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Input from Trigeminal Cutaneous Afferents to Neurones of the Inferior Olive in Rats

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Summary. Extracellular recordings were obtained from inferior olivary neurones of the rat. The responses of fifty neurones evoked by electrical stimulation of a branch of the trigeminal nerve were recorded. Maxillary nerve stimulation was most effective. The response was characterized by an early discharge (single spike and wave, typically with latencies between 16 and 30 msec) and a weak late discharge which followed a period of inhibition of about 100 msec. Half of the neurones responded to one branch of the trigeminal nerve only whereas the other neurones displayed a varying degree of convergence, including sometimes a convergence from limb nerves. Forty-nine olivary neurones were tested for cutaneous receptive fields. Ten out of these had small receptive fields (<20% of the contralateral face) and a low threshold to mechanical stimuli. Twenty neurones which had larger receptive fields responded also to low-threshold or to medium-threshold (i.e. non-nociceptive) mechanical stimuli. None of the neurones displayed receptive fields more extensive than half of the contralateral face and some of the larger fields had a small, low-threshold focus. Olivary neurones responding to electrical stimulation of trigeminal nerves or mechanical stimulation of the face were located in the medial segment of the olivary complex (dorsal accessory and principal olive). A few cells only were located in the lateral segment.

It is concluded that neurones of the inferior olive receive a substantial input from trigeminal afferents and are capable of transmitting precise somatotopical information to the cerebellum.

Key words: Inferior olive – Trigeminal nerve – Rat

Introduction

In a previous study on cats, the organization of trigeminal input to the cerebellar cortex *via* the climbing fibre system was investigated with micro-elec-

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trophysiological techniques (Miles and Wiesendanger, 1975a, b). One of the main results of that study was that over 80% of the Purkinje cells activated by electrical stimulation of trigeminal nerve branches were also readily excited by *gentle* mechanical stimulation of the facial skin. Almost half of these cells exhibited small receptive fields covering less than 20% of the ipsilateral face. It thus appeared that the climbing fibre pathway is capable of transmitting precise information concerning location and intensity of mechanical stimuli. It is likely that most if not all of the responses with the typical complex spike recorded in the Purkinje cell layer were mediated *via* the inferior olive. We now have extended the above mentioned studies by recording trigeminally-induced responses directly from olivary neurones. As could be expected in view of the fact that each Purkinje cell receives an input from only one climbing fibre, essentially the same types of responses were seen in olivary cells as in the cerebellar cortex. In particular, it will be shown that neurones of the inferior olive do indeed transmit precise spatial information from the face area, a property which is not consistent with some reports based on electrical nerve stimulation. So far no electrophysiological studies on facial input to single olivary neurones have been reported, and the literature on trigemino-olivary connections is scant (Stewart and King, 1963; Tiwari and King, 1974).

The rat was selected for the present experiments because of the known large representation of the face in central structures in this animal. The results demonstrate the importance of trigeminal projections in this species also at the olivary level.

Material and Methods

Thirty Wistar albino rats with body weights in excess of 250 g were used. The rats were anaesthetized with 100 mg/100 g body weight urethane. The ventral surface of the medulla oblongata was exposed for exploration of the inferior olive. Microelectrode penetrations were made perpendicularly through this ventral surface to a maximal depth of about 1.5 mm. The sites were selected according to the landmarks of arteries (basilar, inferior cerebellar) and stereotaxic co-ordinates (see also Fig. 4). Branches of the ophthalmic, the mandibular (lingual or mental) and maxillary nerves were prepared for electrical stimulation with pairs of fine needle electrodes insulated except at the tip, which were brought into close contact with the nerve and fastened to the surrounding tissue. Optimal stimulation of these trigeminal branches was achieved by monitoring evoked potentials in the contralateral face area of the cerebral cortex before starting recordings in the olive, and frequently during the experiments to ascertain the adequacy of the nerve stimuli. In a few experiments, a similar pair of needle electrodes was lowered into the depth of the pars intermedia of the contralateral cerebellar hemisphere for identification of the olivary cells by antidromic invasion. This test was later abandoned when post-mortem histological examination showed that all sites at which characteristic complex spikes (see Results) were recorded lay within the olive. Often the microelectrode picked up the activity of several units (Fig. 1A) even with tip diameters as fine as 2 μm. The capillaries were filled with 2 M NaCl-solution saturated with Fast Green dye for marking recording sites (Thomas and Wilson, 1965). Cardiovascular pulsations were diminished by covering the recording area with agar. Activity from olivary neurones was identified during the experiment according to stereotaxic co-ordinates and the depth of recording, the characteristic shape of field and action potentials (Fig. 1A), and the low spontaneous discharge rate (see Results); histologically, by means of the Fast Green marks deposited at the sites of recordings or at the deepest point of an electrode penetration. Serial sections of the brainstem at the level of the inferior olive stained with thionin were analysed routinely.

