in receptive field position from the ventral trunk to the wing of the arm and then distally to the wing of the metacarpal bones and fingers. These recording sites represent a small fraction of the 400 sites that were used to determine the organization of S-I in this animal. These data and those from the other bats are consistent with the conclusion that the distal forelimb representation of the bat is shifted caudally in S-I and S-II, compared with other mammals.

Apart from alteration in the representation of the trunk, which may be related to the unusual location of the forelimb representation, other parts of S-I and S-II correspond to the basic mammalian pattern. As in other mammals, differing amounts of cortex are related to different body parts. For example, a considerable amount of cortex is devoted to the hairs of the face, which are important sensory organs in bats and most other mammals. The free first digit of the wing is well represented, as is the anterior wing or pro-wing (the part of the wing that would most often encounter objects). The representation of the forelimb and associated wing membrane constitutes over one-fifth of S-I, a proportion similar to that found in Microchiropteran bats[7].

In common with many other mammals, bats have several sensory representations in the cortex. Besides S-I and S-II, we obtained clear evidence for a third somatosensory representation caudal to S-I. The organization of this field was not fully determined because it was not reliably driven, and it appeared to be quite sensitive to anaesthesia. The auditory cortex contained at least two representations of tone frequency. Anatomically revealed projections from the primary visual cortex, V-I, indicated at least two additional visual area: V-II and a visual area in more temporal cortex. Our finding of multiple representations of the senses in the cortex of a Megachiropteran bat follows a wealth of data on multiple representations in the cortex of advanced mammals[8]; analogies with these studies suggest that the bat has a moderately advanced brain.

We have shown that the placement of the distal forelimb is altered in S-I and S-II of bats; this may help to elucidate the constraints that determine the location and orientation of body parts in somatotopic maps. In view of the recent evidence that the nervous system of the owl has a 'computational' map of auditory space which can be obtained only indirectly from the sensory periphery, as the latter is tonotopic but not spatiotopic[9], it seems possible that one such constraint is the need to maintain an appropriate relationship between different brain maps of extrapersonal space. If the relationship between the somatotopic map and other brain maps of extrapersonal space has been constrained during phylogeny or ontogeny to depend on the habitual spatial orientation of the animal's body, this may explain the reversed representation of the bat's limbs and digits, as these are caudal to the head during flight or above it during rest, in contrast to most other mammals in which the forelimb is used under, or in front of, the head.

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1. Adrain, E. D. *J. Physiol., Lond.* **100,** 159-191 (1941).
2. Woolsey, C. N. & Fairman, D. *Surgery* **19,** 684-702 (1946).
3. Darian-Smith, I., Isbister, J., Mok, H. & Yokata, T. *J. Physiol., Lond.* **182,** 671-689 (1966).
4. Nelson, R. J., Sur, M. & Kaas, J. H. *J. comp. Neurol.* **184,** 437-490 (1979).
5. Kaas, J. H. *Physiol. Rev.* **63,** 206-231 (1983).
6. Jepsen, G. L. in *Biology of Bats* Vol. 1 (ed. Winsatt, W. A.) 1-64 (Academic, New York, 1970).
7. Zook, J. M. & Fowler, B. C. *Soc. Neurosci. Abstr.* **8,** 38 (1982).
8. Woolsey, C. N. *Cortical Sensory Organization* Vols 1-3 (Humana, Clifton, New Jersey, 1982).
9. Knudsen, E. I. & Konishi, M. *J. Neurophysiol.* **41,** 870-884 (1978).

