# HUMAN MEMORY AND BENZODIAZEPINE-INDUCED AMNESIA: THE EFFECT OF TWO DIFFERENT TYPES OF ANXIOLYTICS ON MEMORY PERFORMANCE IN MICE

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## Introduction

Many theories have been formulated to explain memory from the functional viewpoint. It has frequently been fractionated into short-term and long-term components (Waugh and Norman, 1965; Atkinson and Shiffrin, 1968) although there is disagreement about whether apparent differences between the two components reflect the existence of two different storage systems or a single general memory system with different types of encoding (Lister, 1985). It is clear that, despite the successes obtained, the multi-store approach can no longer be regarded as an adequate theoretical conceptualisation of the architecture of the memory system (Eysenck, 1988). However, this oversimplification can provide a useful approach for describing the amnesic effects of benzodiazepines.

Benzodiazepines are a class of anxiolytic drugs which as a side effect have been shown to produce loss of memory both in animals (Soubrie et al, 1976) and humans (McKay and Dundee, 1980). Much of the studies carried out so far have been devoted to diazepam and lorazepam but recent findings suggest that this effect is common with all the members of the class. The amount of impairment depends on the half-life of the particular drug (Roth et al, 1984), the dose and the route by which the drug is given (O'Boyle, 1988) but is independent of the type of task involved (Izquierdo et al, 1990).

Benzodiazepines do not impair all processes of memory functioning (Lister et al, 1988). What is observed is a drug-induced anterograde amnesia in which there is an impairment to acquire information under the influence of the drug. Retrieval from short-term memory and long-term memory is not affected, possibly suggesting a defect in consolidation [a process by which memory passes from the short-term to the long-term store] (Brown et al, 1982).

The mechanism by which anterograde amnesia induced by benzodiazepines occurs is still very much unclear. It is thought that the benzodiazepine receptors located on the GABA-benzodiazepine receptor complex play a major role in producing amnesia (Nabeshima et al, 1990). The role of GABAergic transmission is also evident in the apparent memory deficit produced by benzodiazepines. GABAergic antagonists such as picrotoxin are able to counteract the deleterious effects on memory produced by benzodiazepines (Thiebot, 1985).

## Methodology

The experiment was designed so as to evaluate the effect of two different types of anxiolytics on acquisition and retrieval of information in mice using passive-avoidance paradigm, hot water acting as the aversive reinforcer. By comparing the effects of these drugs, which produce their anxiolytic effect via different physiological mechanisms, it can be postulated whether an interaction at the GABA-benzodiazepine receptor complex has any effect on memory.

The experiment was divided into two parts -

Part I - Learning stage in which the mice had to exit, in a specific amount of time, from a particular opening after being placed in a maze.

Part II - Administration of the drug followed by the performance of the maze in which the mice had to exit from a different opening than that described in part I.

### Part I

**Animals:** Subjects were 24 female albino mice having an average weight of 37.04g. Mice were kept 3 per cage in an animal house maintained at a temperature of 22±2°C with a day/night cycle of 12-12 hours provided by two 60W lamps placed 1.5m above the cages (lights on: 7.00 am, lights off: 7.00 pm). The mice were marked on the tail (to facilitate identification) with 10% picric acid and 10% carbol fuchsin. All the animals were acclimatised to the surrounding environment for a period of 1 week. During this period, food and water were provided ad libitum.

**Maze:** Detailed diagram of the maze is enclosed.

**Procedure:** The animals were starved overnight and the experiments were performed one cage after the other in a random sequence. All experiments were performed each day between 9.00 am - 12 noon.

