Maturation of Excitatory Synaptic Transmission of the Rat Nucleus Accumbens From Juvenile to Adult

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Kasanetz F, Manzoni OJ. Maturation of excitatory synaptic transmission of the rat nucleus accumbens from juvenile to adult. J Neurophysiol 101: 2516–2527, 2009. First published February 25, 2009; doi:10.1152/jn.91039.2008. Precise control of synaptic strength is critical for maintaining accurate network activity and normal brain functions. Several major brain diseases are related to synaptic alterations in the adult brain. Detailed descriptions of the normal physiological properties of adult synapses are scarce, mainly because of the difficulties in performing whole cell patch-clamp recording in brain slices from adult animals. Here we present the portrait of excitatory synapses and intrinsic properties of medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a central structure of the mesocorticolimbic system, from youth (P14) to adulthood (P120). We found that intrinsic neuronal excitability decreased over development, mainly due to an enhancement of potassium conductance and the consequent reduction in membrane resistance. The ratio between paired-pulse synaptic responses was similar in juvenile, adolescent, and adult MSNs, suggesting that the probability of neurotransmitter release was unaltered. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)–mediated excitatory postsynaptic currents (EPSCs) decayed more slowly in adult MSNs. Akin to AMPAR, NMDAR EPSCs decayed more slowly in adult MSNs than AMPAR in juvenile and adult NAc. As a consequence, electrophysiological descriptions of excitatory synapses in the adult brain are scarce and remain partial at best.

The nucleus accumbens (NAc) is an essential component of the mesocorticolimbic system that plays a prominent role in motivation and reward. Excitatory synapses from the prefrontal cortex, hippocampus, and amygdala converge on individual medium spiny neurons (MSNs), which coordinate the outflow of information from the NAc (French and Totterell 2002, 2003; Groenewegen et al. 1999; O’Donnell and Grace 1995). Several major psychiatric diseases, such as schizophrenia and addiction, are accompanied by profound modifications of these glutamatergic systems (Kalisvaart et al. 2005; Kauer and Malenka 2007; Tan et al. 2007). Electrophysiological recordings in NAc slices from juvenile and adolescent rodents have shed light on the physiology of glutamatergic synapses in this nucleus and revealed various forms of synaptic plasticity (Pennartz et al. 1993; Robbe et al. 2002a,b; Thomas et al. 2000, 2001). As a first step toward the understanding of the physiological and pathological functions of the adult NAc, we established a developmental portrait of excitatory synapses and MSN intrinsic properties in the core of the NAc (Zhang and Warren 2008). Using a simple adaptation of classical slice preparation procedures, we performed whole cell recordings from MSNs in slices from juvenile (P14–P21, the age at which most electrophysiology in slice is done), adolescent (P32–P42), and adult (P120–P200, the age at which most behavioral studies are done) rats. The data show that adulthood was characterized by a reduced intrinsic excitability, a selective slowing of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) excitatory postsynaptic current (EPSC) and marked heterogeneity of synaptic AMPAR/N-methyl-D-aspartate receptor (NMDAR) ratios.

METHODS

Animal treatment

Animal experiments were conducted in strict compliance with European directives (86/609/EEC) and French laws on experimenta-
tion and use of laboratory animals (Authorization number 3307016). Rats (male, Sprague–Dawley) were housed, grouped, and acclimatized to laboratory conditions (12-h light/dark cycles) and had unrestricted access to food and water.

Slice preparation

Rats were anesthetized with halothane or a mixture of ketamine (100 mg/kg) and xylazine (1 mg/kg) and decapitated. The brain was sliced (300 μm) in the coronal plane using a vibratome (IntegraSlice, Campden Instruments, Loughborough, UK). Slicing parameters were as follows: blade advance speed, 0.15 mm/s; blade vibration frequency, 60 Hz; blade vibration amplitude, 0.8 mm. Slices were maintained in a sucrose-based physiological solution at 4°C (in mM: 87 NaCl, 75 sucrose, 25 glucose, 5 KCl, 21 MgCl₂, 0.5 CaCl₂, and 1.25 NaH₂PO₄). These parameters were essential for the quality of the preparation. Immediately after cutting, slices were stored for 40 min at 32–35°C in a low-calcium artificial cerebrospinal fluid (low-Ca ACSF) that contained (in mM): 140 NaCl, 11 glucose, 2.5 KCl, 2.4 MgCl₂, 1.2 CaCl₂, 23 NaHCO₃, and 1.2 NaH₂PO₄, and was equilibrated with 95% O₂-5% CO₂. Slices were then stored in low-Ca ACSF at room temperature until recording. For recording, slices were placed in the recording chamber and superfused (1.5–2 ml/min) with ACSF (same as low-Ca ACSF, with the following exception: 2.4 mM CaCl₂ and 1.2 mM MgCl₂). All experiments were performed at 32–35°C. The superfusion medium contained picrotoxin (100 μM) to block γ-aminobutyric acid type A receptors. All drugs were added at the final concentration to the superfusion medium.

