

## THE NATURE OF AFFERENTS FROM THE LARGE DORSAL NECK MUSCLES THAT PROJECT TO THE SUPERIOR COLLICULUS IN THE CAT

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### SUMMARY

1. A 3.5–5 mm passive stretch of dorsal neck muscles at velocities of 50 mm/sec, a stimulus adequate to excite most neck muscle spindles, was usually ineffective in eliciting unit discharge in the superior colliculus. The sudden release of muscle tension was effective and excited fifty-seven of sixty-seven units tested.

2. When electrical stimulation of neck muscle nerves was used, a stimulus strength sufficient to excite Group III muscle afferents was usually required to elicit unit discharge in the superior colliculus.

3. It is concluded that the projection from neck muscles to the superior colliculus largely takes origin in Group III afferents whose function remains to be determined. Some contribution to the projection to the superior colliculus from proprioceptive afferents served by larger fibres cannot be ruled out.

### INTRODUCTION

It is well established that afferents from the large dorsal neck muscles controlling head movement project to the superior colliculus. In experiments on chloralose-anaesthetized cats, it has been possible to show that electrical stimulation of nerves from the large dorsal muscles of the neck, splenius, biventer cervicis and complexus readily activate units in the superior colliculus (Abrahams & Rose, 1975*a*; Rose & Abrahams, 1978). In the small sample examined, units in the superior colliculus activated by electrical stimulation of neck muscle nerves were found to require activation of the more slowly conducting fibres (Abrahams & Rose, 1975*a*). Based on the assumption that the afferent fibre spectrum of these nerves resembles that of hind-leg muscle nerves, it was suggested that the projection to the superior colliculus from large dorsal neck muscles originated in the secondary endings of muscle spindles.

A variety of recent investigations have provided evidence that such a conclusion may not be justified. Recent examination of the properties of receptors in the large dorsal neck muscles of the cat (Richmond & Abrahams, 1979*a*) showed that in general the conduction velocities of neck muscle spindle afferents were below that of the more

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commonly studied hind-leg receptors, and Golgi tendon organ afferents cannot be separated on the basis of conduction velocity. The afferent fibre spectrum of nerves serving the large dorsal neck muscle has also been examined (Richmond, Anstee, Sherwin & Abrahams, 1976) and found to be unlike that of hind-leg nerves. Instead of the trimodal distribution commonly seen in hind-leg nerves (cf. Boyd & Kalu, 1979) there is an essentially unimodal distribution with a large peak in the 4–6  $\mu\text{m}$  (Group III) range. Further, it has recently been shown that stimulation of neck muscle nerves just above threshold is adequate to excite Group III as well as Group II fibres (Abrahams & Yokota, 1979).

A number of observations have also been made recently with micro-electrodes placed into the superior colliculus of the conscious cat (Straschill & Schick, 1977; Harris, 1980). None of these experiments has reported the presence of unit activity in the superior colliculus which would accord with an input from neck muscle spindles. For these reasons we have examined in greater detail the origin of projection of neck muscle afferents to the superior colliculus in the anaesthetized cat. Two kinds of experiments have been performed, one in which the large dorsal muscles of the neck have been stretched at velocities adequate to excite virtually all the spindles and the second in which very careful determination has been made of the electrical threshold of stimulation necessary to elicit unit response in the superior colliculus.

#### METHODS

Experiments were performed on twenty-two cats anaesthetized with chloralose (60 mg/kg *i.v.*) after the induction of anaesthesia with ethyl chloride and ether. In nineteen cats, both the neck muscles biventer cervicis and complexus were dissected free from their insertion onto the skull and freed from surrounding tissue without separation of the muscles and leaving the nerves and blood supply intact. The muscles could then be subjected to controlled ramp and hold stretch as previously described (Richmond & Abrahams, 1979*a*). The parameters of stretch and tension used in the present experiments were based on those known to be very effective in increasing spindle activity and initiating Golgi tendon organ afferents in complexus and biventer cervicis (Richmond & Abrahams, 1979*a*) and were usually 3.5–5 mm stretch at 50 mm/sec.