The experimental protocol included electrical stimulation of the trigeminal nerve branches contralateral (sometimes also ipsilateral) to the recording site and of limb nerves (contralateral axilla and sciatic nerve). Receptive fields of single neurones were then tested with a blunt probe (fine glass rod) or, when no response was elicited by gentle stimulation in the face area, with pin pricks. Selected unitary responses to nerve stimulation were recorded as dot rasters (not illustrated) or taped for subsequent statistical analysis on a PDP-12 computer (poststimulus time histogram, cumulative distribution; Wyss and Handwerker, 1971). For this purpose the spikes were carefully isolated by using appropriate high-pass filters and a window discriminator.

Results

Electrical Stimulation of Branches of the Fifth Nerve

Figure 1A illustrates some typical recordings from neurones of the inferior olive. Field potentials elicited by a train of electrical stimuli applied to a trigeminal branch had a typical latency of 12 msec.

The discharge of single units was characterized by a negative spike followed by a smaller negative wave. Rarely, the primary spike was followed by a burst of up to 5 small secondary spikes. Spontaneous activity was low and ranged between 1 and 3.5 impulses per second (mean = 1.8 imp/sec; see also prestimulus time histograms in Fig. 1B, C). The response to a train stimulus consisted of a single spike, sometimes followed by a later spike. The characteristic cycle of excitation-inhibition- weak late excitation was clearly seen in poststimulus time histograms and cumulative distribution functions (Fig. 1B, C).

The number of cells responding to nerve stimulation and the latency histograms are shown in Figure 2. Eighty percent of the response latencies to trigeminal nerve stimulation were between 16 and 40 msec. The latencies to forelimb nerve stimulation and to hindlimb nerve stimulation were slightly longer. Electrical stimulation of the maxillary nerve was most effective. The response latencies of caudal units appeared to be longer than of rostral units: at 3.4 mm the mean latency was 21 msec; at 3.6 mm 18 msec; at 4.0 mm 31 msec and at 4.25 mm 31 msec.

Half of the 38 neurones tested for convergence by means of electrical stimulation of the various nerves responded to only one branch of the trigeminal nerve. Three neurones received convergent input from an ipsilateral and a contralateral branch, nine neurones from two or three branches of the contralateral trigeminal nerve (Fig. 1C), and seven neurones from the sciatic or forelimb nerves and one or more trigeminal branches.

Pure inhibitory effects have not been observed. In view of the very low spontaneous discharge rate, however, it would be necessary to average the activity following several hundred stimulus presentations or to record intracellularly in order to detect weak inhibitory responses.

Receptive Fields

A receptive field within the contralateral face area was determined for 49 neurones of the inferior olive. Thirty of these neurones could accurately be lo-

