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Effect of Amitriptyline on Avoidance Learning in Rats Following Olfactory Bulb Ablation[1]

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CAIRNCROSS, K. D., M. G. KING AND S. P. M. SCHOFIELD. *Effect of amitriptyline on avoidance learning in rats following bilateral olfactory bulb ablation.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1063–1067, 1975. – It has been established that following bilateral olfactory bulb ablation there occurs a performance deficit in rats exposed to aversive learning procedures. Associated with the behavioural deficit, there occurs a reduction in total cortical norepinephrine (NE). If the behavioural deficit observed is a sequitur or correlate of the NE reduction, then drug therapy aimed at increasing NE availability in the cortex should overcome the reduction in performance. Amitriptyline increases NE availability by inhibiting uptake mechanisms and increases the rate of synthesis of NE. Rats, previously bulb ablated, were treated with amitriptyline over a 10 to 14 day period and tested in aversive situations. It was demonstrated that the drug treated rats showed improved performance early in acquisition, and that the performance improvement was maintained when the treatment period was extended to 14 days. These results indicate that amitriptyline was inducing a true pharmacological effect, and that the improved performance could be correlated with increased NE availability in the cerebral cortex.

Anosmia Aversive conditioning Norepinephrine Amitriptyline Neocortex

RECENT studies have drawn attention to effects following bilateral olfactory bulb ablation on the aversive learning of rats [16,19]. It has been shown that this surgical procedure adversely affects the learning and retention of aversively motivated behaviour. Compared with sham-operated rats, bulb ablated animals were slower in escaping from the aversive CS following fear conditioning. When the instrumental task was changed to one-way avoidance learning the bulb ablated animals were again significantly slower in avoiding but the deficiency was not nearly so marked as when testing was done with the CS alone. King and Cairncross interpreted this effect as a motivational, rather than a learning deficit in the bulb ablated animals, suggesting that it was correlated with depressed levels of cortical norepinephrine (NE) [16]. This suggestion received further support when it was demonstrated that following bulb ablation the NE content of the total cortex was reduced. The reduction was not confined to the olfactory projection areas of the pyriform cortex [10].

The supposition was made therefore, that drug treatment aimed at increasing NE availability should improve the performance decrement previously demonstrated in anosmic animals. A suitable agent for such a study is the tricyclic anti-depressant drug, amitriptyline. Previous studies have shown that amitriptyline and related tricyclic anti-depressant drugs have the ability to potentiate the peripheral actions of NE [2, 6, 8] and on the basis of these observations it was suggested that this property was the basis of their therapeutic action. More recent work has demonstrated that the potentiating action of the tricyclic group of drugs is not confined to peripheral mechanisms but applies also to central adrenergic systems [11, 18, 21]. In using amitriptyline as a pharmacological tool, the means whereby the drug potentiates the actions of NE must be considered. Recent studies would suggest that amitriptyline and other tricyclic antidepressant drugs produce their initial therapeutic effect by inhibiting the re-uptake of NE released in response to a nerve impulse reaching the synaptic region [4,7]. In other words, the tricyclic group of drugs inhibits the $Uptake_1$ mechanism described by Iversen [14,15]. The end result of such an action would be to increase the availability of NE to the post-synaptic receptor. If the performance decrement described relates to a reduction in neuronal NE then increasing the availability of NE on a short term basis, should improve the performance of anosmic rats on a short term basis. Such a hypothesis is tested in Experiment 1.

However, a further possibility must be considered. This relates to other studies which indicate that a reduction in the amount of NE available for release by a nerve impulse modifies or governs the amount of NE synthesised by the nerve itself [1,17]. The rate limiting step in the synthesis of NE relates to the activity of the enzyme, tyrosine hydroxylase [17]. In the presence of therapeutically active concentrations of anti-depressant drugs, inhibition of the $Uptake_1$ mechanisms could be expected to reduce NE availability. This circumstance would limit the effects of end product inhibition, and so remove the constraints on tyrosine hydroxylase. The end result would be to increase the rate of NE synthesis in the nerve ending. Such an effect would

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be on a continuing basis as prolonged amitriptyline administration would ensure that end product inhibition did not apply.

