J. Physiol. (1982), **327**, *pp.* 65–78
With 1 *plate and* 8 *text-figures*
Printed in Great Britain

AN IONOPHORETIC STUDY OF THE RESPONSES OF RAT CAUDAL TRIGEMINAL NUCLEUS NEURONES TO NON-NOXIOUS MECHANICAL SENSORY STIMULI

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(*Received 4 December 1981*)

SUMMARY

1. Extracellular recordings of the responses of single caudal trigeminal nucleus neurones to non-noxious and noxious facial stimuli and to ionophoretically applied L-glutamate, L-aspartate and acetylcholine were made in urethane anaesthetized rats.

2. Neurones excited by non-noxious mechanical stimuli were located primarily in the magnocellular part of nucleus caudalis, whereas neurones excited by both noxious and non-noxious stimuli were located either ventromedially to the magnocellular part of nucleus caudalis or superficially to the substantia gelatinosa.

3. Both L-aspartate and L-glutamate were found to excite all neurones tested in nucleus caudalis. In contrast, however, acetylcholine was found to excite only 31 % of the neurones tested.

4. Responses of nucleus caudalis neurones to non-noxious sensory stimulation were not antagonized by the excitatory amino acid antagonist D-α-aminoadipate, but were antagonized by *cis*-2,3-piperidine dicarboxylate and γ-D-glutamylglycine, two excitatory amino acid antagonists with a broader spectrum of action.

5. It is concluded that the chemical synaptic transmitter of non-nociceptive mechanoreceptive primary afferent fibres to nucleus caudalis may be a ligand for an excitatory amino acid receptor other than a D-α-aminoadipate-sensitive receptor. The synaptic receptor may thus be of the kainate or quisqualate type, and the transmitter possibly L-glutamate, L-aspartate or an as yet unidentified substance.

INTRODUCTION

The caudal trigeminal nucleus (n. caudalis) receives afferent input from the facial regions, and appears to have anatomical (Gobel, Falls & Hockfield, 1977) and physiological (Price, Dubner & Hu, 1976; Yokota & Nishikawa, 1980) similarities to the dorsal horn of the spinal cord. Although extensive single-unit studies of n. caudalis have been made in the cat and primate (see Anderson & Matthews, 1977), and to a lesser extent in the rat (Nord & Kyler, 1968), little is known about the nature of the neurotransmitter substances which may be used by primary afferent fibres projecting to this nucleus. As clinical studies suggest that n. caudalis may be of importance in the processing of sensory information related to nociception (Sjöqvist, 1938; Lisney, 1981) it can be seen that identification of the neurotransmitters

involved in initiating the responses of n. caudalis neurones to both noxious and non-noxious sensory stimuli would be of great value in our understanding of the mechanisms of trigeminal pain perception.

There is neurochemical evidence (Johnson & Aprison, 1970; Roberts & Keen, 1974) that the excitatory amino acids L-glutamate and L-aspartate may be primary afferent neurotransmitters, and in particular, transmitters used by large diameter low threshold primary afferent fibres projecting to the dorsal horn and dorsal column nuclei (Roberts, 1974; Roberts & Hill, 1978). Recent studies using specific agonists and antagonists indicate that there may be three receptor types at which excitatory amino acids act, and these receptors have been defined in terms of the agonists *N*-methyl-D-aspartate, kainate and quisqualate (Watkins & Evans, 1981). We have recently shown (Salt & Hill, 1981*b*) that there appear to be two receptors in n. caudalis, one sensitive to *N*-methyl-D-aspartate and the other to kainate or quisqualate. We have subsequently examined the effects of a number of excitatory amino acid antagonists on the responses of n. caudalis neurones to non-noxious sensory stimuli, and thus suggest that the monosynaptic excitation of n. caudalis neurones by non-noxious mechanical stimulation of hair and vibrissae follicle afferents is mediated by a transmitter substance acting on a kainate/quisqualate type of excitatory amino acid receptor. Preliminary reports of some of these results have been previously communicated. (Hill & Salt, 1980; Hill, Salt & Watkins, 1981; Salt & Hill, 1981*a*).

METHODS

Animal preparation

Experiments were performed on male albino rats (MRC Porton strain) weighing between 250 and 500 g. Anaesthesia was induced with halothane (5% in oxygen) and continued with urethane (1·25 g/kg, I.P.) and/or halothane (0·5–1·5% in oxygen). A tracheal cannula was inserted to enable the animal to be artificially ventilated, and an external jugular vein was cannulated for the administration of drugs. The head of the rat was fixed in a stereotaxic frame, and the caudal medulla was exposed by removal of the overlying musculature, atlanto-occipital membrane and dura mater. The e.e.g. and e.c.g. were recorded, and the rat's body temperature was maintained at 36–37 °C by means of a thermostatically controlled heating pad.

