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THALAMOCORTICAL AND CORTICOCORTICAL EXCITATORY POSTSYNAPTIC POTENTIALS MEDIATED BY EXCITATORY AMINO ACID RECEPTORS IN THE CAT MOTOR CORTEX *IN VIVO*

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Abstract—Intracellular recordings were made from neurons in the motor cortex of an anaesthetized cat, together with iontophoretic application of excitatory amino acid receptor agonists and antagonists, in order to evaluate the role of such receptors in excitatory postsynaptic potentials evoked from stimulation of afferent and recurrent pathways *in vivo*. Excitatory postsynaptic potentials which were evoked by stimulation of the ventrolateral thalamus were found to be largely insensitive to antagonism by *N*-methyl-D-aspartate receptor antagonists, although they were susceptible to blockade by the non-*N*-methyl-D-aspartate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione. Increasing the ventrolateral thalamus stimulation frequency from 0.5 or 1 to 5 Hz caused an increase of evoked excitatory postsynaptic potential amplitudes and number of action potentials. These augmented excitatory postsynaptic potentials remained insensitive to application of *N*-methyl-D-aspartate antagonists. In contrast, recurrent excitatory postsynaptic potentials evoked by stimulation of the pyramidal tract were found to be sensitive to *N*-methyl-D-aspartate receptor antagonists and/or non-*N*-methyl-D-aspartate receptor antagonists in some neurons.

These results demonstrate the involvement of both *N*-methyl-D-aspartate- and non-*N*-methyl-D-aspartate receptors in synaptic responses of cat motor cortex neurons *in vivo*, and that the synaptic pharmacology of the thalamic input may differ from that of the local recurrent pathways.

The functional anatomy and physiology of the cat motor (precruciate) cortex has been extensively studied.[4–6,10,17] In particular, the electrophysiology of layer V pyramidal tract (PT) neurons has been investigated by several groups of workers, both *in vivo* and *in vitro*.[4,6,24,27] These neurons can be sub-divided into two groups on the basis of the conduction velocity of their axons, as assessed from antidromic stimulation of the PT axons.[27] Such antidromic stimulation also often evokes excitatory postsynaptic potentials (EPSPs) in PT neurons, arising from recurrent collaterals. It is also possible to evoke EPSPs orthodromically in these neurons by stimulation of the ventrolateral thalamus (VL).

There is considerable electrophysiological and pharmacological evidence in favour of the hypothesis that excitatory amino acid receptors mediate local[16,26,29] and thalamically-evoked EPSPs in the cerebral cortex.[1,2,9,13,19,23] The local connections between layer II/III pyramidal neurons have been exhaustively studied *in vitro*.[29–31] In addition, there are also neurochemical data which indicate that an excitatory amino acid may be the transmitter of thalamocortical and corticofugal fibres.[11,12,28] The role of the various excitatory amino acid receptors in mediating EPSPs in the cortex under *in vivo* conditions is, however, still largely a matter for speculation, based on the available *in vitro* work and on the studies which have been carried out *in vivo* using extracellular single-neuron recording techniques. We have therefore carried out a series of intracellular recording experiments in the precruciate cortex of anaesthetized cats. We have applied selective excitatory amino acid receptor antagonists iontophoretically and studied their effects on EPSPs evoked by VL and PT stimulation in order to determine the contribution of *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA) receptors to such EPSPs. Some of these results have been communicated in abstract form.[15]

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP7, D-2-amino-7-phosphonoheptanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPP, 3-[(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; NMDA, *N*-methyl-D-aspartate; PT, pyramidal tract; VL, ventrolateral thalamus.

EXPERIMENTAL PROCEDURES

Animal preparation

Experiments were performed on 13 cats of either sex, weighing between 3.4 and 4.8 kg. Surgical procedures have been described in detail previously.[14] Initial surgery was

carried out under barbiturate (Brietal) anaesthesia (10 mg/kg, i.v., supplemented as necessary), following which the animals were mounted in a stereotaxic frame. Bipolar stimulation electrodes were lowered vertically into the VL (coordinates: AP +10.0, L 4.0, V +2.0) and the PT (coordinates: AP+7.0, L 6.0, V−4.0),[3] following which the ipsilateral precruciate cortex was exposed with a small craniotomy. Cats were then paralysed by a single injection (40 mg/kg gallamine, i.v.) and ventilated with 0.5% halothane in oxygen. Blood pressure and end-tidal pCO_2 were monitored continuously. The halothane tension was adjusted throughout the experiment to maintain adequate anaesthesia, as judged from stable blood pressure and pCO_2 recordings, and from the absence of reflexes once the paralysing agent had worn off. In some experiments the electrocortigram was recorded from two stainless steel electrodes, and this was used to assess depth of anaesthesia.

Recording and iontophoresis

Electrode assemblies for intracellular recording and extracellular iontophoresis were constructed from borosilicate glass capillaries (Clark Electromedical), as described previously.[14] Intracellular electrodes were cemented to seven-barrel iontophoretic assemblies (tip diameter 6–12 μm) so as to protrude by 40–70 μm. The intracellular electrodes were filled with 1.6 M potassium citrate, and the iontophoretic barrels contained one each of the following substances in most experiments (occasionally two barrels were filled with the same antagonist to enhance the probability of successful ejection). NMDA (0.1 M, pH 8–9), AMPA (0.1 M, pH 8–9), 3-[(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (CPP, 0.1 M, pH 8–9), D-2-amino-7-phosphonoheptanoic acid (AP7, 0.1 M, pH 8–9), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 mM, pH 8–9), Pontamine Sky Blue dye (2% in 0.5 M NaCl/0.5 M sodium acetate). In addition, one barrel contained NaCl (165 mM at pH 8–9) for the purpose of current and pH controls.

