no quantification of the postnatal sequence of maturation in the electrophysiological changes in the OCM Mns. This study proposes to answer two main questions: Is the temporal sequence of changes with postnatal development similar for the different physiological properties? If changes were to be found in this neuronal population, would they follow the same time course as those reported in genioglossal Mns or other neuronal populations?

**EXPERIMENTAL PROCEDURES**

**Surgery and solutions**

Experiments were carried out in Wistar rats (5–140 g) of both sexes, from 1 to 40 days postnatal (P1–P40). The experiments were performed in accord with Directive 86/609/CEE of the European Community Council, the Spanish Real Decreto 223/1988, and Seville University regulations on laboratory animal care. All efforts were made to minimize the number of animals used and their suffering. Rats were anesthetized with sodium pentobarbital (50 mg/kg), and quickly decapitated. The brainstem was removed from the skull and placed in a dish filled with ice-cold sucrose–artificial cerebrospinal fluid (ACSF). The midbrain was glued to a Teflon block, submerged in a chamber filled with ice-cold sucrose–ACSF, and transversely sectioned at 300 μm. Slices including OCM were firstly incubated in a chamber containing cold sucrose–ACSF for 35–45 min, and then transferred to a second chamber containing ACSF at a temperature of 21 ± 1 °C. Slices were allowed to recover for more than one hour before any recording. Single slices were finally transferred to the recording chamber and superfused at 2 ml/min (MPII, Harvard Apparatus, Holliston, MA, USA) with ACSF bubbled with 95% O2–5% CO2 (pH 7.4; 21 ± 1 °C). The composition of ACSF was as follows (data are in mM): 126 NaCl, 2 KCl, 1.25 NaH2PO4, 26 NaHCO3, 10 glucose, 2 MgSO4, and 2 CaCl2. For sucrose/ACSF solution, the 126 NaCl was substituted by 240 mM sucrose.

**Electrophysiological recordings and analysis**

Glass micropipettes were visually placed in the region of the OCM nuclei by a bipolar microelectrode made of stainless steel wire of 25 μm, insulated in glass micropipettes whose tips were about 75 μm apart. This microelectrode was placed in the nerve at about 2 mm from the nucleus. The micropipettes used for recordings were filled with a 3 M KCl (40–70 MΩ) solution.

All Mns included for analysis showed a stable resting membrane potential of −55 mV or more negative, an action potential larger than 60 mV, and fired repetitively in response to depolarization pulses of one second. Several parameters were analyzed: resting membrane potential, input resistance, time constant, rheobase, depolarization voltage, duration and amplitude of the action potential, duration and amplitude of the mAHP, and firing rate. Figs. 2–6 illustrate the raw data (open symbols) for the different studied parameters along the postnatal period. Filled symbols in these figures indicate the mean values of the data grouped as follows: first (P1–P5), n=28; second (P6–P10), n=27; third (P11–P15), n=29; fourth (P16–P20), n=24; and fifth (P20–P40), n=26. The duration in days for the age groups was similar to that reported in hypoglossal nucleus (Nunez-Abades et al., 1993; Viana et al., 1994), thereby enabling a comparison between the two motoneuronal pools, as well as with neurons in other nuclei (see Introduction).

Resting membrane potentials were measured as the difference between the intracellular and extracellular potentials after withdrawing the recording electrode from the cell. Input resistance was determined by passing through the microelectrode a series of negative current pulses (500 ms duration, 1 Hz) with 0.1 nA increments, and storing both the injected pulses and the voltage membrane response. Input resistance was calculated off-line as the slope of the voltage–current plot. When there was evidence of an inward rectification or sag, the voltage value used for this latter plot was that at the peak (for details see elsewhere, Carrascal et al., 2005). The membrane time constant was obtained by recording the membrane response to 0.1 nA hyperpolarizing current pulses (500 ms duration, 1 Hz) and fitting the averaged record of 60 sweeps to a single exponential curve (see Fig. 2E).

The rheobase was the minimum current amplitude injected (50 ms pulse duration at 1 Hz) that generated action potentials in 50% of the cases. The depolarization voltage was the increment in membrane potential required to bring the cell to the action potential threshold. To determine the spike threshold, the action potential recording was differentiated; spike onset was the value of the membrane potential at which the first derivative surpassed 10 V/s. Single action potentials were evoked by depolarizing current pulses of 100 μs. Spikes were averaged (eight sweeps) to measure their characteristics: amplitude and duration. Amplitude was the voltage increment between the resting level and spike peak voltage. The value of the duration of the action potential was determined as the width of the spike at its half amplitude (see also Nunez-Abades et al., 1993). In addition, the slopes of the rising and falling phases of the action potential were measured. The averaged action potential was also used to determine the amplitude and duration of the mAHP. The amplitude was the voltage increment between the resting membrane potential and the most negative value reached during the AH. The duration was calculated as the time spent from the intersection of the repolarizing phase of the action potential with the resting membrane potential to the recovery of the resting level (Fig. 5A). The repetitive firing rate was evoked by depolarizing current pulses (1 s, 0.5 Hz) with 0.05 nA increments. The instantaneous frequency was calculated as the reciprocal of interspike interval duration. For each current pulse, the instantaneous frequency was plotted along the maintained depolarization (Fig. 6B). The steady-state firing frequency was the average of the instantaneous frequencies during the last 500 ms of the pulse. For each Mn, the relationship between the steady-state firing frequency and injected current was represented (F/I plot) to calculate the slope, termed frequency gain. The maximum firing rate was defined as the highest frequency achieved by Mns before they began to fail in the discharge.