Sensory stimulation

Non-noxious sensory stimuli usually consisted of movement of facial hairs or vibrissae with a camel hair brush or with an automatically driven air jet, the position, amplitude and frequency of which could be adjusted to be optimal for the activation of the neurone involved. Light pressure (< 16 g) with von Frey hairs was also used as a non-noxious search stimulus, although it was not used in the pharmacological studies. Electrical stimulation via subcutaneous needle electrodes within the receptive field of a neurone was performed in a number of experiments in an attempt to activate sensory fibres electrically and hence estimate their conduction velocities. Noxious mechanical and thermal stimuli (Ayliffe & Hill, 1979; Hill & Salt, 1981*a*) were also used to characterize further the responsive properties of neurones.

Recording and analysis

Extracellular single neurone recordings were made using either a single glass micropipette (tip diameter about 1 μm, filled with 2·5% Pontamine Sky Blue dye in 1 M-NaCl) or multi-barrelled ionophoretic electrode assemblies. Ionophoretic assemblies were made as described prevously (Salt & Hill, 1981*b*), and recordings were made either through the centre barrel (filled with 4 M-NaCl) of a six or seven barrelled micropipette (tip diameter 5–10 μm) or through a single micropipette glued alongside a multi-barrelled pipette so as to protrude by 15–50 μm. Tip separations of more

than 50 μm were not regularly used, as preliminary experiments showed that excessively high agonist application currents were necessary to produce excitation of neurones. One barrel of the multi-barrelled pipette always contained 1 M-NaCl, and was used for automatic current balancing. The remaining barrels each contained one of the following: Na L-aspartate (0·5 M, pH 8·5), Na-L-glutamate (0·5 M, pH 8·5), acetylcholine chloride (ACh) (0·5 M, pH 3·0), Pontamine Sky Blue (2·5 % in 1 M-NaCl), and the excitatory amino acid antagonists Na D-α-amino-adipate (DAA) (0·1 M, pH 8·5), Na *cis*-2,3-piperidine dicarboxylate (PDA) (0·1 or 0·2 M, pH 8·0) and Na γ-D-glutamylglycine (DGG) (0·1 M, pH 8·0). Amino acids and their antagonists were ejected as anions, and ACh as a

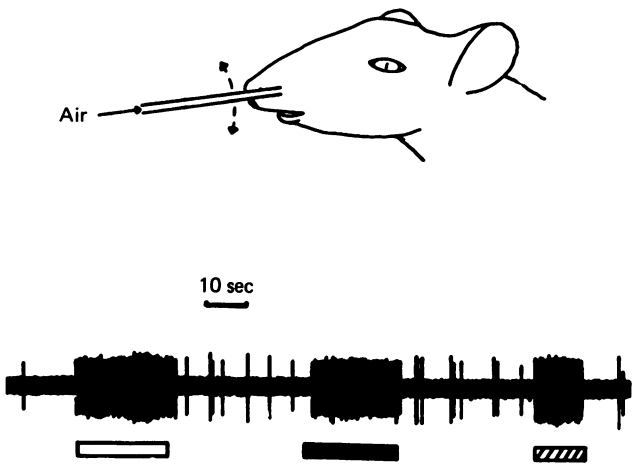

Fig. 1. Photographic record of action potentials recorded from a n. caudalis neurone in response to sequential ionophoretic applications of L-glutamate (35 nA; □), L-aspartate (50 nA; ■), and to physiological stimulation of vibrissae afferent fibres (■). The stimulation is performed by a moveable air jet played over the vibrissal pad, as shown.

cation. Retaining currents (10–20 nA) of opposite polarity to the ejection currents were used to prevent drug diffusion from electrodes.

The electrode was advanced in 1 μm steps by a digital hydraulic microdrive, and all penetrations were made between the obex and the first vertebra, and between 1·5 and 3 mm lateral to the midline. The surface of the medulla was stabilized using a perspex pressor foot, and a 3 % agar in 0·9 % saline solution was poured over the medulla and pressor foot to reduce cooling, dehydration and movement. Single ended recordings or differential recordings between the micro-electrode and an electrode screwed into the frontal bone of the rat were made, and signals were amplified and filtered (100 Hz to 10 kHz band width). Selected action potentials could be gated, on the basis of both height and width (Barnett & Millar, 1979), to produce pulses which were fed into a ratemeter and counter, and on-line computer, A Biomac 1000 or PDP 11–03 computer with a Cambridge Electronic Design 502 laboratory interface was used to generate peristimulus time histograms, and photographic records could be made using a Medelec camera. The output of the ratemeter and action potential counter were recorded on a multi-channel chart recorder, together with e.e.g. and skin temperature.

Non-noxious facial stimulation, and, if using an ionophoretic electrode, continuous ejection of an excitatory amino acid, were used as search stimuli. Responses of neurones to the applied sensory stimuli were noted, and the position and extent of receptive fields were mapped whenever possible. In ionophoretic experiments, automatically timed, sequential applications of agonists and sensory stimulation (using the airjet) were made (Fig. 1) and responses challenged with concurrent ionophoretic applications of antagonists.

