Exp Brain Res (1989) 77:646–652

Experimental Brain Research

Function of non-NMDA receptors and NMDA receptors in synaptic responses to natural somatosensory stimulation in the ventrobasal thalamus

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Summary. Sensory synaptic responses of rat ventrobasal thalamus neurones were challenged with iontophoretic applications of the excitatory amino acid antagonists CNQX and CPP. CNQX, applied with currents which were selective for non-NMDA receptors, antagonised responses of VB neurones to both 10 ms and 2000 ms air jet stimulation of the peripheral receptive field. In contrast, CPP only antagonised the latter type of response. These results suggest a differential involvement of excitatory amino acid receptors in sensory synaptic transmission to the ventrobasal thalamus, with an initial synaptic component being mediated by non-NMDA receptors (including kainate receptors), and a further NMDA receptor-mediated component being manifested upon maintained sensory stimulation. The expression of this latter component appears to be largely dependent upon the integrity of the non-NMDA receptor-mediated component.

Key words: Excitatory amino acids – Thalamus – Somatosensory system – CNQX – CPP

Introduction

Excitatory amino acid receptors have been shown to be involved in synaptic transmission in many areas of the vertebrate central nervous system (For reviews see Watkins and Evans 1981; Mayer and Westbrook 1987). The availability of potent and selective antagonists for excitatory amino acid receptors of the N-methyl-D-aspartate (NMDA) type has enabled a tentative division of synaptic receptors into two groups: those involving NMDA receptors, and those which appear to be mediated by excitatory amino acid receptors of a type other than the NMDA type, presumably kainate and/or quisqualate receptors (Watkins and Evans 1981; Mayer and Westbrook 1987). The characterisation of the non-NMDA receptor-mediated response has however depended heavily upon the use of broad-spectrum excitatory amino acid antagonists, such as kynurenic acid, which block both NMDA receptors and non-NMDA receptors (Perkins and Stone 1982; Mayer and Westbrook 1987). This approach unfortunately leaves open the possibility that those responses which appear to be mediated by non-NMDA receptors are in fact mediated by both non-NMDA receptors and NMDA receptors. A more satisfactory approach would be to use antagonists which are both potent and selective for non-NMDA receptors, and indeed such antagonists have been described recently (Honoré et al. 1988).

In the ventrobasal thalamus (VB), synaptic responses to stimulation of somatosensory afferents can be divided into those that are sensitive to NMDA antagonists and those that are resistant to these blockers, but can be antagonised by kynurenate (Salt 1986, 1987). In particular, it appears that the initial, short latency, response of VB neurones to afferent stimulation is mediated by non-NMDA receptors whereas maintained activity in the afferent pathway generates a post-synaptic response which has a substantial NMDA receptor involvement (Salt 1986, 1987). The contribution of non-NMDA receptors to synaptic responses however requires further clarification. The potent novel non-NMDA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Honoré et al. 1988) offers the opportunity to study the contribution of non-NMDA receptors to VB synaptic responses directly. We have therefore studied the effects of this antagonist on responses of VB neurones to sensory stimulation and iontophoretically

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applied excitatory amino acids. Some of these results have been presented previously in preliminary form (Salt 1988).

Methods

Experiments were performed on adult male Wistar rats, anaesthetised with urethane (1.2 g/kg, i.p.), as described in detail previously (Salt 1987). Extracellular single neurone recordings were made from VB neurones through the central barrel of seven-barrel glass iontophoretic electrodes. This barrel was filled with 3M NaCl, while each of the remaining barrels was filled with one of the following: Na kainate (0.1 M pH 8), Na quisqualate (25 mM in 150 mM NaCl, pH 8), Na N-methyl-D,L-aspartate (NMA, 0.1 M, pH 8), Na 3-(2-carboxypiperazine-4-yl)propyl-l-phosphanate (CPP, 25 mM in 150 mM NaCl, pH 8) CNQX (1 mM in 50 mM NaCl, pH 8.5), pontamine sky blue dye (2% in 0.5 M NaCl/0.5 M Na acetate), 1 M NaCl. Automatic current balancing was performed in some experiments: no qualitative differences were noted between experiments with and without current balancing. Neurones were characterised as VB neurones on the basis of sensory response characteristics, stereotaxic co-ordinates and in some cases ejection of pontamine sky blue at the recording site (Salt 1987).

