POSTNATAL DEVELOPMENT ENHANCES THE EFFECTS OF CHOLINERGIC INPUTS ON RECRUITMENT THRESHOLD AND FIRING RATE OF RAT OCULOMOTOR NUCLEUS MOTONEURONS

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Abstract—Changes in the electrophysiological and morphological characteristics of motoneurons (Mns) of the oculomotor nucleus during postnatal development have been reported, however synaptic modifications that take place concurrently with postnatal development in these Mns are yet to be elucidated. We investigated whether cholinergic inputs exert different effects on the recruitment threshold and firing rate of Mns during postnatal development. Rat oculomotor nucleus Mns were intracellularly recorded in brain slice preparations and separated in neonatal (4–7 postnatal days) and adult (20–30 postnatal days) age groups. Stimulation of the medial longitudinal fasciculus evoked a monosynaptic excitatory potential in Mns that was attenuated with atropine (1.5 μM, a muscarinic antagonist). Mns were silent at their resting membrane potential, and bath application of carbachol (10 μM, a cholinergic agonist) induced depolarization of the membrane potential and a sustained firing rate that were more pronounced in adult Mns. Pharmacological and immunohistochemical assays showed that these responses were attributable to muscarinic receptors located in the membrane of Mns. In addition, compared to control Mns, carbachol-exposed Mns exhibited a higher firing rate in response to the injection of the same amount of current, and a decrease in the current threshold required to achieve sustained firing. These latter effects were more pronounced in adult than in neonatal Mns. In conclusion, our findings suggest that cholinergic synaptic inputs are already present in neonatal Mns, and that the electrophysiological effects of such inputs on recruitment threshold and firing rate are enhanced with the postnatal development in oculomotor nucleus Mns. We propose that cholinergic input maturation could provide a greater dynamic range in adult Mns to encode the output necessary for graded muscle contraction. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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

It is widely accepted that the different brain areas are not mature at birth, and the adult neuronal phenotype is postnatally shaped by genetic and environmental factors (Steljes et al., 1999; Van Aelst and Cline, 2004; Wu et al., 2007). The oculomotor system has been extensively stud-
lizarations and biphasic responses (rapid hyperpolarizing response followed by a slower depolarizing one) in other neuronal populations (Good et al., 2007; Ye et al., 2009).

It has recently been demonstrated in oculomotor nucleus Mns of young adult rats that the activation of cholinergic receptors by carbachol has two effects. First, membrane potential depolarization is produced along with sustained neuronal firing—which is not silenced upon return of the membrane potential to precarbachol values via DC current injection. Second, a higher firing rate than control is produced in response to depolarizing current steps of the same amplitude (Nieto-Gonzalez et al., 2009). These responses are of a postsynaptic nature, that is attributable to the activation of cholinergic receptors located in the membrane of the Mns. Therefore, it has been concluded that cholinergic synaptic inputs play an important role in determining the recruitment threshold and firing rate of oculomotor nucleus Mns (Nieto-Gonzalez et al., 2009). In view of these findings, we sought to determine whether cholinergic synaptic inputs exert different effects on the recruitment threshold and firing rate of these Mns as a function of postnatal age.

**EXPERIMENTAL PROCEDURES**

**Surgery and solutions**

Experiments were carried out on Wistar rats. All studies were performed in accordance with the European Community Directive 2003/65, as well as with the Spanish Royal Decree 120/2005 and University of Seville regulations on the care of laboratory animals. Rats were anesthetized with sodium pentobarbital (50 mg/kg, Sigma-Aldrich) and quickly decapitated. The methods used to obtain the slices, recordings, and analysis are described in detail elsewhere (Carrascal et al., 2006; Nieto-Gonzalez et al., 2007, 2009). In brief, brain slices (thickness 300 μm) including the oculomotor nucleus were incubated in a chamber containing cold sucrose-artificial cerebrospinal fluid (ACSF) for 35–45 min, and then transferred to a second chamber containing ACSF maintained at a constant temperature of 33 °C. Single slices were transferred to the recording chamber and superfused at 2 mL/min (Harvard-MPII) with ACSF bubbled with 95% O₂ - 5% CO₂ (pH 7.4; 33 °C). The composition of the ACSF was as follows (data are in mM): 126 NaCl, 1.25 Na₂HPO₄, 26 NaHCO₃, 10 glucose, 2 MgSO₄, and 2 CaCl₂. For the sucrose-ACSF solution, the 126 NaCl was substituted by 240 sucrose. The following drugs (all from Sigma-Aldrich) were used in this study: carbamylcholine chloride (carbachol, 10 μM), atropine sulfate (1.5 μM), pirenzipine dihydrochloride (2 μM), and tetrodotoxin (TTX, 1 μM). The time taken to completely exchange the recording chamber was about 50 s, and bath application of drugs was always for >2 min. Drugs were usually applied to only one Mn per slice; if it was necessary to apply more than one drug, the slice was washed with ACSF solution for >20 min (Nunez-Abades et al., 2000; Nieto-Gonzalez et al., 2009).

