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N-Methyl-D-aspartate induces recurrent synchronized burst activity in immature hippocampal CA3 neurones in vitro

A.E. King, E. Cherubini and Y. Ben-Ari

INSERM Unité 29, Paris (France)

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Slices of hippocampus prepared from rats aged 1–10 days have been used to examine the chemosensitivity of CA3 pyramidal neurones to *N*-methyl-D-aspartate (NMDA). Superfusion of NMDA excited all neurones tested at all ages including the first day postnatal. In the majority of neurones this excitation was associated with the induction of a period of burst firing which disappeared on removal of NMDA. These bursts took the form of paroxysmal depolarizing shifts (PDSs) with a large amplitude depolarization and a high frequency discharge of spikes. The amplitude but not the frequency of occurrence of the PDSs was influenced by changes in the membrane potential and they could be abolished by either a high divalent cation medium or tetrodotoxin. Their occurrence was synchronous with an extracellularly recorded discharge. The NMDA induced excitation and the induction of the PDSs was attenuated by selective NMDA receptor antagonists D-aminophosphonovalerate (10–50 μM) and D,L-aminophosphonoheptanoate (20–30 μM). The results indicate that chemosensitivity to NMDA develops prenatally and that activation of NMDA receptors can in immature CA3 pyramidals induce recurrent synchronized burst activity.

INTRODUCTION

N-methyl-D-aspartate (NMDA) is a potent excitant of mature hippocampal CA1 and CA3 pyramidal neurones[2,7,8,19,22]. In these and other central neurones NMDA induces interictal burst discharges and it is suggested that the receptors to this agonist may participate in the generation of epileptiform activity[4,18,19]. However, no information is available on the sensitivity of immature hippocampal pyramidal neurones to NMDA. This is of considerable interest since in the immediate postnatal period the hippocampus is subject to rapid ontogenetic change and similarly the epileptogenic properties of its neurones are modified during development[9,24–26]. Furthermore, emerging evidence indicates that NMDA receptor activation plays a critical role in some aspects of nervous system maturation[13]. Due to their intrinsic properties[10] and novel synaptic circuitry[14,16] CA3 pyramidal neurones possess remarkable pacemaker properties. As part of a series of investigations into the properties of NMDA receptors during development of the hippocampus, the sensitivity of immature rat CA3 pyramidal neurones to NMDA was assessed using intracellular recording in vitro.

MATERIALS AND METHODS

Experiments were performed on the immature hippocampal slice prepared from animals aged 1–10 days (day of birth taken as day 0). Male or female Wistar rats were lightly anaesthetized with ether, then sacrificed by cervical dislocation. The brain was removed and submerged in oxygenated Krebs for isolation of the hippocampus from which slices approximately 600 μm thick were cut using a McIlwain tissue chopper. Slices were incubated for at least 1 h before use in oxygenated artificial CSF (composition in mM: NaCl 126; KCl 3.5; NaH_2PO_4 1.2; $MgCl_2$ 1.3; $CaCl_2$ 2; glucose 10; $NaHCO_3$ 25; pH 7.3 in 95%

Correspondence: A.E. King. Present address: Department of Anatomy and Developmental Biology, University College London, London WC1E 6BT, U.K.

O_2/5% CO_2) and transferred as required to a separate recording chamber which received artificial CSF at a minimum rate of 2.5 ml/min and was maintained at an average temperature of 34 °C. High divalent artificial CSF was made by increasing the concentration of magnesium to 6 mM and calcium to 4 mM.

Drugs were dissolved in the appropriate artificial CSF and superfused via a 3-way tap system. Drugs used were: NMDA, D-aminophosphonovalerate and D,L-aminophosphonoheptanoate (D-AP5 and AP7 respectively, Cambridge Research Biochemicals) and tetrodotoxin (TTX, Sigma).

Intracellular recordings were made from pyramidal cells in the CA3 region using microelectrodes filled with 4 M potassium acetate or 3 M potassium chloride which had a final resistance of 80–120 MΩ. In some experiments extracellular microelectrodes filled with 2 M NaCl (1–5 MΩ resistance) were used to obtain field recordings. These were placed at a distance from the intracellular electrode close to the pyramidal cell layer in either the proximal or apical dendrites.

