Postnatal Development of Electrophysiological Properties of Nucleus Accumbens Neurons

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Belleau, Marc L. and Richard A. Warren. Postnatal development of electrophysiological properties of nucleus accumbens neurons. J Neurophysiol 84: 2204–2216, 2000. We have studied the postnatal development of the physiological characteristics of nucleus accumbens (NAcb) neurons in slices from postnatal day 1 (P1) to P49 rats using the whole cell patch-clamp technique. The majority of neurons (102/108) were physiologically identified as medium spiny (MS) projection neurons, and only these were subjected to detailed analysis. The remaining neurons displayed characteristics suggesting that they were not MS neurons. Around the time of birth and during the first postnatal weeks, the membrane and firing characteristics of MS neurons were quite different from those observed later. These characteristics changed rapidly during the first 3 postnatal weeks, at which point they began to resemble those found in adults. Both whole cell membrane resistance and membrane time constant decreased more than fourfold during the period studied. The resting membrane potential (RMP) also changed significantly from an average of −50 mV around birth to less than −80 mV by the end of the third postnatal week. During the first postnatal week, the current-voltage relationship of all encountered MS neurons was linear over a wide range of membrane potentials above and below RMP. Through the second postnatal week, the proportion of neurons displaying inward rectification in the hyperpolarized range increased steadily and after P15, all recorded MS neurons displayed significant inward rectification. At all ages, inward rectification was blocked by extracellular cesium and tetra-ethyl ammonium and was not changed by 4-aminopyridine; this shows that inward rectification was mediated by the same currents in young and mature MS neurons. MS neurons fired single and repetitive Na+/K+ action potentials as early as P1. Spike threshold and amplitude remained constant throughout development in contrast to spike duration, which decreased significantly over the same period. Depolarizing current pulses from rest showed that immature MS neurons fired action potentials more easily than their older counterparts. Taken together, the results from the present study suggest that young and adult NAcb MS neurons integrate excitatory synaptic inputs differently because of differences in their membrane and firing properties. These findings provide important insights into signal processing within NAcb during this critical period of development.

Introduction

The nucleus accumbens (NAcb) is an important telencephalic region that receives its most important inputs from the prefrontal cortex, hippocampal formation, entorhinal cortex, amygdala, and midline thalamic nuclei (Groenewegen et al. 1980, 1982, 1987; Jayaraman 1985; Kelley and Domesick 1982; Kelley and Stinus 1984; Kelley et al. 1982; Krayniak et al. 1981; Newman and Winans 1980; Phillipson and Griffiths 1985). The primary output of the NAcb is to the ventral pallidum (Hakan et al. 1992; Yang and Mogensen 1985; Zahm and Heimer 1990), which is known to be involved in the activation of voluntary movements (Heimer et al. 1994; Swerdlow and Koob 1987). The NAcb is believed to be a center for the integration of limbic and motor systems (Mogensen et al. 1980). It appears to be involved in reinforcement aspects of behavior (Cador et al. 1991; Carlezon and Wise 1996; Joseph and Hodges 1990; Wise and Bozarth 1987).

Many NAcb neurons receive glutamatergic inputs from diverse sources (Finch 1996; O’Donnell and Grace 1995) that must be harmoniously integrated for proper output to be generated by NAcb projecting neurons (O’Donnell and Grace 1995). Presumably, the anatomical substrate for this functional integration is achieved during development through competition/cooperation interactions between the different inputs to the NAcb following Hebbian rules (Hebb 1949).

The activity of single neurons largely depends on their synaptic inputs and their membrane intrinsic properties. These two aspects of neuronal organization are still immature at birth in several regions of the neuraxis, and they develop interdependently during the postnatal period. The influence of the environment during this period has been recognized since the pioneering work of Wiesel and Hubel (Wiesel and Hubel 1963, 1965) in the visual system. Today, it is widely believed that inappropriate neuronal activity during a critical period will lead to permanent impairment of function (e.g., Yuste and Sur 1999). Whereas there is an extensive body of literature on the developmental plasticity of sensory systems, comparable studies of limbic structures are few. Moreover, there is a growing body of evidence suggesting that diseases such as schizophrenia may be the result of a disturbed development of limbic structures (Falkai and Bogerts 1993; Weinberger and Lipska 1995). Recently, an animal model using early postnatal lesion of the subiculum, a limbic structure that has been found to be abnormal in postmortem tissue from schizophrenic patients, mimics much of the symptomatology of schizophrenia excluding higher brain functions symptomatology (Flores et al. 1996a; Lipska et al. 1993; Weinberger and Lipska 1995). One
area in which neurochemical changes have been observed in that model is the nAcb (Flores et al. 1996a).

It is probable that the functional maturation of the nAcb depends on a fragile equilibrium between its different inputs, the disturbance of which would probably lead to pathological states (Lipska et al. 1993, 1998; Weinberger and Lipska 1995). The aim of the present study was to describe the postnatal development of the physiological properties of nAcb neurons to understand their role in the integration of afferent inputs.

METHODS

Rat pups 1–5 day old (P1–P5) were anesthetized by hypothermia, and P6–P49 rats were anesthetized with methoxyflurane vapor in a closed environment. The animals were then quickly decapitated. The brain was taken out and submerged in artificial cerebrospinal fluid (ACSF) at 4°C containing (in mM) 126 NaCl, 26 NaHCO₃, 10 dextrose, 3 KCl, 1.3 MgSO₄, and 1.25 NaH₂PO₄ with a pH of 7.4 when bubbled with a gas mixture of 95% O₂–5% CO₂. Four hundred micrometer-thick slices containing the nAcb were cut with the brain tilted 15° from the parasagittal plane using a vibroslicer (Campden Instruments). Slices were transferred to a submerged-type recording chamber and continually superfused with ACSF at room temperature (20–22°C) at a rate of 1.5 ml/min. The nAcb was visualized under a stereomicroscope (Leica) using the anterior commissure, the neostriatum, the septum, and the ventricles as landmarks based on Paxinos and Watson (1986). Slices were incubated for at least 1 h before recording.