**Electrophysiological recordings and data analysis**

All recorded neurons were identified as Mns by their antidromic activation from the root of the third nerve and by the collision test (for details see Carrascal et al., 2006). The micropipettes used for recordings were filled with a 3 M KCl (40–70 MΩ) solution. Mono- synaptic potentials were elicited by stimulating the region of the medial longitudinal fasciculus close to the boundaries of the oculomotor nucleus. Metallic bipolar microelectrodes (tips ~300 μm apart) were constructed from 25 μm diameter stainless steel wire insulated in glass micropipettes. Cathodal pulses of 150 μs were used, and the current adjusted at subthreshold and increased to suprathreshold values. Recordings were stored on videotape (Neuro-Corder, Neurodata Instruments, PA, USA), and subsequently played back and acquired with a PCI-6070E card (National Instruments, Austin, TX, USA) for off-line analysis. All Mns included for analysis showed a stable resting membrane potential of ~55 mV or more-negative, an action potential amplitude larger than 60 mV, and fired repetitively in response to suprathreshold depolarizing current steps of 1 s. In addition, negative current pulses (0.1 nA, 500 ms, 1 Hz) were used to determine the membrane input resistance of each Mn. With the exception of the exposure of Mns to TTX, the effects of the pharmacological manipulations were reversible. The data for any given Mn in response to the different drugs were accepted only if the membrane potential and spike amplitude returned to the control value (measured when the Mn was impaled) after a wash-out period.

We focused our analysis on Mns (n=36) showing a phasic-tonic discharge in response to suprathreshold current steps (Carrascal et al., 2005; Nieto-Gonzalez et al., 2007). Two separate postnatal age groups of Mns were used: neonatal and adult. The former were obtained in rats of 4–7 postnatal days and the second from young adult rats (20–30 postnatal days) of both sexes. These age groups were selected taking into account the fact that the passive membrane properties of Mns are already established by postnatal day 4 (Carrascal et al., 2006), but the dendritic field is immature (Carrascal et al., 2009). Mns from both age groups were included for analysis when their input resistance was 40–80 MΩ. To investigate the effects of the activation of cholinergic receptors we used carbachol at 10 μM. The effects of carbachol on the membrane potential and firing frequency for each Mn were quantified before exposure (control condition), during perfusion, and after wash-out with ACSF. The effects on membrane potential were measured as the difference between resting membrane potential and spike threshold at a steady tonic rate. To assess whether the membrane potentials during DC current injection were correct, we routinely monitored the bridge-balance over the course of the experimental session (for further details see Nunez-Abades et al., 1993). To determine whether carbachol responses were only attributable to the activation of postsynaptic cholinergic receptors, TTX (1 μM) was added to the extracellular solution to block voltage-gated Na⁺ channels and, thereby, to inhibit spike generation and synaptic transmission. The repetitive-firing frequency was evoked by depolarizing current steps (1 s, 0.5 Hz) with 0.1 nA increments. The steady-state firing frequency was the average of the instantaneous frequencies during the last 500 ms of the current step. For each Mn, the relationship between the steady-state firing frequency and injected current amplitude was represented (I–F plot). We defined current threshold as the intensity of stimulating current capable of eliciting maintained repetitive firing at 20 spikes s⁻¹ (Nieto-Gonzalez et al., 2007, 2009).

All statistical analyses were carried out on the raw data. Significant differences between the Mns before (control) and during bath application of carbachol were determined by using a one-way ANOVA. This test was also used to determine differences between neonatal and adult age groups in response to exposure to drugs. The significance level was established at P<0.05. All data are reported as mean±standard error.