To establish the absolute threshold for electrical stimulation of neck muscle nerves, an Ag/AgCl ball recording electrode was placed on the most rostral C3 rootlet just distal to the point of entry into the dorsal columns. The anterior and posterior biventer cervicis C3 nerve branches (Richmond & Abrahams, 1975*a*) were then stimulated with 50  $\mu\text{sec}$  pulses. Threshold is defined as the voltage necessary to produce a just detectable response with the aid of the averaging technique from eight or sixteen successive stimuli (Neurolog type 741).

Varnished tungsten micro-electrodes were stereotaxically placed in the superior colliculus. Stimulus thresholds for recording responses in the C.N.S. to peripheral nerve stimulation are reduced when a brief stimulus train is employed (cf. Abrahams & Rose, 1975*b*). To establish thresholds, the stimulus consisted of a train of four 50  $\mu\text{sec}$  impulses at 2 msec intervals. The frequency with which stimuli were applied was usually one stimulus train each 4 or 8 sec. When inconsistent responses were seen, the threshold voltage was defined as that voltage midway between the highest voltage at which no unit response was elicited in the superior colliculus and the lowest voltage which caused a consistent response to five successively applied stimulus trains. When the threshold of neck nerve stimulation necessary to elicit unit discharge in the superior colliculus had been established, the signal recorded from the dorsal root entry zone to single pulse stimuli was averaged and photographed. The absolute threshold voltage for neck muscle nerve stimulation was then immediately re-determined.

Unit response in the superior colliculus to the electrical stimulation of nerves tends to have a variable latency, particularly when the stimulus strength is close to threshold and it was a common finding that the latency then increased. Where latencies are reported, they are the elapsed time

between the delivery of the first stimulus pulse in a train and the onset of consistent discharge where a suprathreshold stimulus strength (usually  $10 \times$  threshold) was used.

The temperature of the animal was maintained by a heated blanket and kept close to  $39^\circ\text{C}$ . The neck muscle nerves were kept moist with a silicone pool, and this pool was held close to body temperature by the use of a radiant heat lamp. The animals were immobilized with Flaxedil (gallamine triethiodide, Poulenc) and artificially ventilated at a rate which sustained end-tidal  $P_{\text{CO}_2}$  at a value of close to 4%. The maximum depth of each electrode penetration in the superior colliculus was identified by placing a small electrolytic lesion ( $10\text{--}20\ \mu\text{A}$  for 10 sec, electrode cathode). At the termination of the experiments, the brain was perfused first with saline and then with 25% formalin. After immersion in 25% formalin for 3–7 days,  $30\ \mu\text{m}$  sections were cut on a freezing microtome and stained with Luxol Fast Blue and Cresyl Violet (Klüver & Barrera, 1953). An approximation of recording sites was obtained by reconstruction of the electrode track making a 10% allowance for shrinkage.

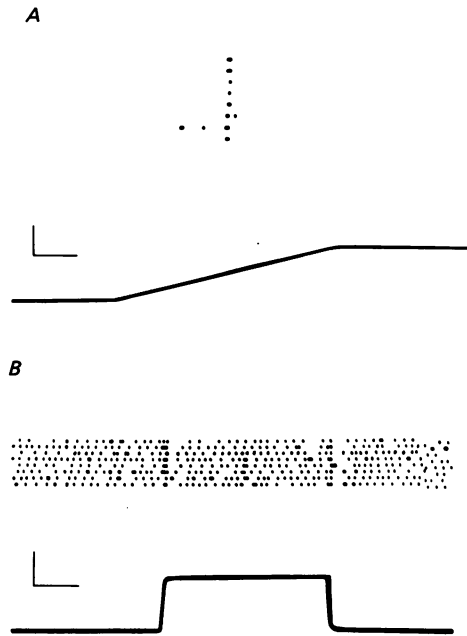


Fig. 1. *A*, brief burst discharge recorded from unit in superior colliculus to eight successive stretches of dorsal neck muscle. Horizontal bar, 50 msec; vertical bar, 2 mm calibration of movement transducer. *B*, effect of eight successive stretches of dorsal neck muscles on spontaneously discharging unit in superior colliculus. Horizontal bar, 200 msec; vertical bar, 2 mm calibration of displacement transducer.