After reflection of the dura, recording electrodes were introduced into the precruciate cortex at an angle of approximately 50° to the horizontal using a stepping microdrive. In some experiments the cortex was stabilized with a pressor-foot surrounding the electrode entry point. In all cases the cortex was prevented from drying with a covering of mineral oil. Neurons were examined for their responses to PT stimulation (0.1–0.3 ms, 0.6–7.0 mA) and to VL stimulation (0.1–0.3 ms, 0.3–10.0 mA), and neurons which responded with an antidromic action potential to PT stimulation were classified as PT neurons. Stimulation intensities at both sites were adjusted to as to reliably evoke postsynaptic depolarizations relative to resting potential (presumed to be EPSPs as they often triggered action potentials) and inhibition defined as either hyperpolarizations relative to resting potential or periods during which there was a stimulus linked inhibition of spiking (see Fig. 1, presumed inhibitory postsynaptic potentials, IPSPs) in the neuron under investigation, and were found to work reliably throughout the course of individual experiments. For pharmacological investigations, stimuli were presented at either 0.5, 1, 5 or 10 Hz and challenged with iontophoretically applied excitatory amino acid antagonists. Wherever possible, the effects of the antagonists were also investigated on iontophoretically applied agonists on the same neuron in order to verify that the antagonist ejection currents were appropriate for selective antagonism of either NMDA receptors or AMPA receptors.

Membrane and action potentials were recorded using an Axoprobe-1A multipurpose microelectrode amplifier (Axon Instruments Inc., 1429 Rollins Road Burlingame, CA 94010, U.S.A.) combined with a headstage HS-2 series ($\times 0.1$ for R_{in} 30–300 MΩ). Conventional d.c. signal amplification techniques were used, and recordings of membrane potential were stored on FM tape for subsequent off-line analysis by computer. This could digitize and produce averages of EPSP responses, and calculate the area under the EPSP curve for individual responses. Such area measurements were used in order to quantify the effects of antagonists on EPSP responses, and at least 10 sweeps were used in these computations. In order to calculate the statistical significance of antagonist effects on EPSPs, the areas under the curve of individual EPSP sweeps were calculated and used to perform a Student's t-test.

Wherever possible, recording sites were marked by iontophoretic ejection of dye (Pontamine Sky Blue). At the end of the experiments, the cat was deeply anaesthetized and the brain was perfused with 10% formaldehyde solution in physiological saline via a carotid artery. The brain was then removed for histological verification of stimulation and recording electrode placement.

RESULTS

Characteristics of ventrolateral thalamus and pyramidal tract-evoked postsynaptic potentials

Recordings were obtained from 22 neurons. These had apparent resting membrane potentials of between −50 and −75 mV and action potential amplitudes in the range of 40–75 mV measured from the apparent resting potential. Of these neurons, six were positively identified as PT neurons on the basis of their response to PT stimulation with an antidromic action potential. Examination of the latencies of the antidromic action potentials allowed classification of these neurons into either fast or slow pyramidal tract neurons: of the six neurons, one was classed as a fast PT neuron, the remainder as slow[5,6]. The effects of amino acid antagonists on synaptic potentials were successfully evaluated on 14 neurons (Table 1).

Table 1. Effects of either AP7/CPP or CNQX on responses of individual neurons to ventrolateral thalamus and/or pyramidal tract stimulation

	VL stimulus		PT stimulus	
Cell	AP7 or CPP	CNQX	AP7 or CPP	CNQX
2cx2	NS	58		
2cx3	NS	35	NS	60
7cx1	NS	NS		
8cx1			31	
9cx1	60			
9cx3	NS			
9cx4	NS		17	NS
26cx1			31	
28cx7	NS	15		
49cx3		45		
51cx1	NS	55		NS
51cx3	NS	75		
54cx2	NS			
56cx1		64	NS	77
mean	60 (1/10)	50 (7/8)	26 (3/5)	69 (2/4)

Values are percentages of control values, as determined from area under the curve calculations, and in all cases the effect was significant at the $P < 0.05$ level. "NS" represents data where the antagonist was tested, but no statistically significant effect was observed. The computed mean at the bottom of each column was calculated from the individual values in the table (excluding NS values), and the numbers in parentheses show the number of these values out of the total number of cells tested with the relevant antagonist.