All statistical analyses were carried out on the raw data. Taking together the data of all age groups for each parameter, first we determined if there were significant differences along development, using a non-parametric Kruskal-Wallis analysis of variance. If there were significant differences, then a non-parametric Mann-Whitney U test between adjacent age groups was applied (i.e. first versus second age groups; second versus third age groups...). In figures and Table 1, the asterisks indicate the statistical significance between adjacent age groups as follows: P<0.05 (*), P<0.01 (**), and P<0.001 (***)

**RESULTS**

Fig. 1A illustrates the experimental design for the functional identification of OCM Mns. The microelectrode was advanced toward the OCM under visual guidance helped
Fig. 1. Functional identification of OCM Mns. (A) Photomicrograph of a transverse section at OCM level, the locations of the bipolar electrode for the electrical stimulation (St) in the third nerve root (IIIln) and the micropipette tip within the OCM are represented. The inset shows an action potential antidromically evoked by electrical stimulation of the IIIln (arrow indicates the stimulus onset). (B, C) We ensured that recorded neurons within the OCM were Mns by the collision test. Two stimuli were used: a brief pulse of intracellular current that always evoked an action potential was the first stimulus (St1), which triggered—with a progressive decrease of latency—a second stimulus (St2) delivered in IIIln. A long interpulse interval evoked two spikes (B), while a decrease in the interpulse interval evoked a failure in the antidromic activation of the somatodendritic component (C); shorter intervals caused a complete failure of the antidromic response (not illustrated). Arrow in B shows initial segment-somatodendritic delay in the rising phase of the antidromically evoked action potential. Abbreviations: PAG, periaqueductal gray; SC, superior colliculus.
Fig. 2. Changes in membrane passive properties in OCM Mns with postnatal development. (A, B) Voltage membrane response of P3 and P8 Mns to negative current pulses. As shown, pulses of the same amplitudes evoked larger hyperpolarizations in the younger Mn. (C) Plot illustrating the current/voltage relationship to determine the input resistance for the two Mns illustrated in A and B. The input resistance ($R_{in}$) was higher in the younger Mn. (D) Scatter plot showing the changes in input resistance as function of postnatal age. (E) At the top is illustrated an average ($n=60$) of the membrane voltage responses to injected current pulses of $-0.1$ nA for a P9 Mn. At the bottom is shown the best-fit exponential ($r=0.98$) for the recorded response; the time constant ($\tau$) was 6 ms. (F) Scatter plot showing the changes in time constant as function of postnatal age. Open symbols indicate data from individual neurons, while filled symbols are the averages of pooled data in the different age groups; the dashed lines are the best fits to the raw data; 1, 2, 3, 4, and 5 in plots D and F indicate age groups; and the number of asterisks or crosses indicates the statistical level of significance: **$P<0.01$, +++$P<0.001$ (see also Experimental Procedures).
with a microscope. This nucleus was distinguished in the slices (300 μm) by its gray color, lying beneath the cerebral aqueduct and close to the midline (Fig. 1A), although the boundaries were not observable with precision in rats younger than P5. Mns were functionally identified within the nucleus by means of antidromic activation (see inset in Fig. 1A) and the collision test. The electrical stimulation of the third nerve root evoked in the Mns an action potential (inset in Fig. 1A) with latencies (difference in time between stimulus onset and spike initiation) of 0.6±0.1 ms (mean±standard error). The collision test was systematically used to establish that the recorded cell was the same as the activated from the nerve root (Fig. 1B, C). A spike was evoked intracellularly with a brief depolarizing current pulse (St₁), which triggered an electrical stimulus delivered in the nerve root with varying latency (St₂). If the interval

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**Fig. 3.** Changes in rheobase and depolarization voltage in OCM Mns with postnatal development. (A, B) Two examples showing the minimum current required, or rheobase, to evoke an action potential in 50% of cases (see records at top) in 3- and 25-day-old Mns. It should be noted that the rheobase was higher in the younger Mn. The records also illustrate the depolarization voltage, which was also higher in the younger Mn. Arrow in B marks the afterdepolarization phase of the spike. (C, D) Scatter plots showing the changes in rheobase and depolarization voltage as function of postnatal age. Open symbols indicate data from individual neurons; filled symbols indicate the average of pooled data in each age group (1–5); and dashed lines indicate the best fit to the raw data. Crosses indicate the level of statistical significance: *P<0.05, **P<0.01 (see also Experimental Procedures).
between the two stimuli was 50 ms or more, the spikes were similar in shape (not illustrated); however when the interval decreased, the action potential showed an initial segment-somatodendritic inflection in the rising phase (arrow in Fig. 1B). Shorter intervals between the two stimuli caused a failure in the antidromic activation of the somatodendritic compartment (Fig. 1C).

