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Non-cholinergic basal forebrain neurons project to the contralateral basal forebrain in the rat

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Following injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) or the fluorescent tracer fluoro-gold into the magnocellular preoptic area and the horizontal limb of the diagonal band, retrogradely labelled neurons were found in the homotopic region of the contralateral basal forebrain. Labelled fibers apparently arising from these neurons travelled in the stria medullaris and the habenular commissure to terminate in the contralateral basal forebrain. Although the neurons retrogradely labelled with fluoro-gold in the contralateral basal forebrain were similar in size to choline acetyltransferase (ChAT)-immunoreactive neurons, and were intermingled with them, none was ChAT-positive. WGA-HRP injections into the nucleus basalis magnocellularis did not result in retrograde labelling in the contralateral basal forebrain. These findings suggest that non-cholinergic neurons may serve as a direct link between the two sides of selective magnocellular basal forebrain regions.

The presence of a projection from selective magnocellular basal forebrain regions to the homotopic regions of the contralateral basal forebrain has been known since early studies using degeneration [14] and tritiated amino acid autoradiography in the rat [17]. More recently, similar contralateral basal forebrain projections have been confirmed using retrograde tracing in cats [9] and primates [9, 15]. During the course of a study on the brainstem afferents to the magnocellular basal forebrain in the rat [16], we re-confirmed the projection connecting the two sides of the basal forebrain using retrograde axonal transport. Furthermore, the distribution and morphology of the retrogradely labelled neurons suggested the possibility that at least some of these neurons might be cholinergic. In the present communication, we report the results of studies employing retrograde transport of wheat germ agglutinin-conjugated horseradish peroxidase (WGA–HRP) following injections into selective regions of the magnocellular basal forebrain, and double labelling combining retrograde fluoro-gold transport and choline acetyltransferase (ChAT) immunofluorescence. The re-

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sults indicate that basal forebrain neurons projecting to the contralateral basal forebrain are not cholinergic.

Under pentobarbital (50 mg/kg i.p.) anesthesia, WGA–HRP (1% in saline, 0.025–0.05 μl, Sigma) was pressure-injected unilaterally into magnocellular basal forebrain regions including the magnocellular preoptic area (MgPA) and the horizontal limb of the diagonal band (HDB) [17] at the level of the decussation of the anterior commissure ($n=6$), or the nucleus basalis magnocellularis ($n=6$) in male Wistar rats, 150–250 g in body weight. The MgPA/HDB region has also been referred to collectively as a part of the nucleus of the horizontal limb of the diagonal band (e.g. ref. 14). Coordinates for the MgPA/HDB were: AP 7.7–7.8, L 2.0–2.2, D +0.3 to +0.9; and those for the nucleus basalis magnocellularis were: AP 7.2, L 2.7, D +2.8, all in reference to the interaural zero, with the incisor bar set at −4.2 mm. Six additional rats received an injection of fluoro-gold (4% in saline, 0.05 μl, Fluorochrome) into the MgPA/HDB. Injections were made over 10 min, and the injection needle was left in place for an additional 10 min. Histological processing for WGA–HRP histochemistry, and double labelling with retrograde fluoro-gold and ChAT immunofluorescence has been described in detail elsewhere [16]. Briefly, after a survival period of 1–2 (WGA–HRP) or 4–5 days (fluoro-gold), rats were overdosed with pentobarbital and perfused with an aldehyde fixative. Following cryoprotection, the brains were cut at 30 μm on a freezing microtome; sections containing WGA–HRP were subjected to histochemical reaction using tetramethylbenzidine as the chromagen [11]. Sections for double labelling were processed for immunofluorescence using a well-characterized rat monoclonal antibody to ChAT whose specificity has been previously documented [5]. Goat anti-rat IgG conjugated with Texas red or TRITC was used as the secondary antibody.

Six WGA–HRP injections included the MgPA/HDB. Of these, two were restricted to the MgPA/HDB region, three included parts of the olfactory tubercle, and one included the nucleus of the olfactory tract. In all these cases, retrogradely labelled neurons were found in the contralateral magnocellular basal forebrain at the level of the decussation of the anterior commissure rostrally, and the supraoptic nucleus caudally (Fig. 1). Larger numbers of retrogradely labelled neurons were seen following injections including the lateral, as compared to the medial, aspect of the MgPA/HDB. Rostrally, retrogradely labelled neurons were scattered within the contralateral MgPA/HDB (Fig. 1A, B). Caudally, labelled neurons increased in number, and formed a compact cluster dorsolateral to the supraoptic nucleus (Fig. 1C, D). Some additional labelled neurons were seen in the substantia innominata and lateral hypothalamus (Fig. 1D). Labelled fibers could be seen to course dorsally from the cell cluster (Fig. 2A), traverse the lateral preoptic area and lateral hypothalamus, and ascend in the stria medullaris, from which they entered the habenula; heavy punctate labelling was seen in the lateral part of the lateral habenula (not shown). Labelled fibers crossed the midline via the habenular commissure, and descended in the stria medullaris to enter the injection site in the contralateral basal forebrain. In addition to this pathway, labelled fibers were seen crossing the midline in the retrochiasmatic area; however, these fibers did not appear to arise from retrogradely labelled neurons