# An *N*-methylaspartate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine?

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**It has been proposed that three major receptor subtypes subserve the putative transmitter role of glutamate and aspartate in the mammalian central nervous system. One subtype is classified by the specific agonist *N*-methylaspartate (NMA)[1-4] and the specific antagonist 4-amino-2-phosphonovaleric acid[4]. It has been shown recently that excitation of neurones by NMA is also selectively reduced by dissociative anaesthetics such as ketamine and phencyclidine[5,6] and by sigma opiates[7], drugs of abuse with common psychotomimetic properties[8,9]. Responses to NMA have an unusual voltage relation[10,11] which may result from a voltage-dependent block of the activated channel by physiological concentrations of magnesium[12-14]. No synaptic potential with properties similar to those of responses to NMA, however, has yet been reported. We describe here an excitatory postsynaptic potential (e.p.s.p.) evoked by electrical stimulation of the white matter and recorded intracellularly from pyramidal cells in slices of rat somatosensory cortex. This e.p.s.p. has the appropriate voltage relation and sensitivity to $Mg^{2+}$ and ketamine to be an NMA receptor-mediated synapse and a potential central site for the psychotomimetic actions of ketamine.**

Recordings were first obtained from coronal slices of cortex, maintained in standard medium containing 1 mM $Mg^{2+}$. Cells ($n = 37$) were selected according to the following criteria. They maintained resting membrane potentials (RPs) $> -75$ mV, action potentials $>70$ mV with overshoots of 10-30 mV and thresholds 15-30 mV positive to RP, and input resistances apparently larger when measured with depolarizing than with hyperpolarizing pulses. Depolarizing pulses evoked slow depolarizations that elicited fast spikes. These properties are similar to those reported previously for cortical neurones *in vitro*[15-18]. The cells could be activated antidromically from the corpus callosum, indicating that they were pyramidal cells. Full somatic invasion by the antidromic spike was, however, achieved only when the cells were depolarized by 20-30 mV. All recorded cells were 100-300 μm from the pial surface.

Electrical stimulation of the ipsilateral callosum with low stimulus strengths (0.2 ms; <10 V) evoked a small, long-latency e.p.s.p. whose voltage relation differed from those of p.s.ps reported previously in that it increased in both amplitude and duration as the membrane was depolarized and decreased as the membrane was hyperpolarized from RP (Fig. 1). In 16 cells, it was possible to evoke such an e.p.s.p. alone; in others, it appeared as a component of a more complex postsynaptic response. Repetitive stimulation (1-2 Hz) of the callosum resulted either in no change in the amplitude of the low-threshold e.p.s.p., or in a small diminution. As described previously[19,20], stimulation of the callosum with higher stimulus strengths (0.2 ms; <30 V) led to a complex series of p.s.ps, lasting >100 ms. Typically, a large, short-latency e.p.s.p., which decreased in amplitude as the membrane was depolarized, was followed by one or more inhibitory potentials that were negative-going at membrane potentials 10-20 mV positive to RP.

All cells tested ($n = 15$) depolarized in response to pulses of NMA (1-4 s, 40-100 nA). These responses decreased in amplitude with hyperpolarization (Fig. 1), as observed in studies of the responses of other neurones to NMA[10,11]. In contrast, responses to glutamate showed a more conventional voltage relation (Fig. 1).