Sensory stimulation was achieved with an electronically gated air-jet which could be directed at a small area of hairy skin or a single vibrissa. All sensory stimuli and iontophoretic ejections of excitatory amino acid agonists were presented in regular timed cycles. Action potentials were gated by a window discriminator and timed and recorded by a computer system. This allowed the generation of peri-stimulus time histograms of neural activity. Response magnitudes were quantified by counting the number of action potentials in a given response. The effects of antagonists on sensory stimuli and agonists were noted during the continuous ejection of antagonists throughout one or more cycles of stimuli (Salt 1987). Wherever possible, several ejections of antagonists were made onto a given neurone in an attempt to achieve the most selective effects possible.

Excitatory amino acid agonists were purchased from Sigma. CPP and CNQX were generous gifts from Sandoz (Berne) and Ferrosan (Soeborg), respectively.

Results

Recordings were made from thirty-four VB neurones, obtained from eighteen rats. All of these neurones responded to deflections of hairs or vibr-

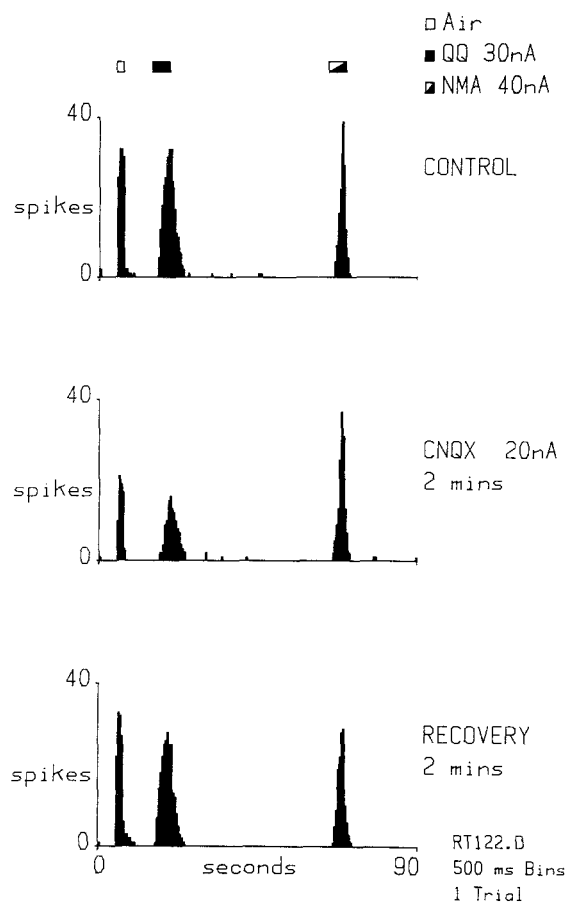

Fig. 1. A series of peristimulus time histograms (psth's) showing the responses of a ventrobasal neurone to stimulation by a 2000 ms air jet directed at the receptive field, and by iontophoresis of quisqualate (QQ) and NMA. The vertical axis calibration is the number of action potentials (spikes) counted into successive 500 ms epochs (bins). The time and duration of each stimulus in all three records is indicated by the bars above the upper histogram. The upper record is a control record, below which are shown responses obtained during the concurrent ejection of CNQX with a current of 20 nA: the antagonist ejection had commenced 2 min before the start of this record. Note that CNQX reduced responses to both air jet stimulation and quisqualate iontophoresis, but had little effect on the response to NMA. The effect of CNQX was reversible: the lower record was taken 2 min after the end of the CNQX ejection

Table 1. Percentage reductions from control values of responses to excitatory amino acids and the two types of air jet stimuli caused by the antagonist CNQX. **A** Neurones where the effects of CNQX on kainate were not studied. **B** Neurones where kainate was used in addition to NMA and quisqualate. Values are means ± standard deviations of n neurones. Values of n in parentheses indicate that the antagonist was only tested on the appropriate sensory response on a sub-set of neurones

