MIDBRAIN EVOKED RESPONSES RELATING TO PERIPHERAL UNMYELINATED OR 'C' FIBERS IN CAT[1]

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INTRODUCTION

AFFERENT PATHWAYS in the central nervous system of the cat have been shown to be activated by groups of fibers of different size in peripheral nerves (1, 5, 8, 10, 11). This has been accomplished by utilizing the known relationship between peripheral nerve size, threshold for excitation, and velocity of conduction (3, 7, 9) with recording of evoked responses to single and repetitive stimuli at various sites in the spinal cord and brain. Evoked activity in the reticular formation of the caudal medulla oblongata incident to stimulation of isolated small unmyelinated 'C' fibers in peripheral nerves was the subject of a previous report (6). This study is concerned with representation of activity in the ventral tegmentum of the midbrain resulting from repetitive stimulation of isolated 'C' fibers in peripheral nerves.

METHOD

Forty adult cats weighing from 2 to 3 kg. were anesthetized with cyclopropane, given atropine 0.2 mg./kg. intramuscularly, a tracheotomy performed, and a femoral artery and vein cannulated with polyethylene tubing for continuous blood pressure recording and drug administration. A stainless steel needle electrode insulated with Lucite to within 60–120 μ of a 10 μ tip was inserted by means of a micromanipulator through a craniectomy with the animal in a stereotaxic instrument. Superficial radial, saphenous and peroneal nerves were exposed and bipolar copper electrodes separated by 1 cm. were placed on the nerves 70–100 mm. apart. Cyclopropane was discontinued and Flaxedil 4 mg./kg. was given intravenously. The animals were maintained on artificial respiration. In addition to continuous blood pressure recording, the rectal temperature was constantly monitored and maintained above 34°C.

After allowing 45 to 60 minutes for the preparation to come into a steady state, the recording electrode was inserted into the ventral tegmentum of the midbrain (A 4.5, L 1, H -4) with adjustment to allow for maximal evoked responses from peripheral nerve stimulation previously described by Collins and O'Leary (4). Recording was effected through a cathode follower and condenser-coupled amplifiers to one beam of a dual-beam oscilloscope. The other beam was used for monitoring the peripheral nerve action potential. Trains of 0.5 msec. square wave stimuli of intensities ranging from 0.04 V. (group A-beta threshold) to 24.0 V. (well above 'C' threshold) were introduced at a frequency of 50/sec. from an American Electronics Laboratory stimulator. The train duration ranged from 200 to 500 msec. Subsequently a third bipolar electrode was placed on the nerve between the distal stimulating and proximal recording electrode. Short bursts (6–12 sec.) of faradic shocks

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from a Harvard inductorium were delivered to the intermediate electrode for the purpose of reversibly blocking the larger fibers and isolating the small unmyelinated fibers according to the method described by Bishop (2). Delay in appearance of the evoked responses was measured from the first stimulus artifact of the train. At the end of each experiment, the recording locus in the midbrain was electrolized and identified in serial stained sections.

Results

The area of recording (Fig. 1) lies beneath the rostral portion of the superior colliculus, ventral to the oculomotor nucleus and periaqueductal gray matter at the dorsomedial margin of the red nucleus, just above the brachium conjunctivum. A ventral tegmental spike-evoked response, shown previously to be related to activation of the gamma-delta fibers of peripheral nerve (4), was localized within a 0.5 mm. movement of the electrode. The flare of spike discharges relating to multiple or trains of stimuli was recorded from a more extensive area, the limits of which will be the subject of a future communication.