If the performance decrement indicated can be reversed in increasing the rate of synthesis of NE, then the improvement of performance in aversive situations would not be confined to a short term event, but would be sustained over a prolonged period. Experiment 2 is designed to test such a possibility.

METHOD

In designing experiments to test the premise certain documented facts relating to the mode of action of amitriptyline must be considered. These relate particularly to the half-life of the drug, and to the means of metabolic degradation of the drug itself. The rat, as an omnivore, metabolises amitriptyline and other tricyclic antidepressant drugs in a manner similar to man [3,20]. However, the precise mode of action of amitriptyline as an antidepressant drug remains equivocal. The weight of available evidence would suggest that the tricyclic antidepressant drugs require up to 3 days to achieve a therapeutic concentration in the extra-neuronal space. This relates to the fact that the administered drug is not itself the metabolic-active moiety, but rather the N-monomethylated metabolite is the active molecule [3].

The plasma half-life of the drug is less than 12 hours [20]. Thus to achieve and to maintain an active concentration of the drug within the animal brain it is necessary to treat the animal twice daily. Further, the pharmacological properties of amitriptyline are such that the administered dose of the drug must be carefully calculated. The tricyclic antidepressant drugs if administered in excess of their therapeutic dose produce a sedative rather than an antidepressant action [5, 12, 13]. Thus, the administered dose of the drug must be strictly calculated if the premises outlined in Experiments 1 and 2 are to be tested realistically. Relating to the fact that the precise mode of action of amitriptyline and related tricyclic antidepressant drugs remains unproven, it is essential that the clinically efficacious human therapeutic dose be adhered to in animal experiments which use this drug as a pharmacological indicator. Such a conclusion is reinforced through empirical studies of dose levels examined from this laboratory.

It is this reason which governs also the time course of the experiment. Amitriptyline in the clinical situation takes 10–14 days to improve motivation in a depressed patient [13]. This extended therapeutic period relates to the pharmacological activity of the drug. As this is associated with changes in NE availability and synthesis, the length of time the drug is administered is a crucial factor in the two experiments.

Animals

Seventy-two male albino Wistar rats aged 80–100 days at the start of the experiment and weighing 250–350 g were used. During the course of the experiment all rats were caged individually and housed in a room with 12 hr light-dark cycle and the temperature regulated to 21° ± 1°C. Food and water were provided ad lib.

Apparatus

The automated shuttle-box, described elsewhere in detail [16] was of clear Plexiglas construction balanced on a fulcrum with an electronically operated guillotine door separating the arms of the box. Internal dimensions of the box were 80.0 × 15.5 × 9.5 cm. The floor was of stainless steel grids. The CS was a buzzer with an 88 db rating measured within the shuttle-box. The UCS was a constant voltage 100 VAC (0.2 mA approximately), 50 cps footshock delivered through a sequential scrambler to the grid floor. Latency of each cross-over response was automatically recorded on a Sodeco printing timer to an accuracy of 0.01 sec. The shuttle-box was housed in a sound-attenuated cubicle in which light and temperature were controlled.

Procedure

All rats were subjected to bilateral olfactory bulb ablation. Each rat first received an intramuscular covering injection of 120,000 IU of bicillin. Animals were then anaesthetised with an IP injection of Equithesin at a dose of 0.33 ml/100 g bodyweight. The head of the rat was then secured in a stereotaxic apparatus and the frontal bone exposed by a mid-line skin incision. Two holes were drilled 2–3 mm either side of the midline at a point corresponding approximately to the posterior margin of the eye. The olfactory lobes were then completely sectioned using a blunt probe. Desquaspon was placed in the holes and the wound sutured. A period of 28 days postoperative recovery was allowed before injections of amitriptyline were started.

Operated animals (An) were allocated randomly to a drug group (Am) or a placebo group (P). Each rat in the Am group received twice daily IP injections of 1.5 mg/kg bodyweight of amitriptyline. P animals received twice daily IP injections of isotonic saline.

Depending on the experiment, animals were tested after either 2, 6, 10 or 14 days of injections. At the completion of behavioural testing rats were immediately decapitated and the brain dissected on ice. Olfactory lobe section was confirmed as previously described [16].

