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THE EFFECT OF LITHIUM CHLORIDE ON ONE-TRIAL PASSIVE AVOIDANCE LEARNING IN RATS

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1 Expression of a one-trial passive avoidance learning response in rats was examined following injections of lithium chloride or sodium chloride before and after initial training and before the first day of testing. Five tests were given at daily intervals, 24 h after training being the time of the first test.

2 Lithium given before the first day of testing impaired response expression on the first and all subsequent days of testing; the rate of extinction was unaffected.

3 Given both before and immediately after initial training, lithium impaired response expression on the first day of testing but slowed down the subsequent rate of extinction, leading eventually to improved performance on the fifth day, as compared with placebo-treated control subjects.

4 The results are interpreted in the light of the hypothesis that lithium impaired the central processing of sensory information.

Introduction

In recent years an increasing number of reports has appeared indicating that lithium salts may produce behavioural effects in animals (see reviews by Johnson, 1972, 1975a, 1975b). Whilst it is still too early for the results of such studies to be reliably related to the therapeutic effects which lithium is known to have in conditions of manic-depression (Gershon & Shopsin, 1973) the findings are intriguing enough to warrant further investigation. Attention has recently been directed to the effects which lithium ions may have upon the establishment of memory traces immediately after a learning experience: the results of several studies in this area have led to conflicting interpretations.

Mark & Watts (1971) and Watts & Mark (1971) showed that when lithium chloride was injected directly into the forebrain of day-old chicks 5 min before they were trained in a one-trial passive avoidance learning situation, the retention of subsequent avoidance responses was impaired. The investigators related this effect to a lithium-induced inhibition of short-term memory traces. The same test situation was subsequently used by Benowitz & Sperry (1973) but with rather different results: retention of avoidance responding seemed to be less impaired when testing was done 20 min after training than when the training-test interval was as long as 24 hours. Since, on the basis of many studies involving electroschock- and drug-induced modification of learning and memory (e.g. McGaugh & Madsen, 1964; Kumar, Stolerman & Steinberg, 1970), short-term memory traces may be regarded as being largely replaced by consolidated long-term traces by 20 min after learning, these results seemed to indicate that lithium effects, if they were indeed upon memory traces of any kind, were probably not upon the short-term type. Benowitz & Sperry (1973) therefore suggested that the lithium effect might be upon a behaviourally inactive, convert type of trace, distinct from the short-term trace, and which functioned as a necessary precursor of long-term memory; this new type of trace had first been proposed by Benowitz & Magnus (1973).

Johnson & Barker (1972) reported effects of lithium chloride on escape-avoidance learning in rats, which they related to a possible drug action on processes interfering with short-term memory consolidation: the experiments described below produced findings which can be similarly interpreted and which may provide the basis for an alternative explanation of the results of the Benowitz & Sperry (1973) study.

Methods

Thirty male rats of the Roman Control strain were used in the present investigations. All rats were aged approximately 100 days at the start of the experiment. They were housed 3 to a cage and provided with water and food *ad libitum*.

Apparatus

The apparatus consisted of a shelf, 10 cm front to back × 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 seconds. A guillotine door could be lowered in front of the shelf to restrain animals either on the shelf or on the shock bars. The whole apparatus was bounded by black Perspex walls 30 cm high and topped by a clear Perspex hinged lid. Room fluorescent lighting was used throughout.

Procedure

On day 1 each subject was placed upon the shelf, the guillotine door raised after a 10 s delay, and the time taken for the animal to step down and place all four feet on the shock bars was recorded with a hand-operated stop-watch. The guillotine door was then lowered to prevent the rat returning to the shelf. Shocks were delivered to the rat's feet immediately after the door had been lowered. The subject was then removed by hand from the apparatus and returned to its home cage. The step-down latency was again measured, in the same apparatus, at 24 h intervals, on days 2 to 6. A cut-off criterion of 10 min was established but was not in fact needed. Shocks were not given in the test sessions and passive avoidance responses acquired on day 1 were thus allowed to undergo extinction.

