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A Rapid Method for the Regional Dissection of the Rat Brain[1]

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HEFFNER, T. G., J. A. HARTMAN AND L. S. SEIDEN. *A rapid method for the regional dissection of the rat brain.* PHARMAC. BIOCHEM. BEHAV. 13(3) 453–456, 1980.—A method is described for the rapid dissection of seventeen areas of the rat brain. Regions from fresh unfrozen brain tissue are dissected from coronal brain slices obtained with use of a cutting block. This method is applicable to pharmacological and behavioral studies which require the dissection of numerous brains during short time intervals.

Brain dissection Catecholamines

STUDIES on the functional role of central neuronal projections require the use of a brain dissection procedure which provides consistent samples of nerve terminal fields. To date, this requirement has been met either by restricting investigation to relatively large brain areas which are easily differentiated by eye [2] or by punching cylindrical tissue samples from frozen [7] or unfrozen [3] brain sections. Although tissue punching techniques enable dissection of individual brain nuclei, a considerable investment of time is required to prepare the tissue (either with a cryostat or a vibratome) prior to obtaining tissue samples.

The present report describes a rapid method for dissection of the major nerve terminal fields of central catecholaminergic projections in the rat brain. Coronal slices from fresh brain tissue are first obtained with use of a cast aluminum cutting block. Brain regions are then dissected from the faces of these slices using common neuroanatomical landmarks. This method requires approximately 10 min for the dissection of up to seventeen brain regions.

METHOD

Materials

The brain cutting block (Fig. 1) is fashioned from an 8 cm length of aluminum alloy (1-1/4 in. bar stock, type 2024-T351, Williams and Company, Pittsburgh, PA). A trough (12.0 mm wide and 11.0 mm deep) cut along the length of this block accommodates brains from rats 30 days of age or older. Channels (12.0 mm deep) are cut through the sides and base of this trough with use of a circular screw slotting saw (2-3/4 in diameter, 0.014 in base width, Cleveland Twist Drill Co., Cleveland, OH). These channels (spaced by 1.5 or 2.0 mm) permit the insertion of standard single edge razor blades at right angles to the longitudinal axis of the trough.

Procedure

Brains are rapidly removed from rats, severing the cerebral axis at the level of the parietal bone. A brain is placed on its dorsal surface in the trough of the cutting block (Fig. 1). During use, the cutting block is kept cold on crushed ice. Razor blades (kept on ice) are carefully inserted through the cutting channels slicing the brain at right angles to the sagittal axis. The brain is positioned such that an initial razor blade can be inserted tangential to the most posterior aspects of the olfactory tubercles. This initial razor blade slices through the sagittal plane of the brain at the level of the body of the anterior commissure (Fig. 2.4; Fig. 18 from the atlas of König and Klippel [4]). The position of the initial razor blade serves as a reference point from which brain sections are obtained. Three razor blades are inserted anterior to the first blade, along the rostral extent of the brain, at intervals of 1.5 mm. Two razor blades are then inserted posterior to the first blade, along the caudal extent of the brain, at intervals of 2.0 mm. If the substantia nigra and ventral tegmentum are to be obtained, two additional razor blades are inserted posterior to the most caudal blade at intervals of 1.5 mm. The brain is thus divided into 9 sections (numbered rostrally to caudally) (Fig. 1). The razor blades are removed from the block with coronal brain slices adhering to their surfaces and are placed on a glass plate suspended on crushed ice. Brain regions are then dissected from these slices (Fig. 2) with use of fine iris scissors (George Tiemann and Co., Long Island City, NY). Tissue is taken bilaterally for all brain regions.

The *olfactory bulbs* are separated from the frontal poles in the first brain section (Fig. 2.1). The *frontal cortex* includes the frontal poles, cortical tissue from section 2 (see Fig. 2.2), as well as the cortical tissue superior to the rhinal sulcus from sections 3 and 4 (Fig. 2.3 and 2.4). The *remaining cor-*

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tex includes cortical tissue superior to the rhinal sulcus from sections 5–9 (Fig. 2.5 and 2.9).

The *nucleus accumbens* and *olfactory tubercle* are dissected from the rostral surface of the third brain section (Fig. 2.3). A wedge of brain tissue is obtained by cutting along two lines: one extending from the base of the lateral ventricle, through the anterior commissure to the medial edge of the lateral olfactory tract and the other connecting the base of the lateral ventricle and the base of the brain. The olfactory tubercle (located on the base of this wedge) is then trimmed from beneath the nucleus accumbens (see Fig. 2.3).