Pipettes were pulled from thin wall borosilicate capillary glass with a P-87 micropipette puller (Sutter Instrument). The pipettes had a resistance between 5 and 13 MΩ. Pipettes were aimed at the nAcb under direct visual guidance. The amplifier bridge balance was optimally adjusted. The output of the amplifier was fed to a LFP 200A DC amplifier/filter (Warner Instruments) and digitized at 0.5 to 10 kHz by a real-time acquisition system Digidata 1200 (Axon Instruments). Data acquisition and offline analysis were done with the pClamp 6.0 software (Axon Instruments).

During current-voltage (I-V) curve recordings, two to four sweeps at the same current level were routinely averaged on-line. Voltages were measured at the end of a current step episode of about 400 ms, and the input resistance (Rᵢ) was calculated in the linear range of the I-V curve around the holding membrane potential. The membrane time constant (τᵢ) was calculated by fitting a simple exponential to an hyperpolarizing voltage response having amplitudes between 3 and 8 mV to minimize the activation of voltage-gated ion channels. Action potential characteristics including amplitude, duration, and threshold as well as spike train patterns, were examined with supra-threshold depolarizing current pulses of varying duration and amplitude.

To compare the physiological characteristics of nAcb neurons with dorsal striatal neurons, the hyperpolarization sag and the afterhyperpolarization were measured as follows using the methods of Kawaguchi (Kawaguchi 1992, 1993). The %sag was measured as 100 × (Vpeak – Vend)/(Vpeak – Vhold). The sag latency was the time difference between the onset of the current pulse and the time of Vhold. The amplitude and latency of the spike afterhyperpolarization (AHP) were defined as the voltage and time differences, respectively, from action potential threshold to the dip voltage of the AHP. Results are presented as means ± SE. Statistical analysis was performed using Sigmastat (SPSS) and Statistica (Stat softened) softwares. When necessary, raw data were logarithmically transformed to fulfill the requirements of parametric statistical tests (Sokal and Rohlf 1995).

RESULTS

Whole cell recordings from 108 nAcb neurons were made in slices from P1–P49 rats. Because of the young age of some animals, it was easier to identify the core than the shell region of the nAcb and, therefore most neurons (91/108) were recorded in the former, whereas few (17/108) in the latter. However, we found no marked differences between the electrophysiological properties of core and shell neurons from animals of the same age groups, and data from both subnuclei were pooled in subsequent analyses.

Electrophysiological identification of cell types

Four types of neurons have been physiologically characterized in the dorsal striatum (e.g., Kawaguchi et al. 1995), and anatomical studies suggest that comparable classes of neurons are present in the nAcb (Meredith et al. 1989, 1992; Pickel and Chan 1990; Zahm 1992). Medium spiny (MS) neurons form 90–95% of the neuronal population of the nAcb, and most of the neurons recorded in the present study displayed the electrophysiological characteristics that have been attributed to MS neurons (e.g., Kawaguchi 1997). Indeed, 102 neurons (94%) were considered of the MS type because they displayed a slowly depolarizing ramp at all developmental stages when depolarized to or slightly above firing threshold with an intracellular current injection (Fig. 1A, arrow in middle panel). This is a characteristic of MS neurons, but not of any other nAcb nor dorsal striatum interneurons (O’Donnell and Grace 1993, 1995; Pennartz and Kii I 1991; Uchimura et al. 1989a). In addition, most of these (n = 85; 83%) exhibited substantial instantaneous inward rectification when hyperpolarized from RMP with intracellular current injection (Fig. 1A, arrowhead in left panel), another characteristic of MS neurons. In some MS neurons, a small depolarizing sag (usually <1%) was observed when large hyperpolarizing current pulses were injected (Fig. 1B, arrow in left panel). Sags in MS neurons were never of comparable magnitude to those found in large aspiny cholinergic (LA) neurons (Kawaguchi 1992, 1997; Kawaguchi et al. 1995), and the characteristics of the sags did not change significantly with age. The remaining putative MS neurons (n = 17; 17%) lacked inward rectification (e.g., Fig. 4A). They were all recorded in slices from animals younger than P16.
characteristic of immature MS neurons (see and, consequently, the absence of inward rectification was considered a characteristic of immature MS neurons (see Passive membrane properties).

Of the remaining six neurons, three displayed a large and slow depolarizing sag in response to hyperpolarizing current pulses (Fig. 1B, arrow in left panel), a large amplitude and long duration AHP (Fig. 1B, arrow in middle panel), and a regular firing pattern (Fig. 1B, right). These characteristics distinguish them from MS neurons.

The other three neurons exhibited the characteristics of fast spiking (FS) neurons. All three neurons lacked significant amount of inward rectification (Fig. 1C, left), displayed comparatively short-duration action potential (Fig. 1C, middle) and high-frequency spike trains with little adaptation (Fig. 1C, arrow in right panel). One of these displayed an interrupted firing pattern in response to large depolarizing currents (Fig. 1C), whereas the other two exhibited a fast decaying spike train (not shown). Only the 102 putative MS neurons were retained for further analysis and developmental considerations.