Electrophysiology

Whole cell patch-clamp recordings of visualized MSN were made in coronal slices containing the NAc. Two intracellular solutions were used, based on cesium methane-sulfonate (for voltage clamp) or K⁺ gluconate (for current clamp), as follows (in mM): 128 cesium methane-sulfonate (CH₃OSO₄) or K⁺ gluconate, 20 NaCl, 1 MgCl₂, 1 EGTA, 0.3 CaCl₂, 2 Na⁺ ATP, and 0.3 Na⁺ GTP, buffered with 10 Hepes (pH 7.3, osmolality 290–300 mosM). Electrode resistance was 4–6 MΩ. To evaluate the access resistance (Rₐ), a 2-mV hyperpolarizing pulse was applied before each EPSC. Rₐ was not compensated and cells were rejected if Rₐ was >25 MΩ or changed >20% during the experiment. The potential reference of the amplifier was adjusted to zero prior to breaking into the cell. Whole cell patch-clamp recordings were performed with an Axopatch-200B (Molecular Devices, Sunnyvale, CA). In current-clamp experiments, the resting membrane potential was measured as soon as the whole cell configuration was achieved and a 13-mV estimated junction potential (using the junction potential tool of Clampex 10, Molecular Devices) was subtracted from all membrane potential measurements. Data were filtered at 1–2 kHz, digitized at 10 kHz on a DigiData 1332A interface (Molecular Devices), collected on a PC using Clampex 10, and analyzed using Clampfit 10 (Molecular Devices). To evoke synaptic currents, stimuli (100-μs duration) were delivered at 0.067 Hz (otherwise stated) with a glass electrode filled with ACSF and placed at a distance >150 μm in the dorsomedial direction.

Data acquisition and analysis

To perform current–voltage (I–V) curves and to test the excitability of MSN, a series of hyperpolarizing and depolarizing current steps were applied immediately after breaking in the cell. Membrane resistance was estimated from the I–V curve around resting membrane potential (RMP). To directly measure the inwardly rectifying potassium currents (I_Kir), MSNs were voltage clamped at −70 mV and a 50-ms step to −150 mV was applied; then, cells were depolarized to −45 mV with a ramp pulse of 0.6 mV/ms (Gertler et al. 2008; Shen et al. 2007). Whole cell capacitance was estimated in voltage-clamp recording from the membrane relaxation after a 2-mV, 100-ms hyperpolarizing step. Sampling rate was increased to 20 kHz and signals were filtered at 10 kHz to better resolve the fast transient. A biexponential function was fit from the peak to the steady state of the capacitive transient and a weighted decay time was calculated. Then, membrane capacitance was calculated as depicted in Fig. 1G. Depolarizing current steps (40 ms long) were used to induce calcium-mediated plateau potentials.

The AMPAR/NMDAR ratio was measured from the EPSC evoked while holding cells at +40 mV. The AMPAR EPSC was isolated after bath application of the NMDAR antagonist d-2-amino-5-phosphonovaleric acid (d-APV, 100 μM). The NMDAR EPSC was obtained by digital subtraction of the AMPAR EPSC from the dual (AMPAR + NMDAR) EPSCs.

Rise times of AMPAR- and NMDAR-evoked EPSCs were fitted with a single-exponential function. To fit decay times of AMPAR EPSCs, a double-exponential decay function was used. NMDAR EPSCs were recorded at +40 mV and decay times were estimated by dividing the area by the peak of the responses.

Spontaneous EPSCs (sEPSCs) were recorded in the whole cell voltage-clamp configuration using AxoScope 10 (Molecular Devices). sEPSCs recorded at −70 mV were detected using a template of sEPSCs generated from averaging several typical synaptic events with Clampfit 10 (Molecular Devices). The template was slid along the data trace one point at a time. At each position, this template was optimally scaled and offset to fit the data. A lower-amplitude threshold of 6 pA was applied, equivalent to 2.5SD of baseline noise. sEPSCs mediated by both AMPAR and/or NMDAR were recorded at +40 mV and detected with digitally designed templates (Molecular Devices). For dual sEPSCs, a template with rise and decay times of 3 and 150 ms, respectively, was used. A lower-amplitude threshold of 16 pA was applied. The specificity of these parameters in detecting only dual sEPSCs was confirmed in each cell by sliding the template along recordings made in the presence of 100 μM d-APV. Only cells in which the number of events detected in the presence of the NMDAR antagonist was reduced by >95% were used. AMPAR sEPSCs at +40 mV were isolated in the presence of APV with a template with rise and decay times of 1.2 and 4 ms, respectively. A lower-amplitude threshold of 9 pA was applied.

The frequency of dual sEPSCs was low (in Hz; juvenile [Jv]: 0.6 ± 0.06; adolescent [Ado]: 0.21 ± 0.02; adult [Ado]: 0.11 ± 0.02). The increased baseline “noise” of signals recorded at +40 mV, generated by opening of NMDAR by ambient glutamate (Herman and Jahr 2007; Le Meur et al. 2007; Sah et al. 1989), prevented the resolution of many of the smaller events. To ensure the sampling of enough events, dual sEPSCs were recorded for 10 min in each cell.

When compared with recordings made at −70 mV, AMPAR sEPSCs at +40 mV were less frequent (in Hz; Jv: 3.9 ± 0.7, Ado: 3.1 ± 0.6, Ado: 1.6 ± 0.4) and smaller. This could be due to the reduced driving force of the synaptic currents and the slightly greater baseline noise that forced us to increase the lower amplitude detection threshold. Because sEPSCs at +40 mV were greater in juveniles, more events were detected.

Asynchronous EPSCs (asyn EPSCs) were recorded at −70 mV with an extracellular solution where CaCl₂ was replaced by the same concentration of SrCl₂. Synaptic events were detected using a template as stated earlier. Asyn EPSCs were collected during a segment comprising 50–160 ms after afferent stimulation (0.1 Hz for 10 min). To verify that asyn EPSCs represent quantal events arising from stimulated synapses, their frequencies and amplitudes were compared with those of sEPSCs detected in a 300-ms prestimulus time window.

Drugs

Picrotoxin was from Sigma (St. Quentin Fallavier, France); 6,7-dinitroquinoxaline-2,3-dione (DNQX) and d-APV were from Tocris...
**RESULTS**

Whole cell recordings were performed in MSNs located in the NAc core in rat brain slices at three developmental time points: juvenile (Juv; P14–P21, n = 67 cells), adolescent (Ado; P32–P42, n = 63 cells), and adult (Adu; P120–P200, n = 85 cells). This classification was made on the basis of studies that, according to a number of neurobehavioral characteristics, estimate that adolescence in rodents spans approximately P28–P42 (Spear 2000).

