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Postnatal Development of Glutamate Receptor-Mediated Responses in the Neostriatum

Key Words

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Abstract

Three experimental approaches were used to examine the maturation of N-methyl-D-aspartate (NMDA) receptors in the neostriatum and compare their developmental profile to that of the non-NMDA receptors [α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA)]. The first, and least conventional approach utilized infrared videomicroscopy to measure NMDA-induced swelling in single cells in a brain slice. The results demonstrated that NMDA receptors display an incremental pattern of postnatal development with no responses at postnatal day (PND) 3, weak responses at PND 7, the largest responses by PND 14 and slight decreases at PNDs 21 and 28. At PNDs 3 and 7, KA-induced cell swelling was proportionately greater than NMDA-induced cell swelling suggesting earlier maturation of this non-NMDA receptor subtype. The second approach used whole-cell patch clamp analysis to examine NMDA currents and compare their maturation to AMPA/KA-induced currents. Though the data are still preliminary, a very similar developmental pattern emerged. NMDA-induced currents were small and developed slowly after PND 7. In contrast, AMPA/KA-induced currents were larger and appeared to develop earlier. Finally, dizocilpine (MK-801) binding was measured in homogenates of neostriatal tissue. The ontogeny of binding resembled a step function with increases between PNDs 3 and 7 and PNDs 14 and 21. Binding peaked at PND 28 and then declined slightly in the adult (PND 60). The affinity of MK-801 for the receptor did not change during postnatal development. These findings demonstrate the pattern of functional development of glutamate receptors in the neostriatum. The NMDA receptor subtype displays minimal functional development until PND 14. In contrast, neostriatal AMPA/KA receptor function appears to precede NMDA receptor function.

Introduction

Neostriatal cells are innervated by a major glutamate-containing projection from the cortex [McGeer et al., 1977; Fonnum et al., 1981; Meshul et al., 1994]. This pro-

jection forms the main excitatory drive into the basal ganglia. Like many glutamatergic synaptic connections, both α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate (KA) and N-methyl-D-aspartate (NMDA) glutamate receptors (GluRs) contribute to exci-

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tatory postsynaptic potentials (EPSP) recorded in neostriatal neurons [Cherubini et al., 1988; Jiang and North, 1991; Kita, 1996]. Little work has been done on the development of functional regulation of GluRs in this region. Excessive activation of GluRs can be lethal to neurons [reviewed by Choi, 1988; McDonald and Johnston, 1990] and there is evidence that the developing neostriatum may be particularly sensitive to excitotoxic injury mediated by both NMDA and AMPA/KA GluRs [McDonald et al., 1988; 1992]. Furthermore, this excitotoxic response of neurons to GluR activation has been proposed to play a role in the pathology of a number of diseases affecting the neostriatum, some with onset during human postnatal development, including obsessive compulsive disorder [Baxter et al., 1992], schizophrenia [Ulas and Cotman, 1993], Tourette's syndrome [Anderson et al., 1992], Huntington's disease [DiFiglia, 1990] and Parkinson's and Alzheimer's diseases [Ulas et al., 1994]. Thus, understanding the ontogeny of NMDA receptors in the neostriatum and any developmentally regulated changes in their functional properties should prove clinically meaningful.

The developmental role of the NMDA subtype of GluR, in particular, has received a great deal of attention. For example, activation of NMDA receptors alters neurite outgrowth in cultured neurons [e.g. Pearce et al., 1987; Brewer and Cotman, 1989] and is involved in the development of experience-related changes in the visual cortex [Kleinschmidt et al., 1987; Bear et al., 1990] and the cerebellum [Rahid and Cambray-Deakin, 1992; Kumuro and Rakic, 1993; Rossi and Slater, 1993; Farrant et al., 1994]. Evidence is also accumulating that NMDA receptors undergo functional alterations during postnatal development. For example, activation of NMDA receptors in neonatal visual cortex and superior colliculus produces larger responses than similar activation in adult animals [Tsumoto et al., 1987; Kato et al., 1991; Carmignoto and Vicini, 1992; Hestrin, 1992]. Likewise, in the cat caudate nucleus, NMDA-evoked electrophysiological responses are larger at postnatal days (PNDs) 16–35 than in older tissue [Siviy et al., 1991].

In the present study, a multifaceted approach was utilized to study the functional postnatal maturation of NMDA and AMPA/KA receptors in the neostriatum. Three techniques were used to examine, in parallel, the development of GluRs. First, infrared differential interference contrast (IRDIC) videomicroscopy was used to investigate the postnatal development of an excitotoxic response of neostriatal neurons to GluR stimulation. This technique takes advantage of the finding that GluR activation

induces cell swelling, an early event in an excitotoxic cascade that can produce cell death [e.g. Choi, 1988; Rothman, 1992]. Second, whole-cell patch clamp electrophysiological techniques were used to examine the postnatal development of GluR-mediated currents. Finally, binding assays were conducted with homogenates of neostriatal tissue to determine how neostriatal NMDA receptor binding changes throughout this same developmental period.