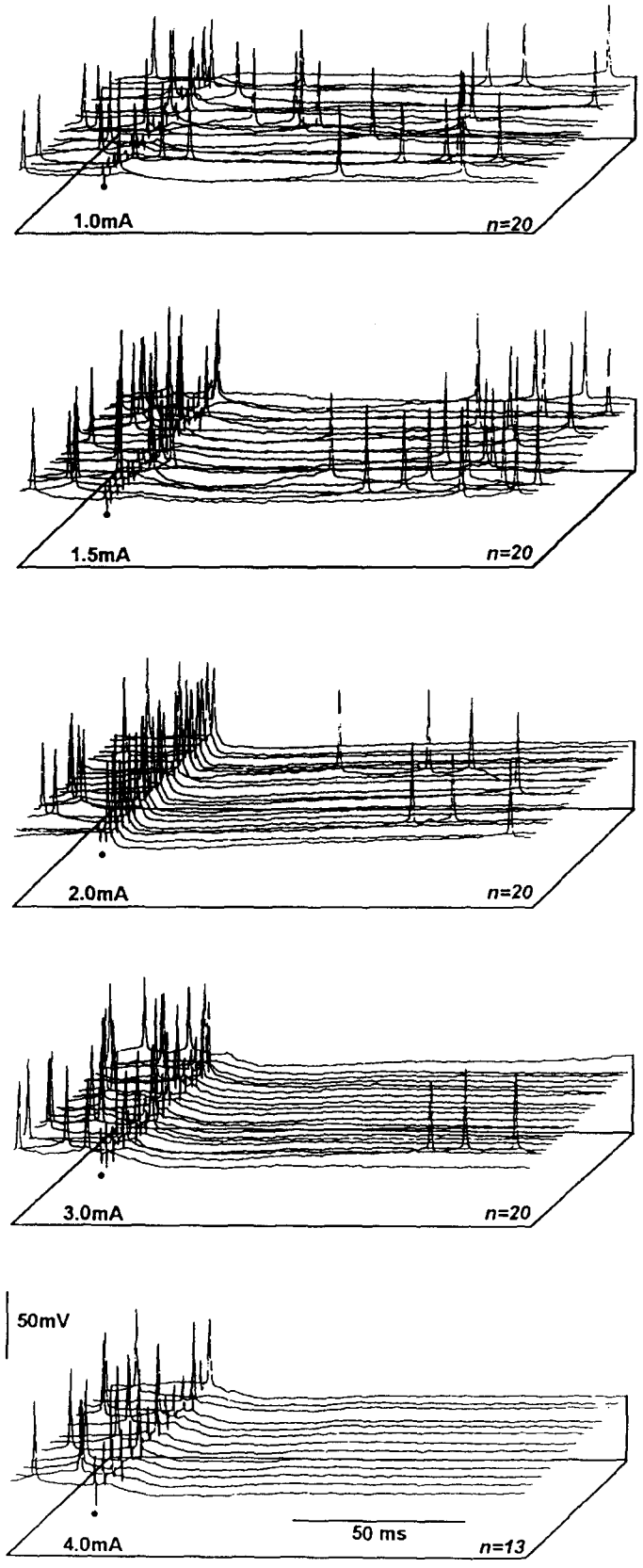

Fig. 1. Responses of a PT neuron to stimulation of the VL with increasing stimulation currents. Each group of records shows 20 individual successive responses to VL stimulation (0.1 ms) at the time indicated by the solid circle below the first record in each group. Increasing the stimulus strength from 1 to 2 mA led to a progressive increase in the size and reliability of the excitatory postsynaptic response, as judged from the amplitude of the EPSP and the increased probability at which action potentials were evoked. Similarly, the inhibitory potentials which followed became more prominent with increasing intensity: it is likely that these potentials were a combination of IPSPs and afterhyperpolarizations. The effect of these was to curtail the EPSP. Further increases in stimulus intensity (to 3 and 4 mA) led to progressively greater curtailment of the EPSPs, as can be seen in the lower two groups of records.

Stimulation of the VL resulted in EPSPs which had onset latencies to stimulation of between 1.8 and 10.8 ms (mean = 4.85 ms) and durations of 17–55 ms (mean = 32 ms). The mean amplitude of such EPSPs was 5.4 ± 7.9 mV (mean ± S.D.). EPSPs which were evoked by PT stimulation had latencies of between 2.6 and 8.1 ms (mean = 5.35 ms) and durations of 12–53 ms (mean = 36 ms). The mean amplitude of these EPSPs was 3.9 ± 2.1 mV. EPSPs resulting from stimulation of either pathway could result in the generation of action potentials, depending on the stimulation intensity, and EPSPs were usually followed by inhibitory potentials which appeared to curtail the EPSPs (Fig. 1).

The effects of varying the frequency of stimulation were systematically investigated for VL-evoked EPSPs on seven neurons. It was found that increasing the stimulation frequency from either 0.5 or 1 to 5 Hz resulted in apparent increases in EPSP amplitudes and this was in some cases associated with membrane hyperpolarization (Fig. 2). Nevertheless, the number of action potentials that were elicited during EPSPs was increased during 5 Hz stimulation. Further increases in stimulation frequency (to 10 Hz) did not produce further amplitude increases, but rather led to apparent reductions in EPSP amplitudes: this appeared to be due to increases in the effect of the following IPSPs.