**Increased intrinsic excitability of MSNs from juvenile rats**

We examined the intrinsic properties of MSNs in current-clamp recordings. All MSNs, regardless of the age of the rat, showed a largely hyperpolarized RMP (Fig. 1A, Table 1) and a strong inward rectification, shown as a shift from linearity in the $I-V$ plots (Fig. 1B). To test the excitability of MSNs, we compared the number of action potentials (APs) elicited by a series of somatic positive current steps (Fig. 1A).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Juvenile</th>
<th>Adolescent</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>$-93.5 \pm 1.0$</td>
<td>$-93.10 \pm 1.0$</td>
<td>$-93.70 \pm 1.3$</td>
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<tr>
<td>AP threshold, mV</td>
<td>$-46.4 \pm 1.0$</td>
<td>$-43.90 \pm 1.4$</td>
<td>$-45.80 \pm 1.2$</td>
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<tr>
<td>AP amplitude, mV</td>
<td>$68.5 \pm 1.5$</td>
<td>$66.80 \pm 3.2$</td>
<td>$68.40 \pm 2.6$</td>
</tr>
<tr>
<td>AP afterhyperpolarization, mV</td>
<td>$9.4 \pm 0.8$</td>
<td>$10.20 \pm 1.0$</td>
<td>$11.90 \pm 0.6$</td>
</tr>
<tr>
<td>AP half-width, ms</td>
<td>$1.3 \pm 0.07$</td>
<td>$1.22 \pm 0.06$</td>
<td>$1.28 \pm 0.06$</td>
</tr>
</tbody>
</table>

Values are means ± SE.

![Graph showing enhanced intrinsic excitability of MSNs from juvenile rats](image-url)
of APs induced was dramatically lower in MSNs from adolescent and adult rats, compared with that from juveniles (Fig. 1C, two-way ANOVA, \( P < 0.001 \) for the age factor, \( P < 0.001 \) for the interaction age vs. current step). The minimal current necessary to evoke AP firing, or rheobase, was lower in juveniles (Fig. 1D; in nA: Juv: 0.19 ± 0.01; Ado: 0.28 ± 0.02; Adu: 0.27 ± 0.01; one-way ANOVA, \( P < 0.05 \)). The differences found in excitability were not due to changes in RMP or AP threshold (Table 1); rather, AP features were similar over age groups (Table 1).

Increased excitability of MSNs from juveniles might be explained, in part, by the higher membrane resistance measured at rest (Fig. 1E; in MO: Juv: 152 ± 18; Ado: 86 ± 9; Adu: 106 ± 8; one-way ANOVA, \( P < 0.01 \)). The conductance in MSNs at rest is composed mainly of the sum of the inwardly rectifying potassium current (\( I_{\text{Kir}} \)) and the linear leaky potassium current (\( I_{\text{Kleak}} \)) (Nisenbaum and Wilson 1995; Uchimura et al. 1989). \( I_{\text{Kir}} \) is voltage dependent, activates rapidly, and shows little inactivation. We quantified MSN \( I_{\text{Kir}}-I_{\text{Kleak}} \) using voltage ramps from −150 to −45 mV (Fig. 1F; also see Methods) (Gertler et al. 2008; Shen et al. 2007). The \( I-V \) relationship showed a similar activation profile and reversal potential of MSN \( I_{\text{Kir}} \) at all ages tested (Fig. 1F). However, \( I_{\text{Kir}}-I_{\text{Kleak}} \) current amplitude was decreased in juveniles, with a peak conductance significantly smaller than that of adolescents and adults (in nS: Juv: 16.0 ± 1.5; Ado: 28.2 ± 2.7; Adu: 27.4 ± 2.1; one-way ANOVA, \( P < 0.01 \)).

Early during development, MSNs do not express \( I_{\text{Kir}} \) and have very high membrane resistance (Belleau and Warren 2000; Tepper et al. 1998). Although all MSNs tested from juveniles expressed inward rectification, it is possible that \( I_{\text{Kir}} \) channel density or distribution was not fully mature. On the other hand, because MSN dendritic arborization is not complete at a juvenile age (Tepper et al. 1998), similar potassium channel density, distributed in a smaller cell membrane surface area, would lead to decreased whole cell currents measured at the soma. We calculated MSN membrane capacitance (Fig. 1G; also see Methods) as an index of cell membrane surface area. Membrane capacitance was smaller in MSNs from juvenile rats (in pF; Juv: 102.3 [79.4–122.7]; Ado: 132.2 [103.3–197.1]; Adu: 174.8 [138.2–223.2]; Kruskal–Wallis test \( P < 0.05 \)). When \( I_{\text{Kir}}-I_{\text{Kleak}} \) conductance was normalized with membrane capacitance, no further differences were observed between the groups (in nS/pF; Juv: 0.16 ± 0.02; Ado: 0.21 ± 0.02; Adu: 0.16 ± 0.02; one-way ANOVA, \( P = 0.13 \)). The data suggest that differences in cell membrane surface area, rather than in density of potassium channels, accounted for the enhanced whole cell \( I_{\text{Kir}}-I_{\text{Kleak}} \) observed in MSNs from adolescent and adult rats. As a consequence, neuronal excitability was decreased.

We also analyzed developmental changes in the expression of outward rectifying potassium currents (\( I_{\text{Kor}} \)) and voltage-gated calcium currents (VGCCs), which modulate MSN firing (Hernandez-Lopez et al. 1997, 2000; Nisenbaum et al. 1994).