Materials and Methods

Preparation of Neostriatal Slices

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind.) 3–60 days old were used. After the animals were killed by decapitation, brains were dissected and placed in cold oxygenated artificial cerebral spinal fluid (ACSF) containing (in mM) NaCl 130, NaHCO₃ 26, KCl 3, MgCl₂ 2, NaH₂PO₄ 1.25, CaCl₂ 1.0, glucose 10 (pH 7.2–7.4). After cutting, transverse sections (350 μ m) were placed in ACSF at 25–27°C for at least 1 h (in this solution CaCl₂ was increased to 2 mM, and 4 mM lactate was added). Individual tissue sections were then transferred to the perfusion chamber in which the slice was held down by nylon threads glued to a U-shaped platinum wire. The slice was submerged in continuously flowing, oxygenated ACSF (25°C) at a rate of 2 ml/min.

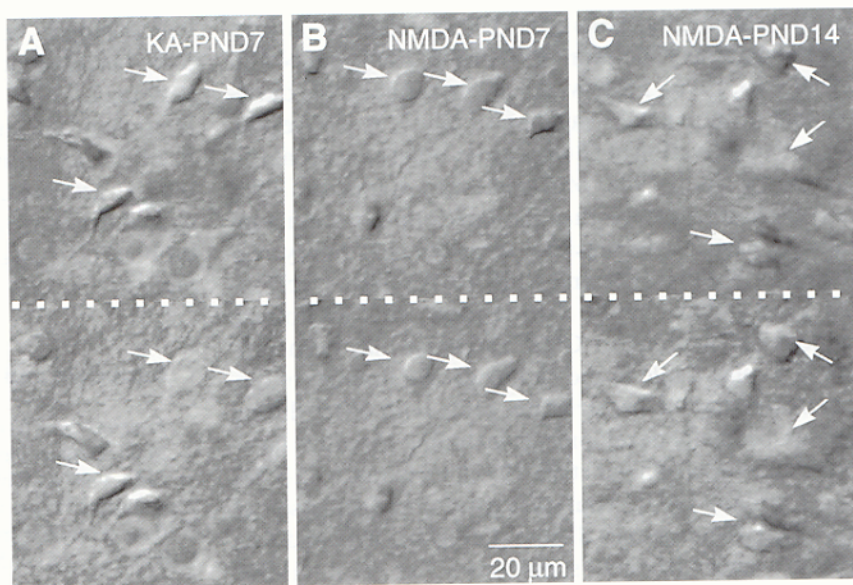
IR DIC Videomicroscopy

Brain slices were viewed with an upright compound microscope (Zeiss Axioskop) using a 40 \times water immersion lens (Zeiss, achroplan, numerical aperture 0.75) and DIC optics. Slices were illuminated with near IR light by placing an IR bandpass filter (790 nm, Ealing Optics, Holliston, Mass.) in the light path. This filter allowed passage of light between 750 and 1,050 nm and thus cut off much of the longer wavelength IR radiation which would heat the tissue. The image was detected with an IR-sensitive CCD camera (Hamamatsu C2400, Tokyo, Japan) and displayed on a video monitor. Analog contrast enhancement and gain control were provided by the camera controller. Digital images were stored on a computer/optical disk for subsequent analysis and additional digital contrast adjustment when necessary. Cells could be visualized to a depth of about 100 μ m below the surface of the slice. In order to quantify changes in response to activation of GluRs, image analysis software (Optimas, BioScan, Edmonds, Wash.) was used to measure cross-sectional somatic area prior to, during and after experimental treatments. Each measurement was made twice and the average value recorded. Measurements were only taken from cells that exhibited clear borders, convex shapes, and phase brightness.

Whole-Cell Patch Clamp Recordings

A slice was placed in the custom-designed chamber attached to the stage of the fixed-stage upright microscope. Cells were visualized as described above for the cell-swelling assay. Whole cell recording techniques were similar to those previously published [Cepeda et al., 1995, 1998]. Electrodes were pulled on a multistage puller (Sutter P97; 1.5 mm o.d. borosilicate capillary glass). The internal solution in the patch pipette contained: K-gluconate 140, Hepes 10, MgCl₂ 2, CaCl₂ 0.1, EGTA 1.1 and Mg ATP 2. The pH was between 7.25 and

Fig. 1. Differential development of NMDA- and KA-induced swelling in cells in neostriatal brain slices visualized by IR DIC videomicroscopy. At PND 7, cell swelling occurs in response to application of KA (**A**) but not NMDA (**B**). However, by PND 14, NMDA also causes swelling (**C**). Top panels show cells in control conditions and bottom panels show cells after 10 min treatment with NMDA or KA (100 μ M). Arrows indicate cells before and after treatment. Calibration refers to all images.



