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CONNECTIONS OF THE MESENCEPHALIC LOCOMOTOR REGION (MLR) IN THE CAT[1]

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ABSTRACT

The cat entopeduncular nucleus (EN), which is the main output of the basal ganglia, is known to project to the mesencephalic tegmentum. We have been able to elicit antidromic responses in single EN neurons from the region of the mesencephalic locomotor region (MLR), then transect (precollicular-postmamillary) the brainstem and elicit rhythmic movements of the limbs by stimulation of the *same* site in the *same* animal. Injections of the fluorescent dye 2,4 diamidino phenylindole 2 HCL (DAPI) into this area induces retrograde labeling of cell bodies in EN and motor cortex. Injections of a tritiated amino acid (leucine) into the motor cortex induce terminal labeling in the area of the MLR. These studies describe convergent projections from EN and motor cortex to the MLR. These connections may be involved in the sequencing and ordering of voluntary movements in which locomotion is necessary.

INTRODUCTION

The internal segment of the globus pallidus in the monkey sends fibers to an area of the caudal mesencephalic tegmentum called the nucleus tegmenti pedunculopontinus (NTPP) (Nauta and Mehler, 1966). This area also is known to receive projections from the precentral cortex in the monkey (Kuypers and Lawrence, 1967). Two electrophysiological studies in the cat reported that entopeduncular (EN) neurons, the homologue of the primate internal pallidum, responded antidromically to electrical stimulation of NTPP. One of these (Filion and Harnois, 1978) reported that 50% of all EN neurons could be driven antidromically from a point along the pallidotegmental pathway (approximately point 1 in Fig. 1). The other study (Larsen and Sutin, 1978) reported that only 8% of EN neurons could be activated antidromically from NTPP (approximately point 2 in Fig. 1).

Physiological studies have established that an animal with a precollicular, premamillary transection (as in line A, Fig. 1) can exhibit spontaneous walking or, at least, locomotion on a treadmill (Orlovsky, 1972). With a precollicular, postmamillary transection (as in line B, Fig. 1), no spontaneous locomotion is seen but must be induced by stimulation of the MLR, a physiologically defined region which includes the cuneiform nucleus (CF) (point 3, Fig. 1) (Mori et al., 1977). With a posterior transection (such as in line C, Fig. 1), no locomotion can be induced (Grillner and Shik, 1973).

The present study was undertaken to determine if EN neurons could be antidromically activated from the same point which, when stimulated following transection (B), would induce locomotion on a treadmill. Some of these results previously have been reported (Skinner et al., 1979). Anatomical studies were also undertaken to confirm our electrophysiological results, and to determine the extent of projections to CF from the motor cortex in the cat.

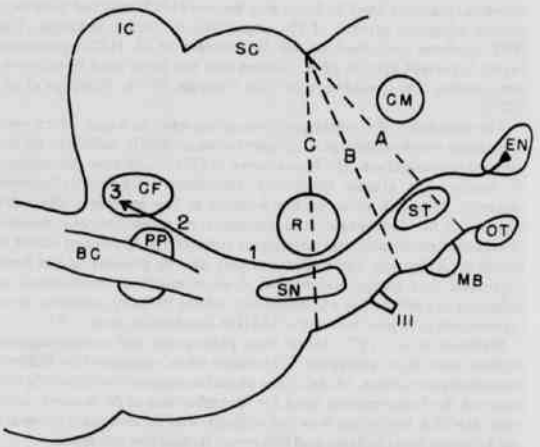

Figure 1. Diagram of part of the cat brainstem. See text for explanation of points 1, 2, 3 and lines A, B and C. BC - brachium conjunctivum, CF - cuneiform nucleus, CM - centre-median nucleus, EN - entopeduncular nucleus, IC - inferior colliculus, MB - mamillary body, OT - optic tract, PP - nucleus tegmenti pedunculopontinus, R - red nucleus, SC - superior colliculus, SN - substantia nigra, ST - subthalamic nucleus, III - oculomotor nerve.