Behavioural procedures. On the day preceding fear conditioning each rat spent 10 min alone in the conditioning box. Twenty-four hr later each rat received 1 hr of fear conditioning comprising 35 presentations of CS and UCS. The CS was a buzzer of 8 sec duration the last 2 sec of which was overlapped by a 100 VAC (approximately 0.2 mA) of scrambled footshock to the grids. The intertrial interval was 90 sec. Conditioning operations were controlled by a minicomputer.

On completion of fear conditioning the rat was returned to its home cage while the switching was changed to administer one-way avoidance learning trials. The buzzer CS (88 db) preceded the UCS (100 VAC at 0.2 mA approximately of scrambled footshock) by 8 sec during which time a cross-over response (avoidance) could be made. If the animal failed to cross during the avoidance interval the UCS onset for 30 sec or until such time as an escape response was made whichever came first. On each trial, CS onset was simultaneous with raising the guillotine door. Closing of the guillotine door, and CS and UCS offset were simultaneous.

During the intertrial interval the animal was returned to its home cage for 50 sec. Twenty-five avoidance learning trials were given.

EXPERIMENT 1

Preliminary experiments previously reported indicates that anosmic rats treated with amitriptyline show an

improvement in aversive learning [9]. In this experiment the early results have been extended to include not only the latency of response, but also the efficacy of response following drug treatment.

Results and Discussion

Analysis of variance for repeated measures [22] was carried out on the cross-over latencies. For each rat, raw latencies were reciprocated (speed) and then averaged over blocks of 5 trials. In addition, analysis of variance for repeated measures was also carried out on the frequency of avoidance, since King and Cairncross [16] had previously shown that avoidance frequency and avoidance efficacy brought out different aspects of the effects of olfactory lobe section in rats. Trials were treated statistically in blocks of 5, giving a maximum of only 5 avoidances for any rat on any block. Because of this, the raw frequency of avoidances was transformed to the arcsin of avoidance proportion so that assumptions underlying the analysis of variance were not violated. Treatment Group means (AnAm, AnP) of performance in terms of speed of avoidance are shown in Fig. 1 and number of avoidances (transformed) in Fig. 2.

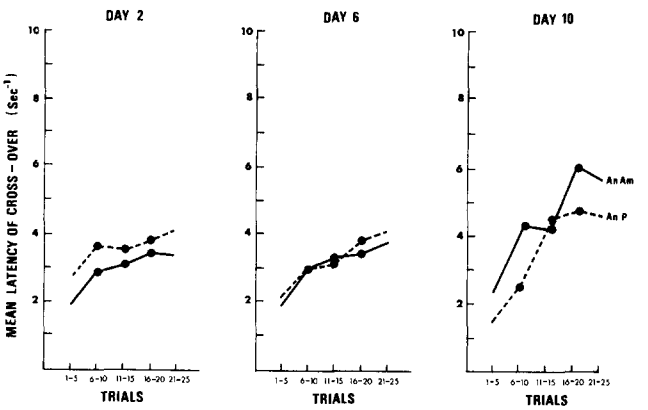

FIG. 1. Acquisition (measured in sec^{-1}) of one-way avoidance learning following fear conditioning in rats whose olfactory lobes had been sectioned. One group was injected with amitriptyline (1.5 mg/kg body weight b.d.) and the other with placebo for 2,6 or 10 days.

The analysis of variance Treatment effect refers to the drug versus placebo conditions (Am, P) and the Trial Blocks effect to Trials 1–5, 6–10, 11, 15, 16–20, 21–25 and the Interaction to the Treatment × Trial Blocks effect (See Figs. 1 and 2).

Analysis of variance of the speed of response (Fig. 1) showed that the Trial Blocks effect was significant on each of the 3 test days: On Day 2, $F(4,72) = 8.25$, $p<0.01$, on Day 6, $F(4,72) = 13.64$, $p<0.01$, and on Day 10, $F(4,72) = 42.45$, $p<0.01$. On Days 2 and 6, neither the Treatment effect nor the Interaction was significant indicating that both P and Am groups acquired the avoidance response at the same rate. On Day 10 however, the Treatment × Trial Blocks effect was significant, $F(4,72) = 2.94$, $p<0.05$. Further testing with the Scheffé test showed that the effect was attributable to a significantly faster performance ($p<0.05$) in the Am drug group on Trials 6–10, (0.441 v 0.265 sec^{-1}) and on Trials 16–20 (0.617 v 0.480 sec^{-1}).