**Passive membrane properties**

Passive membrane properties of nAcb MS neurons changed dramatically during the first few postnatal weeks (Fig. 2). In general, changes were more prominent during the first two postnatal weeks, and the rate of change tapered off during the third postnatal week.

The RMP became more negative with age, averaging \(-59 \pm 3\) mV \((n = 9)\) during the first postnatal week and deepening to \(-84 \pm 1\) mV \((n = 34)\) after P21 (Fig. 2A). There was an even more dramatic change in \(\tau_m\), which decreased fourfold from an average of 143 \pm 16 ms \((n = 7)\) during the first postnatal week to 33 \pm 5 ms \((n = 28)\) after P21 (Fig. 2B). The shortening of \(\tau_m\) was paralleled by a reduction of comparable magnitude in \(R_m\), which decreased from 2,091 \pm 334 M\(\Omega\) \((n = 12)\) to 305 \pm 40 M\(\Omega\) \((n = 34)\) between the first and fourth postnatal week when measured in the nonrectified range around the RMP (Fig. 2C).

Characteristic I-V responses obtained from P7 and P30 MS neurons are shown in Fig. 3. At P7, the I-V relationship was very close to linearity up to \(-140\) mV, more than 80 mV negative to the RMP (Fig. 3A). In contrast, at P30, there was a striking inward rectification that is detectable below \(-90\) mV, only 10 mV negative to the RMP (Fig. 3B). The amount of rectification was estimated by subtracting the \(R_m\) measured in the hyperpolarized range (Fig. 2D) from the one measured around the RMP (Fig. 2C). The results obtained are plotted as a function of postnatal age in Fig. 2E. It is apparent that a large proportion of neurons displayed no inward rectification before 2 wk of age, whereas all older neurons exhibited a significant amount of rectification. Following an abrupt rise during the second postnatal week, the absolute amount of rectification apparently decreased afterward. However, the inward rectification appeared relatively constant throughout the postnatal period studied when it was expressed as a percentage of \(R_m\) measured in the linear range (Fig. 2F). This suggests that the reduction in inward rectification was related to the parallel decrease in \(R_m\) in both linear and rectified ranges (e.g., Fig. 2, C and D). This conclusion is supported by the fact that there was a strong correlation between inward rectification and \(R_m\) \((r = 0.925, P < 0.001, n = 81)\).

During the first postnatal week, the I-V relationship of 90% of neurons \((9/10)\) was linear over an extended range of hyperpolarized voltage responses, showing that young MS neurons lack inward rectification (Figs. 3A and 4A). At the beginning of the second postnatal week, a large proportion of cells began to display an inward rectification in response to hyperpolarizing current pulses. Only 13% of neurons recorded \((6/45)\) between P8 and P15 lacked inward rectification, whereas all neurons \((n = 33)\) recorded from rats P16 and older displayed a significant amount of inward rectification in the hyperpolarized range of voltage responses. To see whether there was a relationship between RMP and the presence or absence of inward rectification, we compared the RMP of neurons of similar ages with and without inward rectification. Cells displaying inward rectification displayed significantly more negative RMP than neurons lacking inward rectification (Fig. 4B). Furthermore, a significant correlation was found between the RMP of rectifying neurons and age \((r = 0.481, P = 0.027)\) but not between the RMP of nonrectifying neurons and age \((r = 0.374, P = 0.170)\), suggesting that the appearance of inward rectification is concurrent with a deepening of the RMP.
Discharge properties

The action potential of nAcbs MS neurons of all ages was characterized by a fast rising phase followed by a slower repolarizing phase (Fig. 5, A and B). When depolarized with positive current pulse, the spike threshold could be positively recognized as an inflection following a slow depolarizing ramp (Fig. 5, A and B, arrows in left panels). The slow depolarizing ramp preceding the action potential was the most consistent physiological characteristic of MS neurons; it was observed in all MS neurons regardless of age. Different potassium, calcium, and/or persistent sodium conductances have been suggested as responsible for this slow depolarization (Kawaguchi 1997), followed the action potential. Some characteristics of the action potential including threshold and amplitude did not noticeably change with age (Fig. 6, A and B). In contrast, there was a striking shortening in the duration at half-amplitude of the action potential, especially during the first 12 postnatal days (Figs. 5, A and B, and 6C), but then it remained relatively stable from P14 until the end of the period studied (Fig. 6C). At all ages, the addition of the Na⁺ channel blocker QX-314 (1 or 2 mM) to the recording pipette internal solution caused a significant reduction in amplitude (from 54.7 ± 1.4 mV, n = 65 to 35.5 ± 3.4 mV, n = 15; t₁ = 5.674, P < 0.001, df = 78) and an increase in duration (from 2.5 ± 0.1 ms, n = 65 to 7.6 ± 1.4 ms, n = 15; t₁ = 6.149, P < 0.001, df = 78) of the action potential showing that voltage-dependent Na⁺ channels were involved in spike generation.

In MS neurons of all ages, a relatively small AHP (9.9 ± 0.4 mV, n = 70), similar to the one found in dorsal striatal MS neurons (Kawaguchi 1997), followed the action potential. There was no statistically significant change in AHP amplitude with age (R² = 0.05, P = 0.07, n = 70), but its latency decreased significantly (Fig. 6D), suggesting that the kinetics of the current underlying the AHP changed during postnatal development.