Actions of N-*methyl*-D-*aspartate and non*-N-*methyl*-D-*aspartate receptor agonists*

Iontophoretically ejected NMDA had depolarizing actions on all neurons which were investigated. Such depolarizations were accompanied by increases in action potential frequency and the occurrence of depolarizing shifts and plateaus. This pattern of action was similar to that which we have previously described in the caudate nucleus,[14] and which has subsequently also been described by others in the cortex, *in vitro*.[7,30] The actions of NMDA were antagonized by either CPP (two neurons) or AP7 (nine neurons) in all cases tested (Figs 3, 4). In contrast to the effects of NMDA, AMPA (eight neurons) or quisqualate (two neurons) did not evoke plateau potentials, although they did depolarize neurons which led to increases in action potential frequency. The antagonist CNQX was tested against responses to AMPA or quisqualate on five neurons. However, although this antagonist was able to reduce responses to AMPA or quisqualate, it also tended to produce reductions in responses to NMDA.

Effects of antagonists on ventrolateral thalamus-evoked excitatory postsynaptic potentials

The responses to VL stimulation of 14 neurons were challenged with one or more excitatory amino acid antagonists. These experiments were carried out with stimulation frequencies of either 0.5 or 1 Hz, although the effects of NMDA antagonists were also studied on EPSPs evoked by 5 Hz stimulation in two cases. Of these 14 neurons, four were positively identified as PT neurons: the synaptic pharmacology of these neurons did not appear to differ from the majority of the VL-responsive group.

The AMPA-receptor antagonist CNQX was ejected onto eight neurons during VL stimulation with currents of between −50 and −250 nA. The VL-evoked EPSP was reduced in seven of these neurons by between 25% and 85% from control values (mean reduction = 50%, Table 1) in terms of the areas under the EPSP curves. In each of these cases, the differences between the EPSPs under

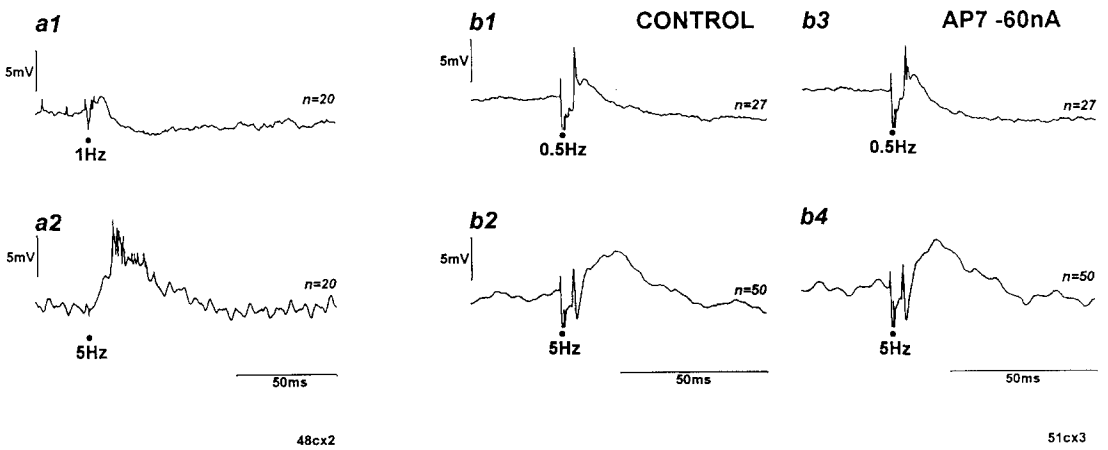

Fig. 2. Potentiation of VL-evoked EPSPs with increases in stimulation frequency. (a) Averages of 20 responses to 20 VL stimuli (3.0 mA) presented at frequencies of either 1 Hz (a1) or 5 Hz (a2). With 1 Hz stimulation, the VL-evoked EPSP was followed by an IPSP. With stimulation at 5 Hz, the EPSP was of greater amplitude and duration, more action potentials appear on the EPSP and no IPSP was evident. (b) Similar data from a second neuron, where VL was stimulated (10 mA) at either 0.5 Hz (b1) or 5 Hz (b2), and again an increase in EPSP amplitude and length was evident with higher stimulation frequency. For this neuron, the procedure was repeated during the iontophoretic application of the NMDA antagonist AP7 with a current of 60 nA (b3 and b4). The antagonist produced no significant effect on the responses to either 0.5 or 5 Hz stimulation.