**Passive membrane properties**

The mean resting membrane potential of all OCM Mns pooled was \(-61.8\pm0.9\) mV. The Kruskal-Wallis analysis of

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**Fig. 4.** Changes in action potential features in OCM Mns with postnatal development. (A) Four records of the action potentials for different-aged Mns. The spikes were synchronized at their peaks (arrow) to illustrate the differences in time of the falling phase (see unfilled arrowheads). Inset: the spikes were synchronized at their onsets (arrow) to illustrate differences in the time course of the rising phase. The illustration also depicts the half-width (dotted line) of the spike to measure the action potential duration (APd, see also Experimental Procedures). (B) Scatter plot showing the changes in duration of the action potential with postnatal age. Open symbols indicate data from individual neurons; filled symbols indicate the average of pooled data in each age group (1–5); and dashed lines indicate the best fit to the raw data. (C) Histogram illustrating the changes in the rising and falling phases of the action potentials. In this histogram are illustrated the mean values and standard errors. Asterisks and crosses indicate the level of statistical significance: *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\) (see also Experimental Procedures).

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**Fig. 5.** Changes in mAHP features in OCM Mns with postnatal development. (A) Records of the mAHP for three (3-, 9-, and 24-day-old) Mns. As shown in these representative examples, the mAHP duration decreased from 172 ms to 100 ms with age, but the amplitude did not. (B) Scatter plot illustrating the changes in mAHP duration with postnatal age. Open symbols indicate data from individual neurons; filled symbols indicate the average of pooled data in each age group (1–5); and dashed lines indicate the best fit to the raw data. Asterisks and crosses indicate the level of statistical significance: *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\) (see also Experimental Procedures).
Fig. 6. Changes in the firing rate in OCM Mns with postnatal development. (A) Two examples of the firing rate for 3- and 21-day-old Mns, which had similar resting membrane potential, at about −62 mV. As illustrated, for sustained depolarizing current (1 s, 0.4 nA) the firing rate increased with age. (B) Plot of the instantaneous frequency for the examples illustrated in A. As shown, the first interspike interval exhibited the highest frequency, then the values declined to the steady-state firing frequency. (C) F/I plots for the Mns whose firing rates are illustrated in A. The slopes indicate the gain in frequency, which are noted in the graph. (D, E) Scatter plots showing changes in frequency gain and maximum firing rate with postnatal age. Open symbols indicate data from individual neurons, while filled symbols indicate the average of pooled data in each age group (1–5). The dashed line is the best fit to the raw data in D, while solid lines connect filled symbols in E. Asterisks and crosses indicate the level of statistical significance: *P<0.05, **P<0.01, **P<0.001 (see also Experimental Procedures).
variance showed that this parameter did not change significantly with postnatal development. Indeed, the means of the resting level for each age group were similar (Table 1). Injection of the negative current pulses evoked membrane potential hyperpolarizations whose amplitudes changed with postnatal development. Fig. 2A, B shows the membrane response of two Mns (P3 and P8) to current pulses with the same amplitude; as illustrated, the membrane response was larger in the younger Mn. The plots of current pulses versus voltage membrane response showed a good linear fit ($r > 0.98$; $P < 0.001$), and the slope of these relationships gave the input resistance (Fig. 2C). The input resistance decreased as a function of postnatal age (see open symbols in Fig. 2D). The best fit to the raw data was a single exponential decay ($\tau = 3.1$ days; Fig. 2D). The time constant of this fit shows that input resistance drastically decreased early during the postnatal development (P1–P5), since 63% of the total decrease was about three days after birth. Two additional findings support the early decay in this parameter. First, the input resistance values were significantly different between the first and second age groups ($P < 0.01$), while non-significant changes were found for the remaining age groups (Table 1). Second, the mean of the input resistance for the first age group was 82.9 MΩ, while for the second one was 63.7 MΩ; it then decayed to a mean value for the fifth group of 53.1 MΩ (see filled symbols in Fig. 2D and Table 1).

Membrane voltage shift follows the time course of several exponential decays. The exponential with the lowest time constant and highest correlation coefficient gives the membrane time constant (Rall, 1960). Fig. 2E shows for a P9 Mn the membrane response (top) and its exponential fit (bottom), elicited by a hyperpolarizing pulse of 0.1 nA. As shown, the membrane response fitted an exponential with a time constant of 6 ms and a high correlation coefficient ($r = 0.98$). The time constant diminished with age (Fig. 2F), and the raw data fitted a single exponential decay ($r = 0.7$; $\tau = 3.9$ days; Fig. 2F). The time constant of this fit indicates that the parameter declined in a short time after birth (P1–P5). In agreement with this result, the decrement in time constant values was significantly different between the first and second age groups ($P < 0.01$), while non-significant modifications were found for the other age groups (Table 1). Furthermore, the mean of the time constant decayed from 9.4–5.9 ms from first to second age groups, respectively; then reached a value of 4.9 ms in the fifth age group (see filled symbols in Fig. 2F and Table 1). Inspection of the modifications in the input resistance (Fig. 2D) and time constant (Fig. 2F) with postnatal age led to the notion that major changes in the two parameters occurred in parallel. In fact, the two variables were significantly related ($r = 0.73$; $P < 0.01$).