Antagonist	Excitatory amino acid agonists			Air jet stimuli		n
	NMA	Kainate	Quisqualate	2000 ms	10 ms	
A CNQX	17 ± 29.7	–	52 ± 9.6	73 ± 26.1 ($n=7$)	88 ± 17.4	12
B CNQX	−4 ± 34.8	82 ± 17.7	12 ± 31.6	65 ± 23.7	79 ± 25.5 ($n=21$)	22

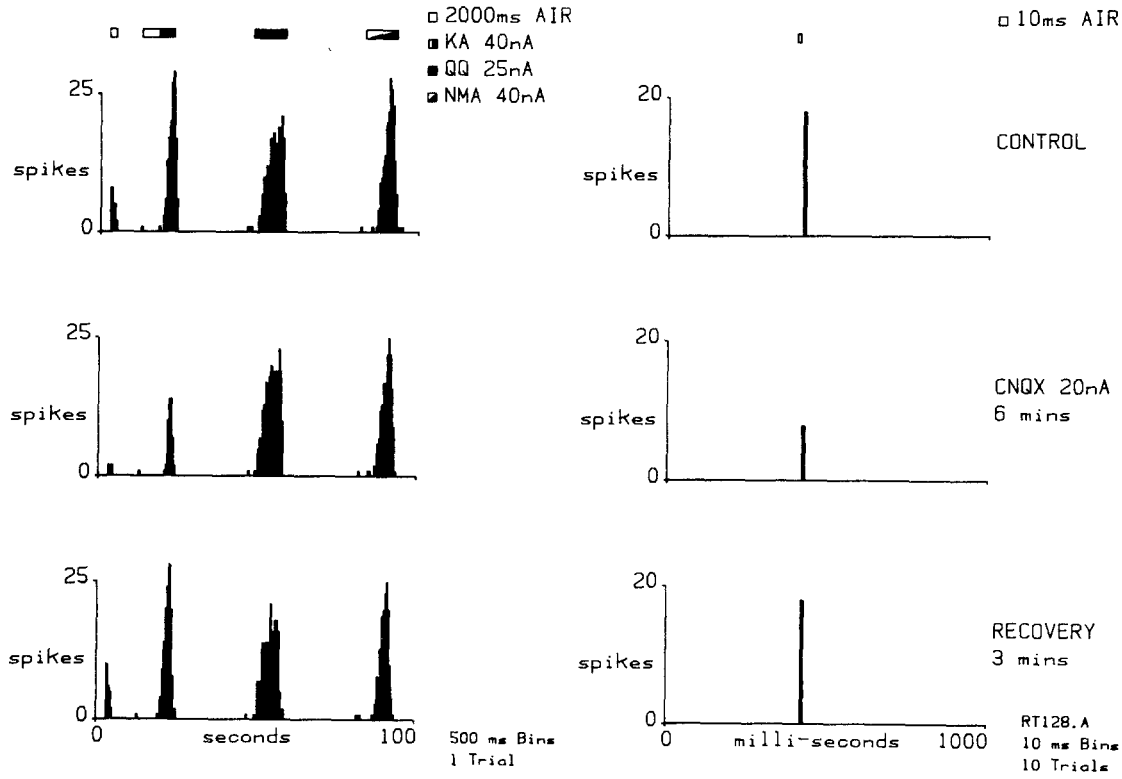

Fig. 2. The left hand column of records are similar to those of Fig. 1, except that responses of a neurone to 2000 ms air jet, kainate (KA), quisqualate and NMA are shown. The upper record is a control, below which is a record obtained during CNQX iontophoresis: note the selective reduction of kainate responses and the concurrent reduction in the response to the 2000 ms air jet. The right hand column of records from the same neurone depicts responses to air jet stimuli of 10 ms duration. Each histogram is cumulative over ten trials with a bin size of 10 ms. Stimuli were presented at a rate of one every two seconds, commencing 25 s before the 2000 ms air jet depicted in the left hand records (see Fig. 3 for a more detailed depiction of the stimulus sequence). The upper record is a control, below which is a histogram obtained during the CNQX ejection: note that the antagonist reduced the responses to the 10 ms air jet stimuli

issae with a blunt probe or air jet. Receptive field locations and sizes were essentially similar to those described previously (Waite 1973; Vahle-Hinz and Gottschaldt 1983; Salt 1987). In all cases the neurones were found to be excited by whichever excitatory amino acid agonists were tested.