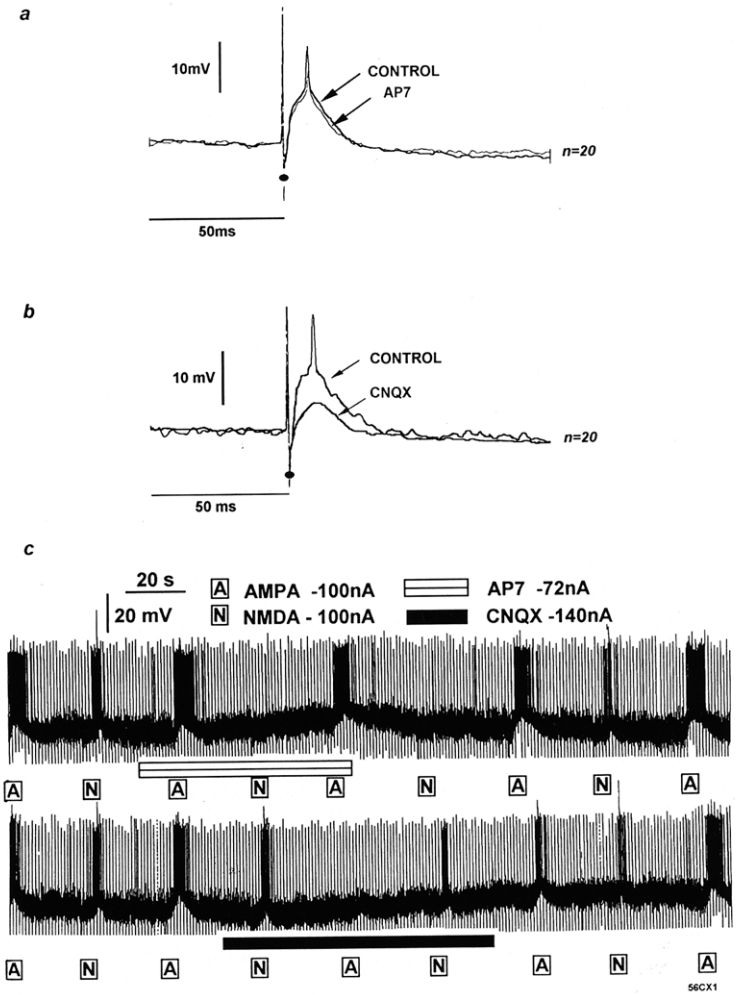

Fig. 3. Responses of a neuron which was responsive to VL stimulation (0.3 ms, 6.8 mA, 0.5 Hz, indicated by the spot), but which was not responsive to PT stimulation, and hence could not be positively identified as a PT neuron. (a) Averages of VL-evoked EPSPs (20 sweeps each), taken during control conditions or during the application of AP7 (72 nA), superimposed upon each other as indicated. This EPSP sometimes gave rise to an action potential, and this is evidenced by the sharp spike on the peak of the EPSP average. Notice that application of AP7 had little effect on the response to VL stimulation, even though the antagonist was able to block responses to iontophoretically-applied NMDA (see below). (b) A similar set of records to those in a, but comparing the EPSPs obtained under control conditions and during application of CNQX (140 nA). CNQX reduced the VL-evoked EPSP substantially. (c) Continuous traces from the same neuron showing responses to successive applications of either AMPA or NMDA, as indicated by the marker bars. The regular vertical deflections seen between agonist applications are the stimulus artefacts and evoked action potentials from VL stimulation (continued throughout the recording). Both agonists caused membrane depolarizations and increases in action potential frequency. Co-application of AP7 (long marker bar) blocked the response to NMDA but not the response to AMPA, and this effect was rapidly reversible. In contrast, co-application of CNQX greatly reduced the response to AMPA.

control conditions and during CNQX ejection were statistically significant at the $P \leqslant 0.05$ level. The antagonist affected both early and late phases of EPSPs, as well as reducing the peak amplitude (Figs 3, 5). This was accompanied by reduction of the number of action potentials evoked by EPSPs (e.g. Fig. 3). The effects of CNQX were reversible upon termination of the iontophoretic ejection. The NMDA receptor antagonists CPP (two neurons) or AP7 (10 neurons) had different actions to CNQX when tested against VL-evoked EPSPs (-50 to -200 nA). On nine of these neurons the antagonists had no significant effect on the EPSP, even though it was possible to antagonize iontophoretically applied NMDA (Figs 3, 5). Two of these neurons were also investigated with 5 Hz stimulation, and in these cases AP7 equally did not reduce the VL-evoked EPSP (Fig. 2). On one neuron, a reduction of the area under the EPSP curve did occur (reduction = 60%), and it appeared that there was a greater reduction in the later EPSP phases compared with the initial phases. In this case there was also a reduction in the number