**Immunohistochemistry**

Immunohistochemical experiments (n=5) were carried out to demonstrate the presence of muscarinic receptors (M₁) in the oculomotor nucleus (for details see Nieto-Gonzalez et al., 2009). Transverse sections of 50 μm thickness were processed to reveal M₁ by confocal laser microscopy. Free-floating sections were incubated for 1 h at room temperature in 3% normal goat serum.
and 0.15% Triton X-100 in PBS, and then in the diluted primary antibody (rabbit anti-M1, Millipore; 1:100) overnight at 4 °C. Finally, sections were incubated with secondary antibody labeled with Alexa 488 goat anti-rabbit IgG (Invitrogen; 1:200) overnight at 4 °C.

RESULTS

All recorded Mns showed stable resting membrane potentials, which were not significantly different between neonatal (−60.6 ± 2.8 mV) and adult (−62.5 ± 3.3 mV) Mns, and required depolarizing current injection to cause repetitive firing. The input resistance was also similar for both age groups (neonatal Mns=61.3 ± 3.7 MΩ and adult Mns=59.7 ± 4.1 MΩ). Exposure of slices to carbachol (10 μM), an agonist of cholinergic (muscarinic and nicotinic) receptors, produced membrane potential depolarization and sustained firing in all neonatal (n=12) and adult (n=12) Mns tested. As shown by the examples in Fig. 1A, B, the response of Mns was a slow depolarization of membrane potential and firing rate in all recorded neonatal and adult Mns tested. As shown by the examples in Fig. 1A, B, the response of Mns was a slow depolarization of membrane potential that resulted in the triggering of action potentials. Once this repetitive firing commenced, the membrane potential continued depolarizing until it reached a stable value; the instantaneous firing frequency also gradually increased, reaching a sustained repetitive firing (11 mV and 13 spikes s⁻¹ for the representative neonatal Mn showed in Fig. 1B). The mean effects of carbachol on membrane potential and firing rate in all recorded neonatal and adult Mns are plotted in Fig. 1C, D. The carbachol evoked a membrane potential depolarization that increased with the postnatal age (Fig. 1C). The mean of the depolarization for the neonatal group was 8.0 ± 0.9 mV, while for the adult group it was 14.9 ± 1.2 mV. This difference was statistically significant. The firing frequency elicited by the exposure of Mns to carbachol also increased concomitantly with postnatal development (Fig. 1D). The mean values of firing frequency evoked by the carbachol was 10.7 ± 1.2 spikes s⁻¹ in neonatal Mns and 17.0 ± 1.8 spikes s⁻¹ in adult Mns, with this increment being statistically significant. In addition, to determine to what extent the firing frequency was dependent on membrane potential, Mns were repolarized to pre-carbachol values with hyperpolarizing DC current injection. Under these conditions the Mns still fired, but at lower frequency to that measured in the period of depolarization (Fig. 1A, B, part 3). When the data from all recorded Mns was pooled for each age group (Fig. 1E), the firing frequency recorded during repolarization was significantly higher in the adult Mns (12.6 ± 1.3 spikes s⁻¹) compared to the neonatal population (7.6 ± 1.2 spikes s⁻¹). Hence, the firing frequency does not depend solely on cell membrane depolarization following carbachol application. Moreover, the mechanisms that induced sustained firing were already present in neonatal Mns but yielded a lower firing rate compared to adult Mns.

Electrical stimulation of the medial longitudinal fasciculus, close to the boundary of the oculomotor nucleus, elicited excitatory postsynaptic potentials (EPSPs) in the recorded Mns (Fig. 2A, B). The synaptic nature of these EPSPs was evidenced by the graded response to increments in current strength and the temporal summation of the evoked responses. Irrespective of the strength of the current stimulus, the EPSPs showed a constant latency of less than 2 ms in all Mns tested. Based on these findings, the recorded EPSPs were considered to be monosynaptic. To investigate if the EPSPs were, at least in part, due to the activation of cholinergic-muscarinic receptors, the bath was perfused with atropine (1.5 μM), a muscarinic receptor antagonist. This treatment produced an observable decrease in EPSP amplitude in all neonatal (n=3) and adult (n=3) Mns studied (Fig. 2C). To elucidate the postsynaptic nature of the effects of carbachol on Mns, brain slices were exposed to TTX (not shown), which blocks action potential generation and synaptic transmission in premotor neurons. Under this condition, the magnitude of the Mn membrane potential depolarization evoked by carbachol was similar with or without TTX, thereby showing that the nature of the response was postsynaptic in both neonatal (n=3) and adult (n=2) Mn populations. Further to this, we studied whether the carbachol-evoked responses were mediated by muscarinic (M1) receptors in neonatal Mns, as proposed in adult Mns (Nieto-Gonzalez et al., 2009). Pirenzepine, a muscarinic (M1) receptor antagonist, inhibited repetitive firing in carbachol-exposed neonatal Mns (Fig. 2D). Furthermore, the effects of pirenzepine on carbachol-treated Mns were essentially similar for Mns from both age groups (n=2 in each case), leading to a block of the firing discharge (Fig. 2E). Finally, we studied the presence of M1 receptors by immunohistochemistry. A large number of immunopositive cell bodies were observed confined to the oculomotor nucleus (Fig. 2F). We conclude that carbachol acts via muscarinic (in particular M1) receptors in the neonatal population in the same manner as for adult Mns.

