Apparent Delayed Enhancement of Memory Following Post-Trial Methylamphetamine Hydrochloride

A recent review[1] of the literature relating to studies involving post-trial administration of drugs to animals in learning situations has noted the apparently ubiquitous finding that central nervous stimulants enhance, whilst central nervous depressants impair, the retention of learned responses. These results have usually been ascribed to drug-induced modifications of a memory consolidation process[2], though the pharmacological bases of such changes are uncertain.

In a recent study involving post-trial administration of methylamphetamine hydrochloride, a central stimulant, WAITE[3] could not find any facilitating effect of the drug on the retention of one-trial passive avoidance learning in rats when the animals were tested 24 h after the learning experience, but when they were retested between 1 and 6 weeks later it was found[4] that those which had received post-trial drug treatment showed clearly enhanced retention of learned avoidance responses as compared with saline-treated controls. The experiment reported here was undertaken in an attempt to replicate these findings.

Methods. The subjects were male rats of the Roman Control (RCA) strain, bred and reared in the Birmingham Psychology Department animal colony, and aged about 100 days at the commencement of the experiment.

Avoidance learning was carried out in an apparatus which consisted of a shelf 10 cm deep × 23 cm wide, elevated 10 cm above a 25 cm square floor comprising parallel metal bars through which a scrambled 0.25 mA A/C (50 Hz) constant current electric shock could be administered to the animals' feet for a period of 5 sec.

Each subject was placed upon the shelf and then the time taken for all 4 of its feet to touch the floor bars (i.e. the step-down latency) was recorded. 20 subjects received shocks immediately after stepping down: they were prevented from making an escape response by a guillotine door which was lowered in front of the shelf. A further 20 subjects received no shock. All subjects whose step-down latencies fell below 5 sec or exceeded 30 sec were rejected and replaced by others. Half of the shocked and half of the unshocked subjects, randomly selected, received methylamphetamine hydrochloride, 5 mg/kg s.c. The remaining subjects received s.c. injections of physiological saline. All injections were given in volumes of 0.1 ml/100 g body weight. The stepdown latencies of all subjects were examined at intervals of 1, 3, 5 and 7 days.

A further 20 subjects, also male RCA rats, were given shocks after making step-down responses but were retested only after a 7 day interval. Half of these subjects received drug injections at the previously noted dosage, the remainder receiving saline.

Results. The experimental data are presented in the Table. Analysis of the results indicated a very clear overall effect of shock treatment ($F = 48.59$; df 1/86; $p < 0.001$) indicating partial learning by the subjects to avoid making step-down responses. The shocked subjects to which saline had been given showed a progressive tendency, over the 4 successive test days, to reduce their step-down latency ($F = 3.06$; df 3/27; $p < 0.05$). That this was due to the extinction of avoidance learning was demonstrated by the fact that the shocked, saline-treated subjects which were not retested until 7 days after learning had latency scores significantly higher than the subjects tested on a previous 3 occasions ($t = 3.046$; df 18; $p < 0.01$; 2-tailed test).

The shocked subjects which had been given post-trial injections of menylamphetamine hydrochloride showed step-down latencies of the same order as those of the shocked, saline-treated subjects on days 1, 3 and 5 after the learning experience, but not only did they not extinguish their learned avoidance responses but in fact showed a tendency to produce increased latencies on the 7th day retest ($F = 5.72$; df 3/27; $p < 0.005$). These findings are also reflected in a significant shock × drugs × days interaction in the analysis of variance ($F = 2.66$; df 3/108; $p < 0.05$) indicating a divergence between the latency scores of the shocked-and-drugged and the shocked-and-saline-treated subjects over the 4 days of retesting.

Subjects given drug injections after initial learning but tested on only 1 occasion 7 days later, produced step-down latency scores which did not differ significantly from those produced by similarly-treated animals which had received 3 previous retests.

Discussion. The experiment confirmed that post-trial methylamphetamine hydrochloride does apparently improve retention of one-trial avoidance learning, not only by eliminating extinction of the response on repeated testing, but also causing it to increase in magnitude 7 days after the original learning experience.

It was thought that a possible explanation might be that retention was in fact improved on retest days 1, 3 and 5, but was offset by a drug-induced stimulation of activity

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Table I. Means and standard errors (in parentheses) of step-down latencies (seconds) for shocked (+) and unshocked (—) rats treated with post-trial methylamphetamine hydrochloride (+) or saline (—) and retested after various time intervals

Treatment		Training-test-interval (days)			
Shock	Drug	1	3	5	7
+	+	238.4 (34.5)	190.1 (39.1)	258.0 (46.8)	367.3 (44.5)
+	—	298.7 (45.8)	303.7 (64.3)	219.2 (37.1)	150.2 (26.1)
—	+	111.9 (37.7)	103.3 (43.4)	104.8 (35.5)	55.0 (15.3)
—	—	93.8 (38.9)	83.5 (28.1)	62.2 (21.5)	82.4 (27.5)
Subjects retested on day 7 only					
+	+				426.3 (51.1)
+	—				335.6 (58.6)