METHODS AND MATERIALS

Fifteen adult cats were anesthetized intravenously with sodium methohexital, a short-acting barbiturate. A four-pronged stimulating comb was placed in the region of MLR, and a glass micropipette introduced into the ipsilateral EN. Subsequent procedures were carried out under locally anesthetized, paralyzed conditions. Artificial respiration was used. Single neurons in EN were isolated extracellularly and their responses to MLR stimulation recorded. Once a single EN neuron was found to respond antidromically to MLR stimulation, the recording site was marked. A precollicular, postmamillary transection was performed and the animal's weight supported by a hammock. Following recovery from paralysis and with stimulating electrodes in place, the limbs were lowered onto a moving treadmill. Stimulation of the MLR was then applied to induce locomotion.

In six other cats, the extent of afferent projections to CF and surrounding area were determined by retrograde neuronal labeling. Under barbiturate anesthesia, injections of 0.1 μl of a 2.5% solution of 4-6-diamidino-2-phenylindole 2 HCl (DAPI) in saline were made into the CF. After four days, the cats were sacrificed, perfused with 10% phosphate-buffered formalin, and the brains placed in cacodylate buffer (pH 7.2) containing 30% sucrose. Sections of 30 μm were mounted from distilled water and air dried without coverslipping. Observation was made with a fluorescence microscope using excitation filters at 360 nm wavelength and observation filters at 430 nm. Labeled neurons containing DAPI fluoresced blue.

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In another series of four cats, motor cortex projections to CF were determined by autoradiography. ^{3}H-leucine was injected (1 μl [50 μCi/μl]) into the axial representation of the motor cortex or into the distal forelimb representation. After four days, the animals were sacrificed and perfused with 10% buffered formalin. Frozen sections of 20 μm were dipped in NTB-2 emulsion and, after six weeks, the sections were developed and stained with hematoxylin and eosin.

RESULTS

Antidromic responses in EN were elicited by electrical stimulation of medial sites within CF, while orthodromic responses could be elicited from more lateral locations in the mesencephalon. The best point for inducing locomotion or rhythmic limb movement in these animals was also invariably located in medial CF. EN neurons activated antidromically (n=7) followed 300-500 Hz stimuli in trains of up to four shocks at a latency of 2.2±0.6 ms (mean and standard deviation). In anterior EN, where most responses were obtained, EN neurons activated antidromically represented 6% of the 119 neurons studied.

In our anatomical studies using retrograde neuronal transport of DAPI, labeled neurons were observed, in descending order of number, in the ventral tegmental area, medial substantia nigra (pars compacta), sub- and hypothalamus, precruciate (motor) cortex, ansa lenticularis and EN. Even though very few labeled EN neurons were observed, their distribution closely matched that of the antidromically identified neurons discussed above.

In our anatomical studies using anterograde transport of a tritiated amino acid, autoradiographic silver grains were found in the ipsilateral CF only in cats injected in the axial representation of the motor cortex. No labeling was observed following injections into the distal forelimb representation. Labeling observed in CF was diffuse, but distinct. These studies indicate that medial motor cortex and pallidal efferents project to the CF.

DISCUSSION

These preliminary studies indicate that a small portion of the pallidotegmental projection descends as far as the CF and appears to terminate within the MLR. From reports in the literature, the CF, at least the dorsal part of NTPP, and perhaps portions of the locus coeruleus all form part of the MLR (Mori et al., 1977). There appears to be a convergence of cortical (Kuypers and Lawrence, 1967), pallidal (Nauta and Mehler, 1966; Skinner et al., 1979) and nigral (Skinner et al., 1979) inputs at this level. Our anatomical studies support this notion.

The functional significance of these pathways remains to be discovered. However observations noted during these experiments provided some indications. When larger currents or higher frequencies than those mentioned were applied to the MLR, considerable extensor rigidity was observed instead of rhythmic alternating movements. This suggests that "overdriving" of the MLR by cortical, pallidal and/or nigral afferents may be involved in certain pathological cases of rigidity. By the same token, lack of input to this region may be responsible for the inability to *initiate* locomotion, such as that observed in Parkinson's disease. We subscribe to the notion that motor cortex and basal ganglia interactions with the MLR may be involved in the "triggering" of locomotion generators, a process either attenuated or exacerbated in diseased states. The basal ganglia have been implicated in the sequencing and ordering of movements (Garcia-Rill et al., 1979). The link between the EN and MLR may subserve the function of initiating sequences of movements in which locomotion is necessary.

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