In order to achieve the great stability which is required for such studies, we had to reduce pulsation of the brain tissue to a minimum. At the same time, it was necessary to provide means for the continuous removal of excess c.s.f. since halothane anaesthesia increases c.s.f. production and thereby raises intracranial pressure. Conventional techniques try to solve these problems separately: pulsation is reduced either by mechanical stabilization of the area of recording (pressors, agar gel, paraffin wax, etc.) or by the application of bilateral pneumothorax and artificial ventilation; intracranial pressure is reduced by the intermittent or continuous withdrawal of c.s.f. from the cisterna magna. This latter procedure in itself enhances pulsation and promotes further c.s.f. secretion. We have found that these techniques could not provide the stability required for lengthy studies.

We have developed a new technique which offers the advantages inherent in recording from a practically closed skull, and at the same time, ensures means for continuous c.s.f. drainage. A small hole (less than 2 mm in diameter) is made in the skull with a dental burr. Then, under microscopic control, the dura is incised, and the incision dilated with a glass probe (the area of cortex exposed measures approximately 1 mm$\times$300 μ). The arachnoid is then ruptured, allowing c.s.f. to leak out, and the microelectrode is introduced under microscopic control.

This technique has several advantages. Firstly, it does not grossly interfere with the intricate physiological relationships between cerebral circulation, c.s.f. production and resorption, and the mechanical stability of the brain tissue. Thus movement of the tissue due to pulsation and raised intracranial pressure is minimized. This ensures a much more stable recording condition than is achieved by conventional techniques. Secondly, the incision in the dura allows a continuous drainage of c.s.f. It is not necessary, therefore, to provide further means for the removal of c.s.f. Thirdly, surgical intervention is reduced to minimum.

Using this technique, we are now able to study isolated single units of unchanging spike amplitude for two hours or more.

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Investigations on the histochemical demonstration of noradrenaline and 5-hydroxytryptamine in the area postrema of the rabbit by fluorescence microscopy

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Modifications of the method for the histochemical identification of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) (Falck & Owman, 1965) are described. Brain tissue containing the area postrema was dissected from control and 5-HT treated animals (Dow, Laszlo & Ritchie, 1972), frozen in isopentane cooled with liquid nitrogen. The frozen tissue samples were stored in liquid nitrogen until freeze dried. Formaldehyde treatment was performed with paraformaldehyde equilibrated at 58% relative humidity, this degree of humidity having been found the most suitable for investigation of the area postrema by fluorescence microscopy. During vacuum embedding the original orientation of the tissue was maintained:

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 UGl 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