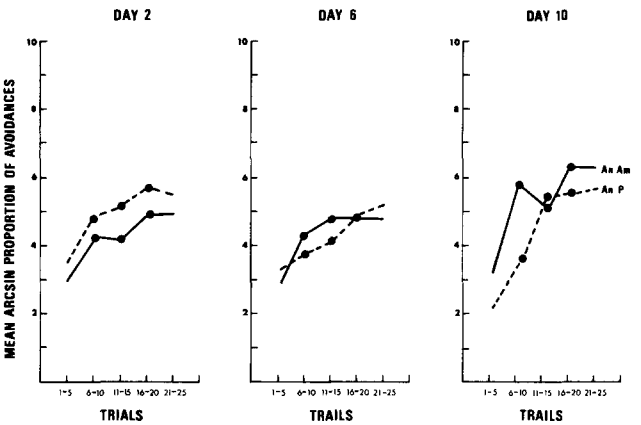

FIG. 2. Acquisition (measured in mean arc sin of proportion of number of avoidances) of one-way avoidance learning following fear conditioning in rats whose olfactory lobes had been sectioned. One group was injected with amitriptyline (1.5 mg/kg body weight b.d.) and the other with placebo for 2,6 or 10 days.

Thus the AnAm group avoided faster than the AnP controls after 10 days of treatment with amitriptyline.

Analysis of variance on the transformed avoidance frequency measures (Fig. 2) showed that the Trial Blocks effect was significant on each test day: on Day 2, $F(4,72) = 10.16$, $p<0.01$, on Day 6, $F(4,72) = 11.47$, $p<0.01$, and on Day 10, $F(4,72) = 41.71$, $p<0.01$. This indicates that in both AnAm and AnP groups the avoidance response was acquired. On both Days 2 and 6 no other effect was significant, indicating that acquisition did not differ between drug and placebo groups. However, on Day 10 the Treatment effect was significant, $F(1,80) = 12.30$, $p<0.01$, the Trial Blocks effect was significant, $F(4,72) = 41.71$, $p<0.01$, and the Treatment × Trial Blocks effect was significant, $F(4,72) = 6.08$, $p<0.01$. Thus on Day 10 the AnAm group performed significantly more advoidances overall than did the AnP group. Further testing with the Scheffe test showed that performance in the drug group was significantly higher ($p<0.05$) on Trials 1–5 (0.781 v 0.519 sec^{-1}) and on Trials 6–10 (1.489 v 0.900 sec^{-1}). Thereafter, however, the AnP group performed as well as the AnAm group. This indicates that the enhancement by amitriptyline of the avoidance response occurred early in acquisition.

In summary, an equivalent therapeutic dose of amitriptyline administered over a 10 day period overcame the performance decrement associated with bilateral olfactory lobectomy. The improved performance in response to aversive conditioning was not apparent on either Days 2 or 6 after the initiation of amitriptyline treatment and therefore can be ascribed to a true pharmacological action of the drug. The assumption can be made therefore, that increasing the short term availability of NE results in a significantly better one-way avoidance performance in the AnAm group of rats compared to the AnP group.

EXPERIMENT 2

The aim of the experiment was to determine whether the short term improvement in performance demonstrated in the AnAm animals in Experiment 1, could be maintained over a relatively prolonged time period. Further, the drug

treatment period was extended from 10–14 days to allow for the enhanced synthesis rate of NE to become effective.

Results

As in the previous Experiment both latency and frequency of avoidance were transformed and subjected to a repeated measures analysis of variance (See Figs. 3 and 4). The only difference between these analyses and the analyses in Experiment 1 was that Trials were blocked in groups of 10 rather than 5 since there were 80 trials.

