Neuroscience Letters, 94 (1988) 88–92
Elsevier Scientific Publishers Ireland Ltd.

NSL 05670

Changes in voltage dependence of NMDA currents during development

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(Received 31 May 1988; Revised version received 7 July 1988; Accepted 19 July 1988)

Key words: Hippocampal slice; Newborn rat; *N*-Methyl-D-aspartic acid current–voltage dependence

N-Methyl-D-aspartate (NMDA), applied by superfusion to hippocampal slices evokes comparable peak inward currents in CA3 pyramidal cells of adult and 0-10 day-old rats. However, NMDA current-voltage plots from immature neurones do not consistently show the region of negative slope conductance characteristic of adult. Therefore at a critical stage of development, NMDA activation may elicit substantial inward currents and Ca^{2+} influx at resting membrane potential.

Activation of *N*-methyl-D-aspartate (NMDA) receptors generates an inward current which is highly voltage dependent [8, 12]. Thus, at resting membrane potential, the NMDA receptor gated ionic channel is not fully operational because of a powerful voltage dependent block by Mg^{2+} [12]. In conditions in which this block is removed, the NMDA receptor-gated ionic channel becomes fully activated, leading to an increase in intracellular calcium [9]. Recent studies suggest that the NMDA receptor channel complex may play a particularly important role in the visual cortex during development since selective NMDA antagonists block experience dependent plasticity in the kitten [7, 14]. To gain a better understanding of the role of NMDA during development, we have recently examined its effects on immature slices using current and voltage clamp techniques. We found that NMDA-evoked inward currents (I_{NMDA}) are less voltage-dependent in immature than in adult neurones. Hence, substantial inward-currents can be evoked by NMDA even at relatively negative holding potentials ($V_m < -60$ mV). By magnifying Ca-influx at a crucial stage of development, this property of immature neurones may facilitate long-term maturational processes. Part of these observations have been reported in brief [2].

Wistar rats, either at an adult stage or during the immediate post-natal period (0–9 days) were used. Transverse slices of hippocampus (600 μm) were cut and transferred to a submerged type recording chamber as described previously [6]. The slices were

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superfused (2.5–3 ml/min) at 34°C with artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 126, KCl 3.5, $CaCl_2$ 2.0, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11. The ACSF was saturated with 95% O_2 and 5% CO_2 (pH 7.3). Microelectrodes filled with 3 M KCl or 2 M CsCl (with tip resistances of 45–60 MΩ) were used for intracellular recordings. CA3 neurones were voltage-clamped using a single electrode voltage clamp amplifier (Axoclamp-2). The sampling frequency was 3–4 KHz, 30% duty cycle. To ensure correct operation of the clamp, the voltage at the head stage amplifier was monitored on a separate oscilloscope. Current/voltage (I/V) plots were constructed directly on a X/Y recorder using slow ramp potentials (in the range of −80 to 0 mV at 1 mV/s). NMDA-induced inward currents were studied in the presence of tetrodotoxin (TTX, 1 μM) to block fast Na^+ conductances and Cs^+ (4 mM) and tetraethylammonium (TEA, 10 mM), to reduce K^+ conductances. NMDA and D-2-amino-5-phosphonovalerate acid (D-APV) were obtained from Cambridge Research Biochemical, Cambridge, U.K.; TTX from Sigma.

Current clamp experiments: bath applications of NMDA (5-10 μM) during the first 10 postnatal days induced in CA3 neurones a membrane depolarization (14+3 mV, $n=51$, $\bar{X}$+S.E.M.), with an increase in firing rate and burst activity. In adult neurones ($n=10$), NMDA induced a depolarization associated with an apparent increase in membrane resistance [4, 5]; in immature neurones ($n=21$) the depolarization was associated with no change in input resistance in 6 neurones and with a decrease (31%) or an increase (39%) in 11 and 4 neurones respectively.

Voltage clamp experiments: superfusion of NMDA (3–10 μM) evoked a slow inward current in both adult and immature neurones. I_{NMDA} was present already a few hours after birth, confirming that NMDA receptors are present early in develop-

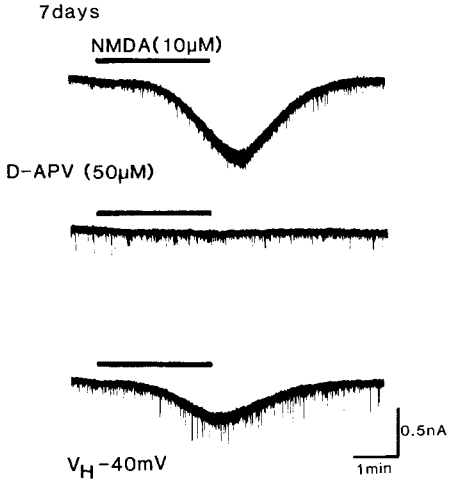

Fig. 1. D-APV-sensitive inward current induced by NMDA. Superfusion of NMDA (10 μM, bars) induced a slow inward current which was reversibly antagonized by D-APV (50 μM). Recording electrode containing CsCl, TTX (1 μM) present throughout the experiment.

ment [3, 13]. At a holding potential between -30 and -50 mV, the inward current induced by 10 μM of NMDA was 0.45 ± 0.04 nA, $n = 16$, $\bar{X} \pm$S.E.M.). As shown in Fig. 1 the NMDA current was blocked by the selective NMDA antagonist D-APV (50 μM). This effect reversed 10–15 min after washing with a control solution.