### Active membrane properties

Fig. 3A, B shows the current pulse required (rheobase) for P3 and P25 Mns to evoke an action potential (top in Fig. 3A), as well as the depolarization voltage. For these representative examples, the values of rheobase and depolarization voltage decreased with postnatal age. Thus, both rheobase (0.3 nA versus 0.15 nA) and depolarization voltage (15 mV versus 7 mV) were higher for the younger Mn. Fig. 3C, D shows the corresponding rheobase and depolarization voltage values respectively for each neuron as a function of age. Along the postnatal days, the rheobase raw data ranged from 0.03–0.97 nA at the beginning of development (P1–P5) and from 0.03–0.35 nA after P20. These data fitted a linear regression (slope $= -0.007$ nA/day; $r = -0.3$). Similarly, the depolarization voltage ranged from 5 to 55 mV for P1–P5 Mns and from 3 to 26 mV after P20 Mns. These data fitted a linear regression (slope $= -0.4$ mV/day; $r = -0.3$). These fits denote that the decay in these parameters was gradual with postnatal development (i.e. no striking change occurred for any particular age). Supporting this notion, the differences for both parameters were significant ($P < 0.01$ for rheobase; $P < 0.05$, for depolarization voltage) only when the first and fifth age groups were compared. The decreasing trend was also observed when the averaged values for each age group were plotted against postnatal age (filled symbols in Fig. 3C and D). Rheobase decreased from 0.29–0.11 nA in (Fig. 3C), and depolarization voltage from 19.7–10.7 mV (Fig. 3D; Table 1). In addition, we found a significant linear correlation between the decrease in depolarization voltage and rheobase ($r = 0.7$; $P < 0.0001$).

### Table 1. Passive and active membrane properties of oculomotor Mns during postnatal development

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age group (P)</th>
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<tbody>
<tr>
<td></td>
<td>1–5</td>
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<tr>
<td>Resting membrane potential (mV)</td>
<td>$-63.1 \pm 1.3$</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>$82.9 \pm 5.1$</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>$9.4 \pm 0.6$</td>
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<tr>
<td>Rheobase current (nA)</td>
<td>$0.29 \pm 0.05$</td>
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<tr>
<td>Depolarization voltage (mV)</td>
<td>$19.7 \pm 2.2$</td>
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<tr>
<td>Duration action potential (ms)</td>
<td>$2.3 \pm 0.10$</td>
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<tr>
<td>Duration mAHP (ms)</td>
<td>$184.8 \pm 6.8$</td>
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<tr>
<td>Frequency gain (spikes s$^{-1}$/nA)</td>
<td>$45.6 \pm 5.6$</td>
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<tr>
<td>Maximum frequency (spikes/s)</td>
<td>$29.8 \pm 4.0$</td>
</tr>
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Asterisks indicate differences between adjacent age groups; crosses indicate difference from first age group (Mann-Whitney U test): $^{*} P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$. 

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Action potential amplitude remained unaltered with the course of postnatal development, while its duration, and rising and falling phases, changed (Fig. 4). Fig. 4A illustrates superimposed the shape of the action potentials from Mns of 2, 9, 18, and 26 days old; as can be observed, there was a trend for the duration of the action potential to decrease with age. In fact, the raw data of action potential duration fitted a single exponential decay \((r=0.7; \tau=9.8\) days; Fig. 4B); 63% of the total decrease in spike duration occurred about 10 days after birth. Inspection of these data in Fig. 4B shows that the changes extended between P1 and P15. Consistent with these findings, the statistical analysis showed major significant differences between the first and second age groups, and between this latter and the third one \((P<0.001)\); and the mean values of action potential duration were 2.3, 1.8, 1.4, 1.3, and 1.2 ms for the five consecutive age groups (Fig. 4B, Table 1).

Fig. 4A depicts that in parallel to the shortening of the action potential there were modifications in both rising and falling phases. To construct this figure, the rising phases were synchronized with the onset of the action potentials (inset in Fig. 4A), while the falling phases were synchronized at the spike peaks. As shown, the difference between these two phases was in their duration (spike amplitude was constant), being shorter for the rising phase.

Irrespective of the age group (Fig. 4C), rising phases were faster (range 25–70 V/s) than falling ones (range 10–40 V/s). There were significant increments between the first and second age groups \((P<0.01)\), and between the second and third ones \((P<0.05\) for the rising phase and \(P<0.001\) for the falling phase; Fig. 4C). The action potential duration, and rising and falling phases changed with a parallel temporal sequence. As was predictable, the action potential duration and rising \((r=0.73)\) and falling phases \((r=0.76)\) were significantly related \((P<0.0001)\).

OCM Mns exhibited marked mAHP. Fig. 5A shows three representative examples of the mAHP for Mns with similar resting membrane potential (about −62 mV), which were 3, 9, and 24 days old. As illustrated, the mAHP amplitude remained almost unaltered at about 7 mV, while the duration changed from 172 to 100 ms. When we analyzed the mAHP amplitude with age group, we did not find any significant difference (not illustrated). In contrast, the mAHP duration decreased with postnatal age, and the data fitted a single exponential decay \((r=0.7; \tau=11.3\) days; Fig. 5B). As shown in Fig. 4B, the changes occurred between P1 and P20. This finding is supported by significant differences between adjacent age groups (1st versus 2nd, 2nd versus 3rd, and 3rd versus 4th; Table 1; Fig. 5B).