## RESULTS

### *Response of units in the superior colliculus and underlying regions to passive stretch of the neck muscles biventer cervicis and complexus*

In the first twelve cats, the recording micro-electrode was introduced into the superior colliculus and advanced while a 2 msec flash was generated every few seconds (Abrahams & Rose, 1975*a*). When a response typical of the superior colliculus was obtained an appropriate ramp stretch was applied to the neck muscles, a technique similar to one successfully employed in experiments on extraocular muscles (Abrahams

& Anstee, 1979). Unlike stretch of extraocular muscles, neck muscle stretch at velocities of 50 mm/sec of 3.5–5 mm extent was clearly not an effective stimulus to unit discharge in the superior colliculus. Few of the thirty-nine units examined in this first series responded to the ramp phase of stretch and almost all were detected because they discharged when the stretch was released and the muscle allowed to return to its resting length. In subsequent experiments (in which a further thirty units were examined), electrical stimulation was first applied to the nerve supplying the muscle being stretched. Once a unit was isolated in the superior colliculus which responded to electrical stimulation of the muscle nerve, it was then tested for its responsiveness to passive muscle stretch.

Of the sixty-nine units tested, only eight exhibited a consistent response to muscle stretch at velocities and tension which could be considered as being in the physiological range. The response observed in six of these units was a brief burst during or just after the stretch (Fig. 1A). Two of the eight units were firing spontaneously and responded to stretch with a transient enhancement of firing frequency followed by cessation or reduction of discharge for about 400 msec (Fig. 1B). In these two units release of stretch produced a similar cycle of events. Of the thirty units whose connexion to neck muscle was established on the basis of electrical stimulation, none responded to stretch at velocities of 50 mm/sec. Unlike the inconsistent response to muscle stretch, the sudden release of muscle tension that occurred at the termination of stretch was an effective stimulus (Fig. 1B) and fifty-seven of sixty-seven units responded at this time by a brief burst. Of the total of sixty-nine units which were tested, seven were located in the superficial layers of the superior colliculus, seventeen in intermediate layers and twenty-two in the deep layers of the superior colliculus. A further eighteen units were located in the tegmentum deep to the superior colliculus, and five in the periaqueductal grey.

Succinyl choline in low doses will cause an enhancement of muscle spindle response (Granit, Skoglund & Thesleff, 1953) and high doses (up to 2 mg/kg) have a similar action in animals heavily dosed with Flaxedil (Richmond & Abrahams, 1979a). In the present experiment, thirty units responding to the release of muscle stretch were tested for their responsiveness after very high doses of succinyl choline (1–6 mg/kg). Twenty-two units were completely unaffected by the injection, and two units ceased discharge before testing could be completed. Five units showed some detectable enhancement of their firing rate at rest and an increased rate of firing during stretch at dose levels of 2–6 mg/kg.

#### *Stimulus strength and the compound action potentials at the dorsal root entry zone*

Previous experiments in which the electrical thresholds of single fibres dissected from upper cervical dorsal roots was examined showed that even the slower afferent fibres in biventer cervicis and complexus had relatively low thresholds (Abrahams & Yokota, 1979). Exclusive activation of the fastest fibres could only be consistently obtained when the voltage was below  $1.2 \times$  threshold. The proportion of Group III fibres activated was substantial as soon as the stimulus strength approached or exceeded  $2 \times$  threshold. Using the compound action potential recorded from the intact anterior C3 rootlet as an index, these data were confirmed (Fig. 2). Compound action

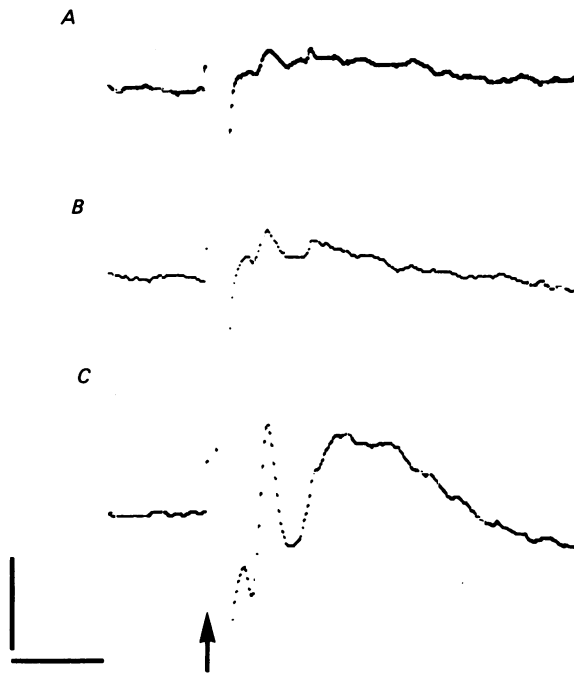


Fig. 2. Compound action potentials from dorsal root entry zone following stimulation of C3 biverter cervicis nerve at *A*,  $1 \times$  threshold; *B*,  $1.2 \times$  threshold; and *C*,  $1.9 \times$  threshold. Note the elevation due to the more slowly conducting fibres. Vertical calibration,  $50 \mu V$ ; horizontal calibration, 1 msec.