First, depolarizing current steps were applied while sodium and calcium conductances were impaired (1 \( \mu \)M TTX and 0.4 mM CdCl\(_2\), respectively). In these conditions, depolarization was limited due to activation of \( I_{\text{Kor}} \) (Fig. 2A), leading to a rectification in the \( I-V \) relationship (Fig. 2B). \( I-V \) curves from juveniles were statistically different from those from adolescents and adults (\( P < 0.0001 \), two-way ANOVA), mainly because of greater depolarization response to moderate amplitude current steps. This may be caused by an increased membrane resistance around rest in juveniles (not shown). However, the level of depolarization achieved when \( I_{\text{Kor}} \) were activated during higher amplitude current steps did not differ between groups. We concluded that differences in \( I_{\text{Kor}} \) were not responsible for the decreased excitability of MSNs from adolescents and adult rats.

Finally, VGCCs were studied in the presence of potassium and sodium channel blockers (25 mM tetraethylammonium, 3 mM 4-aminopyridine, and 0.5 \( \mu \)M TTX; cesium methanesulfonate in the recording electrode). In current-clamp recordings, short current steps induced long-lasting plateau potentials.
in MSNs (Fig. 2, C and D) (Hu et al. 2004). These plateau potentials were calcium mediated and blocked by CdCl$_2$ (0.4 mM; Fig. 2C). Rheobase was smaller in juveniles than that in adolescents or adults (Fig. 2E; in nA; Juv: 0.12 ± 0.01; Ado: 0.22 ± 0.02; Adu: 0.29 ± 0.02; one-way ANOVA, $P < 0.01$).

Other parameters such as amplitude (in mV; Juv: 103.6 ± 3.5; Ado: 98 ± 2.4; Adu: 103.2 ± 4.7; one-way ANOVA, $P = 0.4$), plateau area (in mV × ms; Juv: 71,285 ± 10,793; Ado: 48,211 ± 6,896; Adu: 58,082 ± 9,491; one-way ANOVA, $P = 0.21$), and half-amplitude duration (in ms; Juv: 808 ± 112; Ado: 505 ± 80; Adu: 648 ± 120; one-way ANOVA, $P = 0.21$) did not differ between groups (Fig. 1E). Thus calcium-mediated plateau potentials were more easily recruited in MSNs from juvenile animals, suggesting that VGCCs may participate in the observed enhanced excitability.

Taken together, the data show that the intrinsic excitability of MSNs decreased throughout development, reaching an adultlike phenotype during adolescence. Maturation of morphological features of MSNs, as well as the facilitated recruitment of VGCCs, contributed to this process.

Properties of AMPAR-mediated synaptic transmission during adult development

EPSCs were evoked in MSNs by electrical stimulation of afferent fibers at a distance >150 µM from the soma. We first studied the properties of the AMPAR EPSCs. MSNs were voltage-clamped at −70 mV to avoid activation of NMDARs. Synaptic responses evoked while holding cells at −70 mV were abolished by the specific non-NMDAR antagonist DNQX (20 µM, n = 5; data not shown).

In the NAc, two stimuli applied within a short time interval usually result in the enhancement of the second AMPAR EPSC compared with the first one (Hjelmstad 2006; Pennartz et al. 1991). Paired-pulse facilitation is thought to be a feature of synapses with low initial probability of neurotransmitter release (Hjelmstad 2006). We tested whether the ratio between paired stimuli (paired-pulse ratio [PPR]) changes throughout development (Fig. 3A). Paired stimuli elicited with intervals <100 ms reliably induced equivalent facilitation in juveniles (1.43 ± 0.12 at 50 ms), adolescents (1.68 ± 0.17 at 50 ms), and adults (1.46 ± 0.10 at 50 ms) (Fig. 3B). Thus release probability of excitatory synapses to NAc MSNs, as estimated from the EPSC PPR, remained low at all developmental stages (≤7 mo old).

Next, we analyzed the kinetics of evoked AMPAR EPSCs (Fig. 3C). At all ages tested, the rise times of evoked AMPAR EPSCs were similar (in ms; Juv: 0.93 ± 0.09; Ado: 0.94 ± 0.09; Adu: 1.18 ± 0.07; one-way ANOVA, $P = 0.07$) (Fig. 3D). In marked contrast, adulthood was characterized by a slowing of AMPAR EPSCs. As shown in Fig. 3D, the decay times of AMPAR EPSCs were longer in MSNs recorded in slices from adult rats (in ms; Juv: 3.75 ± 0.20; Adu: 4.73 ± 0.33; Adu: 5.53 ± 0.39; one-way ANOVA, $P < 0.01$).

Kinetics and subunit composition of NMDAR-mediated transmission in the NAc is conserved from youth to adulthood

MSNs were voltage clamped at +40 mV to relieve NMDARs from the magnesium block and NMDAR EPSCs were isolated from the dual-component (AMPA + NMDAR) evoked EPSCs (Fig. 4A; see METHODS). NMDARs are tetramers composed of obligatory NR1 subunits in combination with NR2 (NR2A–NR2D) or NR3. The expression of NR2 subunits is developmentally regulated in many brain regions, including the basal ganglia (Chapman et al. 2003; Dunah and Standaert 2003; Monyer et al. 1994; Sheng et al. 1994; Sinagra et al. 2005). The functional properties of NMDARs, including their kinetics, depend on their subunit composition. We first compared the kinetics of NMDAR EPSCs in the different age groups (Fig. 4A). No differences were observed in the rise (in ms; Juv: 3.18 [2.68–4.43]; Ado: 2.95 [1.15–6.83]; Adu: 3.58 [2.69–4.65]; Kruskal–Wallis test $P = 0.99$) or decay times (in ms; Juv: 209 ± 8; Ado: 205 ± 11; Adu: 221 ± 5; one-way ANOVA, $P = 0.32$) of NMDAR EPSCs (Fig. 4, B and C). We next analyzed the effect of the NR1/NR2B-containing NMDAR-specific antagonist ifenprodil on the evoked EPSCs. Bath application of ifenprodil (3 µM) consistently reduced the amplitude of NMDAR responses in all cells tested (Fig. 4D).
AMPAR/NMDAR ratio increased during life span