Threshold studies were done, comparing the ventral tegmental response to that of the nucleus ventralis posterolateralis of the thalamus. The threshold of the ventral tegmental response was higher than the group A-beta peripheral nerve threshold for the thalamic response and was again related to activation of the group A gamma-delta fibers. In Fig. 2, group A-beta, gamma, delta and group C components activated by increasing intensity of single stimuli are shown in records of the monitored peripheral nerve action potential in A, C, E and G. B, D, F and H illustrate responses to multiple stimuli at the same intensity in the ventral tegmentum of the midbrain. Each tegmental record shows a portion of baseline activity and the shock

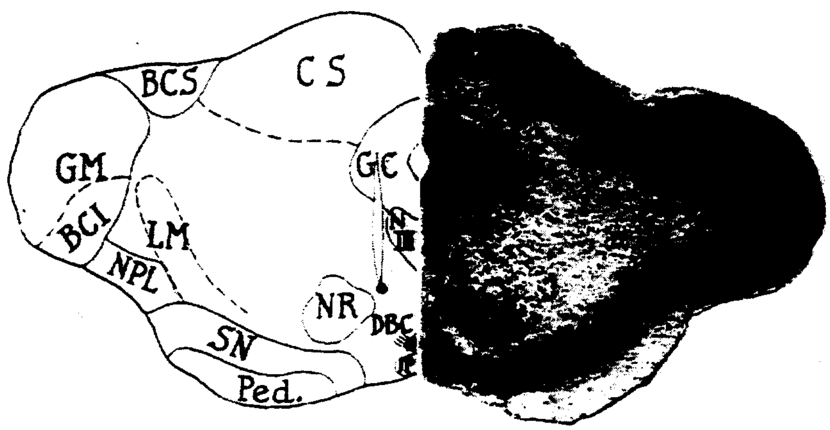

FIG. 1. Transverse section through rostral portion of midbrain of cat. Diagrammatic representation of structures and electrode placement on left. CS, colliculus superioris; BCS, brachium colliculi superioris; GM, corpus geniculatum mediale; GC, griseum centrale; NIII, nucleus oculomotorius; BCI, brachium colliculi inferioris; LM, lemniscus medialis; NPL, nucleus paralemniscalis; NR, nucleus ruber; DBC, decussatio brachiorum conjunctivorum; SN, substantia nigra; IP, nucleus interpeduncularis; Ped., pedunculus cerebralis.