Histological verification of recording site

The locations of recording sites were determined wherever possible by ionophoretic dye ejection from the pipette at the site of a neurone of particular interest (Pl. 1), or at two or more points along an electrode track, following which the animal was perfused with formal saline via the aorta. Fixed tissue was cut into 50 or 100 μm sections using an Oxford Vibratome or a freeze microtome. Sections were then photographed in an enlarger using the unstained sections as negatives. Microdrive depth recordings and location of dye spots were used to reconstruct the electrode track and verify the positions of neurones, which were then plotted on a diagramatic transverse section of the medulla (adapted from the atlas of Palkovits & Jacobowitz, 1974) corresponding to a level of 1 mm caudal to the obex.

RESULTS

Recordings were made from 187 neurones, in n. caudalis and adjacent reticular areas, which were excited by non-noxious sensory stimuli. Other neurones were found in the same animals which did not respond to non-noxious stimuli, but these will be considered in a separate report. Of the 187 neurones, eighty-nine had their response characteristics accurately determined, and were reliably located histologically. The positions of these neurones were used to construct maps of neuronal distribution.

Responses of neurones to sensory stimuli

Of the eighty-nine neurones which were histologically located, fifty-five responded exclusively to non-noxious mechanical stimuli, the remaining thirty-four also responding to one or more of the noxious stimuli used. The characteristics of the responses of these neurones to noxious stimuli will be reported separately. Most of the exclusively non-nociceptive neurones were located within the magnocellular portion of n. caudalis (Fig. 2*A*), whereas neurones which also responded to noxious stimuli were located primarily either ventro-medially to the non-nociceptive neurones, in the reticular areas adjacent to the magnocellular part of n. caudalis, or on the border of or superficially to the substantia gelatinosa (Fig. 2*B*).

Responses of both types of neurones to non-noxious stimuli were essentially similar, and were usually of short duration and rapidly adapting in character. Receptive fields were predominantly small, being restricted to one or two vibrissae or a small area of hairy skin (< 20 mm^2), and these neurones typically fired in bursts of one to five action potentials in a similar manner to that noted previously in more rostral trigeminal nuclei (Shipley, 1974). Neurones also tended to be clustered in groups responding to closely adjacent vibrissae (Shipley, 1974). The response of a neurone to vibrissal stimulation is shown in Fig. 3.

A small number of neurones were found which only adapted very slowly, or not at all, to non-noxious mechanical stimuli. These neurones often had a high spontaneous firing rate, which was dependent on the position of a particular vibrissa or area of the skin. In some cases it was possible to abolish the 'spontaneous' firing using the air jet stimulus. Neurones with similar properties have previously been found in n. caudalis and the dorsal column nuclei of the cat (Kruger, Siminoff & Witkovsky, 1961).

In agreement with other workers (Nord, 1967; Wall & Taub, 1962; Kerr, Kruger, Schwassmann & Stern, 1968; Yokota & Nishikawa, 1980) a somatotopic organization

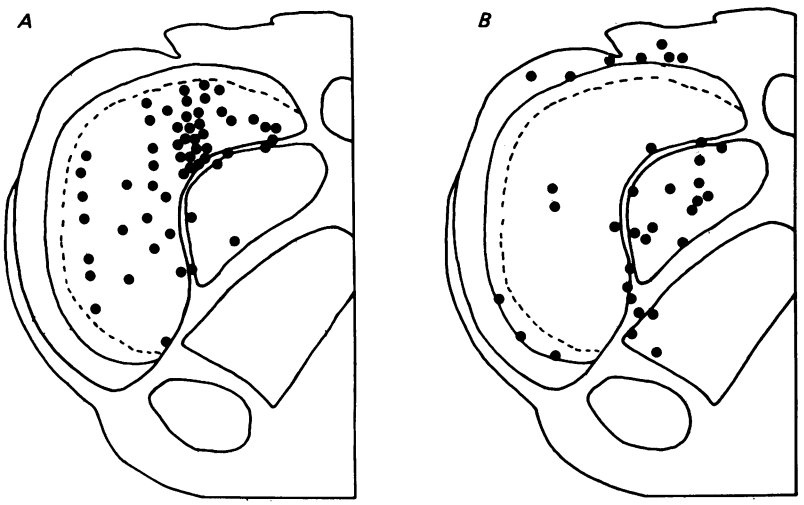

Fig. 2. *A*, locations of neurones responding exclusively to non-noxious sensory stimuli. Note the predominant localization within the magnocellular portion of n. caudalis. *B*, locations of neurones which were responsive to noxious stimuli in adition to non-noxious sensory stimuli. These neurones were located predominantly in the superficial layers of n. caudalis, or in ventro-medial reticular areas. For explanation of the diagrammatic sections, see Pl. 1.