The relative contribution of AMPAR and NMDAR to excitatory synaptic currents is thought to play a role in synaptic integration and plasticity and is an indicator of long-term synaptic plasticity (Malinow and Malenka 2002). We measured the ratio between AMPAR- and NMDAR-evoked EPSCs during postnatal development in the NAc (Fig. 5A). The A/N ratio increased significantly from juvenile to adult rats (Juv: 0.55 [0.44–0.76]; Ado: 0.82 [0.78–1.25]; Adu: 1.08 [0.71–2.11]; Kruskal–Wallis test, P < 0.01) (Fig. 5B). This enhancement seemed to occur preferentially in a subpopulation of synapses. The relative probability histogram showed that A/N ratios distributed around a single mode in MSNs from juveniles (Fig. 5B). In marked contrast, we found that A/N ratios were more heterogeneous in MSNs from adult rats and displayed a bimodal distribution. A subpopulation of synapses displayed lower values, close to those observed in juvenile rats, whereas other synapses showed greater A/N ratios. In adolescent rats, MSNs showed A/N ratios between juveniles and adults values, with a unimodal distribution skewed toward larger values.

Thus a subpopulation of synapses with an augmented contribution of AMPAR over NMDAR to the EPSC emerges during adulthood in the NAc.

Spontaneous synaptic currents are reduced in adult animals

The increase in the A/N ratio could be due to enhanced AMPAR transmission or to a decreased NMDAR component of the EPSC. To distinguish between those possibilities, spontaneous EPSCs (sEPSCs) were recorded. First, AMPAR sEPSCs were isolated in MSNs voltage clamped at −70 mV (Fig. 6A). The amplitude of AMPAR sEPSCs was enhanced in slices from juvenile compared with adolescent and adult rats. The median amplitude was increased (in pA; Juv: 16.7 ± 1.2; Ado: 13.1 ± 0.7; Adu: 12.7 ± 0.7; one-way ANOVA, P < 0.01) and the cumulative distribution of sEPSC amplitudes was shifted toward higher values in juvenile rats (Fig. 6, B and C). In contrast, the frequency of spontaneous events remained variable among ages (interspike interval (ms), Juv: 133 ± 12; Ado: 127 ± 15; Adu: 155 ± 14, one-way ANOVA, P = 0.05 for all groups). The A/N ratios in the sEPSCs were also significantly increased during postnatal maturation (Fig. 6D). The increase in the A/N ratio could be due to enhanced AMPAR transmission or to a decreased NMDAR component of the EPSC. To distinguish between those possibilities, spontaneous EPSCs (sEPSCs) were recorded. First, AMPAR sEPSCs were isolated in MSNs voltage clamped at −70 mV (Fig. 6A). The amplitude of AMPAR sEPSCs was enhanced in slices from juvenile compared with adolescent and adult rats. The median amplitude was increased (in pA; Juv: 16.7 ± 1.2; Ado: 13.1 ± 0.7; Adu: 12.7 ± 0.7; one-way ANOVA, P < 0.01) and the cumulative distribution of sEPSC amplitudes was shifted toward higher values in juvenile rats (Fig. 6, B and C). In contrast, the frequency of spontaneous events remained variable among ages (interspike interval (ms), Juv: 133 ± 12; Ado: 127 ± 15; Adu: 155 ± 14, one-way ANOVA, P = 0.05 for all groups).
FIG. 6. Enlarged AMPAR and NMDAR sEPSCs in MSNs from juvenile animals. A: typical AMPAR sEPSCs recorded at −70 mV in MSNs from juvenile (n = 14), adolescent (n = 13), or adult (n = 16) rats. B and C: AMPAR sEPSCs recorded in MSNs from juveniles depicted a cumulative probability distribution of amplitudes shifted to the right (Kolmogorov–Smirnov [KS] test, P < 0.05) and larger median amplitudes (*P < 0.05, one-way ANOVA). D: in contrast, no changes were observed in the median interevent intervals of AMPAR sEPSCs. E: raw traces of dual sEPSCs recorded at +40 mV (top). The arrows indicate sEPSC onset. Pure AMPAR sEPSCs were isolated after bath application of the NMDAR antagonist D-APV (0.1 mM; bottom). F: NMDAR sEPSCs were estimated from the charge of the dual sEPSCs after AMPAR contribution decayed to zero. Gray lines represent the time window where the NMDAR sEPSC charge transfer was previously normalized by time.

ANOVA, P = 0.3] (Fig. 6D). Next NMDAR sEPSCs were isolated in cells voltage clamped at +40 mV (see METHODS). As shown in Fig. 6, E and F, sEPSCs were composed of both AMPAR- and NMDAR-mediated currents (dual sEPSCs). The NMDAR sEPSC was estimated as the charge transfer of synaptic currents 30 ms after the onset of dual sEPSCs, a time point where AMPAR sEPSCs already decayed to zero (Fig. 6F). NMDAR sEPSCs were largely enhanced in juvenile animals; their charge transfer cumulative probability curve was shifted to the right and their median charge transfer was larger than that in adolescent and adult rats (in pA x ms; Juv: 331 ± 16; Ado: 251 ± 15; Adu: 223 ± 12; one-way ANOVA, P < 0.0001) (Fig. 6, G and H). Following dual sEPSC recording, AMPAR sEPSCs recorded at +40 mV were isolated by the presence of D-APV (100 μM). As observed when cells were voltage clamped at −70 mV, AMPAR sEPSCs were larger in juveniles than those in adolescents and adults (not shown). Furthermore, the ratio between the median amplitudes of AMPAR sEPSCs and NMDAR sEPSCs was increased in MSNs from juvenile rats (Juv: 0.76 ± 0.03; Adu: 0.91 ± 0.06; Adu: 1.03 ± 0.07; one-way ANOVA, P < 0.01) (Fig. 6I).