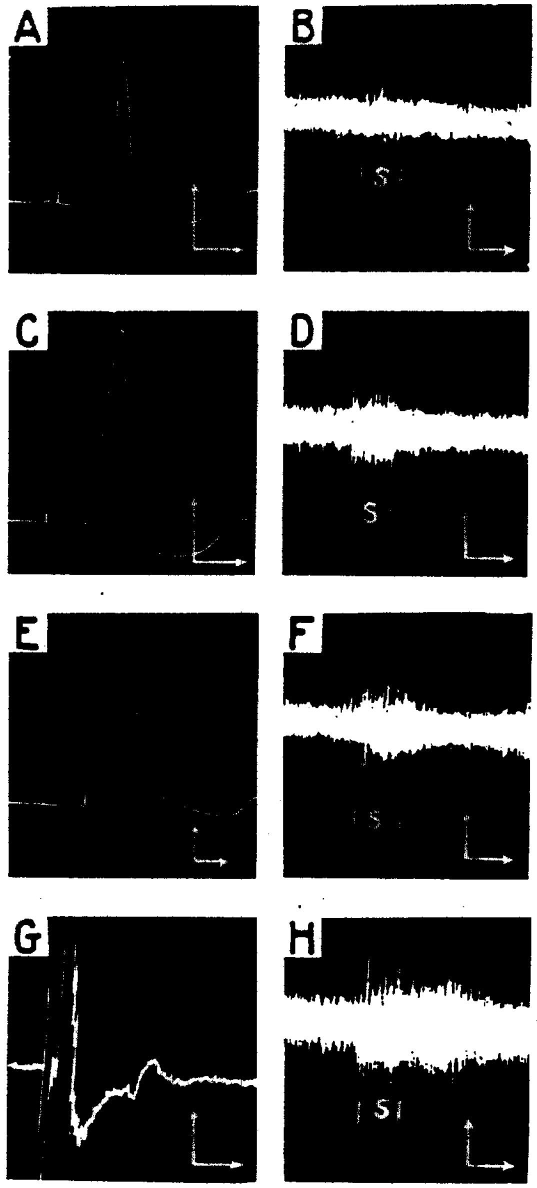

FIG. 2. Oscillographic monitor of left superficial radial nerve action potential for control of stimuli intensity and evoked responses in right ventral tegmentum of midbrain from 250 msec. trains of stimuli (marked S in each illustration) at stimulus voltage and duration (0.5 msec.) of nerve monitor. A, 0.06 V. above beta threshold; B, no single evoked response of flare of spike responses from repetitive 0.06 V. stimuli; C, 0.11 V. above gamma threshold; D, initial single response but no subsequent flare of spikes; E, 0.22 V. above delta threshold; F, initial single response and brief flare of spike responses; G, 20 V. with multifiring A fibers and 'C' wave; H, initial single response and prolonged flare of spike responses. Calibration in A, C, and E, 200 μV. and 1 msec.; in G, 100 μV. and 25 msec.; in B, D, F, and H, 100 μV. and 250 msec.

artifacts near the mid-portion of the record. It is to be noted that the first shock of the train of responses (50 sec for 250 msec.) first evokes a single response at gamma threshold (C, D) but no subsequent flare of spikes. Above delta threshold (E, F), the first evidence of the flare of spikes occurs. Above 'C' threshold (G, H) prolonged spike discharges are recorded as well as the initial single spike response to the first stimulus of the train seen at all intensities above gamma threshold.

It was evident during our threshold and other studies that the response to single shocks above gamma-delta threshold was localized as regards the source of the stimulus whereas the response to trains of stimuli could be recorded equally well in a single locus from all four extremities. There is

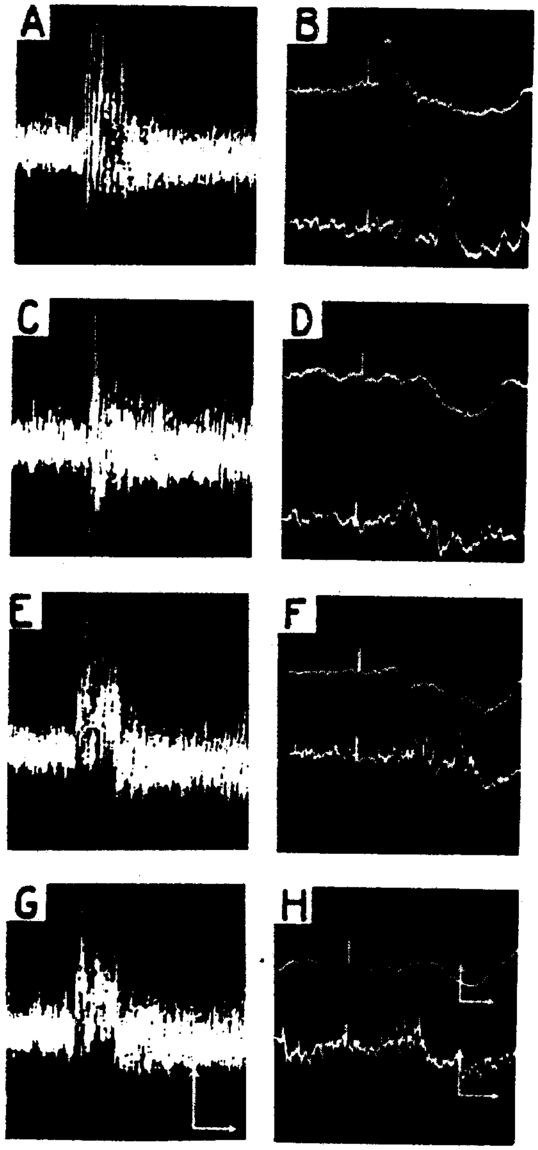

FIG. 3. A, C, E, and G, evoked responses in right ventral tegmentum from multiple 0.05 msec., 24 V., 50/sec. stimuli applied for 250 msec. B, D, F, and H, evoked responses from single 0.5 msec., 2 V. stimuli. Upper line: right thalamus, nucleus ventralis posterolateralis; lower line: right ventral tegmentum of midbrain. A and B from left superficial radial nerve; C and D from right superficial radial nerve; E and F from left superficial peroneal nerve; G and H from right superficial peroneal nerve. Calibrations: left column, 100 μV. and 250 msec.; right column, upper line, 150 μV.; lower line, 100 μV. Horizontal markers: 10 msec.

