Turner (1968) found that promethazine (25 mg) produced significant reduction in c.f.f. and the present study has also shown that the same dose impairs hand-eye co-ordination. The absence of effects on the peripheral components of visual function that were measured would suggest that these effects of promethazine are predominantly, if not wholly, central. No subjective effects of sedation were reported during the 3 h of the experiment when changes in co-ordination were observed, although sedation about 6 h after taking promethazine was noted by all the subjects. This suggests that the hand-eye co-ordination test described, like c.f.f., is able to demonstrate central effects of drugs at a time, or at a dose, when subjective evidence of sedation is not noted. For this reason, and because of its simplicity and the few subjects required, it may prove a useful test in the screening of new drugs for effects on the central nervous system.

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Brain acetylcholine and monoamines during experimental catatonia

Disorders of the extrapyramidal motor system occur in man after large doses of chlorpromazine and reserpine. When given to animals in correspondingly large doses, these drugs bring about a state of catatonic immobility. Bulbocapnine, a drug classically associated with experimental catatonia (De Jong, 1945) also evokes extrapyramidal signs in man (Henner, 1928). It is known that chlorpromazine and reserpine affect the function of monoamine-containing neurons in the brain. We have, therefore, measured the concentrations of 5-hydroxytryptamine (5-HT), noradrenaline and dopamine in the brains of rats made catatonic with bulbocapnine, and also in rats subjected to sound-induced seizures, a procedure which is followed immediately by a period of catatonia (Stainbrook & De Jong, 1943). There is also some evidence that the catatonic state in animals might be associated with an excess of free acetylcholine in the brain, since intracerebral injection of acetylcholine or cholinesterase-inhibiting drugs produces catatonia (Feldberg & Sherwood, 1954; Wada, 1962; Kassil, Latash & Ruthman, 1963). Furthermore, remission from catatonic stupor has been obtained both in animals (Sherwood, Ridley & McCullock, 1952) and in man (Sherwood, 1952) by the intraventricular administration of cholinesterase. We have examined the effect of drug-induced and post-seizure catatonia on the concentration of "free" and "bound" acetylcholine and also the total concentration of acetylcholine in rat brain. No assertions have been made concerning the identity and significance of the "free" and "bound" fractions of brain acetylcholine, but a number of drugs have been shown to affect them differently

(Crossland & Slater, 1968). "Bound" acetylcholine probably represents the stores of transmitter, some of which will be contained in synaptic vesicles, whereas the "free" fraction might be thought of as a mixture of three components comprising newly synthesized acetylcholine in cell cytoplasm, acetylcholine that has been released from neurons and some "bound" acetylcholine which must inevitably be released during the homogenization.

Female Wistar rats, 100–120 g, had catatonia produced by intraperitoneal injection of bulbocapnine, chlorpromazine or reserpine phosphate. Control animals received intraperitoneal injection of 0·9% saline solution. The rats were positioned with their front paws resting on a horizontal rod placed 12 cm above the bench surface, and scored as catatonic if they maintained this unnatural position for at least 1 min. The rats observed in the experiments involving post-convulsive catatonia were all of one particular strain of which approximately 60% were susceptible to sound-induced seizures. A brief exposure to the sound of an electric bell produced convulsions, invariably followed by a period of catatonia. For the estimation of total brain acetylcholine, rats were killed by immersion in liquid air. The frozen brains were crushed and the total acetylcholine was extracted with acid-alcohol. "Free" and "bound" acetylcholine was extracted from the non-frozen brains of rats killed by decapitation. These brains were first homogenized in saline solution to extract "free" acetylcholine and then extracted with acid-alcohol to obtain the "bound" fraction (Crossland & Slater, 1968). The acetylcholine in the final extracts was assayed biologically using the neostigminized frog rectus abdominis muscle. Noradrenaline (Anton & Sayre, 1962), dopamine (Anton & Sayre, 1964) and 5-hydroxytryptamine (Mead & Finger, 1961) were assayed in the brains of decapitated rats. Rats injected with bulbocapnine had the 5-HT extracted from the brain by the butanol-heptane extraction procedure and this interfered with the fluorimetric assay. The bulbocapnine was removed, therefore, by shaking the final brain extract for 1 min with two 5 ml volumes of chloroform.