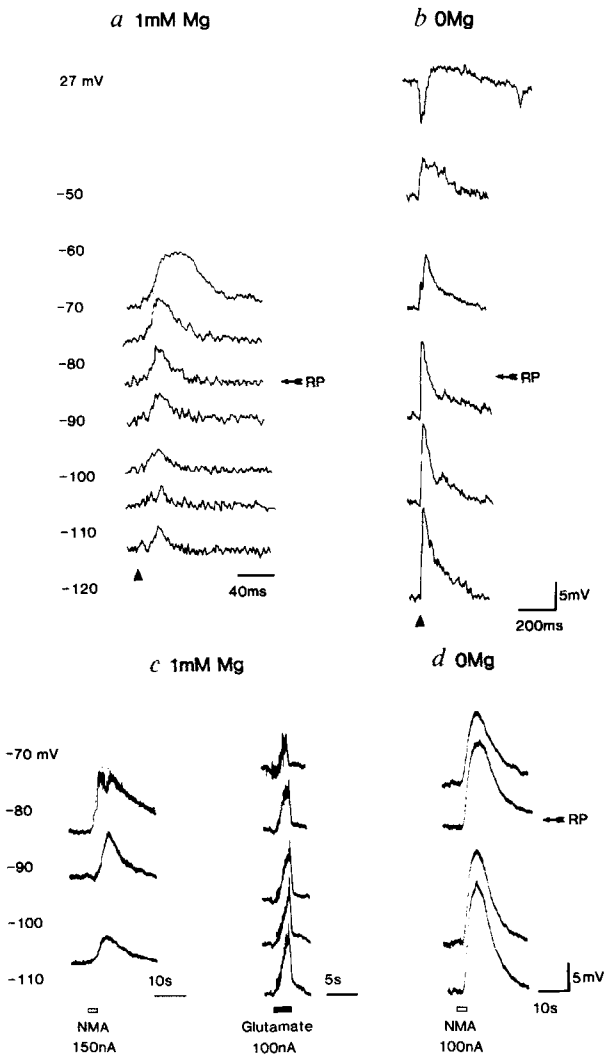


**Fig. 1** Removal of extracellular $Mg^{2+}$ changes the voltage relations of the low-threshold e.p.s.p. and responses to NMA. In the presence of 1 mM $Mg^{2+}$, low-threshold e.p.s.ps evoked in cortical neurones by stimulation of the corpus callosum (▲) (*a*) and responses to iontophoretic pulses of NMA (□) (*c*) decrease in amplitude with membrane hyperpolarization. In contrast, in the absence of $Mg^{2+}$, low-threshold e.p.s.ps (*b*) and NMA responses (*d*) increase with hyperpolarization. Responses to glutamate increase with hyperpolarization even in the presence of $Mg^{2+}$ (*c*). Recordings in *b*, *c* and *d* are from the same neurone. Note the different time scales in *a* and *b* and for responses to NMA and glutamate in *c*. In *d* the NMA current is smaller, but the response is larger than in *c*.

**Methods.** Intracellular recordings were obtained from coronal slices of somatosensory cortex 400 μm thick, from young adult male rats ($n = 15$). Slices were cut using a Vibroslice, transferred to the slice chamber (modified from ref. 24) and maintained at 35 °C at the interface between artificial cerebrospinal fluid (124 mM NaCl, 25.5 mM $NaHCO_3$, 3.3 mM KCl, 1.2 mM $KH_2PO_4$, 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM D-glucose) and warm humidified gas (95% $O_2$/5% $CO_2$). Flow rates were about 0.2 ml $min^{-1}$, bath volume 0.2 ml. Recording electrodes were 3 M K-acetate-filled glass micropipettes of tip resistance 60–120 MΩ. A preamplifier with conventional bridge balance and current injection facilities allowed the membrane potential to be changed. The corpus callosum was stimulated using a bipolar electrode made from twisted 50-μm insulated Ni/Cr wire. *N*-methyl-D,L-aspartate (NMA, 200 mM, *p*H 8), ketamine (50 mM in 150 mM NaCl, *p*H 4) and glutamic acid (200 mM, *p*H 8) were ejected from a multi-barrelled pipette which also included a balance barrel (150 mM NaCl). This pipette was placed close to the tip of the recording electrode and positioned to give the best response of the recorded cell to NMA.