RESULTS

Data are based on intracellular recordings from a total of 51 CA3 neurones in the immature hippocampus prepared from animals aged 1–10 days. The number of cells at each age and the corresponding mean values for the membrane potential and input resistance are presented in Table I. A major difference between neurones of immature and adult hippocampus is the tendency for spontaneous synchronized burst activity in the former[25] especially in the first few days postnatal. The nature of this spontaneous behaviour is the subject of a separate investigation.

Superfusion of NMDA (5–10 μM) for up to 5 min consistently induced pronounced changes in the excitability of all CA3 neurones tested. After 1–2 min of agonist application a depolarization developed often accompanied by an increase in cell firing (Fig. 1). The mean values for the depolarization according to age are presented in Table I. NMDA had a variable effect on cell input resistance; in 4 cells (out of 21 tested) a net increase of 39% was recorded (Fig. 1B) and in another 6 cells there was no net change. Surprisingly, in the other 11 cells a 31% reduction in input resistance resulted (Fig. 1A). Since at least part of this decrease is likely to be due to indirect release of other endogenous factors a further 4 cells were tested in the presence of TTX (1 μM); in one cell a 20% increase resulted while in the other 3 a net reduction of 28% was produced (Fig. 1C).

In many neurones epileptiform burst discharges emerged from this excitation (Fig. 2). These took the form of paroxysmal depolarizing shifts (PDSs) characterized by a large amplitude (10–20 mV) EPSP producing a high-frequency discharge of action potentials and followed by a long lasting (>100 ms) afterhyperpolarization (AHP) with a recurrent frequency of about 0.5–1.0 Hz. In some cases, as in the cell of Fig. 2, this bursting activity was superceded by high frequency cell firing and depolarization. Alternatively it could be sustained for the duration of the agonist application disappearing soon after wash. It should be stressed that while all cells at all ages were excited by NMDA not all displayed this epileptiform burst activity. The percentage of cells at the different ages displaying NMDA-induced PDSs is summarized in Table I. It is apparent from these data that there

TABLE I

Properties of immature CA3 neurones

Values are means ± S.E.M., $n = 51$.

Age (days)	*No. of cells*	*Membrane potential (mV)*	*Input resistance (mΩ)*	*Depolarization (mV) to NMDA (5–10 μm)*	*% Cells bursting after NMDA (5–10 μm)*
1–2	5	−62 ± 2	56 ± 5	10 ± 2	60
3–4	15	−70 ± 3	64 ± 11	9 ± 3	77
5–6	20	−68 ± 3	68 ± 3	14 ± 2	67
7–8	5	−74 ± 4	69 ± 9	23 ± 4	100
9–10	6	−76 ± 4	54 ± 4	14 ± 4	100