Effects of CNQX on excitatory amino acid responses

All of the 34 VB neurones were tested with CNQX. On twelve of these neurones, the effects of CNQX on responses to both NMA and quisqualate were compared. In general, CNQX (8–40 nA) was found to be somewhat quisqualate-selective in this series of experiments, although it must be pointed out that in three cases responses to NMA appeared to be equally or more strongly affected than responses to quisqualate. These findings are summarised in Table 1 A, and an example of a selective antagonism of quisqualate by CNQX is shown in Fig. 1. On the remaining twenty-two neurones CNQX (3–40 nA) was tested against responses to NMA, quisqualate and kainate. The antagonist was found to be selective towards kainate in all of these cases, and indeed, it was possible on many neurones to produce considerable reductions in kainate responses while having little effect on responses to either NMA or quisqualate (Fig. 2). These results are summarised in Table 1 B. It is noteworthy that CNQX appeared to potentiate responses to NMA in a number of neurones. It is important to point out that the selectivity of effects of CNQX against amino acid responses were very dose dependent: small increases in antagonist current beyond a kainate-selective current would typically lead to antagonism of quisqualate responses, and ultimately NMA responses. Indeed, it was difficult to produce large reductions of quisqualate responses without also reducing NMA responses to a certain extent. This is reflected in Table 1 A, where greater reductions of responses to NMA and quisqualate are shown than in Table 1 B. This is a consequence of taking antagonism of quisqualate