The *caudate putamen* and *septum* are dissected from the caudal surface of the fourth brain section (Fig. 2.4) based on their distinct morphological appearances. The caudate putamen includes tissue dorsal to the anterior commissure, ventral to the corpus callosum and medial to the external capsule. The stria terminalis is removed from the ventrolateral borders of the septum. The *globus pallidus* is dissected from the caudal surface of the fifth brain segment (Fig. 2.5) following the landmarks described for the caudate putamen. The most caudal aspects of the caudate putamen are included with the globus pallidus.

The *anterior hypothalamus* is obtained from the caudal face of the fifth brain section (Fig. 2.5) while the *posterior hypothalamus* is obtained from the caudal face of the sixth brain section (Fig. 2.6). The hypothalamic tissue extends dorsally to the columns of the fornix (anterior) or the mammilothalamic tracts (posterior) and laterally to the telencephalic-diencephalic junction. The *thalamus* is also dissected from the caudal face of the sixth brain section (Fig. 2.6), extending dorsally to the hippocampus, laterally to the crus cerebri and fimbria of the hippocampus, and ventrally to the medial lemniscus. The *amygdala* includes the tissue lateral to both portions of the hypothalamus (sections 5 and 6) and ventral to the rhinal sulcus. Wedges of tissue on the dorsomedial borders of the amygdala samples are discarded

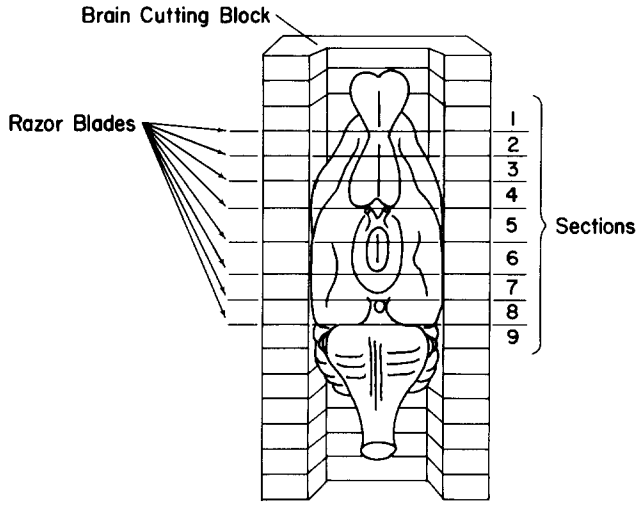

FIG. 1. Diagrammatic representation of brain cutting block illustrating orientation of brain and placement of razor blades to obtain coronal brain sections. The numbers on the right refer to brain sections described in the text and shown in Fig. 2.

(see Fig. 2.5 and 2.6) so as to exclude portions of the striatum and crus cerebri. The amygdala sample includes portions of the entorhinal cortex.

The *hippocampus* is separated from the midbrain and overlying cerebral cortex from sections 7 and 8 (Fig. 2.8 and 2.8) based on its distinct morphological appearance.

The *substantia nigra* and *ventral tegmentum* are dissected from the caudal face of the eighth brain section (Fig. 2.8). The dark oval shape of the substantia nigra is clearly visible on the ventrolateral portions of this section. A horizontal cut is made across the brainstem at the level of the