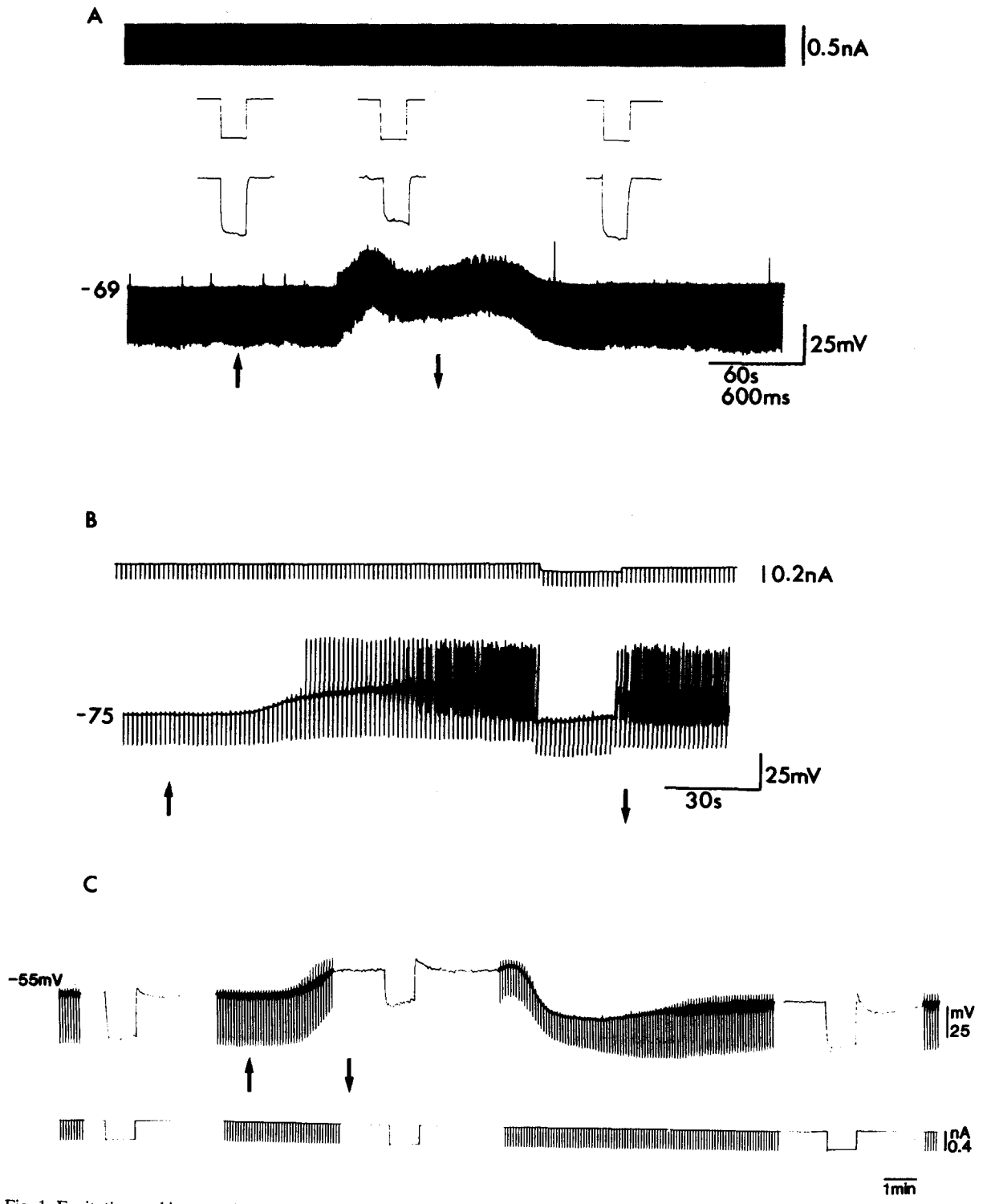

Fig. 1. Excitation and input resistance changes induced in immature CA3 pyramidal neurones by NMDA. The duration of application is indicated in all figures by the arrows; downward deflections are electrotonic potentials elicited by hyperpolarizing current pulses, upper records in A and B, lower record in C. A: NMDA (10 μM) elicits a depolarization associated with a reduction in input resistance in a CA3 pyramidal neurone (postnatal day 6 (P6)). B: NMDA (10 μM) elicits in another CA3 neurone (P9) depolarization with an apparent increase in input resistance. C: in another neurone (P5) in the presence of 1 μM TTX a depolarization and a decrease in input resistance is produced.