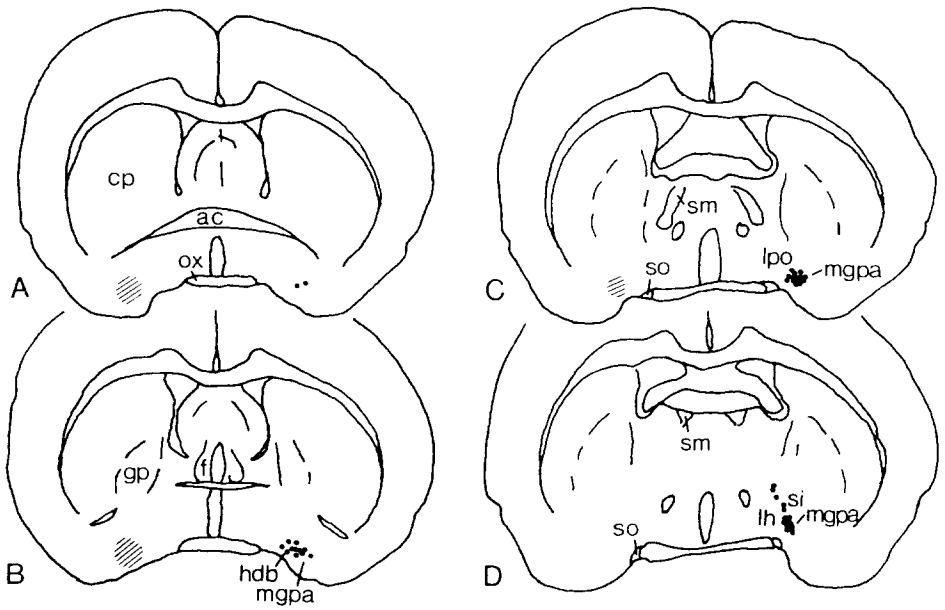

Fig. 1. Distribution of neurons retrogradely labelled in the contralateral basal forebrain (dots on the right side of each section) following WGA–HRP injection (stipling on the left) into the MgPA/HDB region. Sections in A, B are spaced at about 0.45 mm intervals. The number of retrogradely labelled neurons increased from A to D, amounting to 2, 11, 20 and 68, respectively. ac, anterior commissure; cp, caudate-putamen; f, fornix; gp, globus pallidus; hdb, horizontal limb of the diagonal band; lh, lateral hypothalamus; lpo, lateral preoptic area; mgpa, magnocellular preoptic area; ox, optic chiasm; si, substantia innominata; sm, stria medullaris; so, supraoptic nucleus.

in the contralateral basal forebrain. Punctate labelling suggestive of anterograde transport was also seen in the vicinity of labelled neurons in the contralateral basal forebrain (Fig. 2A). WGA–HRP injections into the nucleus basalis magnocellularis ($n=6$) did not result in labelled neurons or fibers in the contralateral magnocellular basal forebrain.

Fluoro-gold injections into the MgPA/HDB resulted in retrograde labelling in the contralateral basal forebrain (Fig. 2B) similar to that seen after WGA–HRP injections (Fig. 1). Examination of double labelling with ChAT immunofluorescence indicated that those neurons retrogradely labelled in the contralateral basal forebrain were similar in size to ChAT-immunoreactive neurons and intermingled with them; however, none was ChAT-positive (Fig. 2B, C). Retrogradely labelled neurons were less frequent than ChAT-immunoreactive neurons at rostral levels (Fig. 2B, C), whereas at caudal levels, usually only several ChAT-positive neurons were seen in or near a cluster of many retrogradely labelled neurons.

The present results confirm previous findings that there is a projection from the MgPA/HDB region of the basal forebrain to the homotopic region of the contralateral basal forebrain in the rat [14, 17], and in addition, indicate that this projection is not cholinergic. Since magnocellular neurons containing glutamate decarboxylase

