Nature (Lond.) 220, 586-587 (1968). LSD 1745/PSI 229

frequency reaches the brain, but the response patterns of higher neurones may be influenced by inhibitory interactions between various auditory inputs[9].

This work was supported by the Anti-Locust Research Centre, London, the Carlsberg Foundation and Statens almindelige Videnskabsfond.

AXEL MICHELSEN

Zoophysiological Laboratory B,
University of Copenhagen,
Denmark.

Received July 29; revised August 23, 1968.

1 Horridge, G. A., *Nature*, **185**, 623 (1960).
2 Horridge, G. A., *Proc. Roy. Soc.*, **155**, 218 (1961).
3 Popov, A. V., *J. Evol. Biochem. Physiol.*, **1**, 239 (1965).
4 Michelsen, A., *J. Insect Physiol.*, **12**, 1119 (1966).
5 Gray, E. G., *Phil. Trans. Roy. Soc.*, B., **243**, 75 (1960).
6 Michelsen, A., *J. Insect Physiol.* (to be submitted for publication).
7 Popov, A. V., in *Evolutionary Neurophysiology and Neurochemistry*, 54 (Nauka, Leningrad, 1967).
8 Adam, L.-J., and Schwartzkopff, J., *Z. Vergl. Physiol.*, **54**, 246 (1967).
9 Yanagisawa, K., Hashimoto, T., and Katsuki, Y., *J. Insect Physiol.*, **13**, 635 (1967).

Behavioural Effects of some Morphine Antagonists and Hallucinogens in the Rat

Corne and Pickering[1] found a good correlation between activity in inducing a behavioural response (head twitches) in mice and the hallucinogenic activity in man of several drugs, which included atropine, hyoscine, lysergide (LSD), mescaline, *N*,*N*-dimethyltryptamine, phencyclidine, psilocin and psilocybin. Drugs that did not cause head twitches in mice included the morphine antagonists cyclazocine (personal communication from S. J. Corne) and nalorphine, both of which are known to cause hallucinations in man[2,3].

While working on morphine antagonists in this laboratory, I noticed that large doses of cyclazocine induced bizarre behaviour in the rat, including side to side head movements, pivoting on the hind feet and walking backwards. Walking backwards has been observed before in the rat after hallucinogenic amphetamine derivatives, mescaline[4] and phencyclidine, and so I have studied several morphine antagonists and other reference drugs for their bizarre behavioural effects in this species, in the hope of obtaining a correlation with hallucinogenic activity in man.

The experimental procedure and method of recording results were as follows. Drugs were administered subcutaneously in a dose volume of 10 ml./kg body weight to groups of five male weanling Wistar rats weighing 40–80 g. Twenty minutes after the drug had been given, each rat was observed in an empty metal food hopper for 1 min. The number of times that the rat turned its head to the right was counted. Next the rat was placed in a shallow translucent plastic box for 1 min. The number of times that the rat pivoted in a semicircle or in a full circle on its hind feet or walked backwards was noted. Each rat was then placed for up to 1 min on the surface of a drum rotating at 1 r.p.m. to test for discoordination[6]. From comparison with counts for untreated control animals, the following criteria were used to determine a positive effect: (1) a rat turning its head to the right twenty times or more in a minute; (2) a rat pivoting in a semicircle twice or more in a minute; (3) a rat walking backwards once or more in a minute. The percentage of rats showing each of these effects was calculated and the mean determined.

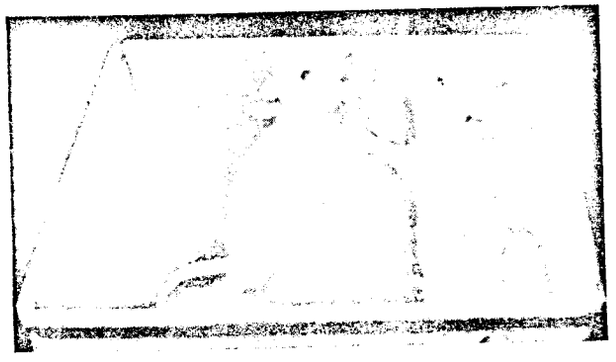


Fig. 1. Photograph of rats fighting 20 min after they had been given 2·5 mg/kg of apomorphine subcutaneously. LSD (5 mg/kg) subcutaneously caused similar effects.