Furthermore, the means of mAHP duration showed a marked decrease between the first \((184.8\) ms) and second \((144.6\) ms) age groups, then a moderate shortening in this parameter continued until the last age group (see filled symbols in 5B and Table 1). Because the shortening of action potentials was parallel to that of mAHP (Figs. 4B, 5B), we tested the relationship between the two parameters; we found a significant co-variation \((r=0.68; P<0.0001)\).

Irrespective of the age group, all Mns discharged repetitively in response to sustained depolarizing current. Fig. 6A, B shows the response for representative P3 and P21 Mns to a current pulse of the same intensity \((0.4\) nA). As illustrated, older Mns showed a higher number of spikes (Fig. 6A) and a higher steady-state firing frequency (Fig. 6B). In addition, we constructed an F/I plot for each Mn (see experimental procedure) that exhibited a good linear relationship; for the cases illustrated, \(r=0.99, P<0.0001\) (Fig. 6C). The slope of these plots gave the frequency gain, which was higher for older Mns (Fig. 6C). The frequency gain values increased linearly with age (slope=0.9 spikes s\(^{-1}\) nA\(^{-1}\)/day; \(r=0.4;\) Fig. 6D). This fit denotes that the increase was gradual with postnatal development (i.e. no striking change occurred for any particular age). Consistent with this finding, the increase in this parameter with maturation was significant \((P<0.05)\) only between the first and fifth age groups (Table 1). Further the mean in gain frequency rise continuously from 45.6–66.5 spikes s\(^{-1}\)nA\(^{-1}\) (Fig. 6D and Table 1).

The values of maximum frequency increased with age (Fig. 6E), and fitted a linear regression (slope=2.5 spikes s\(^{-1}\)/day; \(r=0.8;\) not illustrated). However, the observation of the raw data in Fig. 6E leads to the recognition of two main groups of Mns: one between P1 and P15, and the other after P15. Thus, the maximum firing rate for younger Mns (<P15) ranged from 12 to 78 spikes/s, while for older Mns (>P15) the range was 60–130 spikes/s. This finding is supported by the significant increment found between the third and fourth age groups \((P<0.01;\) Fig. 6E and Table 1). Finally, we investigated the relationship between mAHP duration and firing rate; there was a significant relationship between age-dependent changes in mAHP and frequency gain \((r=0.62; P<0.05)\), as well as with the maximum firing rate \((r=0.71; P<0.0001)\).

**DISCUSSION**

This work studies for the first time the temporal sequence of changes in OCM Mns electrophysiological properties along the entire postnatal development, from birth to adult epoch. We demonstrate significant changes in input resistance, time constant, rheobase, action potential, and firing discharge of the OCM Mns with postnatal maturation. The temporal course of changes was similar for some physiological properties (e.g. input resistance and time constant) but not for all the studied variables. We will discuss these results from three standpoints: first, the physiological bases underlying age-dependent changes; second, the temporal sequence of changes for each physiological property; and third, previously reported results in the genioglossal/hypoglossal Mns and other non-motoneuronal populations. Then, we will discuss present results in the context of the maturation of the oculomotor system, and we will hypothesize about their functional consequences in comparison with those reported in the genioglossal nucleus. However, before addressing these specific issues, we will discuss the advantages and limitations of the method.
All recorded cells analyzed in this study were OCM Mns, as demonstrated by both their antidromic identification and the collision test. All Mns exhibited a stable resting membrane potential (−55 mV, or more negative) with spikes larger than 60 mV and repetitive discharge rate. Despite these criteria, the slice preparation involves cellular lesions in dendritic trees and axons, so we cannot rule out that some results were skewed by the technique. However, cells fulfilling the abovementioned requirements were scarcely damaged, as demonstrated by the combination of the intracellular recording and labeling techniques (Carrascal et al., 2005; Nunez-Abades et al., 1994). In addition, the electrophysiological data reported here using an in vitro approach were similar to those reported from in vivo preparations (Gueritaud, 1988; Durand, 1989). Finally, a major advantage of the present study is that its results are directly comparable with those previously reported with the same method and aims in other motoneuronal pools (Nunez-Abades et al., 1993; Viana et al., 1994), and should provide new insights into the temporal sequence of the postnatal maturation processes. Because we also directly compare present results with data using other procedures, e.g. patch electrodes filled with gramicidin (Baccei and Fitzgerald, 2005), we cannot rule out that some of the differences were attributable to the methods.