Subject selection

All rats showing step-down latencies of less than 5 s or more than 30 s on the initial training trial on day 1 were rejected and replaced by others. In all, 3 rats were rejected on these criteria in choosing the 30 experimental animals.

Injections

All drug injections of either lithium chloride or placebo (sodium chloride), at dose levels of 2.5 mmol/kg body weight, were made intra-peritoneally, 0.1 ml of drug or placebo solution being injected for each 100 g subject body weight. Drug solutions were coded, the code being broken by the experimenter only after the experiment had been completed.

Experimental design

Two drug conditions, lithium chloride and placebo (sodium chloride) were used, injections being made at one of three times relative to the onset of the training shock: 10 min before the shock, 15 s after the shock, or 10 min before the first training trial on Day 2 (i.e. 23 h 50 min after the shock). Five rats, all conforming to the initial selection criteria noted previously, were examined in each of the drug/injection-time conditions. The experimental design was thus a 2 (drugs) × 3 (injection times) × 5 (extinction tests) factorial, with 5 subjects per condition and repeated measures on the third factor.

Results

The findings are shown in Figure 1. Over all there was found to be a clear reduction in response (step-down) latency over the five test trials, $F(d.f.\ 4,96)=31.53$; $P<0.001$, indicating that the avoidance response acquired on the training session underwent progressive extinction throughout the series of test sessions. However, the picture was complicated by the presence of a statistically significant effect of injection time, $F(d.f.\ 2,24)=3.76$; $P<0.05$, and a drug × extinction test interaction, $F(d.f.\ 4,96)=3.72$; $P<0.01$, though the three-way interaction between drugs, injection-times and extinction tests just failed to attain statistical significance at the $P=0.05$ level.

Reference to Figure 1 reveals that whilst the major extinction trend was visible under most experimental conditions it failed to occur to such a marked degree in those animals given lithium chloride injections either before, or immediately after, the training session: in these two cases the animals showed considerably greater retention of avoidance learning on the fifth test session than was exhibited by the corresponding placebo-treated control subjects or by animals given either lithium or placebo before the first test trial. In the animals injected with lithium chloride 10 min before the first test, avoidance responding was reduced on the first and on all four subsequent tests. A separate analysis of variance carried out on the pre-test injection data indicated that there was a significant over-all drug effect $F(d.f.\ 2,7)=4.80$; $P<0.05$, but the drug × extinction test interaction failed to attain statistical significance at the $P=0.05$ level. In all lithium-treated subjects, irrespective of injection time, avoidance responding on the first test session was less marked than that exhibited by placebo-treated control animals, the effects at the three injection times being assessed as significant at the $P=0.05$ level using the Newman-Keuls procedure for making specific comparisons between means (Winer, 1962).

Discussion

Pre-testing injection effects

Johnson (1972, 1975b) has suggested that many of the effects elicited by lithium ions in behavioural test