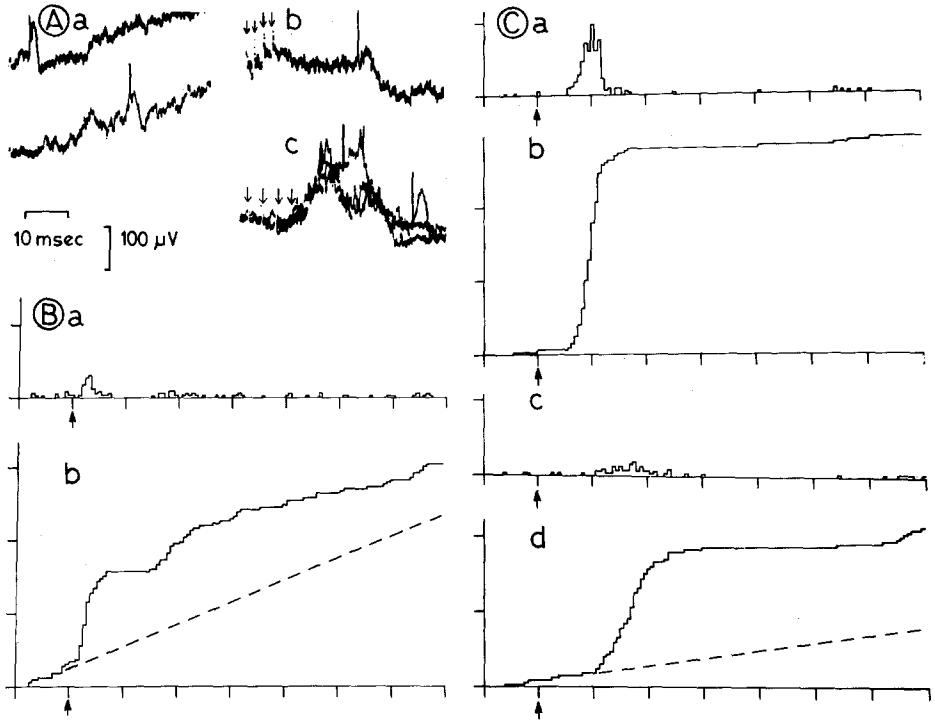

Fig. 1. Activity recorded from neurones of the inferior olive. **A** (a) Spontaneous discharge of two neurones; note characteristic negative spike followed by negative wave. (b) Unitary discharge evoked by electrical stimulation of the maxillary nerve (600 Hz, 4 pulses of 0.2 msec duration and 0.25 mA intensity). (c) Field potential elicited by electrical stimulation of the contralateral ophthalmic branch (400 Hz, 4 pulses, 0.2 msec duration, 0.4 mA intensity). **B** Poststimulus time histogram (a) and cumulative distribution function (b) of an olivary neurone excited by a weak train of electrical stimuli applied to the maxillary nerve; 100 stimulus presentations. Ordinate: 32 counts per division, abscissa: 64 msec per division. Slope of interrupted line indicates level of background activity. Note early excitation (at about 15 msec) followed by a period of suppression of background activity. Weak rebound excitation at about 100 msec. **C** Poststimulus time histograms and cumulative distribution functions of an olivary neurone exhibiting convergent input from the maxillary branch (a, b) and the ophthalmic branch (c, d); 100 stimulus presentations. The response to ophthalmic branch stimulation was weaker and occurred at a longer latency. Ordinate: 32 counts per division; abscissa: 16 msec per division

cated in the olivary complex by means of Fast Green marks. An arbitrary rating of thresholds was done for each neurone: *"low threshold"* meaning reaction to gentle touch or bending of hairs or vibrissae; *"medium threshold"* meaning reaction to stronger but not painful manipulations (pressure with blunt glass rod); *"high threshold"* meaning strong noxious stimulation (pin prick). Receptive fields were further differentiated according to their size. *Small receptive* fields consisted of well defined areas occupying less than 20% of the contralateral face. Particularly sensitive spots were found in the supra-