**Passive membrane properties**

Present results showed a decrement of the input resistance and time constant with postnatal development. The decline in these parameters with development is a characteristic common to most studied neuronal populations, such as those lying in the abducens (Russier et al., 2003), hypoglossal (Nunez-Abades et al., 1993; Viana et al., 1994), tractus solitarius (Vincent and Tell, 1999), vestibular (Murphy and du Lac, 2001), lateral lemniscus (Ahuja and Wu, 2000), and accumbens (Belleau and Warren, 2000) nuclei, as well as in thalamic (Perez-Velazquez and Carlen, 1996) and cortical areas (Zhou and Hablitz, 1996). In genioglossal Mns, the decay in these parameters with age has been attributed to the increase in tonic synaptic inputs, the enlargement of the membrane surface, and the proliferation and/or redistribution in the leak K⁺ channels (Cameron and Nunez-Abades 2000; Cameron et al., 2000; Nunez-Abades et al., 2000). Current hypotheses suggest that the main factor is probably a developmental increase in the expression of a leak K⁺ current that is mediated in part by TASK-1 and TASK-1/3 heteromeric channels (Greer and Funk, 2005; Tailey et al., 2000). Moreover, the K⁺ conductance is modulated by different neurotransmitter systems that also change during the postnatal development (Rekling et al., 2000). A range of evidence suggests that similar mechanisms to those proposed in the genioglossal Mns underlie the changes in input resistance and time constant in other neuronal pools (Belleau and Warren, 2000; Greer and Funk, 2005; Tailey et al., 2000; Vincent and Tell, 1999; Zhou and Hablitz, 1996), and, therefore, we suggest that such processes could also be present in OCM Mns during postnatal maturation.

In addition to the decreases in input resistance and time constant with age, we found a striking decline for both parameters in a short time after birth (P1–P5). Thus, there was a significant difference between the first and second age groups. These findings are also consistent with those reported for the abovementioned neuronal populations, in which both parameters decreased mainly during the first two weeks of development (Ahuja and Wu, 2000; Guido et al., 1998; Nunez-Abades et al., 1993; Perez-Velazquez and Carlen, 1996; Russier et al., 2003; Zhou and Hablitz, 1996). All these data together suggest that input resistance and time constant in most neurons mature early during postnatal development. However, some populations do not follow this temporal sequence of maturation. Thus, the input resistance and time constant for neurons lying in the nucleus of the tractus solitarius decline markedly after the third postnatal week (Vincent and Tell, 1997, 1999). Finally, these parameters did not change with postnatal maturation in some subpopulations of the trigeminal ganglion cells while in others they decreased with age (Cabanes et al., 2002).

**Active membrane properties**

We found here a continuous decrease in rheobase with postnatal age, which contrasts with the increase reported in hypoglossal Mns (Nunez-Abades et al., 1993; Viana et al., 1994). Different trends in rheobase modifications with postnatal maturation have also been reported in other non-motoneuronal pools. Thus, the rheobase increases in older accumbens nucleus neurons (Belleau and Warren, 2000), decreases in cortical pyramidal cells with age (McCormick and Prince, 1987), or is already established at birth in spinal neurons (Baccei and Fitzgerald, 2005). These results led to the suggestion that the postnatal development in this parameter depends on the neuronal pool. Various processes could be underlying the changes in the rheobase. First, in hypoglossal Mns, the increase in the rheobase courses in parallel with a decrease in input resistance (Nunez-Abades et al., 1993; Viana et al., 1994). Second, in accumbens nucleus, the resting membrane potential is more hyperpolarized in adult neurons, requiring a rise in the rheobase to reach the action potential threshold (Belleau and Warren, 2000). Finally, in OCM Mns, the decrease in the rheobase is accompanied by a decrease in depolarization voltage (Carrascal et al., 2005); these changes could be a consequence of the increase in density of voltage-gated Na⁺ channels or of modifications in their kinetics, although other conductances, such as persistent Na⁺ and long-lasting Ca²⁺ currents, can also be participating (Hornby et al., 2002; Li et al., 2004; O’Dowd et al., 1988; Powers and Binder, 2003; Russo and Hounsgaard, 1999). These currents are present in abducens and OCM Mns (Gueritaud, 1988; Russier et al., 2003).

We found in OCM Mns that the durations of the action potential and mAHP decreased with postnatal development. The decay in these parameters is another common trend for all studied neuronal populations (cited above in the section addressing passive membrane properties). It is widely accepted that the shortening of action potential is a...
consequence of the increase in density and/or faster activation kinetics of voltage-gated Na\(^+\) and K\(^+\) channels (Gao and Ziskind-Conhaim, 1998; O’Dowd et al., 1988; Viana et al., 1993b; Straka et al., 2005; Vincent et al., 2000). A decrement in voltage-activated Ca\(^{2+}\) conductance, supporting the action potential afterdepolarization, could also contribute to the decrease in spike duration (Viana et al., 1993a). Furthermore, the decrement in this latter conductance could underlie the decay in mAHP duration, since this phase is dependent on a Ca\(^{2+}\)-dependent K\(^+\) current (Viana et al., 1993b). Therefore, the shortening in mAHP depends on the decrease in spike duration (Carrascal et al., 2005; Nunez-Abades et al., 1993; Viana et al., 1994).