Rats that had been injected with bulbocapnine (50 mg/mg) or chlorpromazine (10 mg/kg) were killed 30 min after injection when the catatonia was most pronounced. After treatment with reserpine (2·5 mg/mg), catatonia was most marked at 12 h. A

Table 1. *Free and bound acetylcholine content of rat brain during catatonia induced by bulbocapnine, chlorpromazine, reserpine and audiogenic seizures.* The rats were killed 30 min after bulbocapnine or chlorpromazine and 12 h after reserpine. The number of animals used are shown in parentheses. Significance of difference from control values:—* $P < 0.05$, ** $P < 0.01$.

Treatment	Brain acetylcholine (μg/g ± s.e.) "Free"	"Bound"	Total
Control	0·21 ± 0·02 (5)	2·92 ± 0·21 (5)	3·13 ± 0·21 (5)
Bulbocapnine (50 mg/kg)	0·24 ± 0·02 (5)	2·86 ± 0·10 (5)	3·10 ± 0·11 (5)
Control	0·22 ± 0·02 (5)	2·91 ± 0·15 (5)	3·12 ± 0·14 (5)
Chlorpromazine (10 mg/kg)	0·19 ± 0·03 (3)	2·56 ± 0·18 (3)	2·75 ± 0·22 (3)
Chlorpromazine (40 mg/kg)	0·14 ± 0·01 (5)**	2·51 ± 0·13 (5)	2·65 ± 0·14 (5)*
Control	0·31 ± 0·07 (5)	2·44 ± 0·15 (5)	2·75 ± 0·17 (5)
Reserpine (2·5 mg/kg)	0·39 ± 0·07 (5)	2·70 ± 0·11 (5)	3·08 ± 0·11 (5)
Control	0·22 ± 0·01 (5)	2·96 ± 0·15 (6)	3·17 ± 0·19 (5)
Audio-seizure (10 min of post-seizure catatonia)	0·19 ± 0·03 (4)	2·39 ± 0·07 (5)	2·52 ± 0·05 (4)

larger dose of chlorpromazine (40 mg/kg) was also used, although this dose produced a flaccid paralysis rather than catatonia. The mean concentration of total acetylcholine showed no statistically significant change in animals rendered catatonic by drugs or by audiogenic convulsions. Bulbocapnine, chlorpromazine and reserpine, in doses sufficient to produce catatonia, were found not to affect either the "free" or "bound" fractions of brain acetylcholine (Table 1). The only significant drug-induced change in brain acetylcholine was that seen after a large dose of chlorpromazine which caused prostration and, at the same time, reduced the "free" acetylcholine and also the total amount (the sum of "free" and "bound"). This is in contrast to the findings of Zeleny, Lindaur & Kosak (1957) who observed a small increase in the total acetylcholine content of rat brain after chlorpromazine. The administration of reserpine has been reported to raise the concentrations of acetylcholine in whole brain (Haas & Wulzinger, 1960; Giarman & Pepeu, 1962) and to cause varying changes in the acetylcholine concentrations of different brain areas (Malhotra & Pundlik, 1959; Beani, Ledda & others, 1966; Malpica, Jurupe & Campos, 1970). We observed only non-significant increases. Convulsive activity induced by various other physical and chemical agents has been consistently shown to result in a fall in cerebral acetylcholine levels (Richter & Crossland, 1949; Torda, 1953; Giarman & Pepeu, 1962). It seems likely, therefore, that in our experiments, the decrease in acetylcholine was a consequence of the audiogenic convulsions rather than of the catatonia. No significant changes were found in the concentrations of cerebral noradrenaline, dopamine or 5-HT during the catatonia induced by bulbocapnine or during post-convulsive catatonia.

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