7.3 and the osmolality was between 280 and 290 mosm. Electrode resistance in the bath was typically 3–6 M Ω . Whole cell recordings were obtained with an Axon Instruments 200A electrometer and monitored on-line with pCLAMP (Ver. 6, Axon Instr.). Cells were approached with slight positive pressure and offset potentials corrected. To minimize changes in offset potentials with changing ionic conditions, the ground path used a 3 M KCl agar bridge to a Ag/AgCl ground well. The pipette was lowered to the vicinity of the membrane keeping a positive pressure. After forming a high-resistance seal (2–10 G Ω) by applying negative pressure, suction with negative pressure was used to break the membrane. While entering the whole-cell mode, a repetitive test pulse of 10 mV was delivered in a passive potential range. Once the whole cell configuration was established, whole cell capacitance was estimated. Whole-cell capacitance and electrode resistance were neutralized and compensated (50–80%) using the pulse. Data acquisition was then initiated. The adequacy of the series resistance compensation was monitored periodically by checking the response to small pulses in a passive potential range.

Iontophoretic Application of GluR Agonists

Iontophoretic applications were produced with Medical Systems Neurophore (5 constant current sources and 1 balancing channel). The balancing channel was used as a collector to provide current control. For iontophoresis, a multibarrelled pipette was placed close (30–60 μ m) to the recording electrode using visual control since both pipettes can be seen simultaneously. The iontophoretic and recording electrodes were held by separate electrode carriers. This configuration minimized electrical interactions between recording and microphoretic electrodes. Each barrel contained one of the following solutions: AMPA (20 mM, pH 8), KA (20 mM, pH 8), NMDA (100 mM, pH 8), and saline for current balancing and controls. Not all drugs were used in each experiment. All drugs were iontophoretically ejected as anions and were prevented from diffusing out of the barrel by a positive holding current of 15–20 nA. In some experi-

ments, current balancing was carried out through a barrel containing 1 M NaCl. No differences were observed between experiments with or without current balancing.

To study the effects of iontophoretically applied GluR agonists, ejection currents were varied between –5 and –300 nA. Neurons unresponsive at –300 nA were considered to be unresponsive for that substance. Antagonists were added to the bathing solution when specificity was assessed. Ejection times ranged from 3 to 5 s. The interval between ejection pulses varied from 2 to 3 min. Controls consisted of the application of negative currents of equal magnitude and duration through the saline-containing barrel of the pipette. In no case did the application of saline either cause changes in holding the current by itself or alter a cell's response to a GluR agonist.

Solutions and Drugs

For cell swelling, slices were continuously perfused and solution exchanges were achieved by a rapid gravity feed delivery system. Pharmacological agents (unless otherwise noted were purchased from Research Biochemicals, Natick, Mass.) included AMPA, DL-2-amino-5-phosphonopentanoic acid (AP5, NMDA receptor antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, AMPA/KA receptor antagonist), KA (Sigma) and NMDA (Sigma). The AMPA/KA GluR antagonist CNQX was dissolved in dimethyl sulfoxide (10 mM stock solution) prior to final dilution.

Receptor Binding

Rats [PNDs 3, 7, 14, 21, 28 and 60 (adult)] were sacrificed by rapid decapitation and their neostriata removed and weighed. Tissue was suspended in approximately 4 ml of 50 mM Tris-HCl buffer (pH 7.4) and homogenized using a Brinkmann Polytron homogenizer (setting 6). The homogenate was brought up to 40 ml and centrifuged for 15 min at 20,000 g for 20 min (4°C). Following centrifugation, the supernatant was discarded and the washing procedure repeated. Membranes were then incubated with Triton X-100 (0.04%) at 37°C

for 15 min followed by centrifugation at 40,000 *g* for 10 min (4°C). Membranes were then washed twice and resuspended in the Tris buffer and stored at -80°C. To provide sufficient tissue suspension volumes for assays, neostriata from different rats were pooled. Tissue pools contained 3–4 tissue samples from rats of the same litter, and each tissue pool was counted as a single observation.

On the day of the assay, neostriatal membranes were washed two additional times with a final suspension in 50 mM Tris-HCl buffer, pH 7.4. Tissue suspensions (0.10–0.60 mg of protein) were added to duplicate tubes containing various concentrations (0.5–400 nM) of [³H]MK-801 (Dupont NEN, specific activity 20.3 Ci/mM) with or without 100 μM TCP. Aliquots from each final tissue suspension were taken and assayed for protein determination. Protein values were obtained using the method of Bradford [1976] with bovine serum albumin as the standard. The final volume of the assay was 1 ml. Incubations were performed at 25°C for 4 h. Following incubation, the samples were rapidly filtered with a Brandel cell harvester and Whatman GF/C filters that have been presoaked in 0.1% polyethylenimine. Filters were washed twice with ice-cold Tris buffer and then placed in scintillation fluid. Radioactivity was measured using a Beckman liquid scintillation counter. Specific binding was defined as the difference in [³H]dixocipine bound in the presence of 100 μM TCP. β_{\max} and K_d values for the saturation experiments were estimated using a nonlinear curve-fitting program (Graphpad). All binding assays were repeated 3 times for each age group.