For the speed measures (Fig. 3) the Trial Blocks effect was significant for both AnAm and AnP groups on each of the 3 test days, indicating that all groups learned the task: On Day 6, $F(7,154) = 10.98$, $p<0.01$, on Day 10, $F(7,154) = 11.35$, $p<0.01$, and on Day 14, $F(7,154) = 24.88$, $p<0.01$. The Treatment × Trials Blocks effect was not significant on Days 6 or 14 but this Interaction effect was significant on Day 10, $F(7,154) = 2.57$, $p<0.05$. This latter effect indicates that the rate of acquisition varied between AnAm and the AnP groups on Day 10. As Fig. 4 shows the interaction arises from a reversal in the rate of acquisition between Trials 30 and 40.

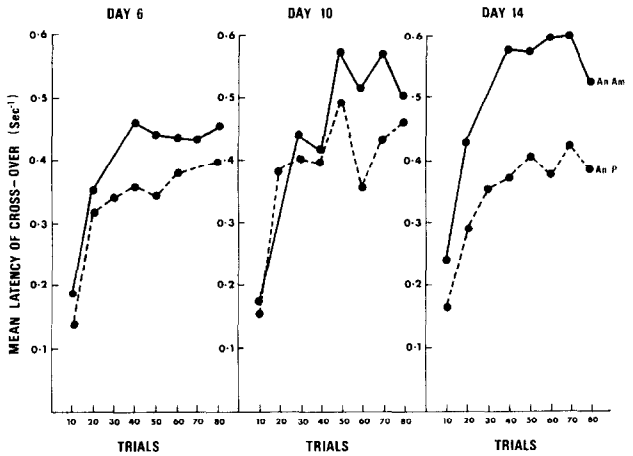

FIG. 3. Performance (measured in sec^{-1}) of one-way avoidance learning following fear conditioning in rats whose olfactory lobes had been sectioned. One group was injected with amitriptyline (1.5 mg/kg body weight b.d.) and the other with placebo for 6, 10 or 14 days.

The Treatment effect (Fig. 3) was significant on Day 14, $F(1,22) = 14.56$, $p<0.01$, but was not significant on either Day 6 or Day 10. Thus on speed measures, treatment with 1.5 mg/kg amitriptyline significantly increased performance in the AnAm as compared with the AnP group but only after 14 days of treatment. The results for Day 14 (Fig. 3) were further analysed using the Scheffé test. The difference between the AnAm and the AnP means was not significant ($p<0.05$) on Trials 21–30, 31–40 and 71–80 and highly significant ($p<0.01$) on the remaining Trial Blocks. The decrease in the extent of performance difference towards the end of the 80 trials is due more to the drug treated animals reducing speed than the control animals gaining in speed of performance. This can be attributed to physical fatigue in the AnAm group.

Similar findings occurred with the avoidance frequency measures (Fig. 4). Analysis of variance revealed a significant Trial Blocks effect for each test day: on Day 6, $F(7,154) = 15.74$, $p<0.01$, on Day 10, $F(7,154) = 23.85$, $p<0.01$, and on Day 14, $F(7,154) = 35.88$, $p<0.01$, indicating that both treatment groups acquired the avoidance task. The Treatment × Trial Blocks effect was significant on Day 6 only, $F(7,154) = 2.05$, $p<0.05$. This effect was barely significant at the 5 percent level and was due to the inexplicable changes in rate of performance between Trials 31 and 50 as indicated by Fig. 4.

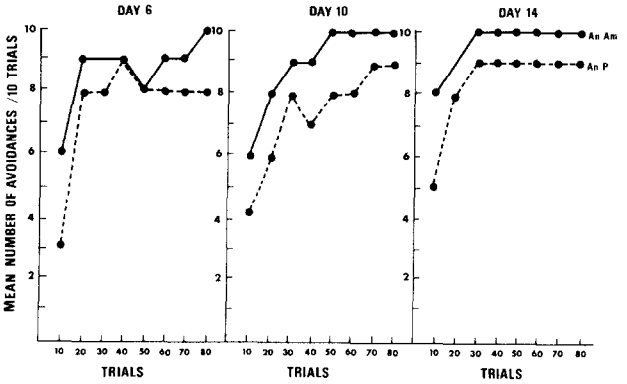

FIG. 4. Performance (measured in mean number of avoidance/10 trials) of one-way avoidance learning following fear conditioning in rats whose olfactory lobes had been sectioned. One group was injected with amitriptyline (1.5 mg/kg body weight b.d.) and the other with placebo for 6, 10 or 14 days.