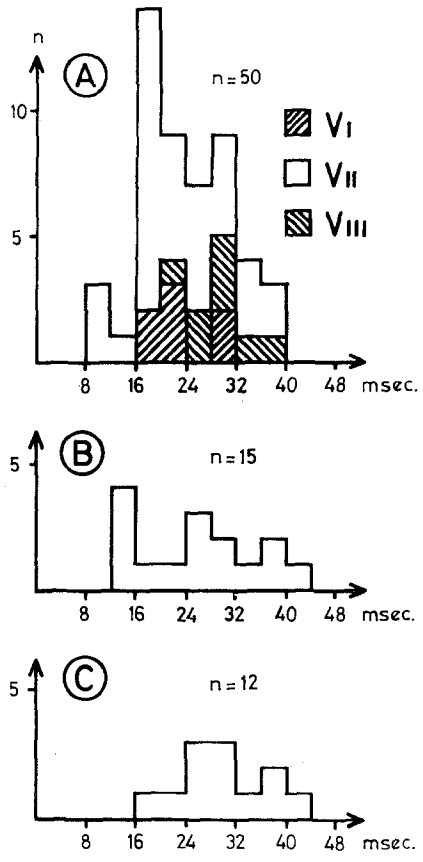

Fig. 2. Latency histograms of neurones of the inferior olive. **A** Units excited by electrical stimulation of the trigeminal nerve branches, **B** of forelimb nerves at the axilla, **C** of the sciatic nerve. V_i, V_{ii}, V_{iii}: units excited by the respective branches of the trigeminal nerve

orbital region, at the tip of the nose and in the region of the vibrissae as well as within the oral cavity. In a few cells an *additional* receptive field which was larger and stocking-like was found on the forelimb (3 neurones) or on the hindlimb (2 neurones). *Medium receptive fields* consisted of an area occupying at most half of the contralateral face. None of the units was excited by stimulation of more extensive areas. Examples of the various receptive field types encountered in four microelectrode tracks are displayed in Figure 3.

Table 1 summarizes the occurrence of these receptive field characteristics. No consistent relation between the size of the receptive field and the threshold is apparent in this table. The pattern of evoked discharges was highly phasic and consisted of a single or a few spikes to the transient stimuli (small taps, touch of vibrissae) and in no instance was a sustained discharge observed in response to a maintained mechanical stimulus. Although these receptive field measurements are not sufficient to give evidence as to the contribution of the various types of mechanoreceptors of the face, they nevertheless strongly indicate that many neurones of the inferior olive have considerable discriminative properties.

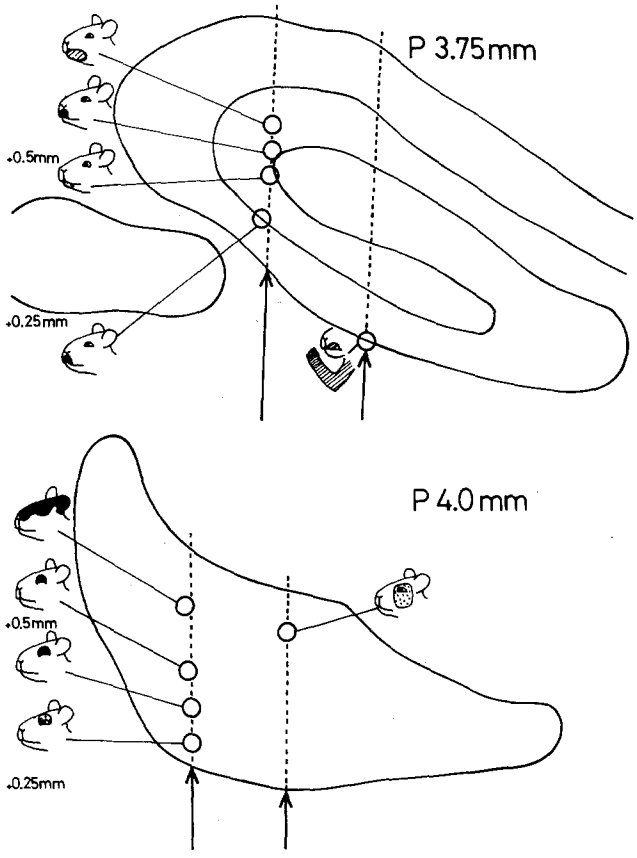

Fig. 3. Types of receptive fields of neurones in the inferior olive. Units encountered in four microelectrode tracks at transverse planes P 3.75 mm and P 4.00 mm (for orientation see Fig. 4). Black: low-threshold receptive field; stippled: medium-threshold receptive field; hatched: high-threshold receptive field (for criteria see text). Reference of depth from the ventral surface on left

