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Absence of transsynaptic transport in cerebello-thalamo--cortical path of the rat

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Abstract. Attempts to visualize the cerebello-thalamo-cortical path in the rat were made with different approaches. We tried (1) double labelling with somatopetal tracing from the motor cortex and somatofugal from the cerebellar nuclei, (2) transmembrane labelling by depositing biocytin or wheat germ agglutinin (WGA)into the motor cortex or cerebellum. WGA was either iodinated with ^{125}I or conjugated with horseradish peroxidase (HRP). The double labelling technique showed an overlap of the tracers in the same thalamic region but no evidence of transsynaptic transport in either direction was obtained. Our results indicate a difference in the organization of this system in primates and rodents, since transsynaptic labelling in the cerebellar nuclei after injections of WGA-HRP conjugate in the monkey motor cortex has been found.

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INTRODUCTION

Monkeys and rats are often used in experimental neurology because of similarity to humans and practical and economical advantages, respectively. These two species showed considerable similarities (Divac 1977, 1984, Aldes 1988) in organization of the two neural systems which interface the cerebral cortex and the motor mechanisms: the basal ganglia and pontocerebellar systems (Kemp and Powell 1971, Allen and Tsukahara 1974, Divac 1977). In the present study we attempted to extend this similarity by demonstrating in the rat transmembrane labelling in the cerebellar nuclei following injections of different tracers into the motor cortex. Similar injections into the deep cerebellar nuclei were made in order to look for transsynaptic transport in the opposite direction: to the motor cortex via the thalamus. Transmembrane labelling of the cerebellar nuclei following intracortical injections of the conjugate between wheat germ agglutinin (WGA) and horseradish peroxidase (HRP) has been reported in the monkey by Wiesendanger and Wiesendanger (1985). Also in other studies WGA labelled with either HRP or radioactive iodine has been shown to cross cell membranes causing labelling in both somatopetal and somatofugal direction (e.g. Fabian and Coulter 1984, review in Jankowska 1985,Baker and Spencer 1986). Since our purpose was to look for transmembrane labelling rather than to establish precise topographic relations in the cerebello-thalamo-cortical path (see: Faull and Carman 1978, Haroian et al. 1981, Schim et al. 1981, Donoghue and Parham 1983, Angaut et al. 1985), we injected large volumes of tracers.

METHODS

SUBJECTS

Eighteen Wistar albino rats of either sex, weighing 150-300 g were used in this study. Younger animals were used in experiments aimed to utilize transmembrane transport of the tracers and older ones when two tracers were simultaneously injected.

MATERIALS

Wheat germ agglutinin was purchased from Sigma and iodinated with ^{125}I (see Acknowledgements). The WGA-HRP conjugate was purchased from Sigma (Type VI, Lots No 31F - 3929 and 3929-2) as well as from Miles (Lot PW-7). Biocytin and bisbenzimide were purchased from Sigma, and avidin-biotin Elite kit from Vector.

PROCEDURES

Two main approaches were employed. First, somatopetal tracers were injected into the motor cortex and the somatofugal tracers into the contralateral cerebellar nuclei. Adjacent sections of the thalamus were examined for the presence of each tracer. Secondly, WGA was injected either into the motor cortex or cerebellar nuclei. In these animals both the thalamus and the cerebellar nuclei or the motor cortex, respectively, were examined. In two animals biocytin was injected into the motor cortex. The thalamus, cerebellar nuclei and pyramidal tract were screened.

In the two-tracer design, two rats received 0.4 µl of 5% bisbensimide into the motor cortex and the same volume of 0.4% ^{125}I-WGA (6.5 µCi in 1 mg of protein on the injection day) into the cerebellar nuclei. After 24 h, these rats received ^{14}C-labelled 2-deoxyglucose 10 µCi/100 g body weight in order to have autoradiograms of the entire sections rather than only of the efferents of the cerebellar nuclei. Forty minutes after 2-DG injections the animals were perfused and the brains immediately frozen and cut in a cryostat. Three alternate series of 20 µm sections were (1) stained with cresyl violet, (2) only dried, coverslipped and charted by means of a fluorescence microscope coupled to an x-y plotter, or (3) placed on cover glass, quickly dried and pressed against a sheet of Kodak SB-5 film which was developed 21 days later.

In the experiments with one tracer only, several approaches were tried. In some animals the tracer was applied to the motor cortex (coordinates in the section on surgical methods). In two rats a lump of 10% polyacrylamide gel, containing 0.4% ^{125}I WGA with the same specific activity as before, was inserted into the premotor area of one hemisphere and the hand motor area of the other hemisphere. The animals survived 5 days, were perfused and their brains cut on the same day in a cryostat. One series of sections was picked up on coverslips which were glued to a cardboard, and pressed against Kodak SB-5 film for 30 days. The cardboard with sections was then photographed unstained and the prints were made to match in size the autoradiograms in order to relate the distribution of radioactivity markings on the developed SB-5 film to the sections. Another series of sections was stained with cresyl violet.