Statistical Analyses

Differences between the average values of experimental and control groups were evaluated using *t* tests or Mann-Whitney rank sum tests when appropriate. Values were considered significantly different if *p* < 0.05. All tests were performed using Sigmaplot (Jandel, San Rafael, Calif.). In the text, values are shown as mean ± SEM.

Results

Cell swelling in response to bath application of NMDA (1–1,000 μM) or KA (1–1,000 μM) was examined in slices from animals PNDs 3–28 (fig. 1, 2). At PND 3, there was no measurable response to NMDA while cells swelled in response to KA (fig. 2). Responses to KA at PND 3 were proportionately small compared to later ages (fig. 2). At PND 7, NMDA-induced cell swelling occurred at the highest concentrations, but was reduced compared to the later ages. Again, KA-induced swelling was proportionately larger than NMDA-induced swelling, especially at the lower concentrations (fig. 2). NMDA and KA produced similar dose-dependent swelling at PNDs 14, 21 and 28. Both NMDA- and KA-induced swelling at PNDs 21 and 28 was slightly smaller than at PND 14. These results form the basis of a developmental time course of GluR-induced responses and suggest that NMDA receptor function is less well developed than KA receptor function during the first 2 postnatal weeks in the neostriatum.

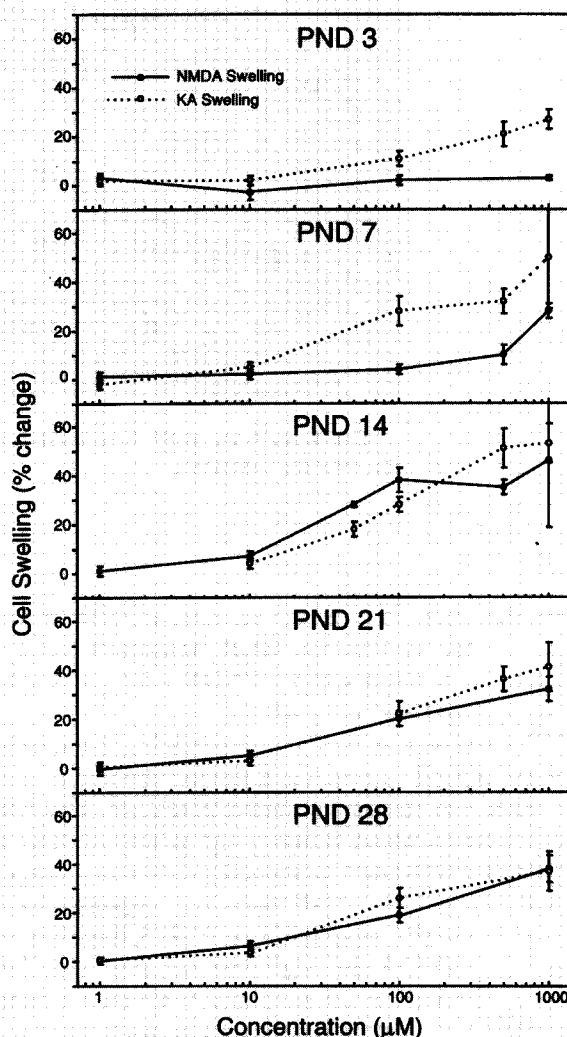


Fig. 2. Neostriatal cells show concentration-dependent swelling in response to NMDA and KA that varies as a function of the age. Each panel shows the concentration-response function for NMDA- and KA-induced swelling of a different age group. Note that at PNDs 3 and 7 KA-induced swelling is more marked than NMDA-induced swelling.

At all ages, the NMDA-induced swelling was blocked by treatment with the competitive NMDA GluR antagonist, AP5 but not by the AMPA/KA GluR antagonist, CNQX. For example at PND 14, NMDA-induced swelling was $27 \pm 2\%$ (100 μM, *n* = 94 cells). In the presence of 50 μM AP5 swelling was reduced to $0 \pm 3\%$ (*n* = 30 cells). This reduction was statistically significant (*p* < 0.001). In