We have studied the voltage-dependence of I_{NMDA} in two ways: by repeating identical applications of NMDA at different holding potentials (V_H); or by determining the steady-state current–voltage plots in the absence and presence of NMDA. In the latter case, the cells were loaded with Cs^+ in order to reduce outward potassium currents and to improve the space clamp. With both types of procedures, I_{NMDA} in adult cells (Fig. 2C, D and Fig. 3A, B) was strongly dependent on membrane potential.

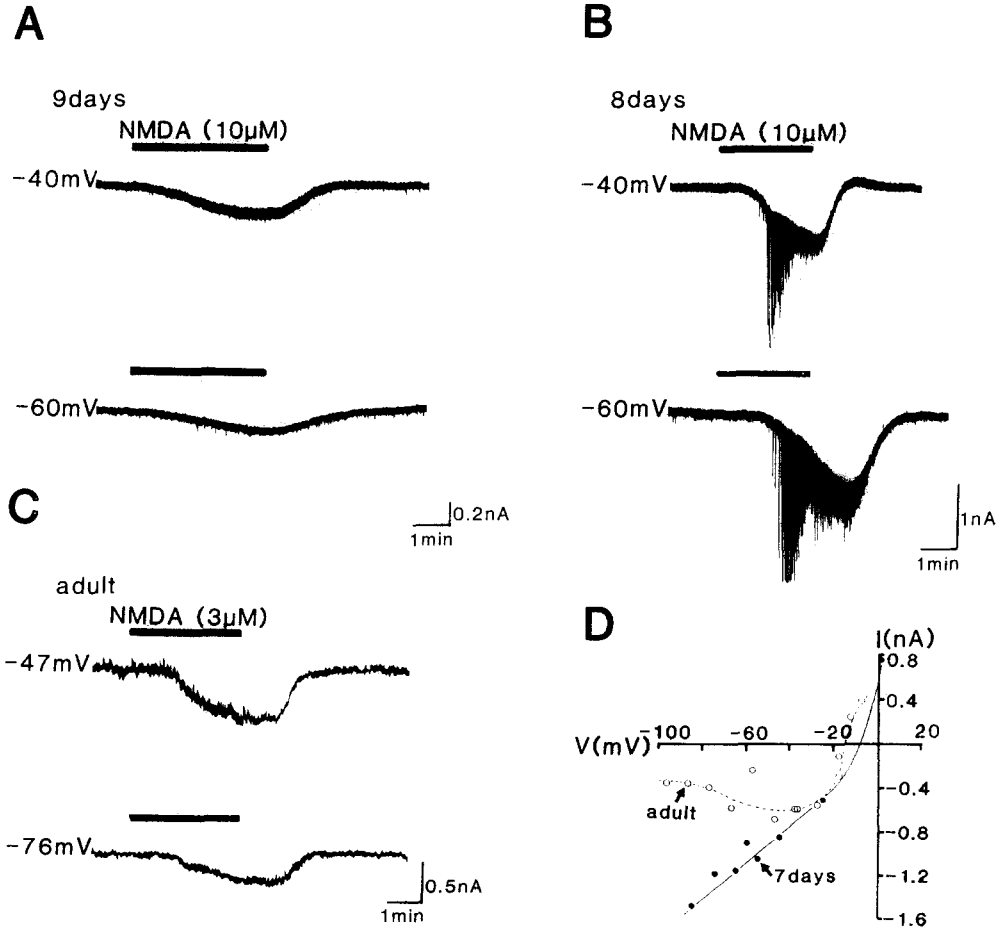

Fig. 2. Voltage dependence of NMDA currents in immature and adult neurones. Membrane currents produced by superfusion of NMDA (bars) at different V_H. Note the increase in I_{NMDA} with hyperpolarization in 7 and 8 day old neurones (B, D) in contrast to opposite changes seen in adults (C, D) and a 9 day old neurone (A). Each point of the I/V plot in D represents the peak of I_{NMDA} at different V_H in an adult and an immature neurone.