Taken together, the data show that spontaneous synaptic currents mediated by both AMPAR and NMDAR are reduced in MSNs from adolescents and adults compared with juvenile rats. Morphological differences in dendrites and spines may have strongly contributed to this developmental downscaling (see DISCUSSION). However, functional changes in AMPARs, NMDARs, or both are necessary to explain the increased A/N ratio observed during adulthood. To better understand this, we analyzed whether A/N ratio of sEPSCs correlated with the
Synaptic currents were evoked in adult MSNs voltage clamped size EPSCs proceeding from the subset of stimulated synapses. The variability of A/N ratio in adults cannot exclude that changes in AMPAR EPSCs contributed to the developmental increase in A/N ratio. Thus a pronounced reduction of NMDAR EPSC determined the developmental increase of A/N ratio. Therefore we recorded AMPAR quantal-size EPSCs from juvenile, adolescent, and adult MSNs. The AMPAR asynchronous EPSCs (see earlier text; Fig. 7C) were measured in the same cell with evoked and spontaneous EPSCs (Pearson’s correlation: Juv: $r = -0.78, P = 0.002$; Ado: $r = -0.91, P = 0.0005$; Adu: $r = -0.95, P < 0.0001$). No significant correlation was observed with AMPAR sEPSCs (Pearson’s correlation: $P > 0.1$ for all groups). The same results were obtained when data from all developmental ages were pooled together (Fig. 7A). Thus a pronounced reduction of NMDAR sEPSCs was the principal cause of the developmental increase in A/N ratio.

In the previous analysis, all the synapses contacting the neuron were sampled and analyzing the median value did not allow us to describe the heterogeneity of A/N ratio observed when recording evoked EPSCs from adult animals (Fig. 5). It is possible that the bimodal-like distribution of the A/N ratio reflected that a subpopulation of synapses were potentiated (more AMPAR EPSCs) compared with other synapses in the same age group (with the same low NMDAR EPSCs). In fact, we found no correlation between the A/N ratio measured in the same cell with evoked and spontaneous EPSCs (Pearson’s correlation: $P > 0.2$ for all groups; Fig. 7A). Therefore we cannot exclude that changes in AMPAR EPSCs contributed to the variability of A/N ratio in adults.

To circumvent this problem, we recorded AMPAR quantal-size EPSCs proceeding from the subset of stimulated synapses. Synaptic currents were evoked in adult MSNs voltage clamped at $-70$ mV and bathed with a medium containing SrCl$_2$ instead of CaCl$_2$. In these conditions, afferent stimulation induced the asynchronous release of vesicles (Fig. 7B) (Oliet et al. 1996; Thomas et al. 2001). The AMPAR asynchronous EPSCs (asynchronous EPSCs) were quantal events, since their amplitudes were indistinguishable from those of sEPSCs measured in the prestimulus period (KS test, $P > 0.5$), whereas their frequencies were increased to $229 \pm 18\%$ (paired t-test, $P < 0.0001$). Furthermore, increasing release probability with paired-pulse stimulation augmented the frequency but did not change the size of async EPSCs (not shown). Once async EPSCs were recorded, CaCl$_2$ was reintroduced in the bathing solution and evoked EPSC progressively increased until a new steady level was reached (typically after 5–10 min) and async EPSCs disappeared (Fig. 7B). MSNs were then voltage clamped at $+40$ mV and the A/N ratio of evoked currents was measured, again subtracting responses after bath application of D-APV to isolate AMPAR EPSCs (see earlier text; Fig. 7C). We found a moderate direct correlation between the A/N ratio and the median amplitude of the AMPAR async EPSCs (Spearman correlation, $r = 0.52, P = 0.05$; Fig. 7D). Next, MSNs were divided in two groups: those with A/N ratio above the median were defined as high A/N, whereas those below were designated low A/N. We compared their async EPSCs and found no differences in their median amplitude (in pA; low A/N: 12.7 8.9–16.3; high A/N: 13.3 12.6–17.9). Kruskal–Wallis test

**Fig. 7.** Reduced NMDAR EPSC determined the developmental increase of A/N ratio. A: A/N ratio measured with sEPSC strongly correlated with the size of NMDAR sEPSC (charge transfer was normalized by time), but not with AMPAR sEPSC or the A/N ratio of evoked EPSC (eEPSC). Pearson’s correlation parameters for the ensemble of cells are depicted. B: sample traces of evoked EPSC in the presence of SrCl$_2$ or CaCl$_2$. Note the presence of async EPSC in the first. C: after recording async EPSC, the A/N ratio of evoked EPSC was measured. Typical traces of EPSCs recorded at $-70$ mV (downward) and at $+40$ mV (upward). NMDAR EPSC was obtained as in Fig. 5. Note the increase of the synchronous EPSC in the presence of CaCl$_2$ compared with SrCl$_2$. Traces represent the average of around 25 events. D: moderate correlation between the A/N ratio and the amplitude of async EPSC ($n = 14$). Spearman correlation parameters are shown. E: MSNs with low and high A/N ratio did not differ in their async EPSC median amplitude or amplitude cumulative distribution (KS test, $P > 0.1$).
Parallel maturation of MSN intrinsic properties

All recorded MSNs showed the general characteristics of mature cells, including hyperpolarized RMP, the presence of inward rectification, and a ramp depolarization with delayed firing during depolarizing current steps (Belleau and Warren 2000). However, “mature” parameters did not appear until adolescence, when membrane resistance and excitability were lowered compared with those in juveniles. Such maturational processes seem to be common to many cell types in the nervous system, as observed in hippocampal and neocortical pyramidal neurons (Frick et al. 2007; McCormick and Prince 1987; Oswald and Reyes 2008; Spigelman et al. 1992; Zhang 2004), neocortical interneurons (Zhou and Hablitz 1996), thalamic (Ramoa and McCormick 1994; Warren and Jones 1997) and striatal (Belleau and Warren 2000; Tepper et al. 1998) neurons.