A major difference between genioglossal and OCM Mns was the extent of the period of major changes for the duration of action potential and mAHP. Thus, in genioglossal Mns, these changes were found along the six days after birth (Nunez-Abades et al., 1993), while in OCM Mns they extended until P15 (spike duration) and P20 (mAHP duration). Because firing rate mainly depends on mAHP duration (Kernell, 1999; Kernell et al., 1999; Piotrkiewicz, 1999; Rekling et al., 2000), comparison of the mAHP data between the two populations of Mns is relevant since it would support a distinct discharge rate in the adult cells. Genioglossal and OCM Mns had similar mAHP durations at birth: about 190 ms, which shortened during the first week to about 150 ms. Thereafter, no further significant decay in mAHP with age was found in genioglossal Mns. In contrast, mAHP in OCM Mns decreased significantly to P20, reaching a value of about 100 ms. Therefore, this distinct temporal pattern of maturation leads to a difference in the magnitude of mAHP duration, which in turn could support a higher discharge rate in OCM Mns (Carrascal et al., 2005). Indeed, the voltage-activated Ca\(^{2+}\)-conductance is lower in OCM than hypoglossal Mns, which should cause a lower activation of Ca\(^{2+}\)-dependent K\(^+\) channels, and thus a shorter mAHP, in OCM Mns (Miles et al., 2004). In addition, an increase of K\(^+\) conductance with development, which contributes to the repolarizing phase of the spike, could also affect mAHP duration (Rekling et al., 2000; Straka et al., 2005). Current evidence in other neuronal populations supports the notion that the most marked shortening in action potential and mAHP is restricted for some populations to initial postnatal epoch (Vincent and Tell, 1999), as in genioglossal Mns, while in others the changes continue until the second/third week postnatal (Murphy and du Lac, 2001; Perez-Velazquez and Carlen, 1996; Zhou and Hahlin, 1996), as here in OCM Mns. In conclusion, the period between P1 and P20 is crucial in the shortening of action potential and mAHP, but the temporal sequence of changes is different from one population to another.

OCM Mns share with hypoglossal (Nunez-Abades et al., 1993; Viana et al., 1995) and spinal Mns (Fulton and Walton, 1986) a tendency to increase the firing rate with postnatal development. This trend is also present in neurons of the lateral lemniscus (Ahuja and Wu, 2000) and vestibular (Murphy and du Lac, 2001) nuclei. In addition, OCM and genioglossal Mns (Nunez-Abades et al., 1993) exhibit a rise in the frequency gain with age, but this trend is not universal (Fulton and Walton, 1986; Murphy and du Lac, 2001). As reported here, the increase in discharge rate with age might significantly depend on a shortening of the mAHP. However, we have found that the most-pronounced changes in mAHP duration were between P1 and P20, whereas frequency gain increased continuously during the entire postnatal maturation. This difference could be supported by the idea that other mechanisms contribute to the discharge properties, such as an increase in the hyperpolarizing-activated mixed-cationic currents and persistent Na\(^+\) conductance, modifications in voltage-activated Ca\(^{2+}\) currents, and induction of A-type K\(^+\) current (Martin-Caraballo and Greer, 2001; Miles et al., 2004; Powers and Binder, 2001; Umemiya and Berger, 1994). Changes in trophic factors and/or neurotransmitters with age could also support the increased firing rate, because they modify the kinetics of these conductances (Gonzalez and Collins, 1997; Kernell, 1999; Kernell et al., 1999; Lape and Nistri, 2000; Perrier et al., 2002; Vinay et al., 2000).

OCM Mns not only tended to fire at higher frequency but also modulated their discharge over a wider range of input amplitudes with postnatal development. Similar findings have also been reported in hypoglossal and spinal Mns (Fulton and Walton, 1986; Nunez-Abades et al., 1993; Viana et al., 1995), as well as in non-motoneuronal populations (Ahuja and Wu, 2000; Murphy and du Lac, 2001). We have also found that postnatal changes in mAHP were significantly related to modification in maximum firing rate. However, the increase in maximum firing rate with age should not be primarily dependent on mAHP, but on the structure of the Na\(^+\) channels related to the duration of the inactivation phase (Hilber et al., 2002). Consistent with this proposal, the major changes in mAHP (P1–P20) and maximum firing rate (P16–P20) occurred in different periods. Finally, two main patterns of changes in maximum firing rate with postnatal development have been reported: first, OCM Mns (present data), like vestibular neurons, exhibited a more pronounced increase in the maximum firing rate between P16 and P20, whereas in genioglossal Mns, a continuous rise has been found in this property until the adult stage (Nunez-Abades et al., 1993).

Temporal sequence of electrophysiological changes for OCM Mns in the context of the oculomotor system maturation

Genetic and epigenetic specifications shape the adult neuronal phenotype with development (Hensch, 2004; Steljes et al., 1999). Some of the previous comments are consistent with this notion: first, modification of passive membrane properties could be a consequence of the increase in tonic synaptic inputs with development; and second, modifications in the kinetics of some conductances could be evoked by changes in trophic factors and/or neurotransmitters with age. Therefore, the maturational processes reported here in OCM Mns could depend, in part, on afferents and/or muscles. OCM Mns lead eye movements evoked by vestibular and visual sensory signals (Buttner-
Ennever and Horn, 1997). In rats, the vestibular organ, the sensitivity of the hair cells, and the circuitry transmitting the signal to the vestibular nucleus are ready about one week after birth (Curthoys, 1981; Rusch et al., 1998). Furthermore, the electrical stimulation of the vestibular nerve evokes eye movement in this period (Curthoys, 1979), and the lesion of the hair cells at P7 impairs the development of the extraocular muscle (Brueckner et al., 1999). If visual sensory signals have any role in the development of OCM Mns, it should be later than P12, when the eyelids open. Visual deprivation causes maldevelopment of the extraocular muscles (Brueckner and Porter, 1998) and impairs the vestibulo-ocular reflex (Berthoz, 1975; Collewijn, 1977). In addition, eye movements evoked by visual and vestibular stimuli are performed from about P21 in rodents, although the efficacy of these reflexes increases a few days later to