Nucleus accumbens MS neurons of all ages fired repetitively and usually showed little to moderate adaptation when depolarized with DC injection (Fig. 5, A and B, right). Their firing frequency increased in a quasilinear fashion as a function of the intensity of the injected depolarizing current. Younger neurons, with their RMP closer to firing threshold (e.g., Fig. 2A) and their larger input resistance (e.g., Fig. 2C), required less depolarizing current to fire a first action potential than older neurons. The increase in firing frequency as a function of the amount of depolarizing current injected decreased with age for both the first interspike interval of a train (Fig. 7A) and the average frequency of the whole train (Fig. 7B). When the frequency of the first interval of a train or of the whole train was plotted as a function of injected current, the relationship was almost linear with correlation coefficients all larger than
The slopes of these linear fits were used to quantify the adaptation of MS neurons (Fig. 7, C and D). Those from the spike train were significantly smaller than the slope of the first interspike interval ($t_5 = 4.07, P < 0.001, df = 32$), showing that there was a small but constant adaptation during a train. Adaptation was observed in all neurons, and it did change significantly during postnatal development ($R^2 = 0.082, P = 0.11, n = 32$). Adaptation measured as a percent reduction in frequency from the first interval averaged $6.5 \pm 1.0\%$, showing that adaptation was consistent but not very large.

When depolarized from a holding membrane potential around $-70$ mV or above, MS neurons from animals of all ages fired normal action potentials (Fig. 8A, left). In contrast, some neurons from young animals fired a fast small amplitude action potential when depolarized from a holding membrane potential around $-90$ mV (Fig. 8A, right). In these neurons, sub-threshold depolarizing current pulses from a membrane potential of $-90$ mV consistently evoked a depolarizing hump (Fig. 8A, arrow in right panel), which was absent at $-70$ mV (Fig. 8A, left). The shunting effect of this small depolarizing hump on the Na$^+/K^+$ spike was most striking during a spike train evoked from $-90$ mV. This started with a small action potential followed by normal size action potentials (Fig. 8B, right) comparable to those evoked from $-70$ mV (Fig. 8B, left). The direct comparison of normal and shunted action potentials showed that the rise time of the action potential was little modified by the depolarizing hump, except for a modest but consistent reduction in amplitude with no change in action potential threshold (Fig. 8C). In contrast, the repolarization phase of the action potential was much faster in presumably shunted than in normal action potentials.

The depolarizing hump could also be activated by membrane repolarization occurring at the end of a hyperpolarizing current pulse from RMP, and it evoked a single rebound action potential of small amplitude (Fig. 8D, inset). Overall, depolarizing humps were observed in 17 MS neurons, 15 of which were from animals less than 2 wk old, suggesting that their presence was transient during development.

**Effects of potassium channels blockers**

Previously described MS neurons in both nAcb and dorsal striatum are characterized by a substantial inward rectification, whereas, in the present study, putative MS neurons in slices from young animals apparently lacked any detectable inward rectification. Inward rectification in the hyperpolarized range is sensitive to certain K$^+$ channels blockers in nAcb MS neurons (Uchimura et al. 1989a). To ascertain the absence of inward rectification in young MS neurons and to study the nature of
the inward rectification expressed in juvenile MS neurons, we have investigated the effects of specific K⁺ channels blockers including TEA, 4-AP, and Cs⁺. Of these, Cs⁺ was found to be the most potent, producing a voltage-dependent block of the inward rectification in neurons in which measurable inward rectification was present (Fig. 9A). With long and/or with large hyperpolarizing pulses, the inward rectification often reversed to outward toward the end of the current pulse (Fig. 9B). Similar effects of Cs⁺ were consistently observed in all tested neurons that displayed a substantial inward rectification in the $I-V$ relationship ($n = 10$). In contrast, Cs⁺ produced no detectable effects in neurons in which there was no evidence of an inward rectification ($n = 5$). The effects of Cs⁺ on inward rectification are summarized in Fig. 10. The $R_{in}$ of young neurons lacking inward rectification (Fig. 10A, ○) was not changed and remained linear with Cs⁺ added to the bath (Fig. 10B, ○). In contrast, the inward rectification of older neurons (Fig. 10A, ●) reversed to outward when Cs⁺ was added to the perfusing medium (Fig. 10B, ●). An analysis of covariance showed that the effects of Cs⁺ were statistically significant ($F_d = 10.12, P = 0.004, df = 28$).

TEA also decreased the inward rectification in MS neurons displaying inward rectification ($n = 3$), but in a voltage-independent manner. No inward to outward reversals in the rectification at more hyperpolarized potentials were observed (Fig. 9B; note that the action potentials are truncated because each trace represent the average of 2 sweeps, see Fig. 11B). In two neurons lacking inward rectification, TEA produced no detectable effect in their response to hyperpolarizing current pulses, although it had marked effects on their spiking characteristics (not shown). In contrast to Cs⁺ and TEA, 4-AP had no statistically significant effects on the response of MS neurons to hyperpolarizing current pulses ($t_d = -0.52, P = 0.613, n = 9$) despite its striking effects on the firing characteristics of all neurons tested (Fig. 9C; note that the action potentials are truncated because each trace represent the average of 2 sweeps). It was also tested on three neurons which lacked inward rectification and produced no significant effects on their $I-V$ relationships.