Table 1. BIZARRE BEHAVIOURAL EFFECTS CAUSED BY SOME DRUGS IN THE RAT

Drug	Dose (mg/kg s.c.)	Per cent effect				Per cent discoordinated
		HM	P	WB	Mean	
Cyclazocine	2·5	20	33	50	34	33
	5·0	80	93	80	84	80
Levallorphan	20	20	20	40	27	20
	40	100	40	100	80	100
Nalorphine	320	0	0	20	7	20
Pentazocine	20	0	0	0	0	0
	40	0	0	40	13	70
	60	0	0	80	27	100
Morphine	160	0	0	0	0	100
Profadol	80	0	0	0	0	100
Amphetamine	80	100	80	80	87	100
Atropine	320	60	20	20	33	40
Mescaline	160	40	20	0	20	20
Phencyclidine	0·6	20	20	0	13	0
	2·5	80	100	40	73	100
Psilocybin	80	20	0	20	13	40
	120	60	60	40	53	80
Saline	*	0	0	0	0	0

Doses are expressed as mg base/10 ml./kg body weight. Responses were observed 20–24 min after subcutaneous administration of drug. HM indicates the percentage of rats making twenty or more turns of the head to the right during 1 min; P indicates the percentage of rats that pivoted in a semicircle twice or more during 1 min; WB indicates the percentage of rats that walked backwards, when placed for 1 min in an observation box. The score is the mean of the percentage of rats showing head movements, pivoting and walking backwards. The percentage discoordinated is the percentage of rats that fell from the surface of a drum rotating at 1 r.p.m. during 1 min.
* 10 ml./kg of 0·9 per cent (w/v) NaCl solution.

The morphine antagonists, cyclazocine and levallorphan, as well as amphetamine, phencyclidine and psilocybin, induced marked ataxia and the bizarre behaviour characterized by the three effects described here. The effects all occurred in doses large enough to cause the rats to fall within a few seconds from the surface of the rotating drum. Detailed results in the rat are given in Table 1, in which the doses listed for each drug include those causing the maximum effects. Table 1 shows that for each of the drugs active in this test, the percentage of rats showing bizarre behaviour was similar to that discoordinated, whereas the analgesics, morphine and profadol [*m*-(1-methyl-3-propyl-3-pyrrolidinyl) phenol][7], at the doses shown, discoordinated all rats, but caused little or no bizarre behaviour. Ethanol, too, in 40 per cent (v/v) aqueous solution given orally in a dose of 10 ml./kg discoordinated 80 per cent of rats without causing bizarre behaviour. The morphine antagonist pentazocine, in a dose of 60 mg/kg, caused head and body shakes, walking backwards and discoordination in most rats. The weak activity of nalorphine is consistent with the finding that, unlike other hallucinogenic drugs in these tests, it did not cause hyperthermia in the rabbit[8]. It was surprising that doses of nalorphine as large as 320 mg/kg discoordinated only 20 per cent of rats. The behavioural reactions of rats to hallucinogenic drugs described here were consistent with subjective effects of the drugs in man[9].

Unexpectedly, LSD in doses of 0·3 to 20 mg/kg subcutaneously did not cause all the types of bizarre behaviour described here, but it caused some walking backwards (20 per cent). The most striking effect of LSD at 5 mg/kg was fighting, similar but not identical to that observed after the administration of 2·5 mg/kg of apomorphine subcutaneously (Fig. 1). Apomorphine caused much more aggressive behaviour than did LSD.

In these experiments large doses of drugs known to be hallucinogenic in man, including cyclazocine and levallorphan, caused bizarre behaviour in the rat, characterized by side to side movement of the head, pivoting on the hind feet or walking backwards. A test based on these behavioural effects may be useful in predicting the hallucinogenic activity of new drugs in man.

I thank Dr H. O. J. Collier for advice and encouragement and the Sterling-Winthrop Research Institute for cyclazocine and pentazocine.

C. SCHNEIDER

Department of Pharmacological Research,
Parke, Davis and Company,
Hounslow, Middlesex.

Received August 26, 1968.

[1] Corne, S. J., and Pickering, R. W., *Psychopharmacologia*, **11**, 65 (1967).
[2] Martin, W. J., *Pharmacol. Rev.*, **19**, 463 (1967).
[3] Keats, A. S., and Telford, J., *J. Pharmacol. Exp. Ther.*, **143**, 157 (1964).
[4] Smythies, J. R., Johnston, V. S., Bradley, R. J., Bennington, F., Morin, R. D., and Clark, jun., L. C., *Nature*, **216**, 128 (1967).
[5] Leonard, B. E., and Tonge, S., *Brit. J. Pharmacol.*, **32**, 415 (1968).
[6] Collier, H. O. J., Hall, R. A., and Fieller, E. C., *Analyst*, **74**, 592 (1949).
[7] Winder, C. V., Welford, M., Wax, J., and Kaump, D. H., *J. Pharmacol. Exp. Ther.*, **154**, 161 (1966).
[8] Jacob, J., and Lafille, C., *Arch. Intern. Pharmacodyn.*, **145**, 528 (1963).
[9] Hoffer, A., and Osmond, H., in *The Hallucinogens* (Academic Press, New York and London, 1967).

Relaxation of Anaesthetics in the Presence of Cyto-membranes

WE recently showed that the nuclear magnetic relaxation rate of benzyl alcohol is increased in the presence of erythrocyte membranes and we concluded that this is caused by restriction of the molecular motion of the alcohol molecules imposed by the membrane structure[1]. With changes in alcohol concentration the relaxation rate altered in a characteristic way (Fig. 1), and it was suggested that alterations in membrane structure were induced by the alcohol which might be correlated with the physiological changes found in anaesthetic action.