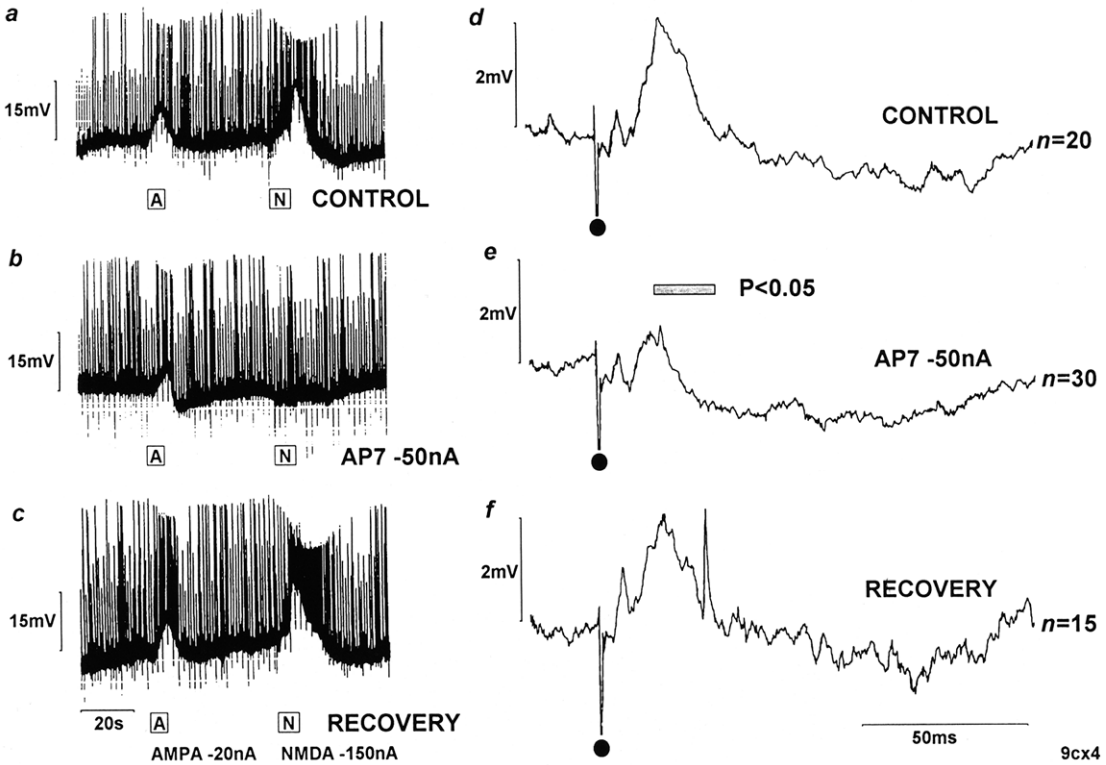

Fig. 4. Records from a neuron which was responsive to PT stimulation (0.3 ms, 0.6 mA, 0.5 Hz) with an EPSP (d–f), but which could not be identified positively as a PT neuron as no antidromic action potential could be evoked. (a–c) Responses to iontrophoretically applied AMPA and NMDA (as indicated by the marker bars) under control conditions, during AP7 application, and following recovery from antagonism, respectively. AP7 produced a selective reduction in the responses to NMDA. (d–f) Averages of EPSP responses to PT stimulation taken under the same conditions as records a–c. Note that during the AP7 application the PT-evoked EPSPs was substantially reduced (e), and this was significantly different from the control record ($P < 0.05$) for the period indicated by the bar above the record. It is evident that the later phase of the EPSP was affected most by the AP7 application.

of action potentials evoked during the EPSP, and a reduction in the frequency of ongoing "spontaneous" action potentials (see below).

Antagonism of pyramidal tract-evoked excitatory postsynaptic potentials by CNQX and AP7

The recurrent EPSP, evoked by PT stimulation, was challenged with CNQX in four neurons. On two of these neurons the recurrent EPSP was unaffected by CNQX, but on the two remaining neurons the EPSP was reduced by 40% and 23% from the control value (area under the curve measurements) (Fig. 5, Table 1). Five neurons were investigated with either CPP (one neuron) or AP7 (four neurons), and in three cases AP7 reduced the recurrent EPSPs by between 69% and 83% (Fig. 4, Table 1). It is noteworthy that although CNQX did not show any preferential antagonism of either early or late phases of recurrent EPSPs (Fig. 5), AP7 did appear to have a more pronounced action on the later EPSP phases (Fig. 4).

Effects of N-*methyl*-D-*aspartate antagonists on ongoing activity*

Pronounced ongoing "spontaneous" activity was seen in 14 neurons. This typically consisted of individual action potentials interspersed with plateau potentials similar to those evoked during iontophoresis of NMDA. This ongoing activity was reversibly reduced during the iontophoretic ejection of AP7 on 12 neurons (Fig. 6).

DISCUSSION

The results presented here represent the first *in vivo* investigation of excitatory amino acid transmission using intracellular recording techniques in the cerebral cortex. The properties of the neurons described here, in terms of their responses to VL and PT stimuli, are similar to those described by several groups who have made intracellular recordings in this cortical area of the cat.[3,5,6,10,27] Only six of the neurons in the present study were positively identified as PT neurons on the basis of their antidromic activation. However, it is conceivable that other neurons in our sample also belonged in this category, but that they were not activated by our electrode placements and stimulus parameters (see also Ghosh *et al.*[10]). Indeed, it is likely that all our neurons which had EPSP responses to PT stimulation were in layers II/III or V/VI, as the recurrent axon collaterals of layer V