Table 1

Total no of cells = 49	low threshold	medium threshold	high threshold
small RF	10	2	8
medium RF	9	11 (2 with low threshold focus)	9 (2 with low threshold focus)

Localization of Olivary Neurones Receiving an Input from Trigeminal Afferents

Longitudinally, the olivary complex was explored from p 3.0 rostrally to p 4.75 caudally (for orientation see Fig. 4). Units with trigeminal input were recorded

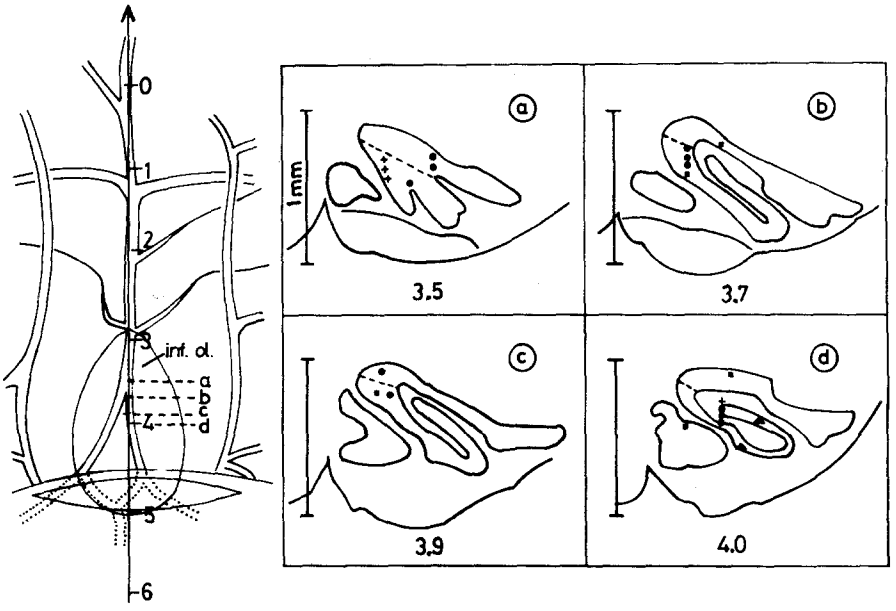

Fig. 4. Location of olivary units and types of receptive fields. *Left:* Diagram of ventral surface of medulla oblongata with the approximate bounderies of the inferior olive (inf. ol.). Posterior distance from stereotaxic zero (= interauricular) plane in mm. Position of transverse sections a, b, c, d shown on the right. *Right:* Transverse sections displaying the outline of the left inferior olive. Numbers indicate posterior plane. Bar on the left (1 mm): depth reference. Dashed line represents boundary between dorsal accessory olive and principal olive. Locations of units and types of receptive fields found for these units are marked with symbols: ● = low-threshold receptive field, ■ = medium-threshold receptive field, + = high-threshold receptive field, ▲ = high-threshold, discontinuous receptive fields including the face and the forelimb

between p 3.5 and 4.75. Most tracks were made between p 3.5 and p 4.0 however, and it is therefore not possible to draw a quantitative conclusion about their distribution in the rostro-caudal extent of the nucleus.

With respect to the laterality, the vast majority of neurones with trigeminal input were located within the medial segment of the olivary complex. Thus, 56 cells were located in the medial portions of the dorsal accessory and the principal nucleus of the olive. Of these cells, roughly half were within the dorsal accessory and half in the principal olive. The distribution within the two subdivisions is only an approximation because the depth of some of the units was estimated on the basis of microdrive readings only. Only a few cells were found in the medial accessory olive (3 units) and in the lateral half of the inferior olive (4 units). The more lateral units tended to have receptive fields with higher thresholds than the more medial units. Olivary neurones selected for Figure 4 were unequivocally localized in histological sections.