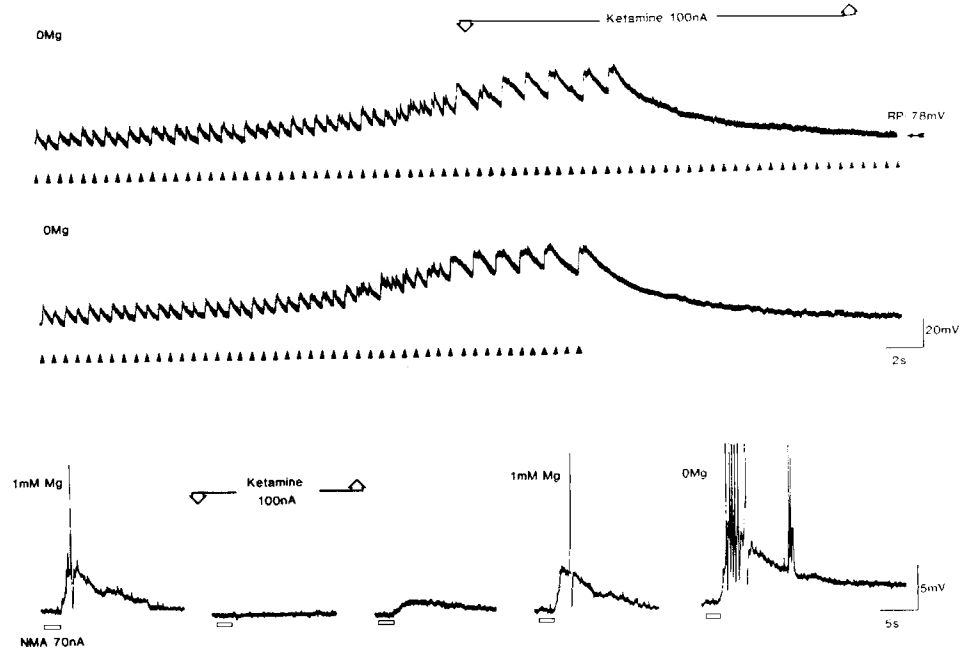


**Fig. 2** Ketamine-induced blockade of both the low-threshold e.p.s.p. and responses to NMA. Intracellular recordings from a cortical neurone. Top record, repetitive stimulation of the corpus callosum (▲) in the absence of extracellular $Mg^{2+}$ leads to potentiation of the e.p.s.p. and a plateau depolarization. A 100-nA current of ketamine blocks the response completely, despite continued stimulation, and the cell slowly repolarizes. Middle record, continuation of the top record after 3 min continuous stimulation, showing reversal of ketamine effects, potentiation of the e.p.s.p. and, on cessation of the stimulation, slow repolarization of the cell. Bottom records, left, response to a 2-s, 70-nA ejection of NMA (□) is totally blocked by a 30-s ejection of ketamine, 100 nA (second record). After 4 min (third record) partial reversal of the ketamine effect is seen and reversal is complete after 5 min (fourth record). Final record shows that removal of extracellular $Mg^{2+}$ leads to an increase in the response of this cell to NMA.

As $Mg^{2+}$ modifies the action of NMA[12-14], these tests were repeated in $Mg^{2+}$-free medium. Within 25–60 min of the changeover, low-threshold e.p.s.ps were measurably larger in amplitude and duration. Repetitive stimulation resulted in a further potentiation of this response, a depolarization plateau (Fig. 2) and, in some cells, bursts of slow potentials, ~20 mV in amplitude and 40 ms in duration, each of which evoked a burst of fast spikes. Potentiated e.p.s.ps could still be evoked several minutes after termination of repetitive stimulation. Similarly, depolarizing responses to NMA were increased in amplitude and duration in $Mg^{2+}$-free medium (Figs 1, 2), so much so that it was usually necessary to decrease the NMA dose to prevent large sustained depolarizations.

The voltage relations of both the low-threshold e.p.s.p. and the response to NMA were also affected by the removal of extracellular $Mg^{2+}$. These responses now increased in amplitude with hyperpolarization (Fig. 1). All changes induced by the removal of $Mg^{2+}$ were reversed within 10–15 min of its re-introduction. Neither the complex p.s.p., the response to intracellular current injection nor the response to glutamate were substantially affected by the removal of $Mg^{2+}$.

