an overall accuracy of 100±5%. Interfering peaks were not found on analysing many samples of urine from many subjects.

A Perkin Elmer F11 gas chromatograph was used. The column was a 100–120 mesh acid-washed celite 545, onto which was coated 5% potassium hydroxide, and 10% carbowax 6000, and packed into a 2 metre 1/8 in o.d. stainless steel tube. The column was silizanised with hexamethyldisilizane at the operating conditions which were as follows: column temperature 140°; injection block temperature 250°; nitrogen flow rate 20 ml/min (measured at room temperature); hydrogen pressure 15 lb/in$^2$; air pressure 25 lb/in.$^2$

Amphetamine was further identified by alteration of its retention time by conversion to its acetone derivative as follows. Acetone (0·5 ml) was added to the ether concentrate in a tapered test-tube and evaporated to about 50$\mu$l on a water-bath at 60°. The concentrate (3–5 $\mu$l) was introduced into the chromatograph; a characteristic shift of the amphetamine peak to the amphetamine: acetone derivative peak was observed (see Fig. 1). The observed shift differentiates amphetamine from other related amines chromatographed in the presence and absence of acetone, e.g. see Fig. 1.

The total time necessary for the analysis of sixteen samples is about 6 hr, which is shorter than that for the previous method (Beckett & Rowland, 1964). Furthermore less sample is required and assays of 0·1 $\mu$g amphetamine base per ml urine may be made without interference from constituents in urine.

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## References

Beckett, A. H. & Rowland, M. (1964). *J. Pharm. Pharmacol.*, **16**, *Suppl.*, 27*T*–31*T*.
Brochmann-Hanssen, E. & Svendsen, A. B. (1962). *J. pharm. Sci.*, **51**, 938–941.
Cartoni, G. P. & Stefano, F. de, (1963). *Italian. G. Biochim.*, **8**, 298–311.

## The pharmacology of hippocampal neurones

SIR,—Little is known of the direct response of cells in the hippocampal cortex to potential cerebral neurotransmitters. We have applied various drugs microelectrophoretically into the environment of these cells and tested their effects on cell firing. This letter reports some preliminary findings.

Our experiments were made on nine adult cats under chloralose-urethane or pentobarbitone anaesthesia. The overlying neocortex was sucked away and the lateral ventricle was filled with 3% agar, and the exposure of the dorsal hippocampus completed. The activity of cells in the CA1 and CA3 fields was recorded extracellularly with the central 2·7 molar NaCl filled barrel of a 5-barrelled glass micropipette. The total tip diameter was 4–8$\mu$. Cell firing was monitored continuously on an oscilloscope and with an audioamplifier. The spikes were also led through a discriminator to a ratemeter the output of which was led to a penwriter.

The other 4 barrels of the microelectrode contained strong aqueous solutions of ionizable drugs. These were expelled from the electrode tip by the passage of appropriate currets.

Over 150 cells have been detected—these either fired spontaneously or could be excited by L-glutamate (confirming unpublished observations by Andersen,

Crawford & Curtis, cited in Crawford & Curtis, 1964). The application of L-glutamate often resulted in the development of high frequency synchronised activity (30–50/sec) making unit recording difficult. γ-Aminobutyric acid had a marked depressant action on all the cells tested. As in the necortex these substances had a characteristic short latency before their effects were observed and their effects ceased within a second of turning off the expelling current.

Nearly 90 cells have been tested with acetylcholine. About half of them were excited. This excitation had a characteristic long latency and persisted for many seconds after stopping the expelling current. Other choline esters, acetyl-β-methylcholine and carbamylcholine, also excited these cells. These cholinoceptive cells are chiefly concentrated in the superficial layers of the hippocampal cortex, between the alvear surface and 1 mm deep. Their receptors are principally muscarinic as atropine selectively blocked the excitatory response to acetylcholine but had no effect on that of L-glutamate, while dihydro-β-erythroidine was ineffective. However, with dimethyl-(+)-tubocurarine the acetylcholine response could occasionally be selectively depressed for many minutes. Further investigation is needed to determine whether acetylcholine is acting on pyramidal cells and has a genuine transmitter action at this site.

Most of the cells reacting to 5-hydroxytryptamine showed a progressive depression and some of them took up to 45 sec before they recovered their former excitability. A small number of cells were excited by 5-hydroxytryptamine.

The responses of hippocampal cells to these substances are qualitatively similar to those described for neocortical cells by Krnjević & Phillis (1963).

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## References

Crawford, J. M. & Curtis, D. R. (1964). *Brit. J. Pharmacol.*, **23**, 313–329.
Krnjević, K. & Phillis, J. W. (1963). *J. Physiol. (Lond.)*, **165**, 274–304.