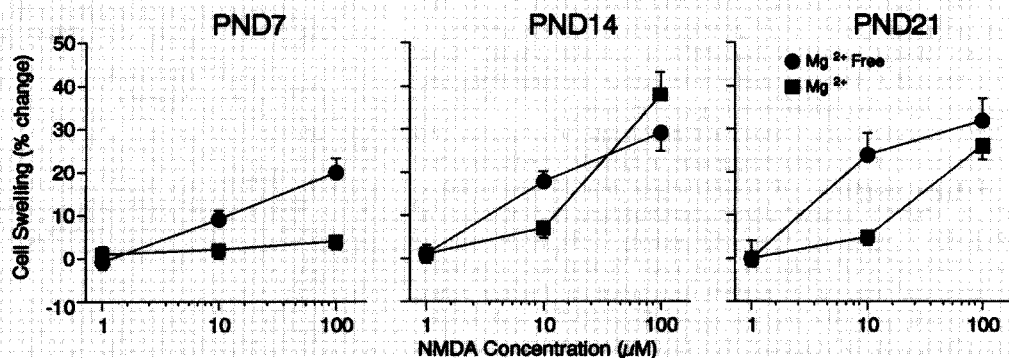


Fig. 3. Mg²⁺-free ACSF increases the magnitude of NMDA-evoked swelling at PNDs 7, 14 and 21, especially at the lower concentrations.

contrast, KA-induced swelling (100 μ M) at PND 14 was blocked by the AMPA/KA GluR antagonist CNQX (5 μ M KA: $28 \pm 3\%$, $n = 46$ vs. CNQX + KA: $-0 \pm 2\%$, $n = 21$, $p < 0.001$) but not by AP 5.

NMDA receptor-induced currents exhibit characteristic voltage dependence such that the ion channel is relatively inactive at resting membrane potentials. This voltage dependence, which is due to the actions of magnesium (Mg²⁺), may be developmentally regulated. In order to test this possibility, NMDA-evoked responses were examined in a Mg²⁺-free solution. At PND 3, there was no response to NMDA in Mg²⁺-free solution (NMDA 1,000 μ M, 10 min: $0 \pm 2\%$ swelling). At PNDs 7, 14 and 21, the Mg²⁺-free solution significantly enhanced swelling induced by NMDA at concentrations of 10 and 100 μ M (fig. 3). However, at the highest concentration examined (NMDA 1,000 μ M), the Mg²⁺-free solution did not significantly alter the swelling (data not shown). The addition of glycine (10 μ M) also did not alter NMDA-induced swelling at any of the ages examined (data not shown).

The development of GluR-evoked currents was examined using whole-cell patch clamp recording and iontophoretic techniques to apply GluR agonists (fig. 4). Recordings were made from neurons at PNDs 3, 7 and 14 to evaluate the development of GluR-induced responses ($n = 4-5$ cells per age group). The same cells were tested for responses to NMDA and AMPA or KA at a holding potential of -70 mV. At PNDs 3 and 7, NMDA-induced currents were small or undetectable whereas AMPA/KA-induced currents were typically larger. By PND 14, both

NMDA and AMPA/KA-evoked currents were larger. At all ages examined, NMDA-induced currents were always smaller than AMPA/KA-induced currents. These electrophysiological findings, though preliminary, provide additional support for findings obtained with the cell swelling assay.

The developmental changes in NMDA responses may be due to age-related variation in receptor number. To examine this possibility, [³H]MK-801 was used to estimate NMDA receptor binding with homogenates of neostriatal tissue at six ages (PNDs 3, 7, 14, 21, 28 and adult). Saturation curves for bound [³H]MK-801 were better fit by assuming two binding sites instead of one (F^2 test, $p < 0.05$). Therefore, curves were resolved into high and low affinity components. However, since the low affinity site accounted for over 95% of the total specific binding at each age, binding densities for both sites have been combined in the analysis. The total β_{\max} values (high and low affinity sites) increased with age (fig. 5). At PND 3, binding was 33% of adult levels. A small but statistically non significant increase was seen in binding sites from PND 3 to PND 7. A significant increase was observed between PNDs 14 and 21. The highest level of binding density (139% of adult levels) occurred at PND 28. In contrast to changing β_{\max} values, dissociation constants (K_d) did not significantly vary across the age groups examined. Thus, the affinity of the receptor for the ligand did not change at the ages examined. There was, however, a trend for the youngest animals to have higher K_d for the high affinity site compared to the other age groups.