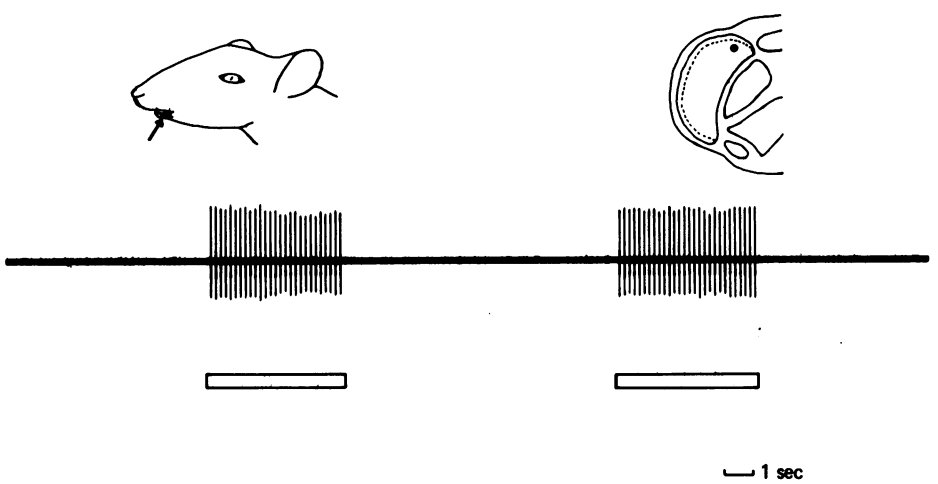

Fig. 3. Responses of a n. caudalis neurone, located as shown, to an oscillating (*ca.* 6 Hz) air jet played on the hairs of the lower lip (arrow). The period of stimulation is indicated by the bars beneath the photographic record. Note the regular firing of the neurone in response to repeated sweeps of the air jet.

of receptive fields of n. caudalis neurones to non-noxious stimuli was seen. Vibrissal representation was very marked, often occupying a large part of the nucleus.

Eleven neurones responding to air jet excitation of hair or vibrissae follicle afferents were also found to be excited by subcutaneous electrical stimulation (Fig. 4). Apparent conduction velocities were established from measurements of peristimulus

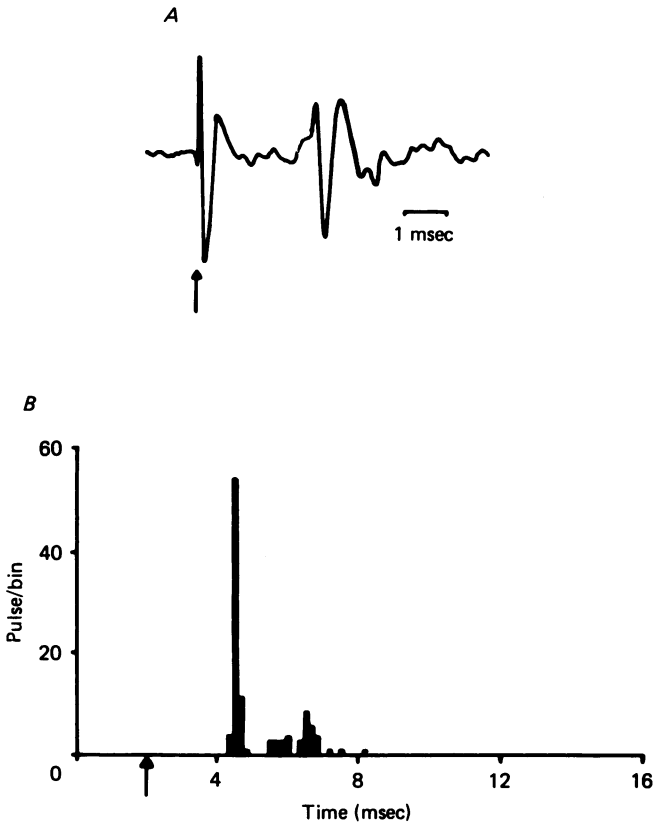

Fig. 4. *A*, single sweep photographic record of a response of a n. caudalis neurone to electrical stimulation (0·7 V, 0·1 msec, applied at the time point indicated by arrow) via subcutaneous needle electrodes. The neurone fires one action potential 2·4 msec following the stimulus. *B*, peristimulus time histogram of the firing of n. caudalis neurone in response to electrical stimulation (0·3 V, 0·1 msec, applied at the time point indicated by arrow). The histogram is constructed from 100 sweeps, and each bin is of 0·2 msec duration.

time histograms or oscilloscope photographs, and the mean value was found to be 19·9 m/s (±1·2 S.E. of mean).

Neurones which could not be accurately located histologically were not found to differ in their responses to non-noxious facial stimuli from those neurones which were located. Hence, results from such neurones were included in the subsequent analysis of the pharmacological studies.