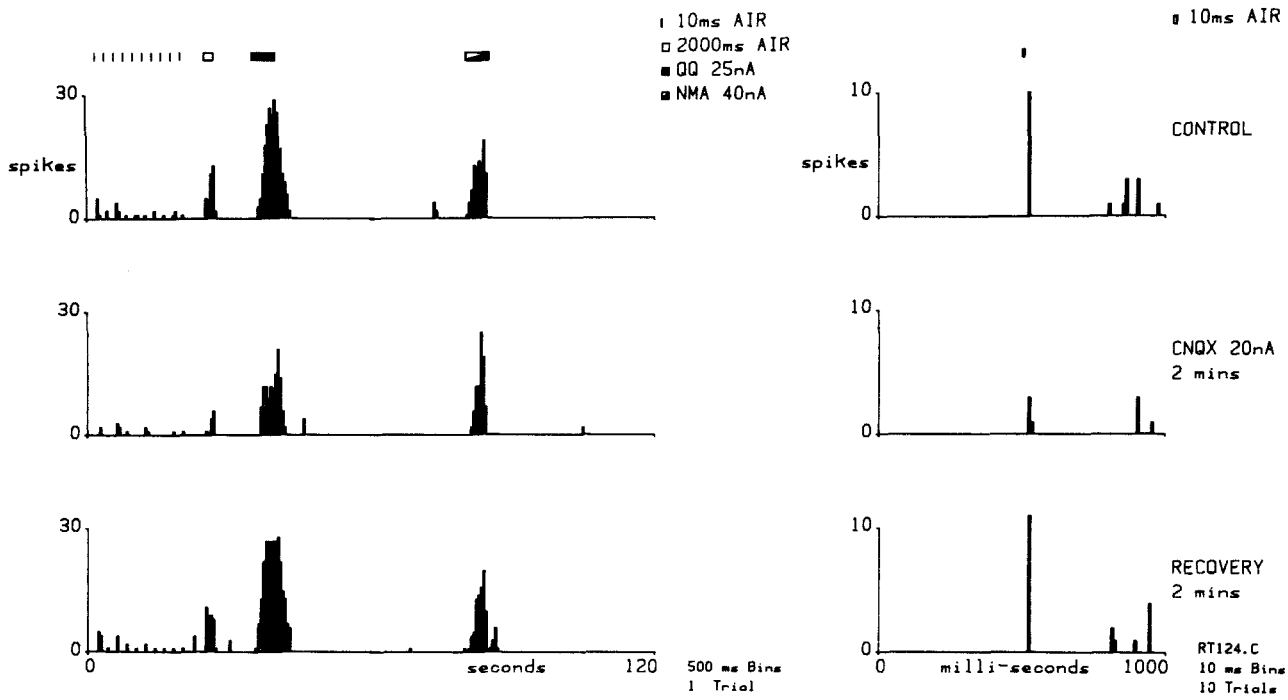

Fig. 3. The left hand column shows similar histograms to Figs. 1 and 2, but also includes action potential spikes recorded during ten successive 10 ms air jet stimuli. These ten responses are also depicted in the cumulative histograms in the right hand column, as in Fig. 2. The upper records are controls, below which are records taken during the ejection of CNQX. Note that CNQX reduced responses to quisqualate whilst having little effect on the responses to NMA. At the same time, responses to both the 2000 ms air jet and the 10 ms air jet stimuli were reduced.

responses as the end-point in the experiments summarised in Table 1 A, rather than antagonism of kainate responses.

Effects of CNQX on sensory synaptic responses

Two types of sensory stimuli were employed in this series of experiments. The first was a continuous air jet of 2000 ms duration directed at the receptive field: this typically produced a maintained neural discharge. The second type of stimulus was a 10 ms air jet, which typically produced a short-latency (10 to 20 ms) response consisting of one or two action potentials (Salt 1987).

CNQX was tested against responses to the maintained air jet stimulus on 29 neurones. It was apparent that such sensory responses were reduced by CNQX when the antagonist was ejected with iontophoretic currents which produced a selective antagonism at non-NMDA receptors. Indeed it was possible to reduce sensory responses with CNQX in the absence of any marked effect on responses to NMA (Fig. 1). Interestingly, sensory responses were antagonised by CNQX to a greater extent than were responses to quisqualate (Table 1 A). Furthermore, on those neurones where CNQX was found to antagonise responses to iontophoretically applied kainate selectively, responses to the maintained sensory stimulus were also reduced (Fig. 2, Table 1 B).

CNQX was also tested against responses to the short duration air jet on 33 neurones, 28 of which had also been tested with the maintained stimulus. These stimuli were repeated at two-second intervals and averaged over either ten or fifteen trials. In all but one case, such responses were antagonised by CNQX ejected at currents that were selective for non-NMDA receptors (Figs. 2 and 3). Indeed, as with the responses to maintained air jet stimulation, reduction of these responses was evident under conditions of selective kainate antagonism (Fig. 2, Table 1 B).

Effects of the NMDA antagonist, CPP

Nineteen neurones which had been studied with CNQX were also studied with the selective NMDA receptor antagonist, CPP (Davies et al. 1986), so that a comparison could be made of the contributions of NMDA receptors and non-NMDA receptors to sensory synaptic responses. CPP (0–6 nA) was found to antagonise responses to NMA with little effect on responses to either kainate or quisqualate. At the same time, responses to the main-