The effects of K⁺ channel blockers on the firing characteristics of MS neurons were also examined. Regardless of age, Cs⁺ produced no significant effects on any characteristics of the action potential including threshold ($t_d = 1.2, P = 0.24, df = 25$), peak ($t_d = 0.36, P = 0.72, df = 28$) duration at half-amplitude ($t_d = 0.45, P = 0.66, df = 24$; Fig. 11A). In contrast, TEA produced marked effects on spike trains. These effects were qualitatively similar to those described by Kita et al. (1985b) in dorsal striatum MS neurons. Following an initial fast action potential (Fig. 11B, arrowhead), the remaining spike train was replaced by a long depolarized plateau that could last several hundred

FIG. 5. Characteristics of action potentials evoked with depolarizing current pulses in MS neurons. Single action potential (left) and firing train (right) in a P1 neuron (A) and a P39 neuron (B). The arrows in A and B (left) indicate the slow depolarizing ramp observed with depolarizing current pulses slightly above threshold, and the bottom dotted line indicates the resting membrane potential. The horizontal scale bar represents 20 ms for single action potential traces and 150 ms for the spike train traces. In A and B, left, only a section of the sweep is shown.

FIG. 6. Characteristics of the action potential as a function of postnatal age. A: threshold. The threshold was measured as the voltage on the rising phase of the response at which the change in slope was maximum in response to just above threshold depolarizing current pulses of 100 or 400 ms (see Threshold labeled dotted lines in the left panels of Fig. 5, A and B). No statistically significant correlation was found between threshold and postnatal age ($R^2 < 0.021, P = 0.270, n = 59$; dotted straight line). B: amplitude. The amplitude was measured from the threshold to the peak of the action potential. No statistically significant correlation was found between the amplitude and postnatal age ($R^2 < 0.032, P = 0.156, n = 65$; dotted straight line). C: duration. The duration was measured at the midpoint between the threshold and the peak of the action potential (Half-amplitude, labeled dotted lines in the left panels of Fig. 5, A and B). A 2nd-order polynomial regression was applied to the data, and a statistically significant negative correlation was found between duration and postnatal age ($R^2 = 0.369, P < 0.001, n = 65$; solid curved line). D: latency of the AHP following the action potential. A 2nd-order polynomial regression was applied to the data, and a statistically significant correlation was found between the latency of the AHP and postnatal age ($R^2 = 0.214, P < 0.001, n = 70$; solid curved line).
milliseconds when large depolarizing current pulses were used (Fig. 11B, arrow). 4-AP also increased the duration of the action potential but produced less dramatic effects than TEA (e.g., Fig. 9C, middle). Before producing its maximal effects on the action potential, 4-AP reduced the latency to the first spike discharge (Fig. 11C) and substantially reduced the amplitude of the AHP (Fig. 11C, arrow) with no apparent effect on the action potential threshold ($t_\text{r} = 1.11, P = 0.29, \text{df} = 16$). All the effects of K$^+$ channels blockers on the membrane and firing characteristics were fully reversible.

**D I S C U S S I O N**

Nucleus accumbens MS neurons were recorded in slices during postnatal development from the day after birth ($P1$) until early adulthood ($P49$). During that period, the basic membrane and firing properties of MS neurons changed dramatically. Soon after birth, they displayed some basic properties of neurons such as negative RMP and the capacity to fire fast Na$^+/K^+$ action potentials. However, several aspects of the membrane and firing properties of young MS neurons were remarkably different from those found in mature MS neurons. During the postnatal period studied, the RMP became more negative and the $R_{\text{in}}$ and $\tau$ decreased severalfold. In addition, young MS neurons fired Na$^+/K^+$ action potentials more readily than older ones and lacked inward rectification during the first postnatal week. By $P49$, the characteristics of MS neurons were not noticeably different from those reported for adult animals. However, our results showed that immature MS neurons do not display membrane potential bistability, which suggests that input/output integration is different in mature and immature nAcB.

**Nature of immature nAcB neurons**

MS neurons are the only known projection neurons in the nAcB, and they are also the most abundant, forming 90–95% of the population. Despite the young age of some of our preparations, the large majority (94%) of recorded neurons were positively identified as MS neurons since they displayed at least one electrophysiological property of MS neurons that is not found in other nAcB neuronal types. In nAcB and dorsal striatum, only MS neurons display a depolarizing plateau preceding the action potential when depolarized from RMP, and a powerful instantaneous inward rectification sensitive to Cs$^+$ when hyperpolarized from RMP (Chang and Kitai 1986; Kawaguchi 1992, 1993, 1997; Kawaguchi et al. 1989, 1990, 1995; Lopes da Silva et al. 1984; O’Donnell and Grace 1993; Pennartz et al. 1991; Uchimura et al. 1989a,b; Yang and Mogenson 1984; Yim and Mogenson 1982, 1988). In the present study, all putative MS neurons in preparations older than $P15$, and 72% of putative MS neurons in preparations from $P1$ to $P15$ animals displayed both characteristics. A subset of neurons from animals $P15$ and younger lacked inward rectification, but they showed a slow ramp depolarization preceding the action potential and, for this reason, were regarded as immature MS neurons. The absence of inward rectification has also been reported in subsets of MS neurons from kitten caudate nucleus (Cepeda et al. 1991) and rat neostriatum (Tepper et al. 1998), supporting the idea that the lack of inward rectification is a characteristic of immature MS neurons.

Six other neurons were classified as non-MS because they had neither of the two physiological characteristics of MS neuron and displayed properties suggesting that they belonged to other neuronal classes types. Indeed, neurons with large depolarizing sag and important AHP have been morphologi-
cally identified as large aspiny cholinergic neurons in the dorsal striatum (Kawaguchi 1993; Kawaguchi et al. 1995), suggesting that cholinergic interneurons have similar physiological properties in the nAcb. The neurons we have named fast spiking neurons have similar physiological characteristics to a class of GABAergic interneurons also found in the dorsal striatum (Kawaguchi et al. 1995).