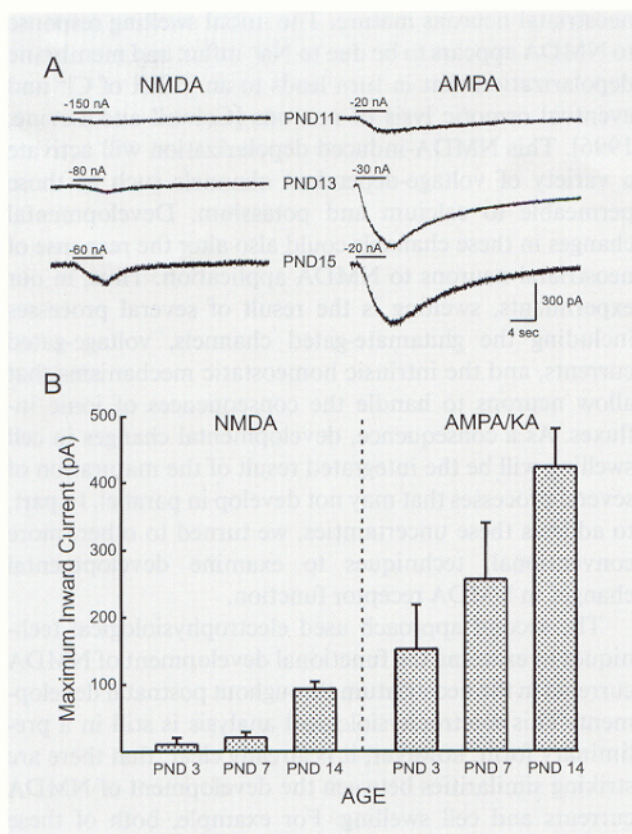


Fig. 4. Response of neostriatal neurons to iontophoretic application of GluR agonists. **A** Examples of inward currents evoked by NMDA and AMPA in the same cells at PNDs 11, 13 and 15. $V_{\text{hold}} = -70$ mV for each cell. At this holding potential, responses evoked by AMPA were always larger than those evoked by NMDA. Note that responses to NMDA appear to develop from PNDs 11–15 while those to AMPA may mature earlier. Iontophoretic currents were: NMDA: -150 , -80 and -60 nA for PNDs 11, 13 and 15, respectively; AMPA: -20 , -30 and -20 nA for PNDs 11, 13 and 15, respectively. **B** Magnitude of NMDA- and AMPA/KA-induced currents varied with developmental age. Plots show mean peak current as a function of age.

Discussion

It is well established that IR DIC videomicroscopy can be used to visualize unstained cells in brain slice preparations [MacVicar, 1984; Dodt and Zieglansberger, 1994]. Previous work has shown that an initial consequence of the application of GluR agonists is cell swelling and that this swelling is an early step in a cascade of events which can eventually lead to cell death [e.g. Choi, 1988; Rothman, 1992]. For the last several years, we have been using

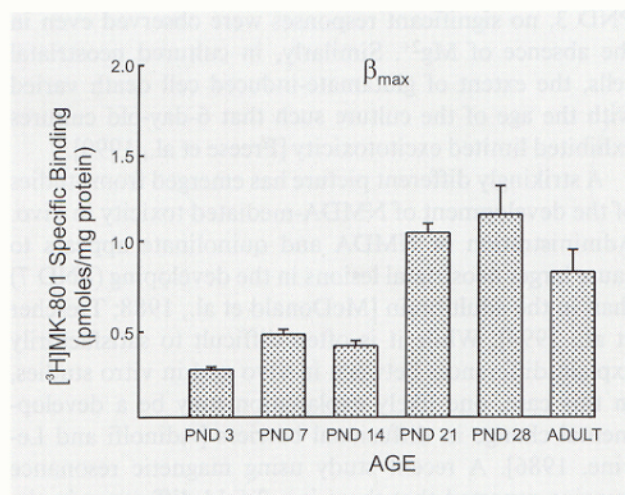


Fig. 5. Developmental changes in NMDA receptor binding. $[^3\text{H}]$ MK-801 was used to estimate NMDA receptor binding with homogenates of neostriatal tissue at six ages (PNDs 3, 7, 14, 21, 28 and adult). The total β_{max} values increased with age. At PND 3, binding was 33% of adult levels. A small step increase occurred between PNDs 3 and 7. A significant increase was observed between PNDs 14 and 21. The highest level of binding density (139% of adult levels) occurred at PND 28.

IR DIC videomicroscopy to examine these dynamic physical changes as a rapid measure of toxicity in a brain slice preparation [Colwell and Levine, 1996, 1997; Colwell et al., 1996; Levine et al., 1998]. We have found that NMDA-induced cell swelling is reproducible, semiquantitative, and, in many cases, a prelude to necrotic cell death [Colwell et al., 1996; Colwell and Levine, 1996]. We also used this assay to examine the excitotoxic consequences of the application of modulators of NMDA receptor function in the neostriatal brain slice. For example, we found that activation of mGluRs as well as activation of D_1 dopamine receptors modulates the NMDA-induced swelling [Colwell et al., 1996; Levine et al., 1998]. To date, the results we obtained with the cell swelling assay have been very similar to those obtained with electrophysiological methods. This correlation encouraged us to use this imaging technique as a functional assay of NMDA receptor development in the neostriatum. We found a developmental pattern of increasing maturation of the NMDA response with robust swelling not observed prior to 14 days of age. Interestingly, at PND 7, significant responses to lower NMDA concentrations ($100 \mu\text{M}$ or less) could only be elicited in the absence of Mg^{2+} . In contrast, at

PND 3, no significant responses were observed even in the absence of Mg^{2+} . Similarly, in cultured neostriatal cells, the extent of glutamate-induced cell death varied with the age of the culture such that 6-day-old cultures exhibited limited excitotoxicity [Freese et al., 1990].