The analysis of the transformed avoidance frequencies (Fig. 4) revealed a significant Treatment effect only for Day 14, $F(1,22) = 4.33$, $p<0.05$. This indicates that AnAm rats avoided shock more than the AnP rats after 14 days of drug treatment which was also the case for speed measures (Fig. 3). The Treatment effect was larger on Day 10 than on Day 14 as Fig. 4 indicates but greater error variance on Day 10 precluded a significant effect. Thus, although the amitriptyline treatment effect was greater on Day 10 the analysis of variance failed to reveal a significant effect because of the greater variability of subjects within treatment groups. Such a response is predictable. On Day 10 of drug treatment, the short term effect of amitriptyline administration, as described in Experiment 1 would be obvious in Trials 20–30. However, the time course of the experiment would not have allowed neuronal synthesis of NE to have become maximal, thus the initial improvement exhibited by the AnAm group would not be maintained following transmitter exhaustion. In short, these results lend support to the premise underlying the execution of Experiment 1, and substantiated in Trials 30–80 in Experiment 2 (Fig. 3). These latter results again demonstrate an early improved performance which could not be maintained.

GENERAL DISCUSSION

The results obtained in Experiments 1 and 2 are most interesting. In the first experiment it was demonstrated that amitriptyline, administered twice daily over a 10 day period, improved performance early in acquisition, but only after the full drug treatment period of 10 days had elapsed. As indicated in the introduction to this paper, tricyclic antidepressant drugs of the amitriptyline type require in excess of 10 days to produce their therapeutic effect in man. The first experiment demonstrated that a similar time lapse must occur in the experimental animal before

behavioural changes can be ascribed to pharmacological activity. Thus, an early improvement in performance of the drug treated animals over the placebo treated group would not have been consistent with pharmacological activity.

The fact that in the first experiment, performance was increased only in the early acquisition requires comment. Reference was made in the Introduction to amitriptyline having two distinct pharmacological actions. Namely, an ability to inhibit the $Uptake_1$ mechanism, and secondly to enhance the rate of synthesis of NE. The second action mentioned is a natural sequitur to the first. Inhibition of the Uptake mechanism will reduce neuronal NE stores, and lift the end product inhibition on tyrosine hydroxylase, thus increasing the rate of synthesis of NE. Both actions, therefore, will increase the availability of NE to the post synaptic receptor, but with different etiology. Inhibition of $Uptake_1$ will produce an increase in NE availability early in behavioural testing, providing the extra-neuronal concentration of the drug has reached effective levels. Such an effect was demonstrated in Experiment 1.

The results obtained in Experiment 2 and presented in Figs. 3 and 4 substantiate the supposition prefaced in the introduction to this paper. The former figure demonstrated a significant improvement in cross-over latency for the amitriptyline treated group of animals as compared with the placebo treated controls. The latter figure shows the maintained improvement of avoidances in the drug treated group. Thus, it would appear that extending drug treatment from 10 to 14 days and increasing the physical demands of the behavioural task improves the performance differential between the amitriptyline treated and the placebo treated groups of rats. Such results would be consistent with the contention that the behavioural criteria studied relate to NE availability. In other words, the motivational deficit previously described for rats in aversive situations following bilateral olfactory bulb ablation, can be overcome by drug treatment designed to increase NE availability in the cerebral cortex.

Further, the results so described would seem to have important clinical implications. The drug regimens undertaken in both the experiments discussed have been derived directly from human studies. The dose of drug administered to the anosmic animals and the duration of the drug treatment are a replication of those found efficacious in the clinical situation [12,13]. Diseases of affect can well be equated to a reduction in individual motivation. In the experimental studies described in these, and previous experiments conducted in our laboratories [9] it becomes increasingly clear that motivation and hence performance can be markedly improved by creating a situation in which the efficiency of NE utilisation is improved. It is not suggested that the experiments described present a total answer to the vexatious question of causation of depressive illness. It is suggested, however, that the results so described offer an experimental basis for new clinical studies which could test the veracity of the hypothesis described in this paper.

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