

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.

REFERENCES

- BRADLEY, P. B. (1968). Synaptic transmission and its relevance for drug action. *Int. Rev. Neurobiol.*, **11**, 1-56.
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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.

REFERENCE

- HERZ, A., WICKELMAIER, M. & NACIMIENTO, A. (1965). Über die Herstellung von Mehrfachelektroden für die Mikroelectrophorese. *Pflügers Arch. ges. Physiol.*, **284**, 95-98.

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

phase by shaking the clear supernatant with water and iso-octane. The catechols are removed by absorption on to alumina and recovered from it using perchloric acid elution. Following this they are extracted using an ion pairing compound dissolved in chloroform and then returned to an aqueous phase by shaking the organic layer with formic acid. The solution is evaporated to dryness and the amines reacted with trifluoroacetic anhydride using methyl cyanide as the solvent.

The 5-hydroxytryptamine is recovered from the alumina supernatant by multiple extractions with a 20% v/v solution of n-butanol in diethyl ether, the resultant solution is evaporated to dryness, and derivatized in a similar manner to that described for the catechols.

The gas chromatography is carried out on a 5% SE-52, coated on 100–120 mesh Gas Chrom Q under temperature programmed conditions, and the derivatives are detected with an electron capture detector containing a nickel-63 source.

REFERENCE

- ANSELL, G. B. & BEESON, M. F. (1968). Rapid and sensitive procedure for the combined assay of noradrenaline, dopamine and serotonin in a single brain sample. *Anal. Biochem.*, **23**, 196–206.

A solid-state iontophoretic current switch

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For microiontophoresis it is necessary to pass very small constant currents through glass microelectrodes. These currents are usually obtained by applying large D.C. voltages (up to 250 v) across resistances of high value in series with the electrode, and the switching of such voltages by mechanical devices can result in considerable switching artefacts which disturb the recordings.

The demonstration consists of a 4-channel Iontophoresis Current Switch, using high voltage transistors and powered by a common mains supply. Each channel is controlled automatically by logic, following a START command (which can be manual or by a start pulse), thus providing precisely timed drug applications and eliminating errors.

The switch uses three transistors only, in a simple circuit. An NPN transistor switches positive voltages and a PNP, negative; a third transistor acts as an emitter-follower, driving the PNP negative switching transistor. Both switches are controlled by a common positive input which is the output of the controlling logic circuit. Outputs from both positive and negative switching transistors are summed, the junction being wired to a high value resistor (500 M Ω) situated remotely, near to the preparation. This arrangement keeps the sensitive parts of the circuit short and minimizes noise due to interference.

The switch has one normal state; when the logic circuit output is "O", the PNP transistor is switched "OFF" and the NPN transistor "ON", and this results in a negative potential appearing at the summing junction. When "1" appears, the reverse happens, producing a positive potential at the junction. The normal state can be arranged by operating a switch, so that either positive or negative ions