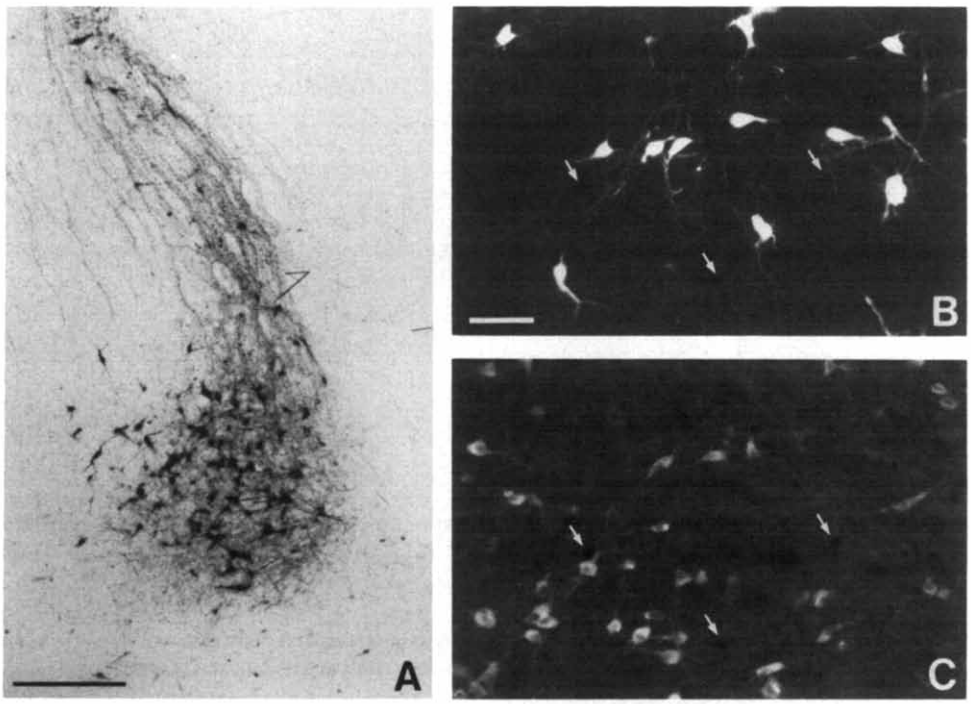

Fig. 2. A: micrograph showing a cluster of neurons labelled with retrograde WGA-HRP dorsolateral to the supraoptic nucleus at about the same level as in Fig. 1D. Dorsal, top; medial, left. Punctate labelling, suggestive of anterograde labelling, is also present in the vicinity of the retrogradely labelled neurons. Note labelled fibers arising from and/or terminating in the cluster of labelled neurons. Bar = 200 μm. B, C: retrograde fluoro-gold labelling (B) and ChAT immunofluorescence (C) of the same section through the MgPA/HDB region at about the same level as in Fig. 1B following fluoro-gold injection into the contralateral, homotopic area. Three pairs of corresponding blood vessels are indicated by arrows as landmarks for orientation. Although retrogradely labelled neurons are intermingled with ChAT-immunofluorescent neurons and similar in size to them, none of them is double labelled. Bar in B is 50 μm, and applies also to C.

(GAD) [2] or γ-aminobutyric acid (GABA) transaminase [13] are found in the MgPA/HDB region, at least some of the neurons giving rise to this commissural projection may use GABA as a neurotransmitter. GAD-immunoreactive terminals have been shown to make synaptic contacts with ChAT-positive neurons in the basal forebrain [19].

Consistent with previous findings [14, 17], the contralateral basal forebrain projection was found to follow a long caudally directed route, i.e. via the stria medullaris to enter the habenula, where it crosses the midline through the habenular commissure to follow the same route rostrally back to the basal forebrain. In addition, heavy anterograde labelling was seen in the lateral aspect of the lateral habenula in the present study. Previous studies using retrograde transport [1, 8] and degeneration [4] have reported that neurons in the nucleus of the diagonal band, preoptic area, and substantia innominata project to the habenula via the stria medullaris [1, 4, 8]. This pro-

jection has been suggested to contain cholinergic and GABAergic components [1, 3, 7, 10]. Taken together, these findings indicate that basal forebrain neurons projecting to the contralateral basal forebrain and the habenula follow the same path as far as the habenula. It is not known whether the terminal labelling seen in the lateral habenula represents axon collaterals of basal forebrain neurons projecting to the homotopic contralateral basal forebrain or a pathway from a distinct group of neurons.

The brainstem afferents to the nucleus basalis magnocellularis and MgPA/HDB regions, including those from cholinergic and aminergic cell groups, are generally similar [16]. These brainstem afferents are bilateral with ipsilateral dominance. The similar [16]. These brainstgem afferents are bilateral with ipsilateal dominance. The contralateral projection originating and terminating in the MgPA/HDB region appears to be specific insofar as only the MgPA/HDB region is involved and is the only known commissural connection between the two sides of the basal forebrain. It is not known whether there is a similar commissural connection in the medial septum or the vertical limb of the diagonal band.

The MgPA/HDB region of the magnocellular basal forebrain contains cholinergic neurons projecting, predominantly ipsilaterally, to various telencephalic as well as diencephalic structures [6, 12, 18]. Although it is yet to be determined whether the projection from the contralateral basal forebrain terminates upon cholinergic neurons and/or non-cholinergic neurons in the same region, the present findings indicate that non-cholinergic magnocellular neurons in the HDB/MgPA may serve as a direct link between the two sides of the basal forebrain.

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