Four rats received lumps of 10% polyacrylamide gel containing WGA-HRP conjugate in the cerebellar nuclei. These animals survived 4 days. After perfusion, the brains were left in 20% sugar in phosphate buffer at 4°C for 24 h, rapidly frozen, and then cut in a cryostat at 20 µm. Two series were reacted for HRP, one series according to the TMB protocol followed by DAB stabilization procedure (Lemann et al. 1985) and the other with DAB alone, according to a modified Malmgren and Olson (1978) recipe.

In four rats single injections of 0.3 µl WGA-HRP conjugate (concentration 2.5% in artificial cerebrospinal fluid) were made into the motor cortex of one hemisphere. The animals were sacrificed 48-96 h after surgery and the brains were processed to reveal HRP as in the previous group. The thalamus and cerebellar nuclei were scrutinized.

In two rats 0.5 µl of ^{125}I-WGA as above was injected into the cerebellar nuclei. The animals survived for 4 days and were processed as was the group in which the same tracers were applied to the motor cortex. In these brains special attention was paid to the radioactivity distribution in the thalamus and its presence in the motor areas of the cerebral cortex.

In two animals 5% biocytin dissolved in Tris buffer pH 7.4, 0.05M was incorporated in polyacrylamide gel and inserted into the motor cortex of one hemisphere. After 48 h the animals were perfused with saline and neutral formalin and cut at 60 µm in a Vibroslice. The sections were reacted with Vector ABC Kit and the diaminobenzidine-Ni procedure.

SURGERY

The animals which survived longer than 24 h were operated in anaesthesia induced by Equithesin (chloral hydrate, pentobarbital and magnesium sulphate, 3.3 ml/kg ip.) and atropine 0.3 mg for each rat. The animals which survived only 24 h were anaesthetized by Ketamine (87 mg/kg ip.) and Rompun (13 mg/kg ip.). The injections of the tracers were made while the head was fixed in a Kopf stereotaxic frame. The coordinates for the motor and premotor cortex were anterior 1.0 (motor) and 4.0 (premotor) mm from bregma, and lateral 1.5-3.0 mm. The coordinates for the cerebellar nuclei were: Posterior to bregma 11-12 mm, Lateral 2-4 mm, Depth 4.0 mm from the dural surface.

HISTOLOGY

Perfusions were performed with the animals in deep Equithesin anaesthesia. (For details on various aspects of methodology see Divac and Diemer 1980, Divac and Passingham 1980, Divac et al. 1987). The fluorescence was studied in a Leitz microscope equipped for epifluorescence. Autoradiograms were processed in MCID picture analysis system (Imaging Research Inc., Canada) to enhance the signal.

RESULTS

Simultaneous use of two tracers showed both of them in the same thalamic region. First, some perikarya were labelled somatopetally from the motor cortex and secondly, the radioactivity indicated the innervation from the cerebellar nuclei (Fig. 1).

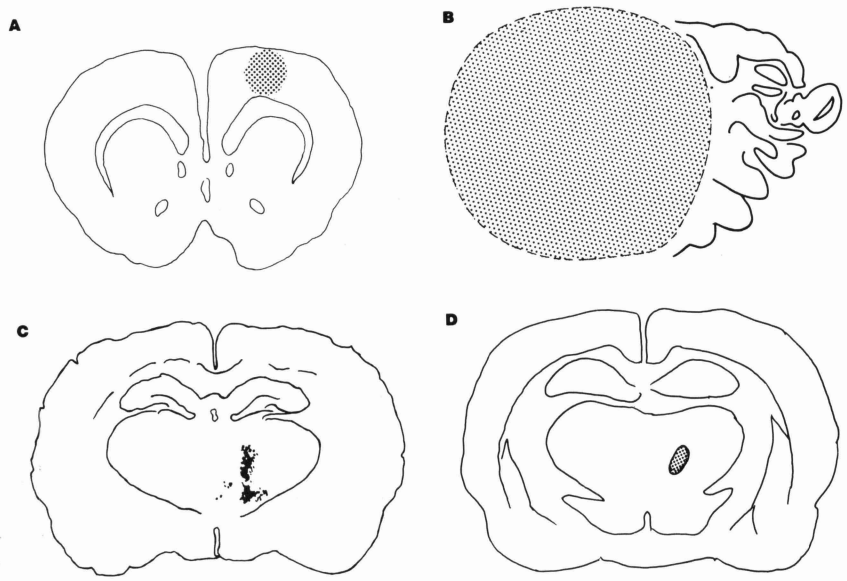
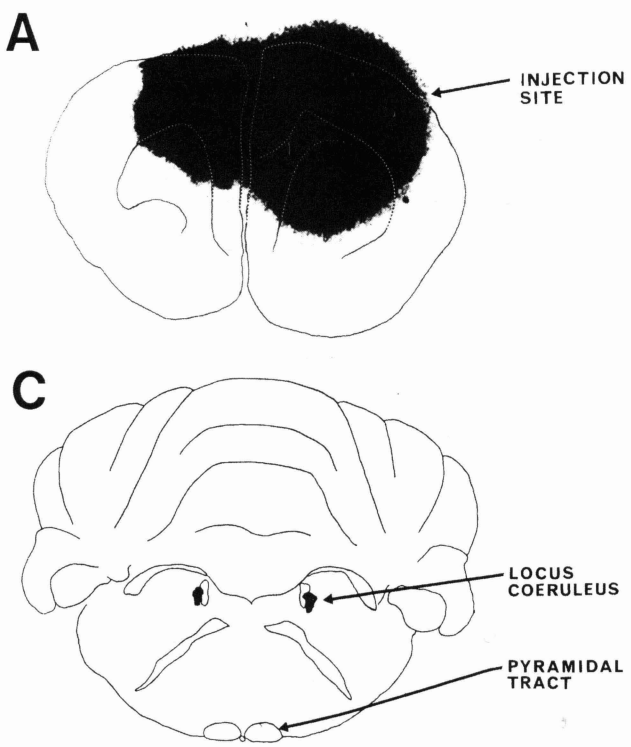