We have now examined this effect with preparations of myelin, synaptosomes and membranes from a largely unmyelinated nerve, the rabbit vagus. Myelin and synaptosomes were prepared by the method of Whittaker[2]. Vagus nerves from the rabbit were desheathed and homogenized in 0·32 M sucrose. The large particles were removed by slow speed centrifugation, and the membrane fraction was centrifuged at 20,000*g* for 15 min and then resuspended in buffer. The measurements of the aromatic line of benzyl alcohol were carried out in a Varian *A*60*A* NMR spectrometer at 25° C as described previously. The results are presented in Fig. 1 as line widths (or relaxation rate), where an increase in line width corresponds to a decrease in molecular motion. The general form of the curves was similar for all membranes and showed an upswing in line width at a concentration above 100 mM. We have previously shown that this corresponds to the concentration at which benzyl alcohol haemolyses intact erythrocytes. Membrane preparations other than erythrocytes gave unstable suspensions, and line width measurements were correspondingly variable; there were also slow changes with time especially at high alcohol concentrations.

We have also examined the behaviour of two other alcohols, neopentanol and trichlorethanol, as well as lignocaine, a cationic local anaesthetic, in erythrocyte membranes (Fig. 2). Neopentyl alcohol shows a similar upswing at high concentration to that found with benzyl alcohol, but at a low concentration the relaxation rate was essentially independent of concentration. The lignocaine curve bears a close resemblance in form to benzyl alcohol, differing only in the effective concentration range and the extent of the line broadening. Trichlorethanol also shows similarities, but the insensitivity of the technique did not permit us to extend the measurements to low concentrations.

The results show that the characteristic changes in the relaxation of benzyl alcohol induced by erythrocyte membranes are confined neither to this type of membrane

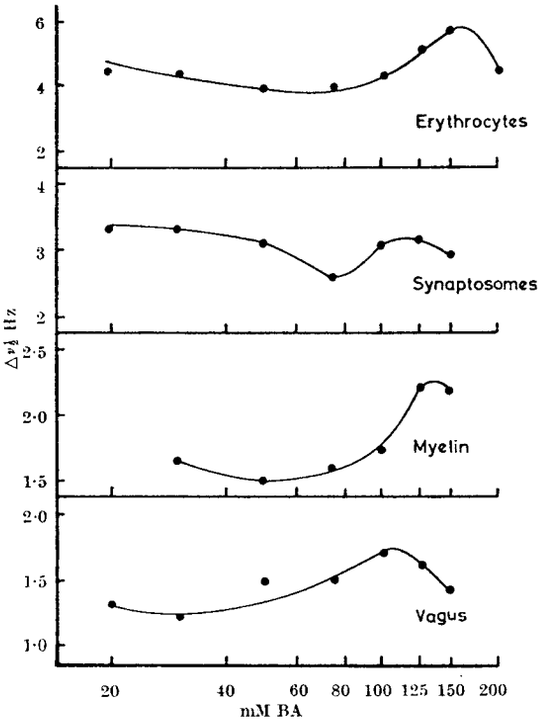


Fig. 1. Line widths ($\Delta\nu_{\frac{1}{2}}$) of the aromatic protons of benzyl alcohol in the presence of various membranes at 1·0 per cent w/v. D_2O buffer containing 100 mM NaCl; 40 mM Na acetate; 10 mM Na phosphates, *p*H 7·2. Measurements at 25° C.

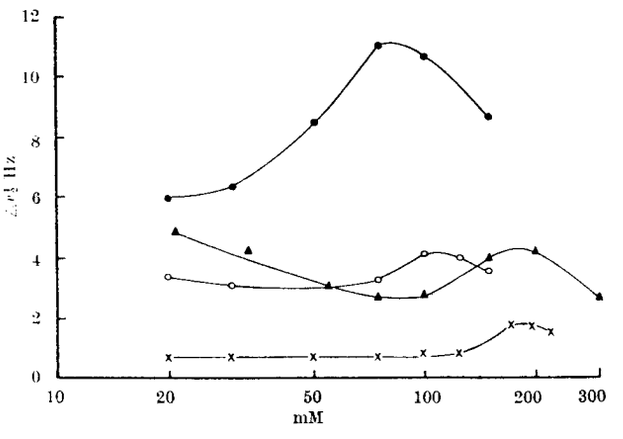
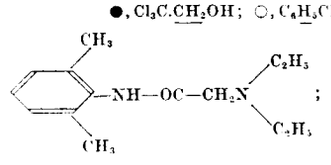


Fig. 2. Line widths of NMR signals from various anaesthetics in the presence of 1·0 per cent erythrocyte membranes. Proton signals measured are underlined. D_2O buffer containing 45 mM NaCl; 30 mM Na acetate; 10 mM phosphates, *p*H 7·2. Measurements at 42° C

●, $Cl_3C.\underline{CH_2}OH$; ○, $C_6\underline{H_5}CH_2OH$;

▲,  ; ×, $[\underline{CH_3}]_3C.\underline{CH_2}OH$.