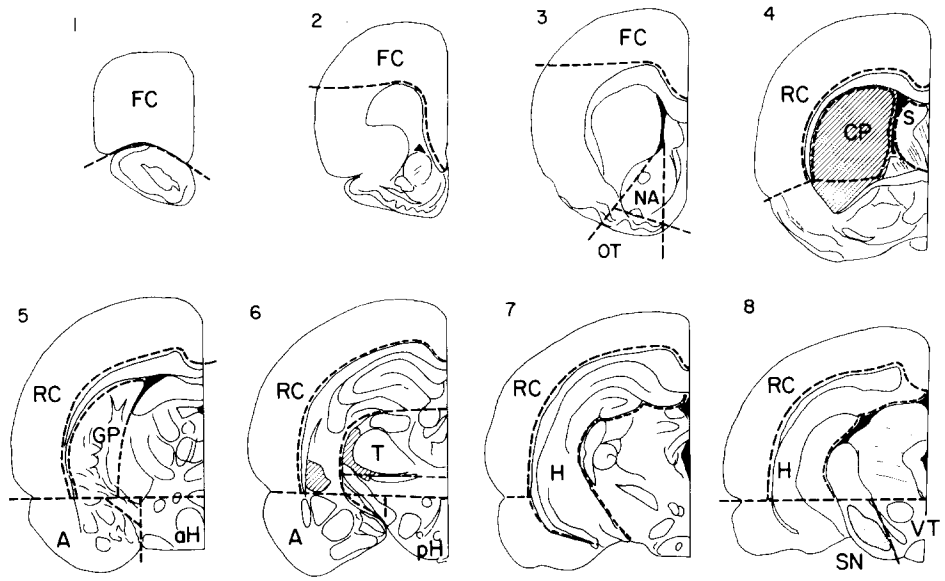

FIG. 2. Diagrammatic representation of coronal brain sections from which brain regions are dissected. Dotted lines indicate borders of brain regions. FC, frontal cortex; NA, nucleus accumbens; OT, olfactory tubercle; S, septum; CP, caudate putamen; RC, remaining cortex; GP, globus pallidus; aH, anterior hypothalamus; pH, posterior hypothalamus; A, amygdala; T, thalamus; SN, substantia nigra; VT, ventral tegmentum; H, hippocampus. Numbers correspond to brain sections shown in Fig. 1.

TABLE 1

REGIONAL CONCENTRATIONS OF NE AND DA IN THE RAT BRAIN

Brain region	Protein* (mg)	NE† (ng/mg protein)	DA† (ng/mg protein)
Olfactory bulb	11.47 ± 0.39‡	1.97 ± 0.14	0.24 ± 0.02
Frontal cortex	23.24 ± 0.52	2.14 ± 0.14	0.49 ± 0.03
Remaining cortex	46.13 ± 0.87	1.92 ± 0.11	0.15 ± 0.02
Nucleus accumbens	1.61 ± 0.10	5.50 ± 0.38	83.51 ± 3.27
Olfactory tubercle	1.39 ± 0.07	4.42 ± 0.24	58.43 ± 3.42
Caudate putamen	4.39 ± 0.19	1.83 ± 0.13	105.12 ± 5.85
Septum	1.37 ± 0.08	4.05 ± 0.23	8.82 ± 0.64
Globus pallidus	4.49 ± 0.31	2.26 ± 0.10	34.48 ± 2.20
Anterior hypothalamus	3.55 ± 0.19	26.01 ± 0.97	2.39 ± 0.12
Posterior hypothalamus	3.49 ± 0.13	19.87 ± 1.81	3.10 ± 0.30
Thalamus	3.69 ± 0.27	3.13 ± 0.18	0.67 ± 0.05
Amygdala	12.96 ± 0.30	2.48 ± 0.13	2.39 ± 0.19
Midbrain§	23.21 ± 0.86	5.51 ± 0.34	2.11 ± 0.13
Substantia nigra	1.38 ± 0.06	1.12 ± 0.11	3.89 ± 0.22
Ventral tegmentum	1.81 ± 0.11	3.88 ± 0.32	7.85 ± 0.53
Hippocampus	11.09 ± 0.60	2.89 ± 0.16	nd¶
Pons medulla	22.17 ± 1.30	6.71 ± 0.34	0.52 ± 0.06
Cerebellum	25.12 ± 1.22	2.60 ± 0.14	nd

*Determined by the Biuret procedure [5].
†Determined by radioenzymatic assay [1].
‡Mean ± SEM of 8 determinations.
§Midbrain contains the substantia nigra plus the ventral tegmentum and is not obtained when the latter two regions are analyzed separately.
¶Not detectable.

rhinal sulcus and dorsal edge of the nigra. After removing the telencephalic tissue lateral to the brainstem, nigral tissue is obtained by extending ventromedially-directed cuts to the base of the brainstem. The remaining tissue lying medial to the substantia nigra and ventral to the horizontal cut represents the ventral tegmentum. The *cerebellum* is then separated from the *pons-medulla* (section 9).

The *midbrain* can be obtained for experiments which do not involve separate analysis of the substantia nigra and ventral tegmentum. In this case, the two most caudally placed razor blades are not used resulting in a total of seven brain sections. After the cerebellum is removed from the brainstem, the midbrain and pons-medulla are separated by a cut at the level of the inferior colliculus. The hippocampus is then reflected and separated from the most caudal section of cerebral cortex.

RESULTS AND DISCUSSION

The dissection procedure provides consistent samples of the brain regions described as evidenced by low variability in regional tissue protein levels and regional tissue concentrations of norepinephrine (NE) and dopamine (DA) (Table 1). The levels of catecholamines in brain areas shown in Table 1 are in basic agreement with previously reported values [9]. The reproducible nature of this procedure stems from the consistency with which the brain sections described can be obtained by use of the cutting block. When using this method, care must be taken to insure correct placement of the initial razor blade. Inaccurate placement of this blade precludes consistent dissection of those brain regions which extend for limited distances along the longitudinal axis of the brain (e.g., nucleus accumbens). Use of the caudal borders of the olfactory tubercles as a baseline for brain sectioning not only provides the correct rostro-caudal reference level (at the body of the anterior commissure) but also enables the correct alignment of the coronal plane of the brain. The limited rostro-caudal extent of the body of the anterior commissure makes it an ideal landmark for confirming the correct placement of the initial razor blade.