Responses of neurones to ionophoretically applied agonists

Both L-aspartate and L-glutamate were found to excite all neurones in n. caudalis to which they were applied. However, only 31 % of the fifty-eight neurones to which ACh was applied were excited. Thus ACh appears to excite a greater proportion of neurones in n. caudalis than in either the dorsal column nuclei (Galindo, Krnjević

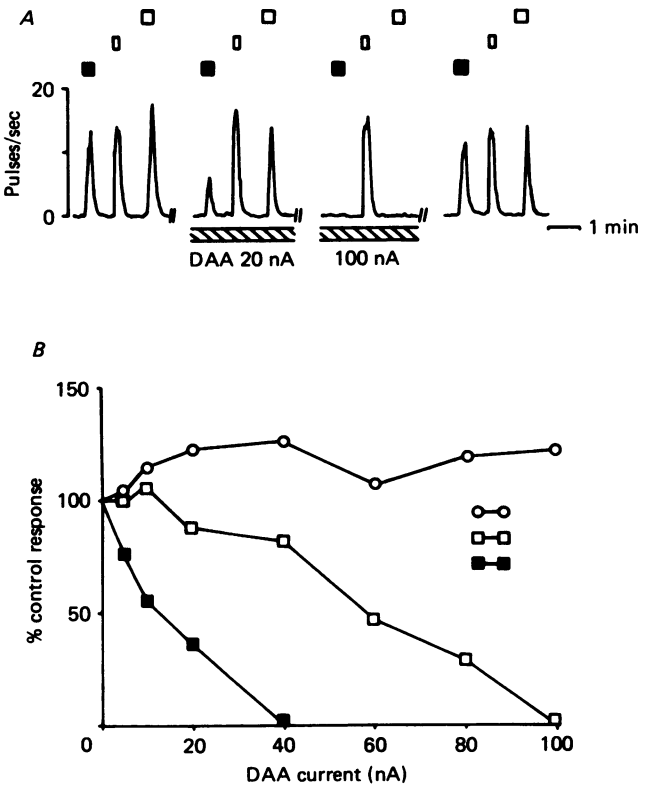

Fig. 5. *A*, analogue ratemeter traces of a n. caudalis neurone excited by sequential application of L-aspartate (45 nA; ■), sensory stimulation (air jet ▯), and L-glutamate (40 nA; □). The responses are challenged with DAA (■), which first antagonizes L-aspartate and then L-glutamate responses. However, responses to sensory stimulation are not reduced. An interval of 10 min occurred between cessation of DAA ejection and recovery of responses to control values. *B*, graphical representation of the data obtained from the neurone shown in *A*. The responses of the neurone to amino acids and sensory stimulation in the presence of increasing currents of DAA are plotted as percentages of control values. ○, air jet; □, L-glutamate; ■, L-aspartate.

& Schwartz, 1967) or the interneurones of the spinal cord (Curtis, Phillis & Watkins, 1961). It was a general finding that higher agonist currents were required to excite neurones when electrode assemblies with protruding recording barrels were used.

Effects of antagonists on sensory responses

Forty-nine neurones were investigated using various excitatory amino acid antagonists. These neurones were recorded at depths which suggest locations in the magnocellular and adjacent reticular areas. In all cases physiological activation of vibrissae or hair follicle afferent fibres was achieved using the air jet, applied at regular intervals, interspersed with applications of one or more agonists (Fig. 1).

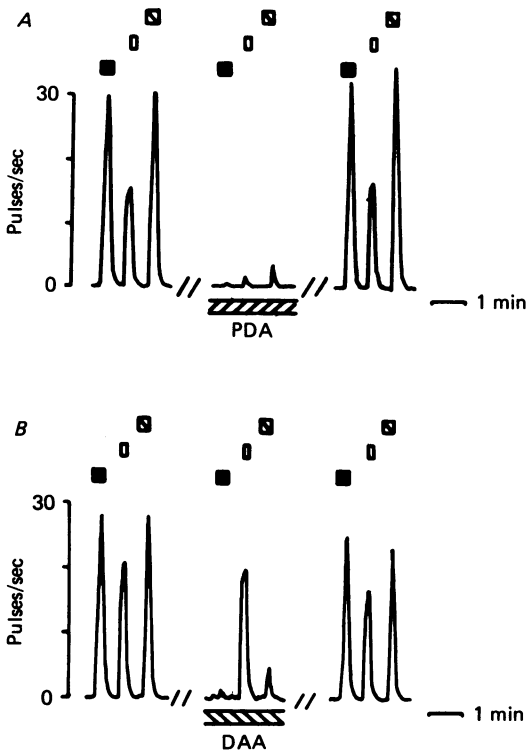

Fig. 6. *A*, analogue ratemeter traces of responses of a n. caudalis neurone to L-aspartate (60 nA; ■), sensory stimulation (□) and L-glutamate (60 nA; ▨). PDA (20 nA, 3 min ejection, ■) antagonizes all three responses, and recovery is seen 7 min after the end of ejection. *B*, responses of the same neurone as in *A*, but challenged with DAA (150 nA, 3 min ejection, ■) Note that only L-aspartate and L-glutamate are antagonized, and recovery is seen 7 min after the end of ejection.