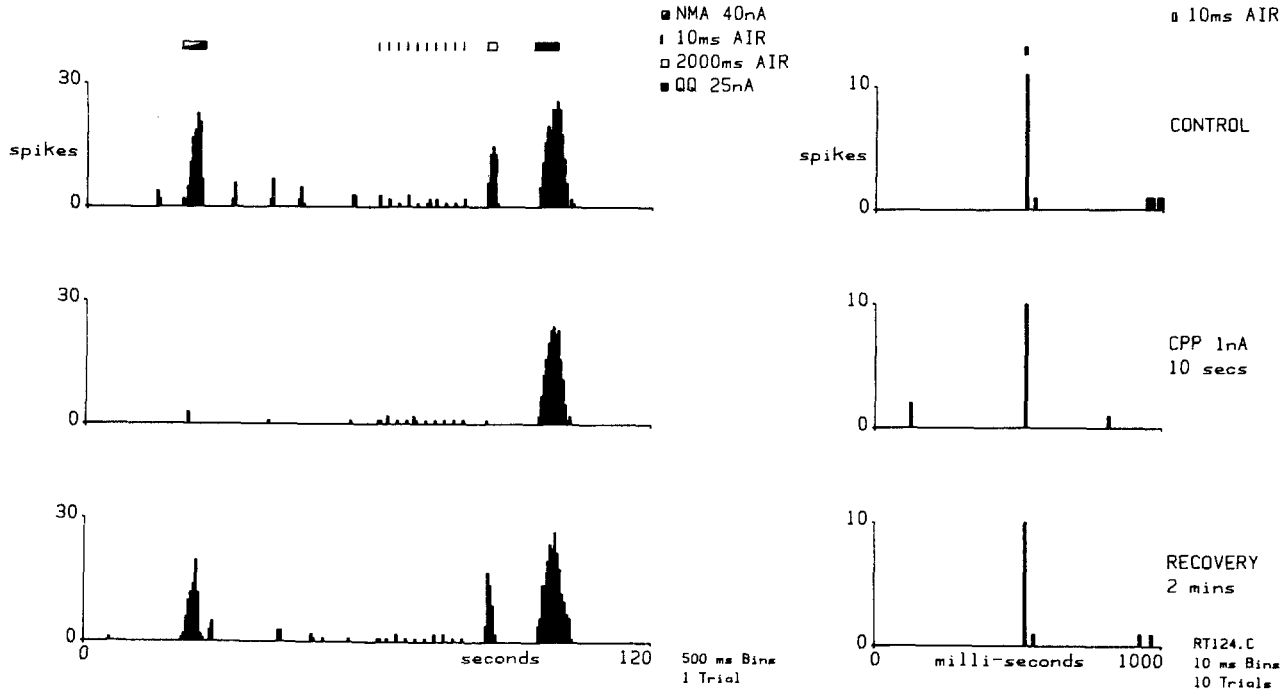

Fig. 4. This shows data from the same neurone as in Fig. 3, and responding to the same cycles of stimuli. Note, however, that the start time of the histograms shown in the left column is shifted compared with Fig. 3. The upper records are controls, below which are shown responses obtained during iontophoresis of the NMDA-receptor antagonist, CPP. This antagonist produced a selective blockade of NMA compared with quisqualate, and at the same time greatly reduced responses to the 2000 ms air jet whilst having no effect on responses to the 10 ms air jet (in contrast to the effects obtained with CNQX on this neurone)

Table 2. Similar table to Table 1, but documenting the effects of CPP on amino acid and sensory responses

Antagonist	Excitatory amino acid agonists			Air jet stimuli		*n*
	NMA	Kainate	Quisqualate	2000 ms	10 ms	
A CPP	91±9.5	–	6±15.8	75±19.6 ($n=3$)	−15±21.0	5
B CPP	98±2.5	7±36.0	3±22.5	69±24.5	−4±19.8 ($n=13$)	14

Table 3. Comparison of the effects of CNQX and CPP on the responses of sixteen ventrobasal thalamus neurones to the two types of air jet stimuli. Values are percentage reduction from control±standard deviation

Antagonist	Air jet stimuli		
	2000 ms	10 ms	
A CNQX	67±24.5	78±27.8	
B CPP	70±24.0	−7±21.4	($n=16$)

tained air jet stimulus were also antagonised, but there was little or no change in the responses to the short duration air jet stimulus (Fig. 4, Table 2).

The effects of both CPP and CNQX on responses to both types sensory stimuli were determined on sixteen VB neurones. These results are summarised in Table 3, and it is apparent that although CNQX was able to antagonise both types of sensory response to a similar extent, CPP only affected maintained air jet responses. Furthermore, CNQX and CPP had approximately the same overall effect on the maintained air jet responses.

