

the sample placed in a metal tray on the surface of solid paraffin was submerged when the latter melted. Paraffin blocks were prepared and 8 μ thick cross sections were cut.

In preliminary experiments the area postrema was located by selective *in vivo* staining with trypan blue (Wislocki & Putnam, 1920; Cappel, 1929). This served as a basis for the location of the area postrema by toluidine blue staining before fluorescence microscopy in adjacent sections.

Sections selected for fluorescence microscopy were flattened on the slide by rolling with a stainless steel rod. Sections were then covered with a cover slip on to which one drop of Entellan (Merck) had been placed.

Before examination by fluorescence microscopy the area postrema on the section was located by reflected tungsten light using the microscope (Zeiss) without an objective, and with phase contrast setting. The section was focused by the optovar ring of the microscope. This procedure eliminates the otherwise unavoidable fading of fluorescence due to ultraviolet light which occurs if the preliminary positioning of the section is carried out by fluorescence microscopy. The fluorescence of the sections was observed with a Schott BG3 as a primary and a 50 (Zeiss) as a secondary filter. The appearance of the area postrema by fluorescence microscopy of the untreated animals was found to be essentially the same as described by Fuxe & Owman (1965).

The fluorescence spectra of different areas of the brain section recorded by using a Schott UG1 as the primary filter, and an interference filter at 2.5 to 5 nm intervals in the transmission light path. From the green fluorescent cells in the area postrema reported to contain NA (Fuxe & Owman, 1965), a fluorescence spectrum different from that of NA in standard droplets was recorded. The possible explanation of this finding is that NA is present in low concentrations in these cells, and the non-specific fluorescence distorted the fluorescence spectrum of the NA derivative. Fluorescence spectra from areas exhibiting specific and non-specific fluorescence were recorded, and differences were found between the two types of fluorescence.

Details of the methodology and illustrations of the results will be demonstrated.

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Use of the iontophoretic and fluorescence histochemical techniques in investigations of the actions of drugs at synapses in the C.N.S.

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The iontophoretic technique is used extensively in investigations of the actions of postulated neurotransmitters, e.g. the catecholamines, and psychotropic drugs

in the central nervous system (Bradley, 1968). The formaldehyde-induced fluorescence technique of Falck, Hillarp, Thieme & Torp (1962) can be used for estimating the catecholamine content of presynaptic terminals in the brains of animals in which iontophoretic studies have been carried out.

The purpose of this demonstration is to show how these two techniques are used in conjunction in this laboratory to differentiate between pre- and post-synaptic actions of drugs and to study the way in which the post-synaptic actions of putative transmitters may be altered by changes in the levels of catecholamines in pre-synaptic terminals. The techniques will be demonstrated and results presented showing how the actions of sympathomimetic amines are affected by depletion of catecholamines in terminals by reserpine or synthesis inhibitors.

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A simple device for the construction of multibarrelled micropipettes

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Multibarrelled micropipettes are used in a number of laboratories for experiments involving iontophoresis. Herz, Wickelmaier & Nacimiento (1965) have described how a vertical micropipette puller can be used to form multibarrelled micropipettes from arrays of glass tubing glued together in metal rings. This demonstration shows how metal springs can be used to clamp arrays of glass tubing in the top chuck of a vertical micropipette puller; the glass tubes are fused together during the pulling process and the resulting electrode is strong enough for normal use, obviating any glueing. Two- to seven-barrelled micropipettes have been made with this machine and it is possible to produce satisfactory electrodes after only two or three days practice.

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Gas chromatographic method for the estimation of noradrenaline, dopamine and 5-hydroxytryptamine

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A procedure has been developed for the estimation of noradrenaline, dopamine and 5-hydroxytryptamine in the rat brain using gas chromatography with electron capture detection. Amounts of the catecholamines as low as 5 ng and of 5-hydroxytryptamine, 10 ng, can be measured in a single piece of brain tissue.

The brain samples are homogenized in n-butanol, according to Ansell & Beeson (1968), and after centrifugation the amines are returned to the aqueous