The timescale of this physiological maturation parallels the morphological development of MSN, as dendritic arborization, spine formation, and synaptogenesis continue until the end of the first postnatal month (Butler et al. 1999; Tepper et al. 1998). These two phenomena are likely to be interrelated. The reduction in membrane resistance (which was the main determinant of the changes in MSN intrinsic excitability) was due to an increase in $I_{\text{Kir}}$ that was correlated with an augmentation of membrane capacitance. The developmental increase in membrane capacitance probably reflects the enlargement of dendritic compartment, the appearance and dramatic increase in the density of dendritic spines, and the elaboration of synaptic inputs (Chronister et al. 1976; Sharpe and Tepper 1998; Tepper et al. 1998). Normalizing $I_{\text{Kir}}$ with the membrane capacitance suppressed the discrepancy between MSNs of different ages, supporting the idea that the morphological expansion of MSNs was at the origin of the divergence in neuronal excitability. On the other hand, the enhanced sEPSC that we observed in MSNs from juvenile animals may correspond to connections made onto dendritic shafts, which are the most abundant synapses at this age (Sharpe and Tepper 1998; Tepper et al. 1998). Synaptic currents generated in dendritic shafts are not attenuated by highly resistant spine necks, resulting in larger spontaneous EPSCs in the cell body (Wilson 1984). Finally, the relationship between morphology and physiology may be bidirectional. The large NMDAR EPSCs and easily recruited VGCCs might serve as activity-dependent signals controlling synaptogenesis and spine arrangement in juvenile MSNs (Butler et al. 1999).

Reduced excitability and decreased size of synaptic inputs in adult MSNs do not necessarily mean less firing in vivo. In vivo, MSN firing is very low at a juvenile age and it increases over postnatal development, reaching the adultlike pattern during adolescence (Tepper et al. 1998). Such changes in MSN firing were correlated with the emergence of episodes of membrane potential depolarization (UP states) (Tepper et al. 1998), which in NAc MSNs are driven by coherent hippocampal activity (O’Donnell and Grace 1995). As during UP states MSN membrane potential is close to the action potential threshold, those depolarizations represent a “gating opportunity” for cortical inputs to induce firing in MSNs (O’Donnell and Grace 1995). Thus whereas MSN firing is mainly dictated by afferent activity, a lower excitability in mature MSNs might filter out weak cortical inputs during UP states, providing a mechanism for detecting finely correlated inputs.

DISCUSSION

A detailed understanding of normal function in the adult brain is a prerequisite for the comprehension of the pathophysiological basis of neurological and psychiatric diseases. Here we present a comparative study of the cellular and synaptic properties of rat NAc MSNs from youth to adulthood. Subtle modifications to classical slice preparation protocols allowed us to obtain high-quality whole cell patch-clamp recordings in brain slices from adult animals (P120–P200). In agreement with previous reports (Belleau and Warren 2000; Tepper et al. 1998), our results indicate that the postnatal development of MSN electrophysiological properties is completed during adolescence. Finally, we provided a detailed description of the basic features of excitatory synaptic transmission in the NAc of juvenile, adolescent, and adult rats. In adult rats, the data revealed a subset of synapses with larger A/N ratios, suggesting that synaptic strength is continuously refined during life, leading to an enhancement of the synaptic contrast across different synaptic inputs.

Whole cell patch-clamp recordings in brain slices from adult rats

Although still scarce, the study of synaptic physiology in slices from adult animals has expanded during the last years. The protocols to prepare slices are rather similar (Benoit-Marand and O’Donnell 2008; Buchanan and Mellor 2007; Cepeda et al. 2003; Conrad et al. 2008; Esposito et al. 2005; Fagen et al. 2007; Koos et al. 2004; Marchionni et al. 2007; Renzi et al. 2007; Tseng and O’Donnell 2004, 2005; van Praag et al. 2002; Zhang and Warren 2008). In our hands, subtle modifications of classical procedures were sufficient to significantly increase the quality of the slices. In particular, we found that a sucrose-based solution for cutting and a fine-tuning of slicing parameters were necessary. Furthermore, we observed that slices improved when animals were anesthetized with a mixture of ketamine/xylazine, presumably because this anesthetic mixture reversibly reduced NMDAR-mediated excitotoxicity. The anesthetic did not alter the recordings. First, the A/N ratio did not change between animals in the same age group anesthetized with halothane or ketamine/xylacine. Second, we reliably induced NMDAR-dependent long-term synaptic plasticity in slices from either group (not shown). Overall, we found that refinements and optimizations of the basic technique, as previously suggested for dendritic patch-clamp recording (Davie et al. 2006), were sufficient to allow whole cell somatic recording in adult animals.

Parallel maturation of MSN intrinsic properties and morphology

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$P = 0.3$). Finally, cumulative probability curves of async EPSC amplitude were not statistically different (Fig. 7E).

These results are consistent with the idea that AMPAR currents play a modest role in shaping the heterogeneity of the A/N ratio observed in MSN from adults. Therefore changes in NMDAR-mediated currents were the main factors determining the developmental increase of A/N ratio of MSNs.
Synaptic portrait of adult MSNs

The frequency of sEPSCs was identical in juvenile, adolescent, and adult MSNs. Since the probability of neurotransmitter release did not change during development (contrary to what was found in the dorsal striatum; Choi and Lovinger 1997), this suggests that the number of functional excitatory synapses was already determined in juvenile MSNs. This is rather surprising, considering that processes of synaptic establishment and elimination are highly active during the third postnatal week (Butler et al. 1999; Gras et al. 2005; Sharpe and Tepper 1998; Tepper et al. 1998). Therefore this may indicate that early connections made onto dendritic shafts are active and that, in juveniles, there is a functional replacement of axodendritic synapses by axospinous inputs (Sharpe and Tepper 1998; Tepper et al. 1998) without changing the number of functional connections.