Discussion

The spectra of activity of CNQX and CPP described in this paper are broadly similar to those described previously by others working in different regions of the central nervous system (Davies et al. 1986; Honoré et al. 1988). It is noteworthy that CNQX was found to be most effective as an antagonist of kainate responses, even though binding

studies (Honoré et al. 1988) and in vitro electrophysiological experiments (Fletcher et al. 1988) would suggest that CNQX has greater affinity for the quisqualate receptor. Nevertheless, iontophoretic experiments in the spinal cord (Honoré et al. 1988) also show a slightly greater reduction of kainate responses than quisqualate responses by CNQX. The reasons for these discrepancies remain to be determined, but it is possible that the greater sensitivity of kainate responses to CNQX is due to an uneven distribution of kainate receptors and quisqualate receptors on the neurone and the non-uniform distribution of the antagonist around the neurone when ejected iontophoretically. Furthermore, as quisqualate does not appear to be the best agonist for what is called the quisqualate receptor (Honoré et al. 1988), it is possible that the effects of iontophoretically applied quisqualate are due to activation of other receptors in addition to the quisqualate receptor. It is however apparent that there is still a need for antagonists which provide better discrimination between kainate and quisqualate receptors.

The finding that sensory synaptic responses of rat VB neurones could be antagonised by CNQX when this antagonist was ejected with non-NMDA receptor selective currents indicates that there is involvement of non-NMDA receptors in these synaptic responses. In particular, the short-latency responses to 10 ms air jet stimuli, which are resistant to NMDA antagonists such as D-2-aminophosphonovalerate (APV) (Salt 1987) and CPP (this study), are antagonised by CNQX. The data obtained with CNQX thus provide direct evidence for the involvement of non-NMDA receptors rather than NMDA receptors in these synaptic responses. Such a conclusion is consistent with previous data obtained in rat VB with the broad-spectrum excitatory amino acid antagonist kynurenate (Salt 1987).

The effects of CNQX on the responses of VB neurones to maintained air jet stimulation are intriguing, because these responses are also very sensitive to both competitive and non-competitive NMDA antagonists (Salt 1987; Salt et al. 1988), as exemplified by Figs. 3 and 4. Indeed, it is apparent from the data obtained from neurones where both CNQX and CPP were tested (Table 3) that the NMDA-receptor mediated and non-NMDA receptor-mediated components of the maintained air jet response do not summate in a linear fashion. This suggests that the integrity of this synaptic response depends on the activation of both NMDA receptors and non-NMDA receptors. It is therefore plausable that maintained afferent stimulation provides a high-frequency synaptic input to VB. The temporal summation of non-NMDA receptor synaptic potentials could then depolarise the post-synaptic neurone sufficiently to allow the development of an NMDA receptor-mediated component by reducing the voltage-dependent blockade of NMDA receptor-mediated responses by Mg^{++} (Mayer and Westbrook 1987). Antagonism of either NMDA receptors or non-NMDA receptors would then be expected to reduce the synaptic response: and this is indeed what is observed in rat VB. An interesting parallel is observed in the lateral geniculate nucleus, the visual homologue of the ventrobasal thalamus: responses to visual stimuli in this nucleus in vivo, consisting of a maintained discharge of action potentials, are sensitive to CPP or APV and CNQX (Moody and Sillito 1988; Sillito et al. 1988; Sillito et al. in preparation), whereas responses to single pulse electrical stimuli, in vitro, are resistant to APV (Crunelli et al. 1987).

A novel finding in this series of experiments is that reductions in sensory synaptic input to VB are evident when CNQX is applied with iontophoretic currents which produce a selective antagonism of kainate responses (Fig. 2). Although the apparent kainate selectivity must be regarded with a degree of caution (see above), this does argue in favour of an involvement of kainate receptors in synaptic responses. Previous findings in VB obtained with the less potent antagonist gamma-D-glutamylaminomethyl sulphonate (GAMS), which was found to be moderately kainate selective, were less conclusive (Salt 1987). It is quite possible that the amounts of GAMS ejected were not adequate to penetrate as far as synaptic kainate receptors: the polar nature of GAMS and its low potency would support this hypothesis. In contrast, CNQX is less polar and much more potent than GAMS (Honoré et al. 1988). CNQX would thus appear to be a superior tool for the investigation of synaptic transmission.