The dissection procedure described is designed for obtaining various nerve terminal fields of central catecholaminergic neurons. Care is taken to provide adequate separation of adjacent brain regions primarily innervated by different midbrain dopaminergic nuclei. Most DA neurons arising in the substantia nigra terminate within the caudate putamen and globus pallidus (the nigro-striatal projection) while DA neurons which arise in the medial aspects of the ventral tegmentum project for the most part to non-striatal forebrain areas (the mesocortical projection) [6]. Although some degree of overlap occurs between these projections, functional [8], as well as anatomical distinctions appear to exist between nigro-striatal and mesocortical DA neurons. For this reason, some dorsal and lateral portions of the nucleus accumbens are excluded so as to prevent contamination of this region (predominantly innervated by mesocortical DA neurons) by caudate-putamen tissue (predominantly innervated by nigro-striatal DA neurons). The frontal cortex sample dissected with this method includes only tissue superior and rostral to the corpus callosum in order to insure that caudate-putamen tissue (containing 200-fold higher levels of DA) is not included. In support of the contention that these regions are adequately separated, we find that electrolytic lesions restricted to the medial aspects of the

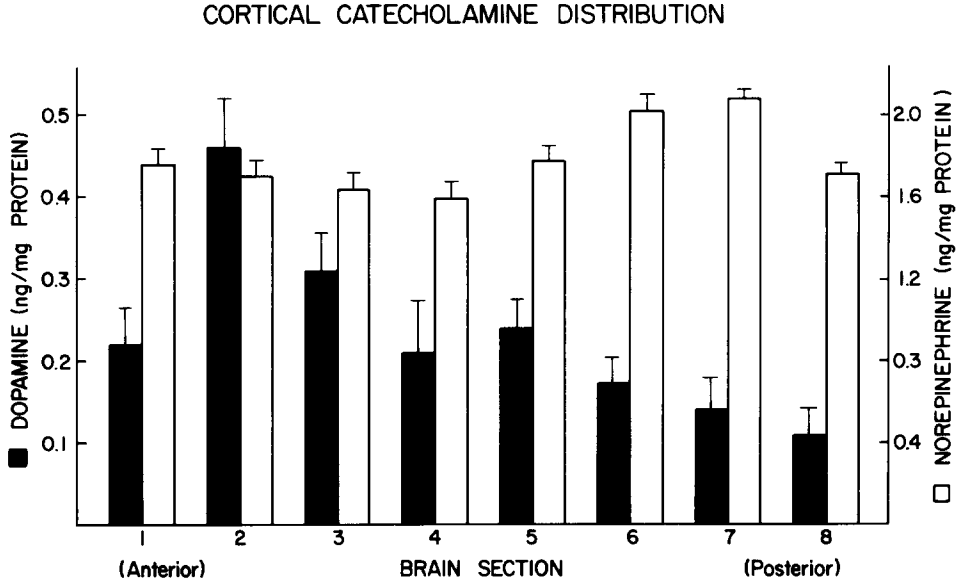

FIG. 3. Regional distribution of NE and DA in cerebral cortex from brain sections shown in Fig. 2.

ventral tegmentum produce 50–80% decreases in the levels of DA within known terminal fields of the mesocortical DA projection (nucleus accumbens, olfactory tubercle, septum and frontal cortex) but reduce DA levels within the caudate putamen by only 10–15%. These results are in accord with the anatomical distinctions made between mesocortical and nigro-striatal DA neurons [6].

The frontal cortex sample includes those rostral cortical areas most densely innervated by mesocortical DA neurons [6]. Analysis of DA and NE concentrations in the cerebral cortex (dorsal to the rhinal sulcus) from each of the nine brain sections reveals a rostral to caudal gradient in the levels of cortical DA, but not NE (Fig. 3). Cortical DA concentration appears relatively high in sections 1–4 (sections contained within the sample designated "frontal cortex") and is progressively lower in more caudal cortical areas. In contrast, similar levels of NE were found in rostral and caudal portions of the cerebral cortex. Portions of the entorhinal cortex are contained within the amygdala sample. Initial attempts to separate this tissue from the amygdaloid nuclei proper resulted in increased variability in estimates of both tissue protein and endogenous levels of DA within the amygdala.

The brain dissection technique described provides a consistent and rapid means of obtaining fresh brain tissue samples. This technique should prove useful in pharmacological and behavioral studies which require the dissection of numerous brains during short time intervals.

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