![Image](image_url)

**Fig. 7.** Co-variation of the electrophysiological properties in OCM Mns with age group. (A, B) Co-variation of the time constant (\(\tau\)) and input resistant with age. (C, D) Co-variation of the spike and mAHP durations with age. A and C are three-dimensional representations, in which the areas crossed by white dotted lines correspond to data interpolated to construct the illustration. B and C are planar projections of the real data represented in A and C.
enable clear vision during self-motion (Faulstich et al., 2004; Lannou et al., 1980). Therefore, the distinct pathways that drive optokinetic and vestibulo-ocular reflexes, including the cerebellar-dependent mechanisms, mature within the first 3–4 weeks (Faulstich et al., 2004). Finally, changes in myelination, neuromuscular junctions, and muscle elements occur during different postnatal periods of the extraocular muscle morphogenesis (Cheng et al., 2004; Porter and Karathanasis, 1999).

We have found that passive membrane properties matured in a short time after birth (P1–P5), while changes in active properties required a longer time scale. Murphy and du Lac (2001) reported similar findings in vestibular neurons. They concluded that changes in membrane properties with development enable fully mature firing properties by the time required for the proper optokinetic response. We may extend such a conclusion to the OCM Mns. However, to establish a relationship in the maturation of OCM Mns as a function of changes in both their afferents and extraocular muscles is speculative. To elucidate i) how the postnatal development in the vestibulo-ocular motor circuitry shapes early changes in physiological properties of OCM Mns, and ii) how active membrane properties of OCM Mns depend on eye opening, would require further research. The influence of the extraocular muscle maturation on OCM Mns should also be studied.

**CONCLUSIONS**

A major difference between genioglossal and OCM Mns was the tendency for the former to increase and the latter to decrease in rheobase with age. In genioglossal nuclei, the lower values in rheobase were at birth, and have been interpreted in functional terms as ensuring the recruitment of most Mns to achieve suckling movements, since this is a critical motor task after birth (Nunez-Abades et al., 1993; Viana et al., 1994). In accord with this hypothesis, the decrease in rheobase with age in OCM Mns would ensure the recruitment of most of these cells after P21, to lead to eye movements (Carrascal et al., 2005).

**REFERENCES**


**A comparison in temporal sequence of postnatal development between OCM and genioglossal Mns: functional consequences**

OCM and genioglossal Mns innervate extraocular and tongue muscles, respectively. A major functional difference between the two muscles is that the former should be ready at P21 (see section above), while the tongue contributes to several motor tasks, such as suckling, swallowing, and respiration as soon as the animal is born. This difference could be underlying the distinct temporal sequence of changes studied here. The most-pronounced decreases in passive membrane properties occurred between P1–P5 and P6–13 for OCM and genioglossal Mns, respectively. The shortening in action potential duration and mAHP occurred in genioglossal Mns during the first week, but not until P15–P20 in OCM Mns. The maximum firing discharge was reached after the third postnatal week in both populations of Mns. Although we have no evidence, we could assume that early processes of maturation in OCM Mns course with the development of both the vestibular signals and extraocular muscle, and that the final active properties are shaped from eye opening until about P21. In this scheme, the earlier shortening of the action potential and mAHP in genioglossal Mns is in accord with certain tongue functions such as suckling, swallowing, and respiration that become mature just after birth. Such changes are followed by modifications in passive properties and maximum firing rate to allow refinements in these motor functions and also to enable other tasks, such as mastication (Berger et al., 1996; Cameron and Nunez-Abades, 2000; Cameron et al., 2000; Nunez-Abades et al., 2000; Kinirons et al., 2003).

**Fig. 7** summarises, in a three-dimensional form some of the main findings reported here. First, input resistance and time constant decreased in parallel in a short time after birth (Fig. 7A). As a result, the data can be clustered in two main groups: Mns with larger input resistance (>60 MΩ) and time constant (>6 ms), corresponding with the first age group, and the remaining Mns (Fig. 7B). Second, durations of action potential and mAHP matured with a similar time course (Fig. 7C). In contrast to passive properties, four clusters of data are distinguished for these active properties, which correspond to the first, second, third, and fourth/fifth age groups (Fig. 7D). Other active membrane physiological properties also changed with different time course, e.g. the rheobase increased in a continuum with age, while maximum firing rate exhibited a more-pronounced change between P16 and P20. Therefore, in response to the first main question of this work (see introduction), we conclude that time constant-input resistance, rheobase, durations of action potential-mAHP, and firing rate followed different temporal sequences of postnatal development. In response to the second main question of this work, the comparison of the present data with those reported in other neuronal populations leads to the conclusion that the pattern of development in the studied electrophysiological properties is unique for each population.

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