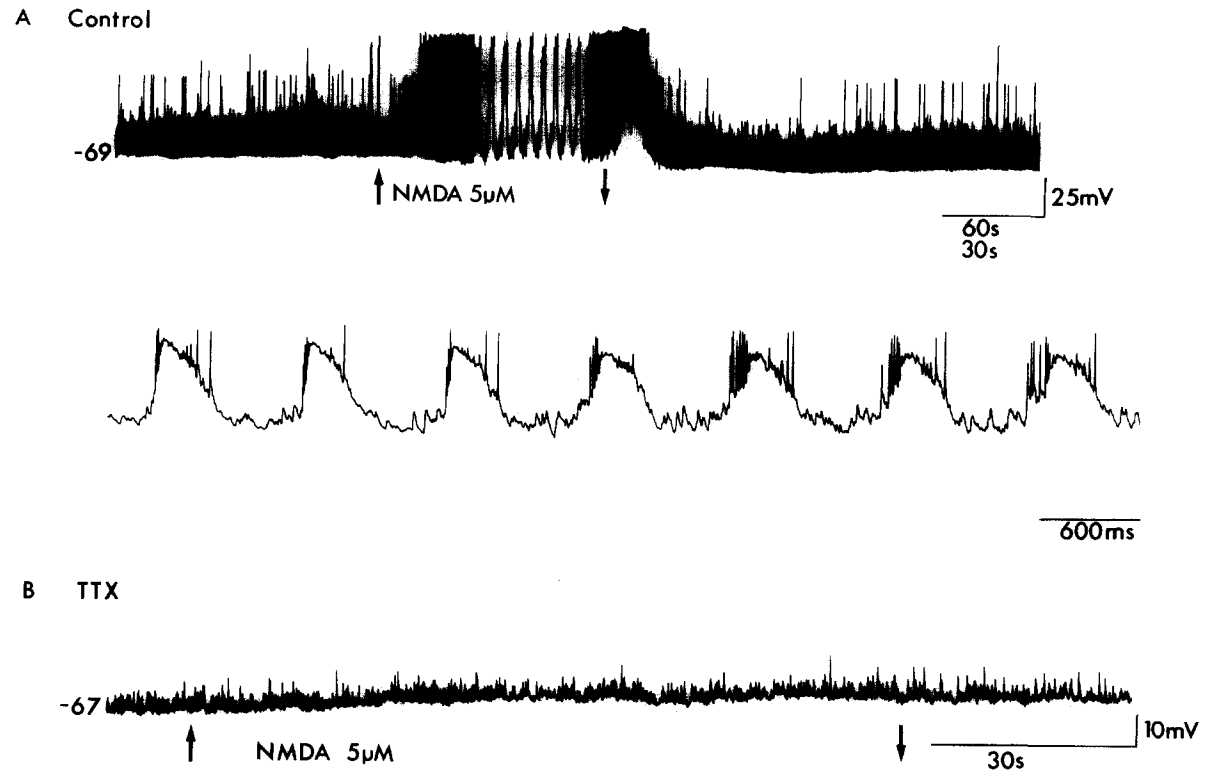

Fig. 2. Excitation and induction of PDSs in a single P4 CA3 pyramidal neurone by NMDA (5 μM). A: slow speed chart record of the depolarization and increased cell firing with subsequent burst firing during application of NMDA. The chart speed was doubled briefly during NMDA application to illustrate bursts. B: accelerated chart record of NMDA induced burst showing typical appearance of the PDSs (see text). C: abolition of burst firing after superfusion of TTX (0.5 μM).

was increased tendency for NMDA-induced burst activity after the first week postnatal. In two cells (one postnatal day 4 and the other day 8) exposure to NMDA precipitated bistable membrane behaviour whereby the membrane potential underwent a sudden depolarization which elicited a high-frequency