At the start of the experiment, the mice were placed in an unfamiliar environment (different cage or isolation cage) in the absence of food and bedding. This constituted the same starting point to each animal while providing a motivational stimulus for the performance of the maze. The housing (home) cage was then attached to exit A of the maze. After 5 minutes in the isolation cage, the mice were placed randomly one after

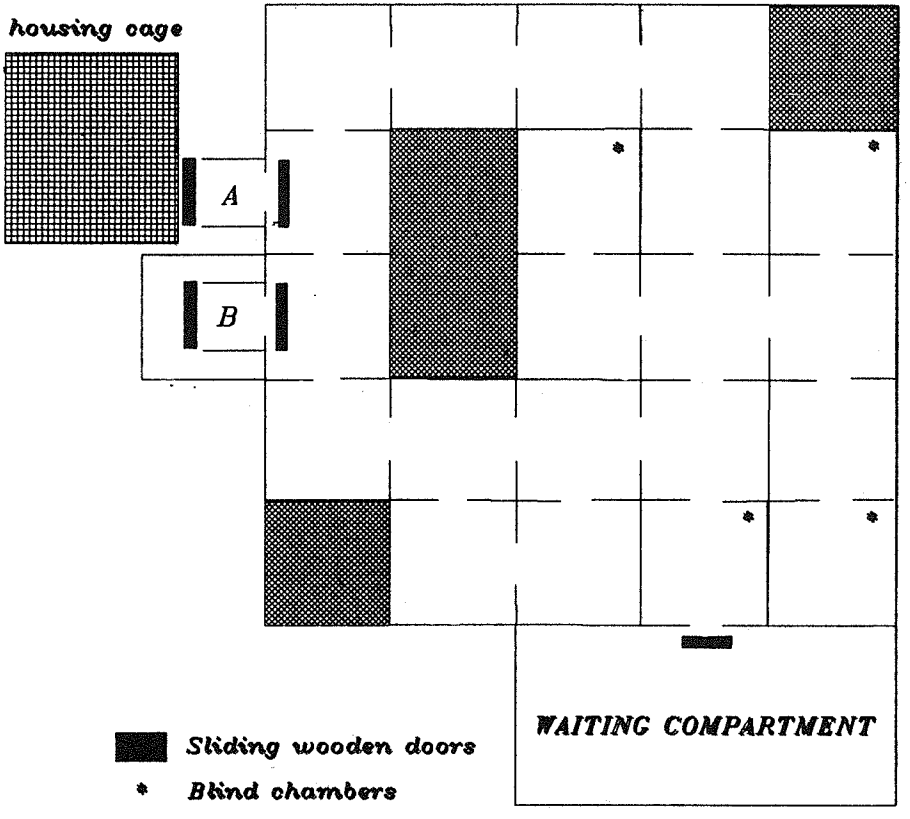


*Figure 1. Maze setup*

the other, in the waiting compartment for 5 seconds, after which they were exposed to the maze by opening the sliding door (which was closed immediately as the animal entered the maze). If the animal went out from exit A, it found itself in its cage in which food was added and it was not disturbed further. If the animal went out from exit B or did not perform the maze (N) in a specified time of 3 minutes, a jet of water (3mls) at 70±2°C was applied on the dorsal part of the abdomen by means of a teat pipette. The animal was then transferred to the waiting compartment for another trial for a maximum number of trials equal to 4. If after the fourth trial the animal did not succeed to move through exit A, it was isolated in a cage deprived of food, water and bedding for 30 minutes after which it was transferred back to its home cage. Both exits A and B had sliding wooden doors which were closed as the body of the animal (but not necessary the tail) entered the respective plastic tube leading to one of the compartments. Animals that successfully entered exit A were left for 10 minutes before being moved to the usual place where they are housed so to completely dissociate the aversive stimulus with maze competition.

The percentage correct responses as a function of the progressive learning sessions together with the total time of journey (±5 sec) were recorded. Maze learning was performed until 85% correct responses or more were obtained in 4 successive learning sessions.

**Part II**

**Drugs**: The two drugs used were buspirone hydrochloride (Laboratoires Bristol, Paris) and lorazepam (Medochemie, Cyprus). Both drugs are classified as anxiolytics, buspirone hydrochloride being an azaspirodecanedione whilst lorazepam is a benzodiazepine. The important difference between these two drugs is that buspirone hydrochloride, unlike lorazepam, does not interfere with the GABA-benzodiazepine receptor complex (Skolnick et al, 1984).