A specific deceleration of the AMPAR EPSC was observed in adults. The time course of the EPSC is determined by several factors, including synchrony of neurotransmitter release, neurotransmitter concentration in the synaptic cleft, and AMPAR gating (Jonas 2000). The fact that AMPAR gating is the major determinant of the decay of postsynaptic currents at small central synapses (Jonas 2000) suggests that various factors such as AMPAR subunit composition may vary from youth to adulthood. There was no change in the kinetics of NMDAR EPSC, arguing against the idea that changes in neurotransmitter release, glutamate spillover, or dendritically attenuated played a major role in delaying the AMPAR EPSC. Finally, in our experimental conditions we did not observe developmental changes in the rise times of AMPAR and NMDAR EPSCs. However, because of the limitations inherent to voltage clamp, subtle changes in rise times might have been overlooked.

The expression of NMDAR subunit composition is finely tuned during early postnatal development (Chapman et al. 2003; Dunah and Standaert 2003; Monyer et al. 1994; Sheng et al. 1994; Sinagra et al. 2005). NR2B-containing NMDAR EPSCs present slower decays (Flint et al. 1997; Monyer et al. 1994) and low concentrations of ifenprodil specifically block NR1/NR2B-containing NMDARs (Dingledine et al. 1999). Our data show that the kinetic properties and the sensitivity to ifenprodil of NMDAR in MSNs are rapidly mature during postnatal life. Ifenprodil did not modify the decay of NMDAR EPSC, suggesting that subunit composition different from NR1/NR2B contributed to the slow deactivation kinetics of NMDARs. Consistent with this idea, heterotrimERIC NR1/NR2A/NR2B and NR2D-containing NMDARs were proposed to mediate synaptic transmission in the dorsal striatum (Chapman et al. 2003; Dunah and Standaert 2003; Logan et al. 2007).

Synapse formation is not achieved during juvenile stages. Thus the changes in EPSC kinetics or A/N ratio observed in adults might originate from newly formed synapses. However, they may also reflect maturation of the connections during adulthood. In support of this idea, both the decay time of AMPAR EPSC and the A/N ratio were “intermediate” in adolescent MSN, suggesting indeed that the proportion of synapses that display the adult phenotype increases progressively during maturation.

The relative contribution of AMPAR and NMDAR to synaptic transmission provides relevant information about the functional state of a synapse. At neonatal stages, immature “silent” synapses almost exclusively express NMDAR and their maturation is mediated by activity-dependent synaptic insertion of AMPAR (Malinow and Malenka 2002; Petralia et al. 1999). Later in development, bidirectional changes in the A/N ratio have been mainly correlated to the expression of long-term synaptic potentiation (LTP: synaptic insertion of AMPAR) or depression (LTD: removal of synaptic AMPAR) (Malinow and Malenka 2002). Activity-dependent plasticity of NMDAR EPSC was observed in many structures, including the NAc (Kombian and Malenka 1994; Lau and Zukin 2007). Here, we found that a decrease in NMDAR EPSC was the main determinant of the developmental changes in the A/N ratio. We propose that synaptic plasticity of NMDAR EPSC plays a major role in the maturation of NAc synapses and the refinement of NAc circuits. Because the ability to experience NMDAR-dependent LTP and LTD will differ between synapses with distinct expression of NMDAR, the plasticity of NMDAR EPSC will signal the switch between high (juveniles) and low (adults) plastic periods (Schramm et al. 2002).

Moreover, in the adult NAc, synapses with high A/N (less NMDAR EPSC) may be less “plastic” than inputs with low A/N ratio. In addition to gaiting plasticity, NMDARs directly control information flow through corticostral circuits (Pennartz et al. 1991; Pomata et al. 2008; Wolf et al. 2005; but see Hu and White 1996). Thus the plasticity of NMDAR EPSC would help selecting specific corticostral channels. Altogether, these mechanisms might allow NMDARs to modulate NAc-related physiological and pathological behaviors (Kelley 2004; Pulvirenti et al. 1992, 1994; Vanderschuren and Kalivas 2000).

In agreement with the present data, it has been reported that NMDAR EPSCs are prominent between the first and third postnatal weeks and decline thereafter (Zhang and Warren 2008). In marked contrast with our data, NMDAR EPSCs were almost undetectable after adolescence. Although the origin of this discrepancy is not clear, it might be attributed to the stimulation of different sets of afferent fibers, as expected in distinct brain slice preparations (coronal vs. parasagittal).

The mechanisms underlying NMDAR-dependent LTP and LTD and the plasticity of NMDAR EPSCs in the NAc are not fully understood (Thomas et al. 2000). In addition, NAc synapses also display forms of synaptic plasticity that do not involve NMDARs, including long-term depression mediated by endocannabinoid and metabotropic glutamate receptors (Robbe et al. 2002a,b). Whether they are developmentally regulated remains to be elucidated. Finally, different populations of MSNs in the NAc have been distinguished based on their projection and subtype of dopamine receptor they express (Lu et al. 1998; Zhou et al. 2003). Subpopulations of MSNs present different morphological and physiological properties (Gertler et al. 2008; Kreitzer and Malenka 2007; Shen et al. 2007), including diverse cellular responses to cocaine (Bertran-Gonzalez et al. 2008; Lee et al. 2006). Thus different types of MSNs with different synaptic behaviors could be distinctly modulated during development.

In conclusion, our study shows that excitatory synapses of the NAc undergo complex modifications before reaching maturity.
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