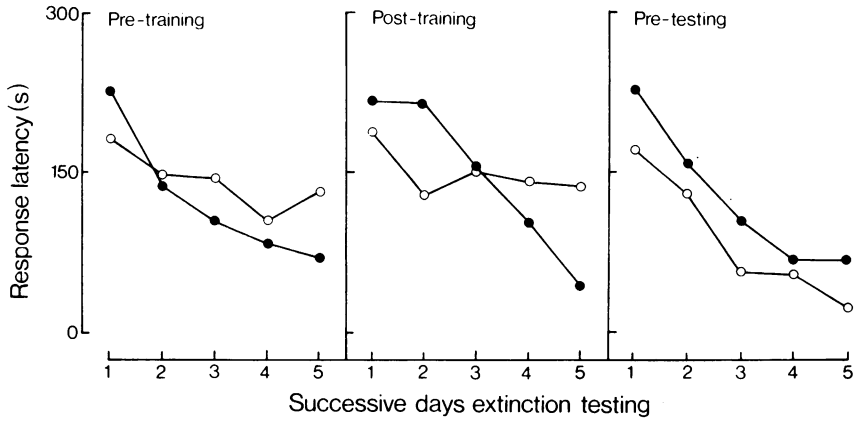

Figure 1 Injections of lithium chloride (O) before and after one-trial passive avoidance training caused impairment of avoidance responding on the first day of testing, but subsequent extinction over the next four test sessions was less rapid than that shown by control subjects. Pre-testing injections led to reduced avoidance responding on all days of testing. (●) = Placebo.

situations may be explained on the basis of a lithium-induced impairment of the efficiency with which an animal analyses and attaches significance, or importance, to sensory input. It was argued that even if such an impairment were only slight, affecting sensory information around threshold levels, this might be sufficient to be reflected in behavioural changes in appropriately designed experiments. On this hypothesis, lithium chloride given before testing might be expected to impair avoidance performance by effectively preventing an animal from processing all the environmental cues needed to elicit the learned response. This would lead to a shortened step-down latency on the first day of testing, as observed in the present experiment.

The reduced latencies occurring on the subsequent four test sessions cannot similarly be explained unless it is assumed that lithium ions are still present in the animal's tissues following the single injection before the first test trial: this seems unlikely. It may be that the early step-down on the first occasion leads to an enhanced extinction effect which is then reflected in subsequent trials and maintained throughout the test series.

Pre- and post-training effects

The explanation of the effects produced by lithium chloride injections given 10 min before, or 15 s after, training on day 1, is not to be found in the drug's effects on the test procedures. In the first place, as previously noted, it is unlikely that much lithium remains unexcreted 24 h after administration, and secondly the effects of peri-training administration differ quite markedly in form from those of pre-testing injection. We must examine the process of response acquisition and retention to elucidate the mechanisms of lithium action.

Only the pre-training injections can affect acquisition processes, but both pre- and post-training injections can operate upon the processes of memory trace establishment and consolidation, provided that the lithium ions are not entirely eliminated from the body and are still able to affect neural processes 10 min after injection and into the immediate post-training period. In view of the similarities between the effects of pre- and post-training lithium injections (see Figure 1) it seems reasonable and parsimonious to relate them to the same mechanisms. Figure 2 illustrates what these mechanisms might be.

Short-term memory traces (stm) are regarded as being established during the training period, subsequently decaying rapidly in the post-training period. Meanwhile, more stable long-term memory traces (ltm) consolidate in the post-training period. Such a model is certainly an oversimplification as far as human memory is concerned (Norman, 1970) but it will suffice for the description of most animal studies on memory formation.

Lithium chloride, it is suggested, produces its effects noted in the present studies by exerting its influence during the period of short-term memory decay and long-term memory consolidation. Two separate effects may be involved.

Firstly, it is well documented (Kumar *et al.*, 1970) that many central nervous depressant drugs may interfere with memory establishment and it has been suggested that this may result from the impairment of