Discussion

Recordings from individual neurones of the inferior olive have disclosed a number of characteristic features which have been reviewed by Armstrong (1974) and by Oscarsson (1973). These features are as follows: 1. Low spontaneous background activity (0.2–2 Hz; 1.8 in the present experiments). 2. Discharges, recorded extracellularly, consisting of a single discharge followed by a silent period lasting about 100 msec and a long-latency rebound discharge; synaptic efficacy may vary in slow cycles of up to 30 sec. All earlier reports of unit activity in the inferior olive have come from cat experiments. In the present investigation the same features were observed in rats. The latencies to trigeminal nerve and limb nerve stimulation in rats also agree well with those obtained in cats if the differencies in conduction time due to the length of nerves are taken into account.

The present experiments revealed a strong input from trigeminal afferents onto neurones of the inferior olive which has not been described before. This observation is consistent with the strong somatosensory representation of the face in other central structures of the rat's brain, and also with the findings of Miles and Wiesendanger (1975a, b) of a trigeminal input, *via* climbing fibres, to the cerebellar cortex of the cat.

A major discrepancy exists with respect to interpretation of olivary function: Oscarsson and collaborators (Oscarsson, 1973) viewed the olive as an important relay for motor command signals and for internal feedback signals from spinal interneurones of segmental reflex paths. It was found that four out of five ascending spino-olivary paths were activated by the so-called flexor-reflex afferents from wide, high threshold receptive fields. The funicular neurones lacked modality specificity and were considered to provide only a crude spatial discrimination. However, Oscarsson and collaborators also identified a fifth ascending pathway situated in the dorsolateral funiculus which had much more precise spatial discriminatory capability.

A different experimental approach, i.e. recording responses evoked by climbing fibres in the cerebellar cortex, led Eccles et al. (1972) and Leicht et al. (1973) to a different conclusion. These authors, using gentle cutaneous stimuli instead of electrical nerve stimulation and statistical analysis of unitary responses were impressed by the capability of the climbing fibre system in terms of precise spatial discrimination. Furthermore, thresholds to natural stimulation often appeared to be quite low (for instance < 100 μm skin indentation). Miles and Wiesendanger (1975b) reported similar observations on trigeminally-evoked responses mediated by climbing fibres in cats.

The present experiments on the inferior olive of rats may partly reconcile the divergent opinions about olivary function. It was confirmed that electrical stimulation of peripheral nerves may reveal a considerable degree of convergence onto olivary neurones (in the present experiments about half of the neurones). This convergence may include inputs from the face and from the limbs. Natural cutaneous stimuli also revealed a population of cells which were activated by noxious stimulation only. Similarly, investigation of climbing fibre-induced responses of Purkinje cells also disclosed a number of neurones

with widespread receptive fields or discontinuous fields covering distal parts of several limbs (Leicht et al., 1973; Miles and Wiesendanger, 1975b). Such cells may represent a subpopulation of olivary neurones with convergent inputs and with a low sensitivity to peripheral stimulation which may well subserve the function envisaged by Oscarsson and collaborators. The overall impression gained from the experiments of Eccles et al. (1972), Leicht et al. (1973), Miles and Wiesendanger (1975b), and now also from the present study is, however, that the olivary complex is more implicated *in the transmission of precise somatotopic information from peripheral receptors*.

It is proposed that the more "sensory" role and the more "motor" role of olivary function are not mutually exclusive. It is likely that the type of preparation and the type of stimulation (electrical stimulation of nerves *vs*. natural activation of receptors) may emphasize more one or the other aspect of olivary function. Some, but not all, olivary neurones were found to fire in synchronized bursts after systemic harmalin injections leading to a 10/sec tremor *via* a cerebellar-bulbo-spinal loop (De Montigny and Lamarre, 1973). Llinás and Volkind (1973) proposed that harmaline-activated neurones of the inferior olive may be viewed as a model of "a motor transient generating system". It would be important to further investigate whether there is a duality of olivary function, viz. "motor" and "sensory", by correlating the peripheral response characteristics and the tremor-generating properties in the same individual olivary neurones.

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