Development of membrane and firing properties of MS neurons

All putative MS neurons recorded prior to P7 lacked inward rectification. Afterward, the proportion of rectifying neurons recorded increased constantly with aging so that all MS neurons displayed inward rectification after P15. This is in contrast with an in vivo study in rat dorsal striatum in which the proportion of MS neurons expressing inward rectification also increased with age but was only 40% in P30–P42 animals and 80% in adults (Tepper and Trent 1993; Tepper et al. 1998). These discrepancies might reflect functional differences between dorsal striatum and nAcb neurons or might be due to differences in recording techniques.

In absolute terms, inward rectification was larger in cells from younger animals and decreased with age (e.g., Fig. 2E) but, since this decrease was paralleled by a decrease in R_{in} of comparable magnitude, it appeared as a somewhat all or none phenomenon readily integrated with other membrane properties and was not a progressive change in membrane properties such as the change in RMP. In contrast, the progressive increase in the proportion of neurons displaying inward rectification during the first two postnatal weeks suggests that the regulation of the expression of this phenotype is age dependent.

Developmental changes in basic membrane properties included a large decrease in R_{in} paralleled by a shortening in \( \tau \). Similar findings have been described in other structures.

![Image](http://jn.physiology.org/)

**FIG. 8.** Low-threshold depolarizing humps. A: responses of a P6 MS neuron to sub- and supra-threshold depolarizing current pulses at a holding membrane potential of −70 mV (left) and −90 mV (right). The arrow in the right panel indicates the depolarizing hump present in the sub-threshold trace which is absent in the left sub-threshold trace. B: trains of action potentials evoked from −70 mV (left) and −90 mV (right). At −90 mV, the 1st action potential of the train was fast and small (arrow) compared with the subsequent spikes of the train; the small action potential was absent when depolarization occurred from −70 mV (left trace). C: expansion of the action potentials shown in A aligned on their firing threshold (dotted line). D: voltage dependency of the low-threshold depolarizing hump elicited at the end of an hyperpolarizing current pulse. The low-threshold depolarizing hump is still discernible following the action potential (arrow). The inset shows the maximal number of action potentials overriding the depolarizing hump that could be evoked with a large hyperpolarization. The vertical scale bar represents 50 mV in A, B, and D, 25 mV in C, and 100 mV in the inset in D. The horizontal scale bar represents 120 ms in A, 300 ms in B and D, 10 ms in C, and 600 ms in the inset in D.

![Image](http://jn.physiology.org/)

**FIG. 9.** Effects of CsOH (A), tetraethyl ammonium (TEA; B), and 4-aminopyridine (4-AP; C) on the membrane properties of MS neurons. A: voltage responses and corresponding I-V relationships of a P12 neuron before (Control), during (CsOH 28 min), and after (Wash 54 min) the addition of 300 \( \mu \)M CsOH to the artificial cerebrospinal fluid (ACSF). B: voltage responses and corresponding I-V relationships of a P37 neuron before (Control), during (TEA 10 min), and after (Wash 25 min) the addition of 25 mM TEA to the ACSF. C: voltage response and corresponding I-V relationships of the same P12 neuron shown in A before (Control), during (4-AP 15 min), and after (Wash 31 min) the addition of 2 mM 4-AP to the ACSF. All traces represent the average of 2 sweeps. In all 3 I-V curve panels: ▲, Control; ■, Blocker; ●, Wash.
throughout the neuraxis (McCormick and Prince 1987; Pirchio et al. 1997; Ramoa and McCormick 1994; Spigelman et al. 1992; Tepper and Trent 1993; Tepper et al. 1998; Warren and Jones 1997; Zhou and Hablitz 1996). The concomitant decrease in R\textsubscript{m} and τ\textsubscript{m} with age implies an increase in ion channel density as previously noted (McCormick and Prince 1987; Spigelman et al. 1992; Zhou and Hablitz 1996), assuming that the specific membrane capacitance does not change with age (Deuchars and Thomson 1995, 1996). Membrane resistance also depends on the surface area of the cell (e.g., Warren and Jones 1997), but we do not have any morphological data to confirm that MS neurons actually grow during the period covered by the present study. In the dorsal striatum, although it was found that MS neurons soma do not grow between P6 and adulthood, there is a large increase in the number of dendritic spines and a thickening of the dendrites (Tepper and Trent 1993; Tepper et al. 1998), suggesting that changes in R\textsubscript{m} and τ are paralleled by an increase in cell membrane surface.

The higher R\textsubscript{m} and thus a smaller electrotonic length in newborns may help MS neurons to integrate synaptic inputs in a more efficient manner; this could compensate for their lack of organization (McCormick and Prince 1987; Spigelman et al. 1992). Our R\textsubscript{m} and τ values are much larger than those reported by others for MS neurons (Kawaguchi et al. 1989; Kita et al. 1984; O’Donnell and Grace 1993). Whereas part of the differences can be attributed to the young age of some of our preparations, much of the differences can be explained by the fact that we used the whole cell recording technique, which consistently yielded much higher values for these parameters than sharp electrode recordings. However, the fact that the experiments were conducted at room temperature may have somewhat contributed the long τ by slowing membrane conductances.

Several possibilities have been advanced to explain why the RMP is more depolarized in immature neurons: these include a lower K\textsuperscript{+} conductance (Spigelman et al. 1992), an inactive Na\textsuperscript{+}/K\textsuperscript{+}/Cl\textsuperscript{−} co-transport extrusion mechanism (Zhang et al. 1991), and an immature Na\textsuperscript{+}/K\textsuperscript{+} ATPase (Fukuda and Prince 1992). In the present study, we found that neurons expressing inward rectification displayed lower membrane potential than neurons of the same age without this characteristic, suggesting that a portion of the hyperpolarization of the RMP is related to or co-expressed with inward rectification.