Twenty-four of these neurones were also tested with noxious stimuli, and six were found to be excited. Of the seventeen neurones whose excitatory responses to non-noxious sensory stimulation were challenged with DAA (5–250 nA) thirteen showed no decrease in response to, or a potentiation of the sensory input, even when application currents and durations of DAA were in excess of those required to antagonize the effects of the exogenously applied amino acids (Figs. 5 and 6, Table 1). On the remaining four neurones tested, only a slight reduction (22–34 %) in the

sensory response was evident. No differences in the effects of DAA were evident whether it was applied from a conventional multi-barrelled electrode (nine neurones) or an assembly with a protruding recording barrel (eight neurones).

In contrast to the effects of DAA, both PDA (20–100 nA) and DGG (20–100 nA)

TABLE 1. Depression of sensory responses of n. caudalis neurones by excitatory amino acid antagonists

	DAA ($n = 17$)	PDA ($n = 22$)	DGG ($n = 11$)
Mean depression of sensory responses (%)	6·6 ± 12·6	57·5 ± 21·1	60·2 ± 22·8
Mean current used	65 ± 65 nA	42·3 ± 18·2 nA	43·2 ± 27·6 nA

All entries are the mean ± S.D. of n values. Both PDA and DGG produce similar mean percentage depressions of the sensory responses, and these values are significantly different ($P < 0·001$, Students t test) from the mean percentage depression produced by DAA.

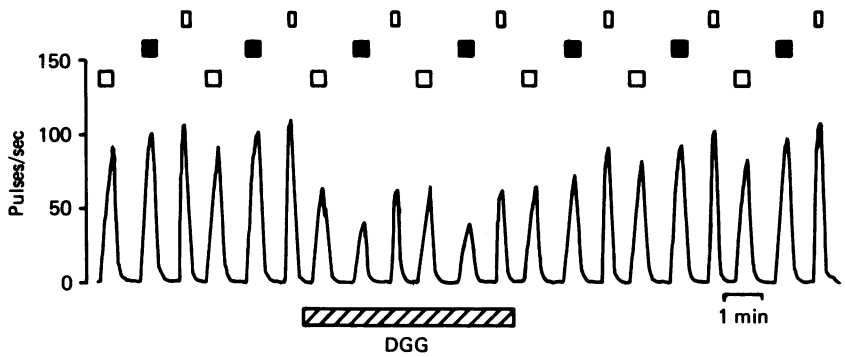

Fig. 7. Continuous analogue ratemeter trace of a n. caudalis neurone excited by L-glutamate (200 nA; □), L-aspartate (120 nA; ■) and sensory stimulation (air jet; ▯). The responses are challenged with continuous DGG ejection (50 nA; ■). It can be seen that DGG reduces all three types of response.

were found to depress responses to sensory stimulation by 24–100% ($n = 22$) and 25–100% ($n = 11$), respectively, on all of the neurones tested (Figs. 6 and 7, Table 1), at similar, or, in a small number of cases, slightly higher application currents than those needed to antagonize the responses to excitatory amino acids. As found with DAA, both PDA and DGG were equally effective whether applied from a conventional electrode or an assembly with a protruding recording barrel. In order to verify the specificity of PDA and DGG for excitatory amino acid receptors, which has been shown in the spinal cord (Davies, Evans, Francis, Jones & Watkins, 1981; Francis, Jones & Watkins, 1980), excitations produced by ACh were challenged with PDA or DGG. In all eleven neurones studied responses to ACh were unaffected by PDA or

DGG, whereas responses to amino acids and/or sensory stimulation were markedly reduced (Fig. 8).

There did not appear to be any pharmacological differences between neurones which were exclusively non-nociceptive and those which also responded to noxious stimuli.