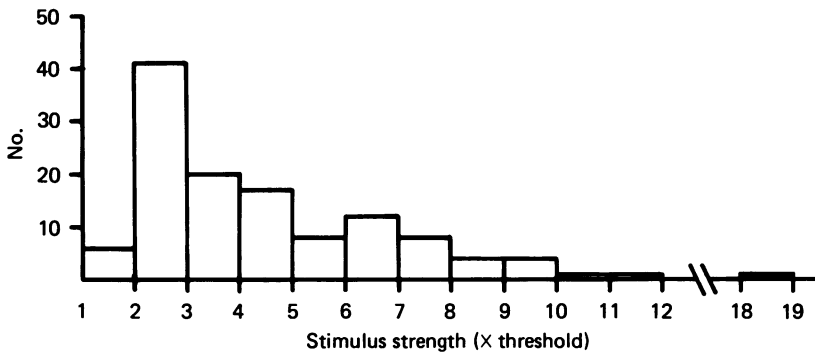


Fig. 3. Histogram of strength of stimulus trains to C3 biverter cervicis required to elicit unit discharge in superior colliculus.

potentials with a substantial Group III component were evident at stimulus strengths close to  $2 \times$  threshold and above.

*Electrical thresholds of biventer cervicis and complexus afferent fibres necessary to activate unit response in the superior colliculus and underlying structures*

A total of 124 units were recorded from in the superior colliculus and underlying structures which responded to electrical stimulation of neck muscle nerves and for which stimuli thresholds could be established. As Fig. 3 shows, only four units had electrical thresholds below  $2 \times$  threshold. The remaining 120 units required electrical stimulation at strengths of  $2 \times$  threshold or above for their excitation. Fifty-eight units were located in the superior colliculus, fifty-nine in the tegmentum deep to the superior colliculus, and seven in the central grey.

#### DISCUSSION

The afferent system taking origin in neck muscles of the cat differs in a number of respects from the more commonly studied hind-leg afferent system. The most obvious characteristic in the large dorsal muscles is the sheer abundance of spindles (Voss, 1958; Cooper & Daniel, 1963; Richmond & Abrahams, 1975*b*) although these spindle densities, which may exceed 100/g, are eclipsed by the densities in the small deep neck muscles which may contain 500 spindles/g or more (Richmond & Abrahams, 1979*b*). The sheer density of the spindle population of neck muscles has tended to overshadow another factor, which is the very large percentage of Group III sensory fibres in neck muscle nerves (Richmond *et al.* 1976). Variability in the content of Group III fibres in afferent nerves has often been noted (Lloyd & Chang, 1948; Boyd & Davey, 1966), ranging from a low content of 16% in the nerve to popliteus to a high content of 46% in tibialis posterior. In neck muscles biventer cervicis has about 55% and complexus about 32% of their fibres in the Group III range (Richmond *et al.* 1976). These are among the highest proportions of Group III fibres so far recorded in muscle nerves.

The present experiments provide evidence that the projection to the superior colliculus from the large dorsal neck muscle probably has a substantial origin in Group III afferents. The electrical threshold needed to elicit a response in the superior colliculus is consistently well above that needed to excite only the fastest afferents. Combining the previous experiments on the electrical threshold of single fibres of neck muscle afferents (Abrahams & Yokota, 1979) with the present work with compound action potentials, it seems certain that significant numbers of neck muscle afferent fibres with conduction velocities below 20 m/sec must be activated to elicit a response in the superior colliculus.