Fig. 1. Diagramatic presentation of the results of simultaneous injections of bisbenzimide into the motor cortex (A) and ^{125}I WGA into the cerebellum (B). In the same thalamic region adjacent sections revealed either bisbenzimide-labelled cell nuclei (C) or intense radioactivity (D).

Thus, at least on the light microscopic level, the cerebellofugal pathway terminates in the thalamic region from which the projection to the motor cortex originates.

In the brains with injections of radioactively labelled WGA either in the motor cortex or cerebellar nuclei, the radioactivity was found in the thalamus but not in the cerebellar nuclei or the motor cortex, respectively. At the ponto-cerebellar level the cortical injections labelled two formations only: locus coeruleus and the pyramidal tract (Fig. 2). In these animals no radioactivity was detected in the cerebellar nuclei (not illustrated).

In the brains in which WGA-HRP complex or biocytin were injected into the motor cortical area, microscopic examination revealed labelling in the



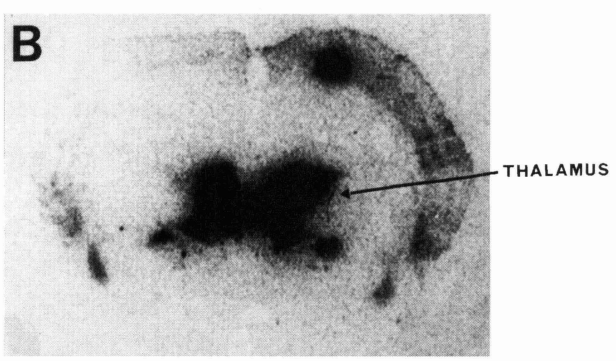


Fig. 2. The results in a rat with very large injection of ^{125}I-WGA into the motor area of both hemispheres (A). The thalamus was intensely labelled (B). Some radioactivity was found also in the locus coeruleus and pyramidal tracts (C). The more caudal sections in which cerebellar nuclei were present showed no radioactivity (not illustrated).