A strikingly different picture has emerged from studies of the development of NMDA-mediated toxicity in vivo. Administration of NMDA and quinolinic acid appears to cause larger neostriatal lesions in the developing (PND 7) than in the adult brain [McDonald et al., 1988; Trescher et al., 1994]. While it is often difficult to satisfactorily explain differences between in vivo and in vitro studies, in this case, one likely explanation may be a developmental change in diffusional barriers [Adinolfi and Levine, 1986]. A recent study using magnetic resonance imaging reported that there is a 2-fold difference in the ability of a labeled tracer to spread through the neostriatum of PND 7 and 21 rats [Campagne et al., 1996]. Thus, changes in diffusional barriers during development may play a major role in determining the extent of NMDA-induced toxicity in vivo but a much smaller role in vitro. Of course, other explanations are possible and a definitive explanation will have to await further studies.

We feel that there are several advantages to using the cell swelling assay for developmental studies. IR videomicroscopy allows us to follow dynamics of excitotoxic response of single cells in neural tissue. Nervous tissue is made up of a heterogeneous cell population and it is clearly important to be able to distinguish between cell types. For example, in the present study most of the cells studied were probably medium spiny projection neurons that make up as much as 95% of the neuronal population in the neostriatum [Gerfen, 1992]. Large interneurons, that are largely resistant to excitotoxic damage, could be identified and were not included in the data set. In addition, because it is an imaging technique, it is possible to measure simultaneous responses from several cells in the same field of view. This allows more rapid progress than would be possible with, for example, electrophysiological techniques. Finally, NMDA-induced swelling is a very early step in a cascade leading to cell death and occurs before the cell has committed to death. Thus, agents that block cell swelling might be better for therapeutic intervention than agents that interfere with a later step in the excitotoxic cascade.

However, there are also some obvious disadvantages to the use of this assay. The principal problem is the uncertainty of the relationship between receptor activation and cell swelling. NMDA receptor activation clearly leads to cell swelling and the magnitude of swelling changes as

neostriatal neurons mature. The initial swelling response to NMDA appears to be due to Na^+ influx and membrane depolarization that in turn leads to an influx of Cl^- and eventual osmotic lysis of neurons [Colwell and Levine, 1996]. This NMDA-induced depolarization will activate a variety of voltage-dependent channels such as those permeable to calcium and potassium. Developmental changes in these channels could also alter the response of neostriatal neurons to NMDA application. Thus, in our experiments, swelling is the result of several processes including the glutamate-gated channels, voltage-gated currents, and the intrinsic homeostatic mechanisms that allow neurons to handle the consequences of ionic influxes. As a consequence, developmental changes in cell swelling will be the integrated result of the maturation of several processes that may not develop in parallel. In part, to address these uncertainties, we turned to other, more conventional, techniques to examine developmental changes in NMDA receptor function.

The second approach used electrophysiological techniques to examine the functional development of NMDA currents in the neostriatum throughout postnatal development. This electrophysiological analysis is still in a preliminary form; however, it is already clear, that there are striking similarities between the development of NMDA currents and cell swelling. For example, both of these functional assays reveal limited responses to NMDA application prior to PND 14. Similarly, in cultured neostriatal cells, a positive correlation has also been found between the development of NMDA-induced currents and toxicity [Koroshetz et al., 1990]. More work needs to be done, but the initial findings suggest that the development of NMDA currents will be correlated with developmental changes in cell swelling.

In other regions of the brain, previous studies have reported that the magnitude of NMDA current increases [Koroshetz et al., 1990; Ujihara and Albuquerque, 1992], decreases [Hori and Kanda, 1996] or is unaltered [Burgard and Hablitz, 1994; Vincent et al., 1996] throughout postnatal development. In both the neocortex and spinal cord, there is evidence that NMDA receptor function develops before that of AMPA/KA receptors [Burgard and Hablitz, 1993; Kim et al., 1995; Ziskind-Conhaim, 1990; Zona et al., 1994]. However, based on our data with the cell swelling and electrophysiology assays, this does not appear to be the case in the neostriatum. The difference appears to be particularly striking at PND 3, at which time we can measure swelling and currents caused by the application of AMPA/KA but not NMDA. These differences may reflect variation in the structure of the

NMDA receptor expressed and differences in the developmental pattern in different brain regions.