To test if the cholinergic inputs modulate the recruitment threshold and firing rate with postnatal development, neonatal (n=8) and adult (n=9) Mns were studied by delivering intracellular depolarizing current steps of the same amplitude before (control) and during bath application of carbachol (Fig. 3A, B). In this latter condition the Mns were repolarized to restore the membrane potential to that observed prior to the carbachol treatment. Both neonatal and adult Mns showed a higher firing frequency in the presence of carbachol; furthermore, carbachol exerted a much more robust influence on the firing rate of adult Mns compared to neonatal Mns. The repetitive firing properties in neonatal and adult Mns as a function of the current intensity of the stimulus (I–F plots) were also studied (Fig. 3C, D). These experiments were carried out on Mns under control conditions and after adding carbachol to the bath. Under both control and carbachol-exposure conditions, the I–F plots exhibited a good linear relationship (Fig. 3C, D). Irrespective of the current intensity, the Mns of both age groups discharged at a higher firing frequency in the presence of carbachol. The slope of the I–F plots increased with age as previously reported by Carrascal et al. (2006). Furthermore, the slopes of the I–F plots showed a decrease in carbachol-exposed adult Mns compared to that seen under control conditions, but this was not the case for neonatal Mns. Fig 3E compares the mean values found in the neonatal and adult populations of firing rates evoked by 0.4 nA depolarizing current steps, before and after the application of carbachol to the recording bath. As shown in the figure, neonatal Mns discharged under control conditions at a rate of
15.1±3.3 spikes s⁻¹ compared to 19.2±4.3 spikes s⁻¹ in adult Mns. When slices were exposed to carbachol, the firing frequency increased in both neonatal (27.4±2.5 spikes s⁻¹), and adult Mns (45.4±4.1 spikes s⁻¹). Significant differences were found in the firing frequency between control and carbachol-exposed Mns in both neonatal and adult age groups. In addition, the increase in firing frequency for the neonatal Mns (12.3±3.3 spikes s⁻¹) was significantly different from that seen in the adult group (26.2±5.1 spikes s⁻¹), demonstrating that carbachol evoked a more pronounced repetitive discharge in response to intracellular depolarizing current steps of the same amplitude in the adult group of Mns. Finally, the current threshold was lower in carbachol-exposed neonatal Mns compared to adult Mns. Fig 3F illustrates the mean threshold values for neonatal and adult populations of Mns, before and after the application of carbachol to the bath.
Fig. 2. The cholinergic response in the rat oculomotor nucleus Ms acts via muscarinic receptors. (A) Superimposed recordings of EPSPs showing graded responses as a function of current stimulus strength. A suprathreshold stimulus is seen to evoke an action potential. (B) Superimposed recordings showing temporal summation of EPSPs with paired stimuli that could trigger an action potential. (C) Superimposed EPSP recordings under control conditions and following exposure to the muscarinic antagonist atropine (1.5 μM) in neonatal (6-day-old) Mn. Note the decrease in EPSP amplitude, even though stimulus current strength was maintained. (D) Recording from a neonatal (5-day-old) Mn showing that pirenzepine (2 μM; a M₁ muscarinic antagonist) blocks carbachol-dependent firing. The framed area includes an expanded recording at the time indicated by the star symbol. (E) Time course of the inhibition of carbachol-dependent firing by pirenzepine in neonatal (5-day-old) and adult (25-day-old) Mns. Dots correspond to instantaneous frequency sampled at a fixed rate. (F) Confocal photomicrograph showing immunopositive cells for muscarinic M₁ receptors located inside the boundaries of the oculomotor nucleus (OCM) in a brain slice from a neonatal (6-day-old) rat. The inset illustrates a higher magnification of the cells indicated by arrowheads. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.
Fig. 3. Response to depolarizing current steps in control and carbachol conditions in oculomotor nucleus Mns. (A, B) Firing discharge evoked by same-amplitude current steps in neonatal (4-day-old) and adult (22-day-old) Mns under control conditions and then exposed to carbachol (10 μM). (C, D) Current-firing frequency (I-F) relationship in a representative Mn of each age group under control conditions (filled circles) and then exposed to carbachol (empty circles). Note that carbachol decreased the recruitment threshold of both Mns. (E) Histogram showing the mean firing frequency evoked by 0.4 nA current steps in control and carbachol-exposed Mns of neonatal and adult rats. (F) Histogram showing the mean current threshold (nA) in control and carbachol-exposed Mns of neonatal and adult rats. Asterisks indicate significant differences between both conditions for each age group.
This figure shows that: 1), the neonatal Mns (0.54 ± 0.06 nA) exhibited a higher current threshold than adult Mns (0.45 ± 0.06 nA) under control conditions; 2), both populations of Mns exposed to carbachol exhibited a significantly decreased current threshold (neonatal Mns = 0.34 ± 0.03 nA; adult Mns = 0.09 ± 0.03 nA); and 3), neonatal Mns showed a significantly lower decrease in current threshold (0.2 ± 0.04 nA) compared to adult Mns (0.36 ± 0.05 nA). In summary, Fig. 3F also shows that carbachol evoked a decrease in the current threshold, which became more pronounced with the postnatal age.