The results of the experiments on stretch of neck muscle are largely but not entirely, consistent with the hypothesis that the projection to the superior colliculus is from Group III fibres. The spindle and Golgi tendon organs of neck muscles are served by afferent fibres almost all of which conduct at velocities in excess of 20 m/sec (Richmond & Abrahams, 1979*a*). Since responses to stretch were seen on occasion, and succinyl choline could facilitate a response there may be some spindle afferent input. However, the dosages of succinyl choline necessary to elicit an effect were

extremely high. In the anaesthetized cat the dose of succinyl choline iodide needed to produce a strong spindle response was in the range 5–20  $\mu\text{g}$  (Granit *et al.* 1953). When the animal is heavily treated with Flaxedil, the dose needed is many times greater (50–2000  $\mu\text{g}/\text{kg}$ ) (Richmond & Abrahams, 1975*b*). The dose needed in the present experiments was as high as 6 mg/kg, a dose which may have an excitatory action on a wide range of muscle receptors (Kidd & Kucera, 1969). It may be argued that the failure to demonstrate a response during stretch could reflect the effects of the anaesthetic, but stretch of extraocular muscles under the same anaesthetic conditions is most effective in eliciting an ordered and reproducible response in the superior colliculus (Rose & Abrahams, 1975; Abrahams & Anstee, 1979). However, it is still necessary to explain the consistently observed response when the tension was quickly released from the muscle. It is possible that such responses could come from perivertebral structures which are rich in receptors, including spindles which are extremely sensitive to the smallest movements (Richmond & Abrahams, 1979*a, b*).

If the afferent fibres to the superior colliculus take origin in the Group III population of complexus and biventer cervicis, the question then arises as to which receptors give rise to those fibres. Experiments on the hind-leg muscles of the cat established that the majority of Group III fibres came from receptors that were pressure-sensitive. Paintal (1960) found that more than two thirds of the receptors he examined in gastrocnemius, soleus, and tibialis anterior were activated by pressure applied directly to the muscle. More than half of those tested responded weakly during a muscle twitch, and less than half responded during tetanus, but weakly. Since the local injection of 6% NaCl was effective in eliciting discharge in these Group III receptors, and this injection in man is painful (Lewis, 1942), Paintal (1960) considered these receptors as 'pressure-pain' receptors.

In a later study, Bessou & Laporte (1961) found the muscle Group III fibres to belong to a family of pressure receptors characterized by differences in receptive field characteristics and adaptation patterns. More recent examination of Group III fibres (Hnik, Hudlicka, Kucera & Payne, 1969; Mense, 1977; Kumuzawa & Mizumura, 1977) has suggested that Group III fibres share with Group IV fibres a special sensitivity to algescic chemicals (histamine, 5-hydroxytryptamine, bradykinin, and  $\text{K}^+$ ) and to temperature. This has been inferred as evidence for a nociceptive role for these afferents by some (Kumazawa & Mizumura, 1977; Mense, 1977), although the  $\text{K}^+$  sensitivity has also been interpreted as evidence for a role for Group III receptors in cardiovascular and respiratory reflex responses to muscular exercise (Hnik *et al.* 1969; Mitchell, Mierzwiak, Wildenthal, Willis & Smith, 1968; Kumuzawa & Mizumura, 1977). Histological examination of receptors served by Group III afferents (F. J. R. Richmond, personal communication) has shown that they come from paciniform corpuscles and free nerve endings. It is possible that some of these afferents are vibration-sensitive and thus contribute to the 'off' response that was so consistently observed.

In recent experiments on the superior colliculus of the hamster, Stein & Dixon (1978) and Stein (1978) have reported the presence of units responsive to cutaneous nociceptive stimuli, and have suggested that these projections may relate to a role of the superior colliculus in eliciting aversive movements to nociceptive stimuli. The

muscle afferent projection to the superior colliculus terminates in part on cells of origin of the tectospinal and tectoreticular systems (Abrahams & Rose, 1975*a*; Rose & Abrahams, 1978). It is not easy to see how non-proprioceptive muscle afferent input to the superior colliculus may be related to the defence reaction which has long been known to be elicited from stimulation of the superior colliculus (Hunsperger, 1956; Spiegel, Kletzkyn & Szekely, 1954; Abrahams, Hilton & Zbrozyna, 1960), and which is the basis of Stein (1978) and Stein and Dixon's (1979) hypothesis. Muscle pain does, however, alter patterns of motor activity and profoundly influences the use of the affected muscle. Perhaps this would explain, in part at least, the significance of a projection of Group III afferents from neck muscle to descending systems to neck motoneurons.

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