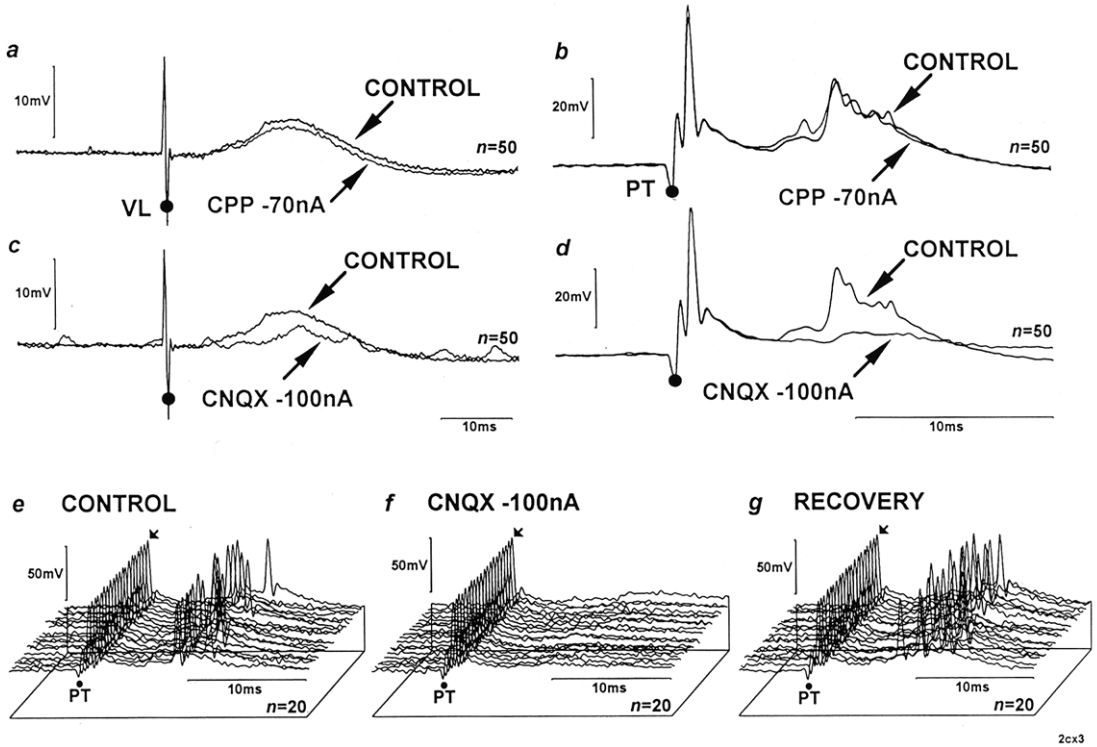

Fig. 5. Responses of an identified PT neuron to either VL (0.3 ms, 0.7 mA, 1 Hz, solid spot) or PT (0.3 ms, 1.0 mA, 1 Hz, solid spot) stimulation. (a, b) Superimposed averaged control responses and responses during co-application of the NMDA antagonist CPP (arrows) to either VL stimulation (a) or PT stimulation (b). Similar records, but comparing control responses and responses during CNQX ejection are shown in c and d. Note that CPP had no significant effect on either type of response, although it was able to block responses to iontophoretically applied NMDA (not shown). In contrast, CNQX reduced responses to both VL and PT stimulation, affecting both early and late phases of the EPSPs. (e–g) Individual sweeps of responses to PT stimulation during control conditions, during CNQX application and following the recovery from CNQX, respectively. The small arrows indicate the occurrence of an antidromic action potential, which can be seen in each sweep of all three records. The recurrent EPSPs and synaptically evoked action potentials are evident in control and recovery records approximately 10 ms after the stimulus, but are blocked during the CNQX application.

pyramidal neurons terminate in these layers but not in other cortical layers.[5,10] The relatively short latency of EPSPs to VL stimulation and their short duration in the majority of cases suggests that these EPSPs are monosynaptic. Such EPSPs have been recorded previously in PT and other precruciate cortical neurons.[6,10] It was evident, however, that in some of our recordings there were longer-latency components to VL-evoked EPSPs. It is not certain what the origin of these EPSPs is, but it is conceivable that they represent responses to recurrent cortical excitations evoked by VL input, and the finding that in some cases the later phases of such EPSPs were sensitive to AP7 would agree with this interpretation (see below). The data presented here, showing that synaptic responses of precruciate cortical neurons are susceptible to antagonism by NMDA antagonists and/or the AMPA antagonist CNQX, indicate the involvement of NMDA receptors and AMPA receptors in both thalamic input and recurrent excitation within the motor cortex.

It is apparent from our data that the VL input onto precruciate neurons is, in general terms, sensitive to CNQX, but insensitive to CPP or AP7. This suggests that the thalamic input to cortical neurons is predominantly mediated by excitatory amino acid receptors of the AMPA variety under the present experimental conditions. It seems unlikely that the resistance of the VL EPSPs to either CPP or AP7 stems from an inability of these antagonists to reach the synapses, because in seven neurons where these compounds were ineffective the same EPSP could be antagonized by CNQX ejected from the same micro-electrode. Previous extracellular recording studies *in vivo* and intracellular studies *in vitro* have also produced data which suggest that the thalamic input to the visual and somatosensory cortices is mediated predominantly via non-NMDA receptors.[1,2,9,13,19,28] This does not, however, exclude the possibility that NMDA receptors may make a contribution to the responses of precruciate neurons to VL stimulation. Indeed, on one neuron, NMDA antagonism did lead to a reduction in the later components of the VL EPSP and