**DISCUSSION**

This work has demonstrated that cholinergic efficacy in the adult Mn phenotype takes place concomitantly with postnatal development. As such, carbachol led to a membrane potential depolarization that triggered action potentials in the oculomotor nucleus Mns, with the magnitude of depolarization and the frequency of firing being higher in adult than in neonatal Mns. The inhibition of synaptic transmission with TTX showed that these responses could be attributed to the activation of postsynaptic cholinergic receptors, that is those located in the Mn membrane. In addition, the carbachol-exposed Mns exhibited a higher firing rate compared to control in response to depolarizing current steps of the same amplitude, and a decrease in the current threshold required to achieve sustained firing. These two effects were enhanced with postnatal age.

The increase with age of the effects of acetylcholine on Mn membrane potential depolarization in the oculomotor nucleus (about twofold in adult Mns compared to neonatal Mns) contrasts with results obtained in the pedunculopontine nucleus where the effects of carbachol are maintained from birth to adulthood (Good et al., 2007), or with those obtained in neurons of the thalamic parafascicular nucleus where the percentage of cells in which carbachol hyperpolarizes the membrane increases with postnatal development (Ye et al., 2009). These comparisons led us to propose that the maturation of cholinceptive properties depends on each neuronal pool. Furthermore, we have demonstrated, in both neonatal and adult oculomotor nucleus Mns, by pharmacological and immunohistochemical assays, that carbachol acts via muscarinic receptors. These receptors are linked to G proteins that give rise to the inhibition of several types of K⁺ currents (Krnjevic, 1993), and the activation of Ca²⁺ conductances and/or non-selective cationic currents such as I₇ currents (Klink and Alonso, 1997b; Yajeya et al., 1999; Fisahn et al., 2002; Honda et al., 2003; Fernández de Sevilla et al., 2008). Some of these conductances may underlie the membrane potential depolarization seen in oculomotor nucleus Mns and should be necessarily different in magnitude as postnatal maturation proceeds, as demonstrated for the hyperpolarization-activated inward current in hypoglossal Mns that undergoes a postnatal increase in current density (Bayliss et al., 1994).