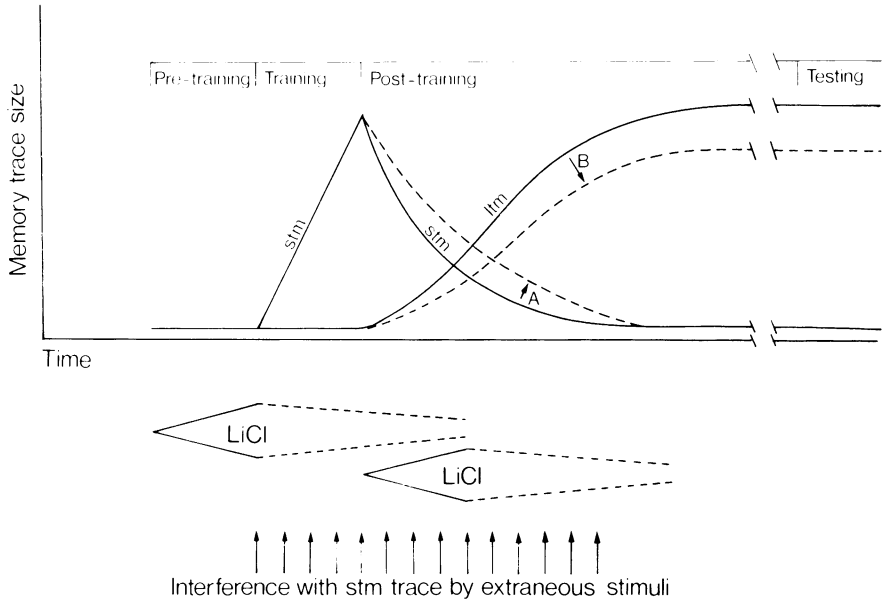

Figure 2 Schematic representation of the hypothesized effects of lithium on memory traces. stm: short-term memory trace; ltm: long-term memory trace; LiCl: effective lithium chloride concentrations after pre- or post-training injection, the pre-training injection continuing to give rise to effective tissue concentrations into the post-training period; A: reduced stm trace decay rate as a result of LiCl blockade of interfering stimuli; B: impaired consolidation of ltm leading to a smaller final ltm trace size.

ltm consolidation. Whilst lithium may not fit entirely happily into a central nervous depressant classification it does nevertheless have some properties characteristic of such drugs and it may be that it has an effect on ltm consolidation (B in Figure 2). This would lead to the formation of a smaller ltm trace and consequently to less marked expression of avoidance learning on the first test trial, as observed in the experiment.

The reduced rate of extinction of the avoidance response, as assessed over the full five-day period, which follows pre- and post-training lithium chloride administration may be related to an effect of the drug upon the decay rate of the stm trace. If an animal is responsive to stimuli arising from its environment in the immediate post-shock period (and it is possible that shock administration leads to heightened awareness and hyperresponsiveness) then it might be that at least part of the stm decay process is due to interference between the trace and new memory traces which arise just after the learning experience but which are irrelevant to it. By blocking, or impairing, the analysis of these extraneous stimuli, as it has been suggested that lithium chloride might do (Johnson, 1972, 1975b), lithium ions could prevent the formation of the interfering traces and delay the stm decay process (A in Figure 2).

Johnson (1970) has suggested that the parameters of stm decay rate may in some way be encoded in the ltm trace so as to determine the stability, assessed as the resistance to extinction, of the latter. In other words, the ltm trace, although reduced in size by the lithium treatment given in association with the learning experience is, at the same time, made more stable and relatively refractory to modification by the extinction process. A similar explanation has been used to account for chlorpromazine effects on memory, chlorpromazine being a drug which, in its therapeutic profile, may have some similarities with lithium salts (Johnson, 1971a, b).

It is possible to use the interpretation suggested above to explain the effects observed by Benowitz & Sperry (1973). If lithium chloride does indeed extend the duration of the stm trace this might still be available, or available to an enhanced degree, 20 min after the learning experience to contribute to the expression of avoidance responding, thus producing a better avoidance response than that observed 24 h post-learning when only the ltm trace remains to be behaviourally active.

Alternative explanations of the findings are also possible. If, in the experiments of Benowitz & Sperry (1973), lithium chloride was still available 20 min after administration, but not 24 h later, the finding of little

impairment of learning at the earlier test time may be related to a state-dependence effect (Overton, 1966) assuming that the lithium was active during learning acquisition as well as in the post-training period.

Secondly, it is still not known whether lithium ions are entirely eliminated 24 h after administration (Fyrö & Sedvall, 1975) and if there were some residual effect of any remaining drug during the testing procedure this might account for the impaired performance on the first test occasion. The lowered rate of extinction could then be related to enhanced ltm formation following protection against stm interference in the manner previously suggested.

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