Ketamine (40–100 nA for 5–30 s) greatly reduced the responses of cortical neurones to NMA (Fig. 2), but not to glutamate, in both 1 mM $Mg^{2+}$-containing and $Mg^{2+}$-free medium. The same doses of ketamine also reduced the low-threshold e.p.s.p. and prevented its potentiation. Even established plateau depolarizations were blocked by ketamine (Fig. 2), although high-threshold p.s.ps were unaffected. These effects of ketamine were reversed 2–5 min after terminating its ejection (Fig. 2). In a further five cells, the competitive NMA antagonist 4-amino-2-phosphonovaleric acid also selectively reduced responses to NMA and the low-threshold e.p.s.p.

The similarity between the properties of the low-threshold e.p.s.p. and the depolarization elicited by NMA, both in their unusual voltage relation and in their sensitivity to $Mg^{2+}$ and ketamine, are consistent with a role for NMA receptors in the synaptic activation of cortical neurones. The unusual voltage relation in the presence of $Mg^{2+}$ and the sensitivity of the responses to $Mg^{2+}$ could be explained by the voltage-dependent block by $Mg^{2+}$ of the activated channel proposed by Nowak *et al.*[13] and Mayer *et al.*[14]. At membrane potentials negative to rest, the block would be powerful and responses much reduced. With depolarization, the block would decrease and responses increase in amplitude. On removal of $Mg^{2+}$, the underlying, conventional voltage relation of the channel would be revealed and responses would increase progressively with hyperpolarization. Although amino acids have been implicated in synaptic transmission[4,21], this is the first intracellular study clearly linking NMA receptors and synaptic excitation. Manipulation of membrane potential and $Mg^{2+}$ levels *in vitro* may reveal similar e.p.s.ps in other brain areas.

Reduction of such cortical e.p.s.ps may contribute to the behavioural effects of ketamine and other dissociative anaesthetics, since the effects of NMA are blocked *in vivo* by doses of ketamine below those required for anaesthesia[5] but similar to those that induce psychotomimetic effects in man[8,22] and delirium in animals[9]. The potency of dissociative anaesthetics and sigma opiates relative to phencyclidine in behavioural tests correlates well with their potency as selective NMA antagonists[23]. Further studies will be required to assess the implications of these results with respect to the psychotomimetic effects of systemically active NMA antagonists.

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1. Curtis, D. R. & Johnston, G. A. R. *Ergbn. Physiol.* **69**, 97–188 (1974).
2. McLennan, H. & Lodge, D. *Brain Res.* **169**, 83–90 (1979).
3. Davies, J. & Watkins, J. C. *J. Physiol., Lond.* **297**, 621–635 (1979).
4. Watkins, J. C. & Evans, R. H. *A. Rev. Pharmac. Tox.* **21**, 165–204 (1981).
5. Anis, N. A., Berry, S. C., Burton, N. R. & Lodge, D. *Br. J. Pharmac.* **79**, 565–575 (1983).
6. Harrison, D. L. & Simmonds, M. A. *Br. J. Pharmac.* (in the press).
7. Berry, S. C., Dawkins, S. L. & Lodge, D. *Br. J. Pharmac.* **83**, 179–185 (1984).
8. Domino, E. F. & Luby, E. D. in *PCP (Phencyclidine): Historical and Current Perspectives* (ed. Domino, E. F.) 401–413 (NPP Books, Ann Arbor, 1981).
9. Jasinski, D. R. *et al. PCP (Phencyclidine): Historical and Current Perspectives* (ed. Domino, E. F.) 331–400 (NPP Books, Ann Arbor, 1981).
10. Macdonald, J. F., Porietis, A. V. & Wojtowicz, J. M. *Brain Res.* **237**, 248–253 (1982).
11. Dingledine, R. *J. Physiol., Lond.* **343**, 385–405 (1983).
12. Ault, B., Evans, R. H., Francis, A. A., Oakes, D. J. & Watkins, J. C. *J. Physiol., Lond.* **307**, 413–428 (1980).
13. Nowak, L., Bregestovski, P., Ascher, P., Herbert, A. & Prochiantz, A. *Nature* **307**, 462–465 (1984).
14. Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. *Nature* **309**, 261–263 (1984).
15. Scholfield, C. N. *J. Physiol., Lond.* **275**, 535–546 (1978).
16. Ogawa, T., Ito, S. & Kato, H. *Brain Res.* **226**, 315–319 (1981).
17. Connors, B. W., Gutnick, M. J. & Prince, D. A. *J. Neurophysiol.* **48**, 1302–1320 (1982).
18. Stafstrom, C. E., Schwindt, P. C. & Crill, W. E. *Brain Res.* **236**, 221–226 (1982).
19. Scholfield, C. N. *J. Physiol., Lond.* **275**, 547–557 (1978).
20. Vogt, B. A. & Gorman, A. L. F. *J. Neurophysiol.* **48**, 1257–1273 (1982).
21. Hicks, T. P. & Guedes, R. C. A. *Expl Brain Res.* **49**, 157–173 (1983).
22. White, P. F., Way, W. L. & Trevor, A. J. *Anesthesiology* **56**, 119–136 (1982).
23. Lodge, D. & Berry, S. C. in *Modulation of Sensorimotor Activity During Alterations in Behavioural States* (ed. Bandler, R.) 503–518 (Liss, New York, 1984).
24. Haas, H. L., Schaerer, B. & Vosmansky, M. *J. Neurosci. Meth.* **1**, 323–325 (1979).