$n = 10$ for each shock/drug condition.

causing the animal to step down from the shelf more quickly: as the stimulant effect wore off so the enhanced retention would become increasingly evident. Alternatively, the drug might produce a delayed effect upon motor activity, causing the subjects to become less active after a period of 7 days, thereby increasing the step-down latencies and producing an artifactual improvement in learned response retention. However both these possibilities were eliminated since it was observed that there were no indications of any effects on motor activity affecting step-down readiness amongst the non-shocked control subjects. Moreover, a subsidiary experiment on a further 10 subjects, 5 of which were given methylamphetamine hydrochloride (in the dose level previously noted) and the remainder given saline, confirmed that no elevated locomotory or rearing activity occurred 1, 3 or 5 days after injection. This does not, of course, rule out the possibility that some other characteristic of the drug obscures elevated step-down latencies on retest days 1, 3 and 5, though it is very difficult to see what the mechanism of this might be. This same subsidiary experiment also showed that no impairment of motor responses occurred 7 days after drug treatment such as would be necessary to explain the elevated latency sores observed in the previous experiment.

Other explanations, not related to changes in motor activity, may also be considered. It may be, for example, that methylamphetamine hydrochloride produces an effect which increases over time and which operated in such a way as to facilitate the expression of the learned response, i.e. to enhance memory retrieval. At a physiological level this might be due to the progressive development of a change in synaptic functioning, or to the build-up of some psychoactive metabolite of the drug. There is, however, no evidence to support either of these proposals.

A second possibility is that the drug affects the process of memory consolidation so as to enhance the establishment of a long-term trace and to make it less susceptible to disruption. However, experimental animal studies[5-7] on memory consolidation have indicated that the transformation of short-term into long-term traces is likely to be essentially completed within a matter of minutes, or at the most within 2 or 3 h, following the learning experience. This does not, of course, mean that other processes of more extended duration might not be involved in the fixation of long-term traces. These extended processes might be susceptible to modification by emergent effects of previous drug administration. In the case of the drug under consideration, there is little known about the form which such emergent effects might take: acute methylamphetamine hydrochloride administration produces catecholamine depletion in nerve terminals, particularly in the hypothalamus, amygdala and hippocampus[8], but the time course of this effect is uncertain, as are the functional implications for memory consolidation.

The phenomenon of a pharmacologically induced apparent improvement of memory occurring after a 7-day delay is very curious, if indeed it is a phenomenon which can be replicated under different experimental circumstances, and its further and closer examination may help to elucidate some of the consolidation processes which occur during memory establishment.

Résumé. L'hydrochlorure de méthylamphétamine, drogue qui était administrée aux rats après qu'ils aient subi une seule expérience d'entrainement, améliorerait la mémoire des animaux testés, mais cet effet se n'est produit que 7 jours après l'expérience.

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Binding of 5-Hydroxytryptamine and Noradrenaline by Rat Lung

Rat lungs in vitro have been shown to remove both 5-hydroxy-tryptamine[1] (5-HT) and noradrenaline[2] (NA) from the pulmonary circulation. Alabaster and Bakhle[1] reported that NA, in concentrations up to 10 times those of 5-HT, failed to influence removal of the latter by lung, suggesting that removal sites for the 2 amines may not be identical, or that their affinity is greater for 5-HT than for NA. In an effort to identify possible binding sites for both amines, radioactive 5-HT and NA were administered simultaneously to rats and the subcellular distribution of each amine determined subsequently in sucrose homogenates of lung.

Male rats (180 to 250 g) were anesthetized with Dial/urethane. Solutions of ^{14}C-5-HT and ^{3}H-NA (New England Nuclear Corporation, specific activities 15.3 mC/mM and 9.95 C/mM respectively) were prepared in 0.9% sodium chloride at concentrations such that 6 µg of 5-HT base and 0.1 µg NA base were infused (via a juglar cannula) per min. This mixture of amines was infused for 10 min, after which lungs were removed either immediately or, in some experiments, 30 min later. Lungs were rinsed in saline, blotted, weighed and homogenized in 0.25 M sucrose (total of 7–8 ml). The ^{14}C-5-HT and ^{3}H-NA in an aliquot of the homogenate was measured as described earlier[2,3]. The homogenate was centrifuged at $3000 \times g$ for 10 min. In some experiments, an aliquot of the supernatant was layered over a linear gradient of sucrose (see below). The remainder of the supernatant was centrifuged at $105{,}000 \times g$ for 30 min. The resulting sediment was resuspended in 2.0 ml of 0.25 M sucrose. 0.5 ml of the suspension was used for measurement of ^{14}C-5-HT and ^{3}H-NA. The remainder (1.5 ml) was layered over an 11.5 ml linear gradient of sucrose, ranging in concentration from 0.25 to 1.0 M and prepared with a Beckman Gradient former. Before making the gradient 2.0 ml of 1.5 M sucrose was added to each tube. Gradients were centrifuged at $132{,}000 \times g$ for 1 h, after which 22 fractions, each of approximately 0.6 ml,

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