essentially no difference in the evoked flare of spike discharges (Fig. 3A, C, E, G) from repetitive stimuli above 'C' threshold but single responses (Fig. 3B, D, F, H) in both the ventral posterolateral nucleus of the thalamus (top line) and ventral tegmentum of the midbrain (bottom line) are markedly altered when, after obtaining maximum responses from an upper extremity, nerves of the other extremities were stimulated. It was the impression of the investigators that there was more overlap of central representation of evoked responses in the thalamus from the contralateral nerve stimulation (*e.g.*, left superficial radial, left superficial peroneal) but in the ventral tegmentum more overlap between the upper extremities (*e.g.*, left and right superficial radials) than from the contralateral fore and hind limb nerves. In both thalamus and ventral tegmentum, maximum single responses were obtained only from a contralaterally stimulated extremity nerve.

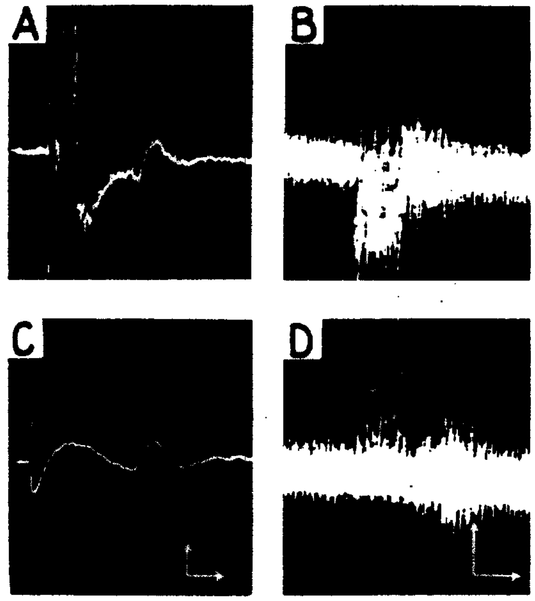

FIG. 4. A: Peripheral nerve recording after single stimulus applied to left superficial radial above 'C' fiber threshold (24 V.) showing multifiring A fiber and 'C' fiber potential. B: Flare of spike responses appearing in ventral tegmentum of midbrain record immediately after shock artifact resulting from a 250 msec. train of 0.5 msec., 50/sec. stimuli at 24 V. applied to left superficial radial nerve. C: Left superficial radial nerve recording after application of induction-coil faradic block of larger fibers showing isolated unmyelinated 'C' fibers potential only. D: Appearance of flare of spike responses 570 msec. after start of 250 msec. train of multiple stimuli (same parameters as in B) resulting from blocking of delta peripheral nerve fibers and showing prolonged evoked response from repetitive isolated unmyelinated 'C' fiber stimulation. Calibration—nerve records, 50 μV., 15 msec.; tegmental records, 100 μV., 300 msec.

Since the threshold studies of the ventral tegmental response to trains of stimuli (Fig. 2) demonstrate activity relating to A-delta fibers as well as 'C' fibers, an electrode connected to a Harvard inductorium was placed between the distal stimulating and proximal recording electrodes and brief bursts of faradic stimulation applied to the nerve. This resulted in a reversible block of conduction in the myelinated fibers. Conduction delays were calculated from the first shock of the train of stimuli in order to have a consistent measurement reference even though successive 6–10 stimuli were necessary to evoke a response. Figure 4 shows the record of the peripheral nerve action potential in A and after the faradic block in C. Figure 4B and D are records obtained from the right ventral tegmentum after trains of 0.5 msec., 50/sec. repetitive stimuli were applied for 250 msec. to the left superficial radial nerve. The delay in appearance of the flare of spikes in D relates to the blocking of the larger A-delta fibers. In 15 animals stimulation of isolated 'C' fibers in the contralateral superficial radial nerve resulted in an average delay between start of the stimuli and appearance of the evoked flare of spike discharges, of 570 msec. with a range of 550–600 msec. Attempts to calculate conduction delay from the saphenous nerve were unsuccessful in that the isolated 'C' fiber stimulation in this nerve gave a response which was attenuated to the extent that measurement of the delay was unsatisfactory. In five animals the contralateral peroneal nerve isolated 'C' fibers were repetitively stimulated and the average delay for the appearance of the ventral tegmental response was 890 msec., with a range of 850–930 msec.