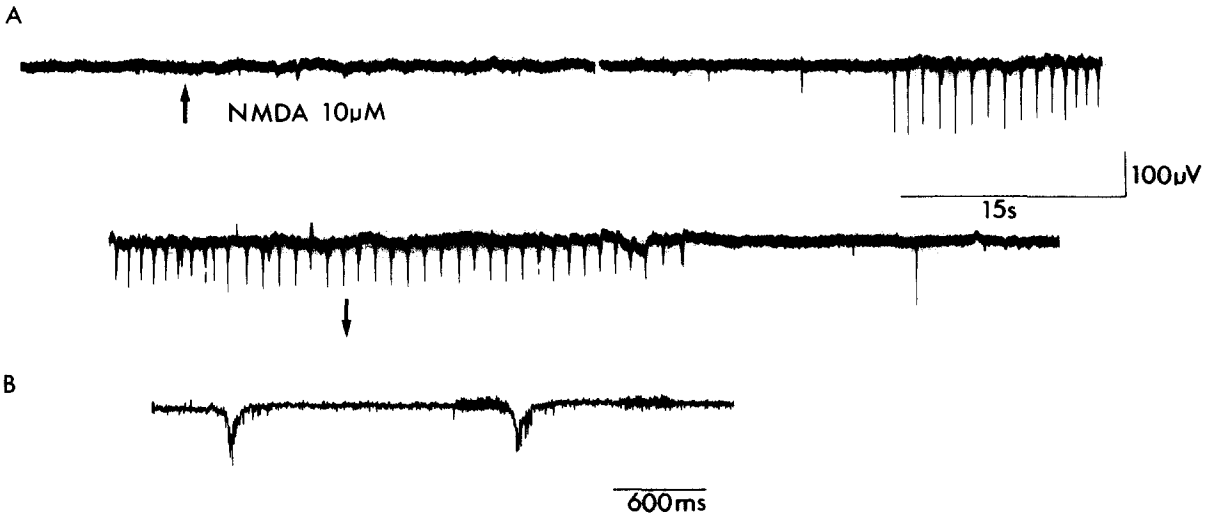

Fig. 3. A: extracellular spontaneous field potentials induced by NMDA in the CA3 pyramidal layer at P4. The chart record is continuous; duration of NMDA application is indicated by the arrows. The electrode was positioned close to the cell body layer within the basal dendrites. B: accelerated record showing appearance of extracellular burst discharge.

spike discharge. This depolarization was sustained for several seconds and subsequently the membrane potential rapidly returned to baseline.

The NMDA-induced bursts were also observed extracellularly using a low-resistance microelectrode placed within or close to the stratum radiatum. An example using a slice prepared from a postnatal day 4 rat is shown in Fig. 3; after a delay of up to 1 min a negatively directed field potential was generated at a frequency of 1–2 Hz which disappeared soon after washing. In some cases where simultaneous intra- and extracellular recording was possible it was apparent that the appearance of the intracellularly recorded PDSs and the extracellular field potential was synchronized (inset of Fig. 4). The PDSs displayed several other characteristics. Firstly, in the majority of cells tested (9/11) the amplitude but not the frequency of appearance of the PDSs was a function of the membrane potential. This point is illustrated in Fig. 4A. In only two cells was the appearance of the PDSs influenced by changes in the membrane potential being abolished by hyperpolarization (Fig. 1B). Secondly, prior application of TTX (0.5–1.0 μM) which will block sodium-dependent conducted activity reversibly inhibited the induction of the PDSs by NMDA. An example of this is shown in Fig. 2B; interestingly in this particular cell, as well as abolishing the PDS's, TTX strongly reduced the associated cellular depolarization indicating that the effect of NMDA was trans-synaptically mediated. However, in 6/8 cells a significant depolarization was retained even in the presence of TTX (see for example the cell of Fig. 1C). A similar effect was produced following superfusion of a high divalent cation artificial CSF (6 mM magnesium:4 mM calcium); an example for a single neurone (postnatal day 4) is shown in Fig. 4B.

In order to test whether the effect of NMDA was mediated specifically via an NMDA receptor its sus-