NMDA receptor binding has also been shown to undergo developmental changes in the central nervous system. Extensive work, using binding techniques, has been done on the development of NMDA receptors in the hippocampus [Baudry, et al., 1981; Tremblay et al., 1988; Morin et al., 1989; Insel et al., 1990; Wright et al., 1994] and visual cortex [Bode-Greuel and Singer, 1989; Erdö and Wolff, 1990; Gordon et al., 1991; Reynolds et al., 1991; Kumar et al., 1994]. In general, NMDA receptor binding is present at birth and increases during the first 2–3 weeks of postnatal development. In the neostriatum, at least two previous studies have examined the ontogeny of the NMDA binding sites. One reported that NMDA binding densities remained constant throughout development [Insel et al., 1990] while the other reported that densities increased gradually until PND 25 [Subramaniam and McGonigle, 1994]. Based on the results of experiments using our cell swelling assay, we expected to obtain results similar to Subramaniam and McGonigle [1994] with low levels of binding at PND 3 which would steadily increase with developmental age. Instead, we found that developmental changes in MK-801 binding resembled a step function from PNDs 3–7 and 14–21. The large increase in binding between PNDs 14 and 21 was presumably due to an increase in receptor synthesis, since there was no corresponding change in receptor affinity over this period. Interestingly, this is the period of maximal synapse formation in the neostriatum. Similar to the previous studies, we did not find any evidence for developmental changes in K_d [Insel et al., 1990; Subramaniam and McGonigle, 1994]. However, we believe that it is appropriate to be cautious in the interpretation of these binding studies. General problems associated with this approach include questions about the specificity of radiolabeled compounds and loss of structural information due to the use of tissue homogenates. This approach will also miss potential developmental changes in the regional pattern of GluR binding [Insel et al., 1990]. Despite these problems, the MK-801 binding experiments provide baseline information about the ontogeny of NMDA receptors in the neostriatum. Taken at face value, the binding data suggest that changes in receptor numbers, but not affinity, can account for some of the developmental changes observed with other functional assays.

An appealing explanation for ontogenetic changes in NMDA receptor-mediated responses is developmentally regulated variation in the expression of the subunits and/or splice variants that make up the receptor. Certainly, there is evidence that the composition of subunits making

up NMDA receptors changes during development and varies between different regions of the brain. Two gene families that encode NMDA receptor subunits have been identified. One family, the NR1 gene, undergoes alternative RNA splicing to give at least 8 receptor splice variants. The second family is comprised of several subunits (NR2A–D) that combine with NR1 subunits to form NMDA receptors. The expression of both gene families has been shown to be regionally specific and developmentally regulated [Watanabe et al., 1992; Pujic et al., 1993; Williams et al., 1993; Monyer et al., 1994; Riva et al., 1994; Zhong et al., 1995]. Furthermore, there is extensive evidence that changes in the composition of NR1 splice variants and NR2 subunits of NMDA receptors result in alterations in the functional properties of the receptor as demonstrated in expression systems [reviewed by McBain and Mayer, 1994; Zukin and Bennett, 1995]. Thus, a reasonable hypothesis is that the developmental regulation of the NR1 splice variants and NR2 subunits contributes to changes in physiological properties of NMDA receptors observed during maturation.

Another important mechanism may be developmental regulation of modulatory sites on GluRs. Developmental changes in the sensitivity of NMDA receptors to glycine and Mg^{2+} have been reported [Ben-Ari et al., 1988; Morrisett et al., 1990; Kleckner and Dingledine, 1991; Williams et al., 1993]. For example, Burgard and Hablitz [1994] report that for neocortical NMDA-evoked currents, Mg^{2+} block increases with age while peak current does not change from PNDs 3–14. In NMDA receptor channels, differences in the sensitivity to Mg^{2+} are determined by the expression of the NR2 subtype. In expression systems, NMDA receptors can be assembled from the NR1 and different NR2 subunits. These expressed receptors show differences in voltage-dependent Mg^{2+} block which are determined by the specific NR2 subunit [Kutsuwada et al., 1992; Monyer et al., 1994; Ishii, 1993]. Channels containing NR2A or NR2B are more sensitive to Mg^{2+} block compared with NR2C- or NR2D-containing channels [Monyer et al., 1994]. Thus, if the NMDA receptors expressed in younger tissue were more sensitive to Mg^{2+} block, then one would predict that young neostriatal tissue contains more NR2A/B than NR2C/D. Based on the cell swelling results, it would be interesting to look at the relative expression of NR2A/B and NR2C/D at the PND 7 time point. Future work will focus on exploring the possibility that such developmentally regulated changes in the expression of NMDA receptor subunits contribute to the observed pattern in the maturation of functional NMDA receptors.

Taken together, the findings obtained from the present studies provide important information necessary to understand functional NMDA and AMPA/KA GluR development in the neostriatum and provide clues for generating rational strategies to treat GluR dysfunction during development.

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