Buspirone hydrochloride and lorazepam were dissolved in 0.9% w/v saline, filtered and injected i.v. in the tail vein (after being warmed to 37.5±1°C) in a volume not exceeding that of 0.2mls. The doses were that of 0.1428mg/kg body weight for buspirone hydrochloride and 0.0357mg/kg body weight for lorazepam. Approximately 0.1mls of saline was administered to the controls.

**Procedure:** The mice were divided into three groups (number of mice was 21 at this stage). The first group acted as the controls (n=8), the second group received buspirone hydrochloride (n=8), and the third group were administered lorazepam (n=7). In principle, the procedure was similar as that used in part I. However, important differences included:

i) The time of stay in the isolation cage was different because different drugs reach peak plasma concentration in different times. For the controls, this was 5 minutes whereas for buspirone hydrochloride and lorazepam, time of stay was 10 and 30 minutes respectively.

ii) The exits were changed so that exit from A lead to the application of the aversive reinforcer whilst exist from B lead the animal to its home cage were it was not disrupted further. This change represented new information to the animal so that if the consolidation process was affected negatively, drugged mice would show a decrease in performance with respect to the controls.

iii) Whereas in part I the learning sessions were performed each day, in part II, administration of the drug followed by the learning session for each group were performed every two days so as to enable sufficient elimination of the drugs from the body and hence minimising the risk of drug accumulation.

iv) Only 6 learning sessions were performed so that the risk of drug tolerance and withdrawal effects that could have developed after long-term administration of lorazepam were reduced to a minimum.

**Behaviour:** The behaviour of the animals both in the isolation cage and in the maze were observed to determine to what extent sedation effects the performance of the animals. Sedation was 'measured' by observing the activity in the isolation cage and in the maze. The motor activity in the maze was also noted

**Statistics:** Data of the percentage correct responses for the different groups of mice (controls, buspirone and lorazepam treated) were analysed for the level of significance using logistic regression.

## Results

**Passive Avoidance Learning in Part I:** Part I of the experiment required the animals to exist from opening A in a specified period of time being 3 minutes. All animals showed complete learning, in accordance with the cirterion mentioned above, after 11 learning sessions (a total of 358 learning trials). The % correct responses calculated by the formula:

$$\% \text{ correct responses} = A/(A+B+N) \times 100$$

where A = exit from A, B = exit from B, N = no exit and (A+B+N) = total amount of trials, showed a considerable increase with subsequent learning sessions giving a value of 95.65% in the 11 learning session meaning that out of 23 learning trials, 22 resulted in a correct response in the first trial. These results together with the considerable decrease in the total time in maze indicated retention in memory.

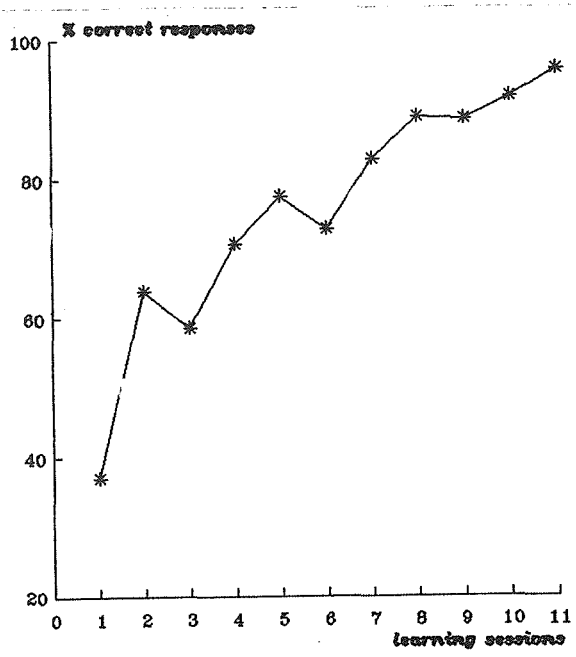


*Figure 2. % correct responses with progressive learning sessions (Part I of experiment)*