As previously demonstrated in adult oculomotor nucleus Mns (Nieto-Gonzalez et al., 2009), we found that both neonatal and adult Mns are silent at rest, but enter into repetitive firing when exposed to carbachol and membrane potential were restored to rest by a hyperpolarizing DC current. These results may reflect that even though the soma is at resting membrane potential, large parts of the dendrites may not be sufficiently repolarized. Although this technical limitation cannot be completely discarded, it is also plausible that depolarization is not the sole mechanism responsible for repetitive firing evoked by carbachol. This proposal is supported by other studies in which, for example, glycine or GABA treatments of neonatal hypoglossal Mns evoke a slight depolarization of the membrane potential, but the same current steps yield firing under control conditions and not when exposed to the neurotransmitter (Marchetti et al., 2002), suggesting an increase in the spiking current threshold. In contrast, in carbachol-exposed neurons of the dorsal column gracilis nucleus, membrane potential depolarization is accompanied by a diminution in firing threshold (Fernández de Sevilla et al., 2006). These latter results are in agreement with the data presented here. In addition, the firing frequency of adult Mns was almost twofold higher than in neonatal Mns, measured when membrane potential was returned to values prior to the carbachol treatment. This finding could be explained by postnatal changes in the neuromodulation of Na⁺ currents underlying spiking (Franceschetti et al., 2000; Cantrell and Catterall, 2001). On the other hand, neonatal and adult oculomotor nucleus Mns exhibited a higher firing frequency in response to depolarizing current steps of the same amplitude when treated with carbachol. Similar results were obtained in other populations of neurons (Brown and Yu, 2000; Alaburda et al., 2002; Chevallier et al., 2006; Fernández de Sevilla et al., 2006; Miles et al., 2007). These studies suggest that muscarinic receptor activation produces an increase in the firing frequency by decreasing Ca²⁺ –dependent K⁺ conductances and I₇,m currents (Lape and Nistri, 2000). The modification of these conductances would lead to a decrease in the post-hyperpolarization phase of the action potential that would result in an increase in firing rate. These mechanisms could be used to explain the increase in firing frequencies observed in the oculomotor nucleus Mns after application of carbachol, not only in adult Mns (Nieto-Gonzalez et al., 2009), but also in neonatal ones (present data).

**Age-dependent enhancement of the cholinergic effects on firing rate and recruitment threshold in the context of the postnatal maturation of the oculomotor nucleus**

The results reported here could be interpreted in the context of the postnatal modification of intrinsic electrophysiological properties of the membrane (Carrascal et al., 2005, 2006). Passive membrane properties decrease during early development (up to 3–4 days postnatal). Since the neonatal age group was comprised of brain slices from 4 to 7-day-old animals, and the input resistance was similar to that of the adult group, it does not seem plausible that the effects of the activation of cholinergic receptors were mediated by modification of this parameter. Active membrane properties, which include rheobase and firing
rate, are gradually modified concomitantly with postnatal development (from birth until 15–20 postnatal days). For instance, the maximum firing frequency increases progressively during the first 3 weeks after birth, reaching values found in adults (Carrascal et al., 2006). As such, the postnatal changes in active membrane properties lead to an increase in the excitability and repetitive discharge with age (Carrascal et al., 2005, 2006). If we could assume that cholinergic synaptic inputs would exert the same influence on neonatal and adult Mns, the rise in excitability and firing rate of the oculomotor nucleus Mns with age support the fact that the effects of the carbachol would be more pronounced in adult Mns.

The results reported in the present work could also be interpreted in the context of cholinergic synaptic proliferation. Indeed, a direct link has been established in spinal Mns between an increase in cholinergic synaptic inputs with postnatal development and the maturation of the motor system that permits weight bearing and locomotion (Wilson et al., 2004). In oculomotor nucleus Mns, two phases (1–10 and 11–30 postnatal days) have been distinguished in the changes of somatodendritic morphometry during postnatal maturation (Carrascal et al., 2009). During the first phase, dendritic complexity and membrane somatodendritic surface area increases, while during the second phase, dendritic elongation takes place—with a simplification of dendritic complexity—and membrane surface area continues to increase slowly. The increase in somatodendritic surface area with development, particularly from 4 days after birth, and the reorganization of the dendritic architecture are probably related to changes in synaptic inputs. In other words, it is plausible that these anatomical changes support the production and / or refinement of synaptic connections with development as reported in other neural systems (Nunez-Abades et al., 1994; Nunez-Abades and Cameron, 1995; Vincent et al., 2004; Wilson et al., 2004). Changes in trophic factors and / or neurotransmission with age could also support the increase in recruitment threshold and firing rate because they modify the kinetics of the conductances underlying such processes (Gonzalez et al., 2007, 2009). Indeed, the recruitment threshold and the slopes of the I–F plots in oculomotor Mns are modulated by cholinergic synaptic inputs. The present work increases our understanding of these processes by showing that postnatal development strengthens the efficacy of cholinergic synaptic modulation on the activity of the Mns. This latter finding could support the fact that the threshold and firing frequency could be dynamically modulated by acetylcholine across a greater range with age. Under such circumstances the level of tonic excitatory cholinergic inputs could contribute to determine the threshold and firing rate required to generate the output necessary for graded muscle contraction.

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