Short-duration action potentials were present as early as P1. With low concentrations of QX-314 in the patch pipette, the action potentials were much wider and of lower amplitude showing that functional voltage-dependant Na\textsuperscript{+} channels are already involved in the depolarizing phase of the action potential at birth. The duration of the action potential decreased by more than half during the period studied, whereas its amplitude and threshold did not significantly change. The average amplitude and threshold measured in the present study are in the range of those reported in nAcb of adult rats (O’Donnell and Grace 1993), supporting our finding that these parameters do not significantly change during postnatal development. In rat dorsal striatum in vivo, the action potential threshold was also found to be constant during postnatal development, although its amplitude was smaller between P6 and P10 than at later ages, but it did not change significantly after that (Sharpe and Tepper 1999). Developmental changes in action potential duration and amplitude have also been found in kitten caudate nucleus (Cepeda et al. 1991). A maturation of sodium channels or an increase in their density might explain why the action potential is shorter in older animals. A parallel maturation of the K\textsuperscript{+} delayed rectifier (K\textsubscript{DR}) and the fast sodium currents on MS neurons, respectively. The middle traces were recorded after wash in periods of 24 min (A), 10 min (B), and 19 min (C). The wash out periods were of 40 min (A), 25 min (B), and 35 min (C). The horizontal scale bar represents 250 ms for A and 125 ms for B and C.

**FIG. 10.** Amount of inward rectification in the hyperpolarized range in the control situation (A) and with Cs\textsuperscript{+} added to the perfusing medium (B). The ordinate represents the amounts of inward rectification calculated as the ratio of the difference between the R\textsubscript{m} measured around the resting membrane potential and that measured at hyperpolarized membrane potentials and the R\textsubscript{m} measured around the holding membrane potential (see Fig. 2). A statistically significant correlation was found between the effects of Cs\textsuperscript{+} on the rectification and postnatal age (R\textsuperscript{2} = 0.493, P = 0.024, n = 10). Cells lacking inward rectification were excluded from the regression.

**FIG. 11.** Effects of CsOH (A), TEA (B), and 4-AP (C) on the firing properties of P12, P37, and P15 MS neurons, respectively. The middle traces were recorded after wash in periods of 24 min (A), 10 min (B), and 19 min (C). The wash out periods were of 40 min (A), 25 min (B), and 35 min (C). The horizontal scale bar represents 250 ms for A and 125 ms for B and C.
current at all ages, in agreement with findings in other brain structures (McCormick and Prince 1987; Ramoa and McCormick 1994; Warren and Jones 1997; White and Sur 1992; Zhang et al. 1991). With aging, more depolarizing current was needed to generate action potentials in MS neurons. Thalamic neurons are similarly affected by age (Ramoa and McCormick 1994; Warren and Jones 1997), whereas, in contrast, the current threshold of cortical pyramidal neurons decreased with age (McCormick and Prince 1987). In the present study, we have identified two factors that could contribute to this phenomenon. First, the RMP became more negative with age, while the spiking threshold remained constant throughout development, meaning that, even with a constant membrane $R_{\text{in}}$, larger currents would be needed to bring the membrane to spiking threshold and to keep it there during sustained activity because of the increased ionic repolarizing strength between spikes. Second, $R_{\text{in}}$ decreased significantly with age, so that more current was needed to reach spiking threshold and this even if the RMP had remained constant. In addition, the significant decrease in AHP latency with age suggests that the amount of current needed to increase spiking frequency during a train had to counteract larger $\text{Ca}^{2+}$-dependent $K^+$ conductances with age.

In a subset of neurons we have observed a depolarizing hump that was activated when the membrane was depolarized from $-90 \text{ mV}$ but not $-70 \text{ mV}$. When activated on release from an hyperpolarizing current pulse, it was sufficient to generate one or two rebound action potential. These characteristics are reminiscent of low-threshold $\text{Ca}^{2+}$ spike (LTS), which has been extensively studied in thalamic neurons and has also been described in a subset of nAcb neurons (O’Donnell and Grace 1993). With sufficient depolarization, the hump was overridden by a somewhat shunted action potential. In contrast, the first rebound spike at the end of a hyperpolarizing current pulse appeared normal and was followed by a depolarizing hump. With larger hyperpolarization, a second, smaller action potential was evoked. One plausible explanation is that the depolarizing hump increased some ionic conductances, possibly $\text{Ca}^{2+}$-dependent $K^+$ current if the depolarizing hump is actually a LTS, that shunt a normal action potential. Nevertheless, the depolarizing humps were of small amplitude and could be compared with LTS found in thalamic relay neurons during early postnatal development (e.g., Warren and Jones 1997). On the other hand, we have not positively identified these as LTS with the use of specific $\text{Ca}^{2+}$ blockers, and, therefore we cannot exclude other possibilities such as the involvement of electrotonic coupling or of dendritic or initial segment spike (Grace 1990).

Overall, changes in membrane and firing properties of nAcb MS neurons occurred mostly during the first 3 postnatal weeks, whereas later, changes could not be discriminated from interneuronal variability. Firing characteristics appeared to reach adult values around P15, whereas membrane properties including the RMP, $R_{\text{in}}$, and $\tau$ appear to mature about 1 wk later. This is in close agreement with similar studies performed in the dorsal neostriatum (Misgeld et al. 1986; Tepper and Tret 1993; Tepper et al. 1998), suggesting that the nAcb and dorsal striatum mature during overlapping postnatal period. As remarked above, the only notable difference is the presence of inward rectification in all nAcb MS neurons after P15, and the implications for this difference are discussed below.