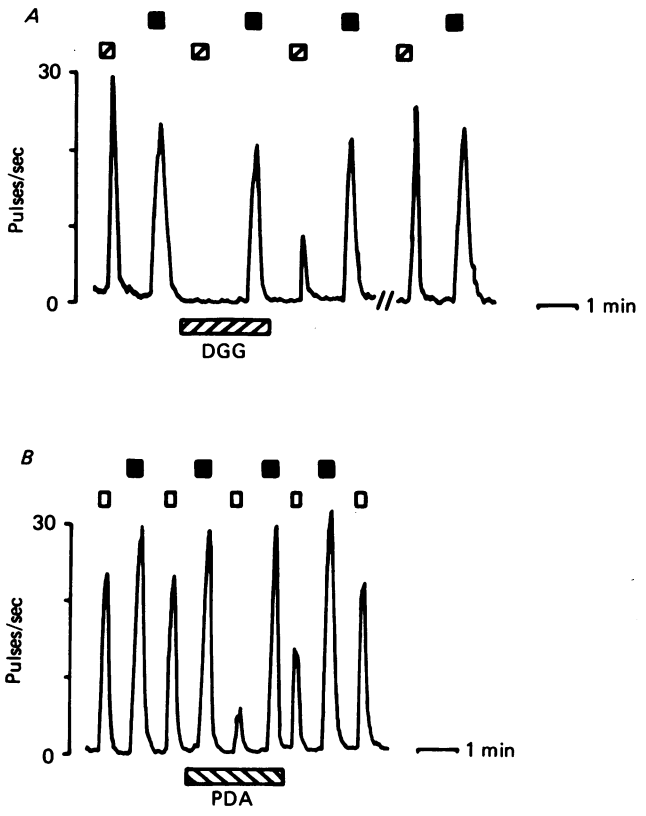

Fig. 8. *A*, analogue ratemeter trace of excitation of a n. caudalis neurone produced by ACh (25 nA; ■) and L-glutamate (45 nA; ▨). It can be seen that DGG (25 nA; ■) antagonizes the L-glutamate responses with very little effect on the ACh responses. The break in the trace corresponds to a 5 min period. *B*, responses of a neurone to ACh (25 nA; ■) and sensory stimulation (air jet; □). PDA (25 nA; ■) antagonizes the sensory response, but not the response to ACh.

DISCUSSION

The responses of n. caudalis neurones to non-noxious sensory stimuli reported in the present study show similarities to those obtained by other workers in the rat (Nord & Kyler, 1968), primate (Price *et al.* 1976) and cat (Mosso & Kruger, 1973; Kruger *et al.* 1961; Sessle & Greenwood, 1976). Similarities with the dorsal horn of the rat spinal cord are also apparent (Menétrey, Giesler & Besson, 1977), with the magnocellular part of n. caudalis corresponding to laminae III and IV of the dorsal

horn (Rexed, 1954), and the adjacent reticular areas to laminae V and VI. This conclusion would be in agreement with the morphological observations of Gobel *et al.* (1977).

The short and relatively constant response latencies of n. caudalis neurones to low-intensity electrical stimulation within their receptive field suggest that this input, which is likely to be the same as that activated by physiological stimulation, is monosynaptic. This is supported by morphological findings in the dorsal horn of the cat (Brown & Noble, 1979). The calculated apparent conduction velocity of approximately 20 m/sec does not take into account synaptic delay, or the fact that the conduction velocity of trigeminal afferent fibres suffers a serious drop in the medulla (Wall & Taub, 1962; Lisney, 1978). When these factors are taken into consideration, the true conduction velocity of these fibres lies, in all probability, in the range of the Aβ, large myelinated, group of cutaneous afferent fibres (Burgess & Perl, 1973).

Previous work (Watkins & Evans, 1981) has shown that there are up to three excitatory amino acid receptors in the spinal cord. However, we have recently shown (Salt & Hill, 1981*b*) that there appear to be only two types in n. caudalis; a *N*-methyl-D-aspartate type and a kainate/quisqualate type. The antagonist DAA is relatively specific towards the *N*-methyl-D-aspartate receptor (Davies & Watkins, 1979; Salt & Hill, 1981*b*), whereas PDA and DGG appear to have a broader spectrum of activity, and will also antagonize kainate and quisqualate responses in n. caudalis (Salt & Hill, 1981*b*). Thus, our finding, presented here, that DAA will not antagonize the responses of n. caudalis neurones to non-noxious mechanical stimulation, suggests that the afferent fibres conveying these signals do not release their neurotransmitter(s) onto a receptor of the *N*-methyl-D-aspartate type. It is possible that the antagonist did not reach sufficient concentration in the synaptic cleft to produce antagonism, but the high currents and long durations of application used in some cases, together with the ionophoresis of the antagonist at a site 20–50 μm remote from the cell body, presumably in apposition to the proximal dendritic tree of the neurone, where afferent fibres may make contact (Brown & Noble, 1979), make this possibility seem unlikely. In addition, it was possible to antagonize the sensory responses with PDA and DGG, two antagonists with a broader spectrum of action. The specificity of these antagonists for excitatory amino acid receptors is indicated by the lack of antagonism of the responses of spinal and n. caudalis neurones to ACh and peptides (Davies, Evans, Francis, Jones & Watkins, 1981; Francis, Jones & Watkins, 1980; Hill & Salt, 1981*b*). Thus, it appears likely that the synaptic receptor in n. caudalis, at least in the magnocellular and reticular areas, is of the kainate/quisqualate type. Whether this receptor is also of importance in the responses of marginal neurones to non-noxious stimuli is a matter requiring further study. It is noteworthy that the *N*-methyl-D-aspartate receptor appears to be involved in synaptic transmission in the spinal cord (Davies & Dray, 1979; Davies & Watkins, 1979), and thus it is possible that there are pharmacological differences between n. caudalis and the dorsal horn of the spinal cord (Salt & Hill, 1981*b*). However, in view of the sensory importance of vibrissae to the rat (Vincent, 1912; Welker, 1964) such a difference may not be surprising.