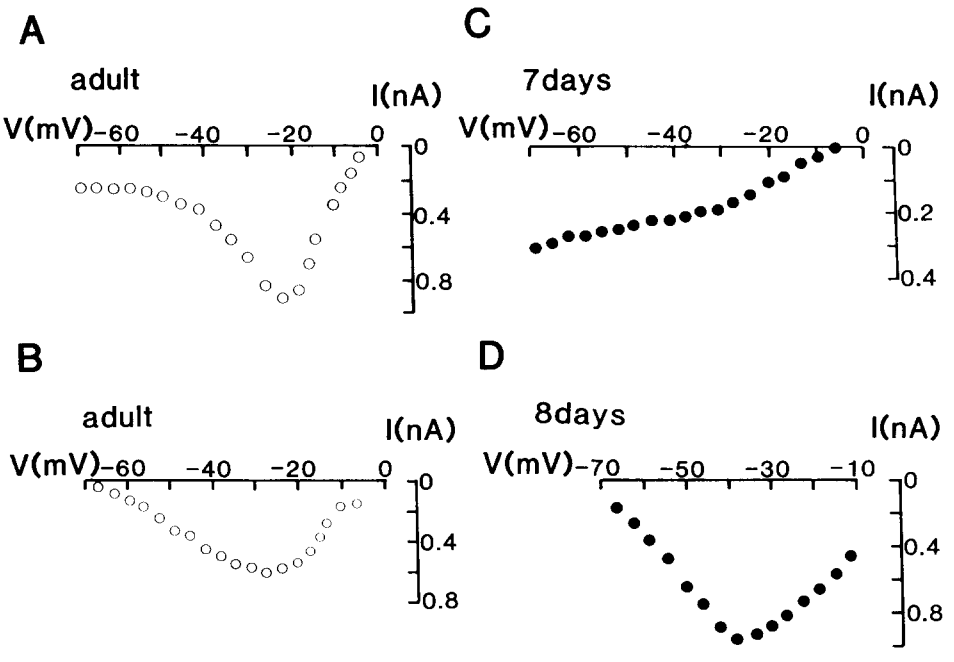

Fig. 3. Currents evoked by NMDA (10 μM) in mature and immature CA3 neurones. The difference between current–voltage plots before and during NMDA in mature (open circles) and immature (closed circles) neurones are illustrated. Note the lack of voltage dependency of I_{NMDA} in the cell shown in C. Recording electrode containing CsCl, TTX (1 μM) present throughout the experiment.

The agonist-induced current decreased at hyperpolarized potentials and increased at depolarized potentials. There was a region of negative slope conductance between -60 and -30 mV (Fig. 3A, B; e.g. ref. 8, 12). For a total of 13 cells, the mean slope was $-12.4+2.35$ pA/mV ($\bar{X}+$S.E.M.), the largest inward current being at $-37.3+3$ mV. The current reversed to outward near 0 mV ($-4.7+2.7$ mV; $n=3$).

The voltage dependence of I_{NMDA} in immature neurones ($n=17$) was more variable. Thus, as shown in Fig. 2, I_{NMDA} was either enhanced (Fig. 2B) or reduced (Fig. 2A) at more hyperpolarized potentials. Similarly, the steady-state current–voltage plots were quite variable: in some instances (Fig. 3D) they showed a region of negative slope conductance comparable to that observed in adult neurons; in other cases they were voltage independent (Fig. 3C). In 6 out of 17 immature cells, the I/V curve obtained in the presence of NMDA showed only a positive slope throughout (Fig. 2D). In the overall population ($n=17$) the mean slope conductance between -60 and -30 mV did not significantly differ from 0 ($-0.89+3.17$ pA/mV). As a result, when V_H was in the resting potential zone (-60 to -80 mV) much larger inward currents were evoked by NMDA in immature ($0.51+0.15$ nA; $\bar{X}+$S.E.M.) than in mature ($0.13+0.034$ nA) cells. In those immature cells that did show a region of negative slope conductance, the peak of inward current occurred at a similar V_H ($-39.2+2.6$ mV) as in mature cells. The reversal of I_{NMDA} ($-8.3+2.08$ mV, $n=11$) was very close to that obtained in adult neurones.

In conclusion, our data show that in immature neurones, NMDA currents are less consistently voltage dependent than in adult cells. Our observations cannot be explained by a poorer voltage clamp of immature neurones since during the first postnatal week, pyramidal cells are relatively small, have only rudimentary dendritic trees, few spines [10] and have a higher input resistance. The shorter electrotonic distance will improve the efficacy of the point clamp. The mechanism underlying the change in voltage dependence in immature neurones is presently elusive, however developmental changes in receptor structure and function are not unprecedented [11]. Whatever the exact mechanism, the weak voltage dependence of NMDA currents in immature hippocampal neurones may lead to an increased calcium influx at more hyperpolarized potentials and thus promote neuronal growth and differentiation [1].

K. Krnjevic received financial support from the Medical Research Council of Canada and is grateful to the Fonds de la Recherche en Santé du Québec for a travel grant. Financial support from DRET is acknowledged.

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