Functional perspective

In vivo experiments have shown that MS neurons exhibit membrane potential bistability and that this characteristic has a profound impact on the signal transfer characteristics of these neurons (Gerfen and Wilson 1996; Kincaid et al. 1998; Nisenbaum and Wilson 1995; Nisenbaum et al. 1994; O’Donnell and Grace 1995; Wilson 1993; Wilson and Kawaguchi 1996; Xu et al. 1991). Nucleus accumbens MS neurons recorded in vivo in adult animals show a pattern of spontaneous activity consisting of brief episodes of firing separated by long periods of silence (O’Donnell and Grace 1993, 1995). Intracellular recordings of MS neurons in vivo in both nAcb (Finch 1996; O’Donnell and Grace 1995; Yim and Mogenson 1988) and dorsal striatum (Buchwald et al. 1973; Calabresi et al. 1990; Finch 1996; Hull et al. 1970; Wilson and Groves 1981; Wilson and Kawaguchi 1996; Yim and Mogenson 1988) have shown that active and silent episodes correspond to two different stable membrane potential states about 20 mV apart: an hyperpolarized silent state around $-80 \text{ mV}$ and a depolarized active state around $-60 \text{ mV}$. MS neurons fire only when in the depolarized state with the spikes often occurring in bursts. Membrane potential bistability in MS neurons appears to be produced by the interplay between afferent excitatory synaptic inputs and intrinsic membrane $K^+$ conductances (Wilson and Kawaguchi 1996). Following lesion or reversible inactivation of hippocampal excitatory inputs in vivo, nAcb MS neurons remain in the hyperpolarized state, suggesting that the depolarized state requires hippocampal inputs (O’Donnell and Grace 1995). In slices maintained in vitro, the membrane potential of MS neurons remains around $-80$ to $-90 \text{ mV}$, corresponding to that of the in vivo hyperpolarized state (Chang and Kitai 1986; O’Donnell and Grace 1994; Uchimura et al. 1989b). No depolarized episodes are seen presumably because active excitatory synaptic inputs to the nAcb are cutoff (O’Donnell and Grace 1995; Wilson and Kawaguchi 1996).

A low RMP such as that found in vitro in mature MS neurons is an essential condition for the appearance of membrane potential bistability in MS neurons. Indeed, a low RMP coupled with the possibility of a stable plateau depolarization of 20 mV will maintain the membrane potential close to spiking threshold so that weak excitatory inputs from other sources will make the cell fire (e.g., O’Donnell and Grace 1995). The membrane properties in young MS neurons are quite different from those present in mature animals, suggesting that membrane potential bistability is unlikely to be the primary mode of input integration in MS neurons during the first postnatal weeks. We have found that MS neurons RMP did not reach a value comparable to adults (around $-80 \text{ mV}$) before the end of the third postnatal week, suggesting that membrane bistability could not be fully developed before that time and that until then MS neurons will respond differently to converging excitatory inputs. Indeed, membrane potential bistability is not consistently observed in dorsal striatum MS neurons in anesthetized rats before the end of the fourth postnatal week when the proportion of MS neurons expressing inward rectification has reached adult levels (Sharpe and Tepper 1999; Tepper et al. 1998). In the nAcb, we have found that inward rectification is expressed in all MS neurons by P16 and later, raising the possibility that bistability appears earlier in the nAcb than in dorsal striatum. In addition, if the expression
of inward rectification is a requirement to membrane potential bistability, than it is potentially expressed in all nAcbs MS neurons but only in a subpopulation of MS neurons in the dorsal striatum.

It has been suggested that inward rectification plays an important role in membrane potential bistability (Wilson and Kawaguchi 1996). Its absence in most cells during the first postnatal week indicates that MS neurons will not display membrane potential bistability during early postnatal life. In MS and some other types of neurons, inward rectification is produced by a voltage-gated K⁺ current similar to I_{Ks} (Nisenbaum and Wilson 1995). This current may also be involved in the maintenance and stability of relatively negative passive RMP (Nichols and Lopatin 1998).

The functional differences between young and mature MS neurons could be important throughout a period during which activity-dependent development and stabilization of synaptic inputs is probably occurring in the nAcbs. The nAcbs receive putative excitatory glutamatergic inputs from various sources that are not fully developed at birth, so the nAcbs is likely to complete its development in parallel with those structures. Our results suggest that young MS neurons require smaller excitatory synaptic inputs to be activated because of their more depolarized membrane potential, N-methyl-D-aspartate (NMDA) receptors will be readily activated favoring Ca²⁺-dependent plasticity. An interesting and challenging question is as follows: what triggers the expression of inward rectification in MS neurons? One possibility is that inward rectification is expressed in MS neurons in an activity-dependent manner following the arrival of a glutamatergic innervation (Moody 2000). Indeed, early postnatal disruption of hippocampal and cortical innervation of the nAcbs results in long-lasting neuro-molecular changes in the nAcbs accompanied by an increase in behavioral sensitivity to psychostimulants (Flores et al. 1996a,b; Lipska et al. 1998). This could be related to changes in K⁺ conductances (Premkumar and Ahern 1995; Rosenzweig-Lipson et al. 1997; Wang and Grahame-Smith 1992).

An electrophysiological characterization of MS neurons of different ages that were deafferented at birth may help to answer this question.

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