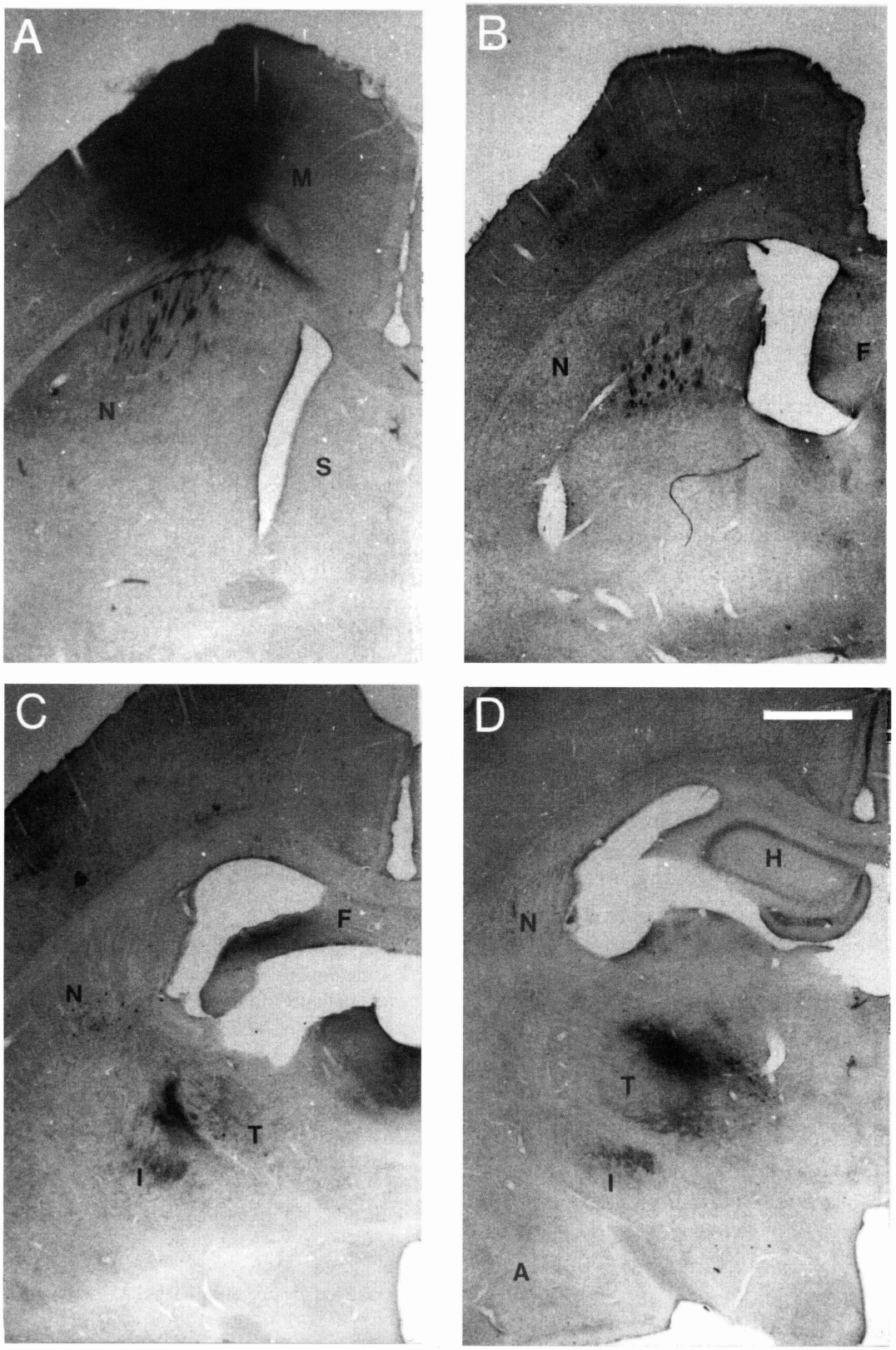


Fig. 3. The results in a rat with injection of WGA-HRP into the motor area. The labelling is strong in axons of the capsula interna, and in the thalamus, but no label was detected in the cerebellar nuclei (not illustrated). Abbreviations: A, amygdala; F, fornix; H, hippocampus; I, internal capsule; M, motor cortex; N, neostriatum; S, septum; T, thalamus.

thalamus but not in the cerebellar nuclei, regardless of the survival time employed (Fig. 3). Biocytin and WGA-HRP were found in the fibers of the pyramidal tract.

DISCUSSION

The overlap of labelling with the somatofugal tracers from the cerebellum and somatopetal tracers from the motor cortex in the thalamus of the rat indicates the existence of connections between the neurons of the cerebellar nuclei and the thalamic neurons with projections to the motor cortex. This result confirms earlier studies in the rat (Faull and Carman 1978, Haroian et al. 1981, Schim et al. 1981, Donoghue and Parham 1983, Angaut et al. 1985). Similar observations were made in the monkey (Percheron 1977, Asanuma et al. 1983).

The cerebello-thalamo-cortical path in the two species seems, however, to differ at least in one respect: In the monkey transmembrane transport of WGA-HRP from the cerebral cortex to the cerebellar nuclei has been reported (Wiesendanger and Wiesendanger 1985), whereas all our attempts to demonstrate the same in the rat have failed.

An obvious explanation of our failure could be inadequate methodology. Casagrande et al. (1990) reported that even different batches of WGA-HRP differ in their ability to be transported somatofugally, somatopetally and transneuronally. This, however, does not seem likely because of the several different approaches and tracers used, including attempts to replicate the Wiesendangers' method with WGA-HRP purchased from Sigma and Miles. Sigma's WGA-HRP is not made with aminocaproic acid which seems to prevent transmembrane transport of the tracer (Russell et al. 1991). Besides, we see invariably intense labelling in the thalamus and the brainstem (locus coeruleus and the pyramidal tract) near the level of the unlabelled cerebellar nuclei.

If no artefacts are involved, we are facing a species difference which may be resolved by comparative electronmicroscopy of the motor thalamus in monkeys and rats.

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