Both L-aspartate and L-glutamate, endogenous ligands of excitatory amino acid

receptors, are mixed agonists and may act at all three types of receptor (Watkins & Evans, 1981), although exogenously applied L-aspartate seems to act almost exclusively at the *N*-methyl-D-aspartate receptor in n. caudalis, and exogenous L-glutamate also appears to have a large part of its action at this receptor (Salt & Hill, 1981*b*). In addition, the finding that it is possible greatly to antagonize responses to these two agonists with DAA, without antagonizing the sensory responses of neurones (see Figs. 5 and 6) suggests that the majority of extra synaptic receptors are of the *N*-methyl-D-aspartate type, and synaptic receptors are of the kainate and quisqualate type. It is of interest that a difference in characteristics between synaptic and extra-synaptic receptors has been shown in both the frog and locust neuromuscular junction (Neher & Sakmann, 1976; Cull-Candy, 1976). However, it is also possible that this phenomenon is explicable in terms of differences in diffusion characteristics of various agonists and antagonists, and the development of selective kainate and quisqualate antagonists would be of great value in providing an answer to this question.

Thus, although the kainate/quisqualate receptor appears to be involved in synaptic transmission, it is not possible to state whether L-aspartate or L-glutamate is the neurotransmitter of the afferent fibres to n. caudalis. Indeed, it is possible that another endogenous amino acid or other, as yet undiscovered, ligand may be the neurotransmitter (Watkins, 1978). In this respect, it is noteworthy that methyl-tetrahydrofolate has recently been shown to compete for cerebellar kainate binding sites (Ruck, Kramer, Metz & Brennan, 1980). However, this substance does not appear to have kainate agonist or antagonist properties in electrophysiological experiments in caudal trigeminal nucleus or spinal cord (Evans, Hill, Salt & Smith, 1982) or accurately to mimic the neurotoxic actions of kainate (Olney, Fuller & de Gubareff, 1981; Roberts, Foster & Thomas, 1981). Clearly, there is a need for further neurochemical experiments to aid the identification of the neurotransmitter(s) acting at these receptors.

We are grateful to J. C. Watkins for the gifts of amino acid antagonists and for helpful discussions. Thanks also to C. E. Allen, C. S. Crozier and P. Darby for skilled technical assistance.

Supported by grants from the Royal Society, Wellcome Trust and the Medical Research Council.

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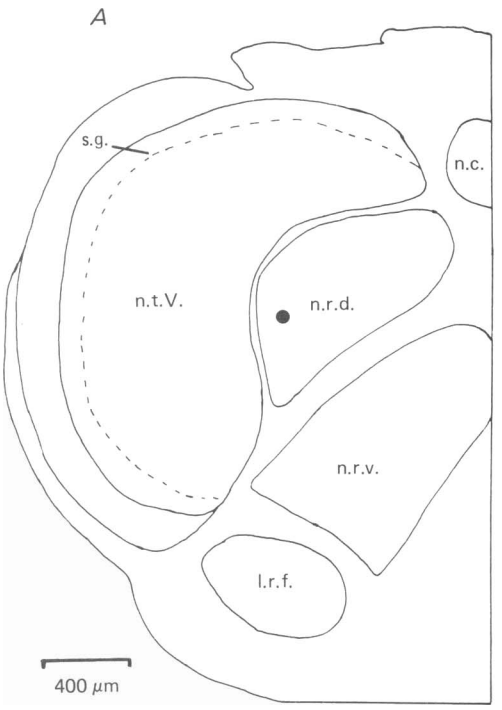
EXPLANATION OF PLATE

A, Transverse section of the rat caudal medulla sectioned at 100 μm on a freeze microtome. The photograph was made by using the section as a 'negative' in an enlarger. A dye spot produced by ionophoretic ejection of Pontamine Sky Blue can be seen near the centre of the photograph as a white mark. *B*, Diagramatic representation showing the location of the main structures in the section. The location of the dye spot is marked on as a filled circle. Abbreviations: n.t.V., trigeminal nucleus caudalis (magnocellular portion); s.g., substantia gelatinosa; n.c. cuneate nucleus; n.r.d., dorsal reticular nucleus; n.r.v., ventral reticular nucleus; l.r.f., lateral reticular formation.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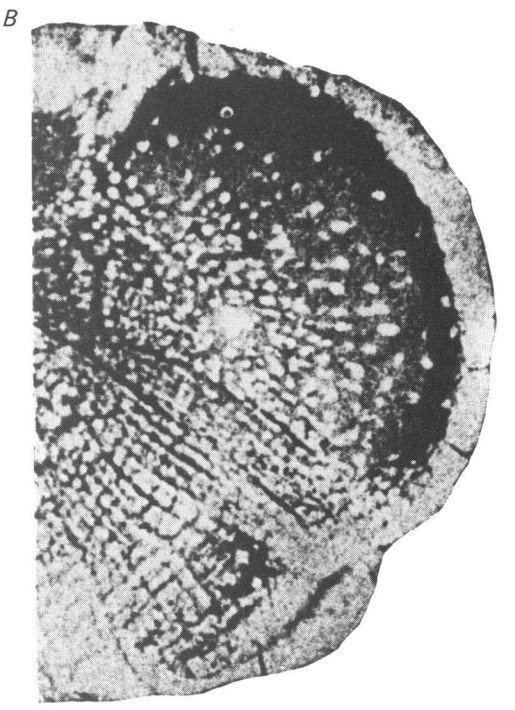
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