In conclusion, the results presented here show that both NMDA receptors and non-NMDA receptors, are involved in afferent synaptic transmission to VB. Non-NMDA receptors play a role in the mediation of the short latency sensory synaptic response, and it is likely that the generation of the NMDA receptor-mediated response component of the maintained response is dependant on the integrity of the kainate/quisqualate receptor mediated input. Although the present data seem to particularly implicate kainate receptors in synaptic transmission, they do not rule out the possibility that quisqualate receptors and possibly other receptor types are also involved in synaptic trans-

mission in VB: the development of more selective quisqualate and kainate antagonists would help to resolve this matter.

Acknowledgements. This work was supported by the Medical Research Council. Thanks to Dr. P.L. Herrling and Dr. T. Honoré for gifts of CPP and CNQX, respectively. Prof. A.M. Sillito provided helpful comments on the manuscript.

References

Crunelli V, Kelly JS, Leresche N, Pirchio M (1987) On the excitatory post-synaptic potential evoked by stimulation of the optic tract in the rat lateral geniculate nucleus. J Physiol 384:603–618

Davies J, Evans RH, Herrling PL, Jones AW, Olverman HJ, Pook P, Watkins JC (1986) CPP, a new potent and selective NMDA antagonist. Depression of central neuron responses, affinity for [^{3}H]D-AP5 binding sites on brain membranes and anticonvulsant activity. Brain Res 382:169–173

Fletcher EJ, Martin D, Aram JA, Lodge D, Honoré T (1988) Quinoxalinediones selectively block quisqualate and kainate receptors and synaptic events in rat neocortex and hippocampus and frog spinal cord in vitro. Br J Pharmacol 95:585–597

Honoré T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE (1988) Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241:701–703

Mayer ML, Westbrook GL (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. Prog Neurobiol 28:197–276

Moody CI, Sillito AM (1988) The role of the N-methyl-D-aspartate (NMDA) receptor in the transmission of visual information in the feline dorsal lateral geniculate nucleus (dLGN). J Physiol 396:62P

Perkins MN, Stone TW (1982) An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 247:184–187

Salt TE (1986) Mediation of thalamic sensory input by both NMDA receptors and non-NMDA receptors. Nature 322:263–265

Salt TE (1987) Excitatory amino acid receptors and synaptic transmission in the rat ventrobasal thalamus. J Physiol 391:499–510

Salt TE (1988) Effects of CNQX on excitatory amino acid and synaptic responses of rat ventrobasal thalamus neurones. Br J Pharmacol 95:755P

Salt TE, Prasad SK, Wilson DG (1988) Antagonism of N-methylaspartate and synaptic responses of neurones in the rat ventrobasal thalamus by ketamine and MK-801. Br J Pharmacol 94:443–448

Sillito AM, Murphy PC, Moody I (1988) The role of N-methyl-D-aspartate and quisqualate receptors in mediating the retinal input to the lateral geniculate nucleus. In: Cavalheiro EA, Lehmann J, Turski L (eds) Frontiers in excitatory amino acid research. Alan R Liss, New York, pp 429–434

Vahle-Hinz C, Gottschaldt KM (1983) Principal differences in the organization of the thalamic facial representation in rodents and felids. In: Macchi G, Rustioni A, Spreadico R (eds) Somatosensory integration in the thalamus. Elsevier, Amsterdam, pp 125–146

Waite PME (1973) Somatotopic organization of vibrissal responses in the ventrobasal complex of the rat thalamus. J Physiol 228:527–540

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Ann Rev Pharmacol Toxicol 21:165–204

Received December 9, 1988 / Accepted April 18, 1989

Note added in proof.

We have carried out experiments recently using the more selective quisqualate receptor agonist, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA). Essentially similar results to those obtained with quisqualate were obtained using AMPA.