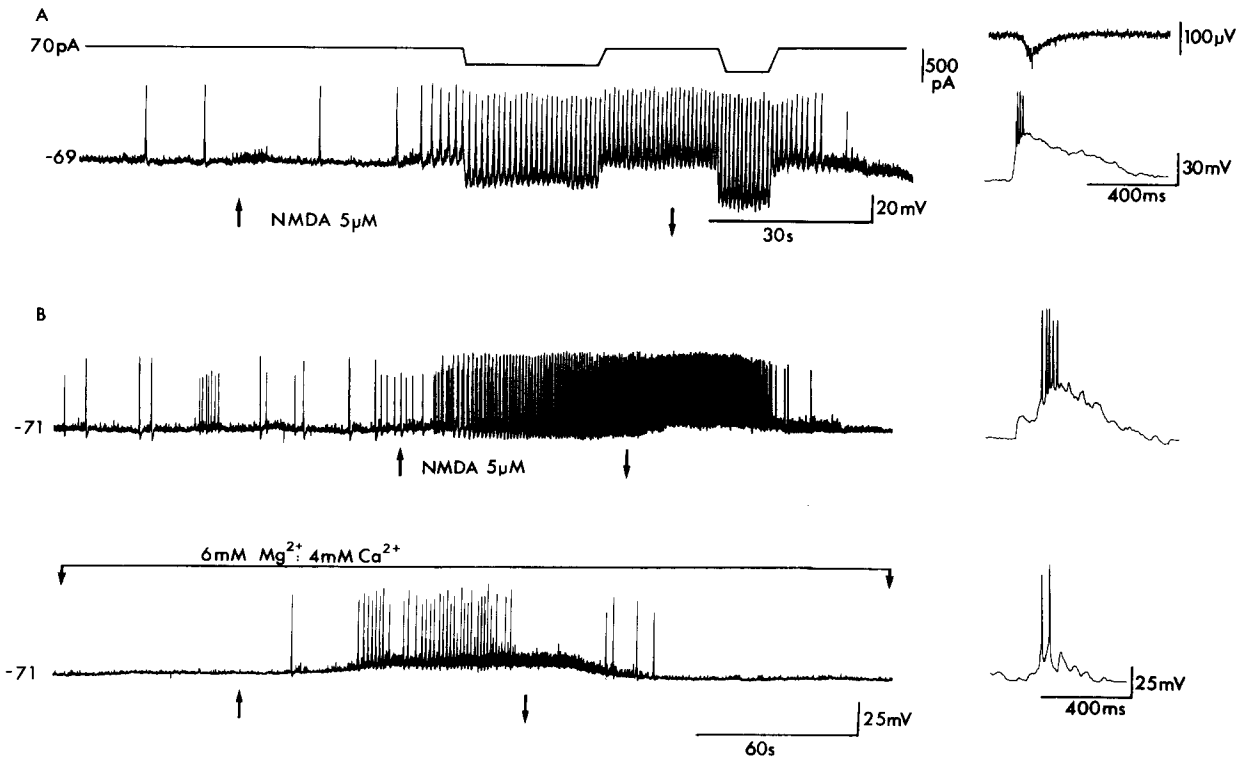

Fig. 4. Network properties of NMDA induced PDSs in immature (P4) CA3 pyramidal neurones. A: inset (right) shows simultaneous extracellular (upper) and intracellular (lower) single sweep records of a burst discharge elicited by 5 μM NMDA. The occurrence of the burst was not altered by membrane hyperpolarization; upper trace is current. B: top trace, excitation and PDSs elicited by 5 μM NMDA in another CA3 pyramidal neurone. Bottom trace, reduction of excitation and abolition of the PDS following superfusion of a high divalent cation (6 mM magnesium:4 mM calcium) medium. The two insets (right) show single-sweep intracellular records of the PDSs in control (upper) versus high divalent cation (lower) CSF. Note the reduction in amplitude and duration of the PDS in high divalent medium although some depolarization is still evident.

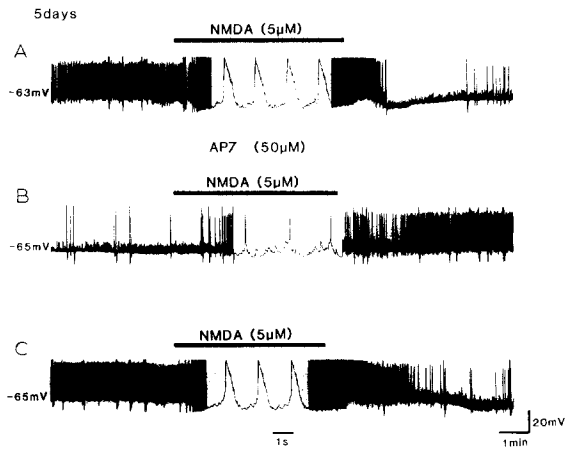

Fig. 5. Partial antagonism of NMDA-induced excitation and PDSs by 50 μM AP7 in a P5 CA3 pyramidal neurone. A: excitation and induction of PDSs by 5 μM NMDA in control medium. B: partial reduction of excitation and abolition of PDSs in the presence of the antagonist. C: recovery after wash of AP7.

ceptibility to antagonism by the selective receptor antagonists D-AP5 (10–20 μM, n = 3) and AP7 (20–30 μM, n = 6) was evaluated. Following superfusion of the antagonist for 5–10 min the depolarization produced by NMDA was reduced compared to control; for D-AP5 the % reduction was 52% (n = 3) while the value for AP7 was 70% (n = 6). Examples of the antagonism of the NMDA induced excitation