DISCUSSION

The finding of evoked central nervous system activity in the ventral tegmentum of the midbrain of the cat relating to 'C' fiber activation of peripheral nerves and the similarity of this evoked response to that seen in the caudal medulla, described in a previous publication (6), suggests a small fiber afferent system in the paramedial reticular formation of the brain stem. Collins and O'Leary (4) demonstrated an evoked response relating to A-gamma-delta peripheral nerve fiber single stimuli in the same ventral tegmental area of the midbrain. Under light Nembutal anesthesia they found a discretely localized response from the contralateral extremity which was abolished by section of the anterolateral quadrant of the spinal cord opposite to the nerve stimulated. We find evoked activity in the same area from single A-gamma-delta stimuli applied to nerves in all four extremities with some degree of central localization relating to each extremity. In contrast, there is equal representation of the evoked flare of spike responses from all four extremities following repetitive stimulation of A-delta and 'C' fibers (see Fig. 4). It has been shown previously (4, 13) that the ventral tegmental response is sensitive to anesthetic agents and this may be the explanation for the discrepancy regarding more precise localization of the single response reported by Collins and O'Leary. The threshold relationship of the evoked single response to the gamma-delta fibers of the peripheral nerves was confirmed.

The conduction delay from nerve to midbrain following stimulation of isolated 'C' fibers was compared with that noted in the previous communication describing the flare of spike responses in the caudal medulla. In the superficial radial nerve, unmyelinated fiber conduction velocity was 1.4 m./sec. Previously we determined that central nervous system conduction velocity was slightly faster at 1.7 m./sec. After measuring many more central conduction delays, a ± 50 msec. error in latency measurements was noted. It has been concluded that in the spinal cord conduction velocity is 1.5 m./sec., approximately that of peripheral nerve conduction velocity. In the brain stem, however, there is a conduction time of 200 msec. for an average distance between the caudal medulla and midbrain ventral tegmental recording points of 25 mm. This reflects a conduction velocity of 0.13 m./sec. Despite possible errors in measurement, this denotes marked slowing in conduction in the brain stem, signifying either smaller fiber size or polysynaptic transmission. Anatomical studies (14, 15) make the latter most likely. It was of interest to note that an evoked potential to a clicking sound and a flash of light was seen in this area as in the paramedial reticular formation of the medulla.

Thus two afferent pathways have been delineated: the first related to the gamma-delta fibers of peripheral nerve, relatively well localized, oligosynaptic, and with a high-voltage response to single or the first of rapidly applied multiple stimuli; the second, related to the A-delta and 'C' fibers of peripheral nerve with no apparent localization as regards the extremity stimulated, with no response to a single stimulus, slow central conduction, and a prolonged train of spike discharge as a response to multiple stimuli.

Summary

Evoked potentials resulting from repetitive stimuli applied to isolated unmyelinated peripheral fibers were recorded from the ventral tegmentum of the midbrain in 40 normal cats. The centrally recorded multiple spike discharges, similar to those evoked in the caudal paramedial medulla oblongata in a previous study, could be activated from trains of stimuli applied to both A-delta and 'C' fibers monitored in somatic nerves. The delayed appearance of the ventral tegmental response to C-fiber peripheral nerve stimulation was indicative of slow central conduction. At this level of the neuraxis no topographical localization from any of the extremities was demonstrable for this response.

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