# A common sequence of calcium and *p*H signals in the mitogenic stimulation of eukaryotic cells

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**When normal quiescent ($G_0$) cells are stimulated by mitogens to enter the cell cycle, the metabolic derepression which occurs[1–8] is similar in a variety of cells. The mechanisms initiating these responses and their relationship to subsequent progression through $G_1$ to DNA synthesis in S phase, however, are generally undefined. The clearest evidence has been obtained in sea urchin eggs[9], where fertilization by sperm causes a rapid, transient increase in the concentration of free cytoplasmic $Ca^{2+}$ ($[Ca]_i$), followed by a sustained increase in cytoplasmic *p*H ($pH_i$). It has been demonstrated clearly that these ionic responses are obligatory for progression to DNA synthesis by the normal pathway after fertilization, although the $Ca^{2+}$ signal can be bypassed by parthenogenetic agents which elevate directly $pH_i$ (for example, $NH_4^+$ ions)[9]. These observations raise the questions of whether other eukaryotic cells show the same sequence of ionic responses when stimulated by mitogens and whether such signals are an obligatory component of their mitogenic pathways. We show here that a common sequence of $[Ca]_i$ and $pH_i$ responses occurs in both quiescent mouse thymocytes and Swiss 3T3 fibroblasts stimulated by appropriate mitogens. Furthermore, 'opportunistic' mitogens (those that do not act on the cells *in vivo*, such as concanavalin A (Con A)[10], the $Ca^{2+}$ ionophore A23187[11] and 12-*o*-tetradecanoyl phorbol 13-acetate CTPA)[12,13]) that are mitogenic for both mouse thymocytes and 3T3 fibroblasts[14], each produce characteristic ionic responses that are the same in both types of cell.**

Previous studies on freshly isolated thymocytes and lymphocytes have shown that a 1.5–2-fold increase in $[Ca]_i$ occurs within 1–2 min of addition of ligands which cross-link mitogen receptors on the cell surface (for example, Con A[15,16], phytohaemagglutinin[16], anti-immunoglobulin antibody[17] and the monoclonal antibody UCHT 1, ref. 18). In thymocytes stimulated by mitogenic concentrations of Con A, this $Ca^{2+}$ signal declines slowly over 24 h[19]. However, we have noted previously that, in freshly isolated thymocytes, there is a population of cells in S phase which is highly active metabolically, consistent with previous reports for related cell systems[20]. When the cells are loaded with quin 2 (ref. 21), the fluorescence signal is weighted in favour of the active cells, which are ~10-fold larger in volume than the quiescent cells. We therefore re-

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