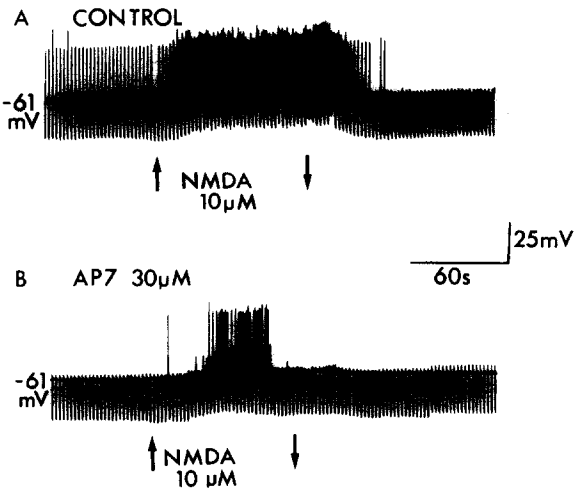

Fig. 6. Partial antagonism of NMDA induced excitation by AP7. A: 10 μM NMDA depolarizes and induces single spike firing in a P5 CA3 pyramidal neurone. Downward deflections are electrotonic potentials in response to hyperpolarizing current injection (record not shown). B: the excitation is reduced by 10 μM AP7.

are illustrated in Figs. 5 and 6. A feature of the antagonism was that in the cells where NMDA induced PDSs these were completely abolished by D-AP5 or AP7. For example, in the cell of Fig. 5 the period of rapid burst firing induced by NMDA in control is eliminated by 50 μM AP7. This effect could be reversed on removal of the antagonist.

DISCUSSION

This study shows that chemosensitivity of CA3 pyramidal cells to NMDA is already established at birth and therefore precedes completion of neurogenesis and synaptogenesis within the hippocampus which occurs postnatally[5,23,24]. These immature neurones respond to NMDA either with a simple excitation manifest as a depolarization and an increase in cell firing or with excitation and subsequent induction of burst firing characterized by depolarizing shifts, the so called PDSs. That this excitation is mediated via a functional NMDA receptor and not a non-specific precursor amino acid receptor is indicated by the effectiveness of the selective NMDA receptor antagonists.

The emergence of burst firing in response to NMDA has been described for adult hippocampal pyramidals[2,8,19,22]. Two classes of neuronal bursts have been proposed[12], one type is termed 'endogenous' due to its reliance only on intrinsic voltage-dependent properties of the neuronal membrane. In this respect it strongly contrasts with the second type called 'network' which is additionally dependent on synaptic interconnections within a group of neurones. In immature hippocampal CA3 pyramidals several features are consistent with the induction of network bursts by NMDA: the voltage dependence of PDS frequency, their synchronous appearance in the extracellular field record and, finally, the abolition of PDSs by TTX which will block sodium-dependent indirectly conducted activity or high divalent cation Krebs which will block poly- (but not mono-) synaptic transmission[6]. However, since a reduction of extracellular magnesium will induce epileptiform discharges which can be offset partially by D-AP5[1,18] it is possible that the abolition of burst firing following elevation of the extracellular magnesium is partly due to antagonism of NMDA-mediated excitation.

This epileptogenic effect of NMDA which is pres-

ent from the first day postnatal is in contrast to the effect of picrotoxin which in the neocortex is not manifest until the second week of life[9]. Similarly, in immature hippocampus up to postnatal day 9 there is a reduced susceptibility to epileptiform activity following convulsant exposure[26]. It has been suggested that the predominance of inhibition in the immature hippocampus may protect against seizure generation[24] but in the CA3 region both inhibitory and excitatory potentials are present from an early stage[25]. In rabbit fetal hippocampus synaptic potentials can be recorded in CA3 neurones from as early as 24 days gestation and synaptic contacts especially on apical dendrites are seen at 21 days gestation[25]. Local synaptic circuits and recurrent excitation in the CA3 region of adult hippocampus are intimately linked to the ability of CA3 to generate and sustain interictal network bursts[14–16]. These local synaptic interactions may be mediated by an excitatory amino acid[17] acting in part via the NMDA receptor. Direct activation of such local circuitry by exogenous NMDA may be sufficient even in the immature hippocampus to precipitate synchronized burst discharges. In many regions of the central nervous system during maturation there is a transient period of proliferation of diffuse connections appearing either as simply excess terminal branches or wholly abberrant collaterals[11,20]. The presence of such divergent pathways, if functional, may facilitate the spread of activity across the synaptic network despite the relative immaturity of the afferent circuitry.

The significance of this early emergence of NMDA sensitivity and its relationship to sensitivity in mature CA3 neurones clearly requires further investigation. However, studies indicating glutamate or NMDA receptor activation as a mechanism for regulation of neuronal growth[3,21] may provide a clue.

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