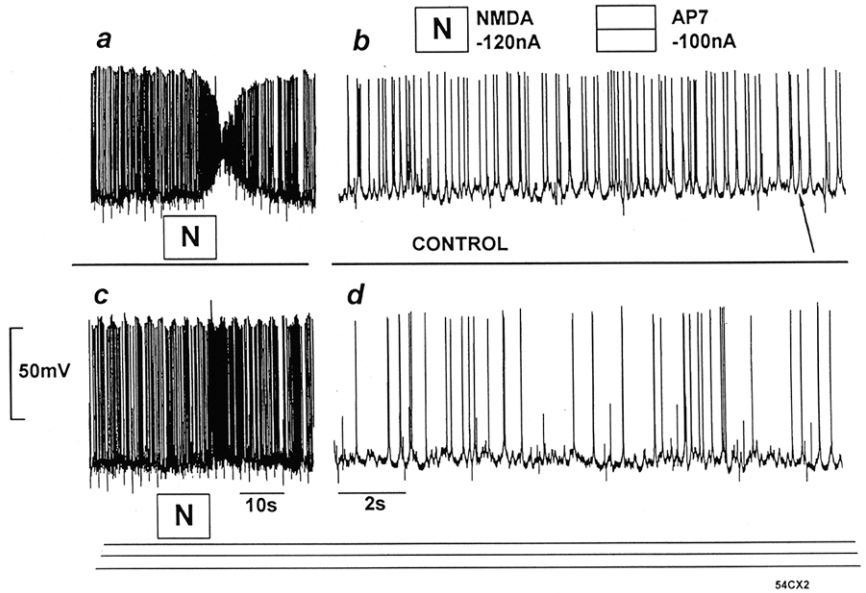

Fig. 6. Effects of AP7 (100 nA) on NMDA and ongoing activity. (a) Control response to NMDA (marker bar). (b) Ongoing activity under control conditions. Note the occurrence of "spontaneous" bursts of action potentials (example indicated by the arrow). (c) Blockade of the response to NMDA during co-application of AP7 (100 nA). (d) Reduction of the ongoing activity during AP7 application.

in the number of action potentials evoked during EPSP, although on this neuron there was also a reduction in the ongoing activity. It may be that, under *in vivo* conditions, NMDA receptor-mediated EPSP components are masked by hyperpolarized membrane potentials in view of the known voltage dependence of such events.[18,20] This seems unlikely, however, given that it was possible to record responses to iontophoretically applied NMDA in our neurons, and that we were able to record PT-evoked EPSPs with substantial NMDA receptor-mediated components. An alternative possibility is that any VL-evoked NMDA receptor-mediated EPSP components are masked by IPSPs, and this possibility has been suggested in the mouse thalamocortical pathway *in vitro*,[1] at least in early development.

It is well known that repetitive stimulation of thalamic afferents to the cerebral cortex leads to augmentation of cortical EPSPs.[21,22] It has been suggested that this may involve NMDA receptors, as the incremental EPSP components have been found to be voltage-dependent in a similar manner to NMDA receptor-mediated responses.[21] However, we found that increasing the stimulation frequency did not change the susceptibility of VL EPSPs to antagonism by AP7, although there was potentiation of the VL-evoked EPSP at 5 Hz stimulation frequency. This indicates that the augmented EPSP components are not mediated by NMDA receptors, and it is possible that this phenomenon depends on some other property of neocortical neurons.[21]

It is intriguing that the effects of CNQX and the NMDA receptor antagonists on the recurrent, PT-evoked, EPSPs differed between neurons. Indeed, both types of antagonist were tested against such EPSPs on four neurons, and in none of these did both antagonists have an effect. CNQX antagonized the recurrent EPSP in two cases, AP7 in one case, and neither antagonist in one further case. This is suggestive of two pharmacologically distinct EPSP populations, but this would require further investigation. It is noteworthy that the recurrent EPSP of cultured rat visual cortical layer V neurons is mediated by both NMDA receptors and non-NMDA receptors,[16] and it has been shown that other recurrent cortical EPSPs or excitations have substantial NMDA receptor components.[2,31]

The reduction of "spontaneous" activity of precruciate neurons by NMDA antagonists is of interest. This activity appeared similar to that evoked by iontophoretic application of NMDA: that is, single action potentials interspersed with plateau potentials crowned with one or more action potentials. It is possible that this activity was a result of leakage of NMDA from iontophoretic pipettes. This seems unlikely, however, for several reasons. Firstly, relatively high iontophoretic currents were needed to eject NMDA in order to produce responses on several neurons which showed ongoing activity: this suggests that the retention currents were adequate. Secondly, similar ongoing activity has been seen by other workers *in vivo* not using iontophoretic electrode assemblies. A more parsimonious explanation is thus that the spontaneous activity is in fact due to ongoing NMDA receptor-mediated synaptic input. A similar suggestion has been made by Fox and Armstrong-James[8] for the rat somatosensory cortex on the basis of extracellular recording of burst activity and

iontophoresis of NMDA antagonists. They further suggested, on the basis of lesion experiments, that the input which generates this activity arises from the intralaminar thalamic nuclei rather than from a specific relay nucleus. Whether this is true in the cat precruciate cortex remains to be established. Interestingly, it has been found that the non-competitive NMDA antagonist, the dissociative anaesthetic ketamine, can reduce the slow (0.3 Hz) membrane potential oscillations and ongoing burst firing of cat cortical neurons *in vivo*, when given intravenously.[25]

CONCLUSION

In conclusion, the work presented here supports the hypothesis that excitatory amino acids are involved in thalamic inputs to precruciate cortical neurons and in the recurrent excitation of the same cells in the adult cat. EPSPs elicited by thalamic (VL) stimulation studied in their natural environment appear to be mainly mediated by AMPA-type receptors, as they were antagonized by CNQX but were largely insensitive to NMDA antagonists. It is, however, evident that NMDA receptors are functional in the present *in vivo* recording conditions: (i) iontophoretically applied NMDA was able to depolarize and excite the same neurons; (ii) ongoing activity was inhibited by NMDA antagonists; (iii) in contrast to VL-evoked EPSPs, some recurrent EPSPs evoked by stimulation of the PT were found to be sensitive to iontophoretically applied NMDA antagonists, indicating the participation of NMDA receptors in the generation of these EPSPs.

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