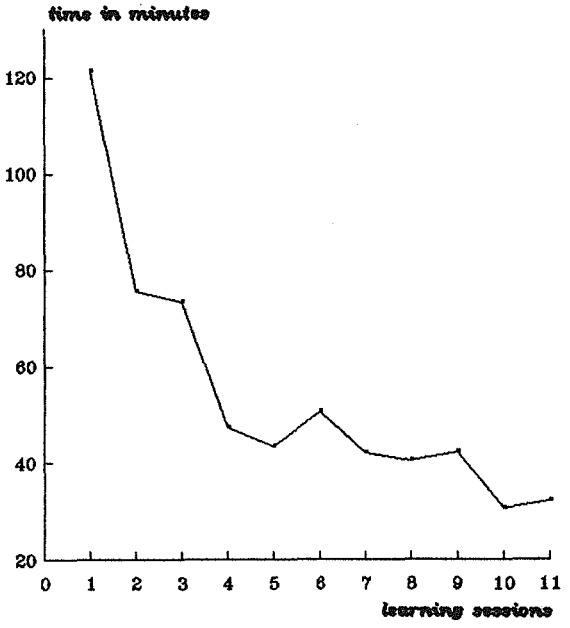


***Figure 3. Total time in maze (Part I of experiment)***

**Passive Avoidance Learning in Part II:** In the second part of the experiment the animals had to exit through opening B to avoid the application of the aversive reinforcer. Buspirone hydrochloride did not impair the acquisition of new information (exit from B and not A) with respect to the controls (coefficient of standard error = -0.0823, 95% confidence interval ranging from 0.425 to 2.20). On the other hand, lorazepam impaired the acquisition of the new information presented, this impairment being significant with respect to the controls (coefficient of standard error = -2.65, 95% confidence interval ranging from 0.151 to 0.757; p for whole group = 0.0065). The time spent in the maze for the lorazepam group was considerably higher than that of the controls but not very much different than that of buspirone hydrochloride treated mice suggesting that although the percentage correct responses for buspirone hydrochloride parallelled those of control, the former spent more time in the maze searching for exit B.

Retrieval of information already present in memory was not affected either by the drugs as in the first learning session of part II, both lorazepam and buspirone-treated mice 'remembered' quite effectively what has been learned in part I of the experiment.

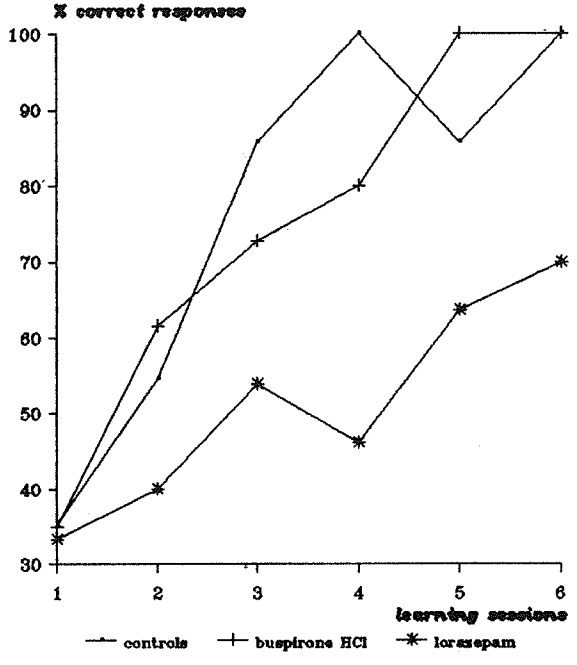


*Figure 4. % correct responses with progressive learning sessions (Part II of experiment)*

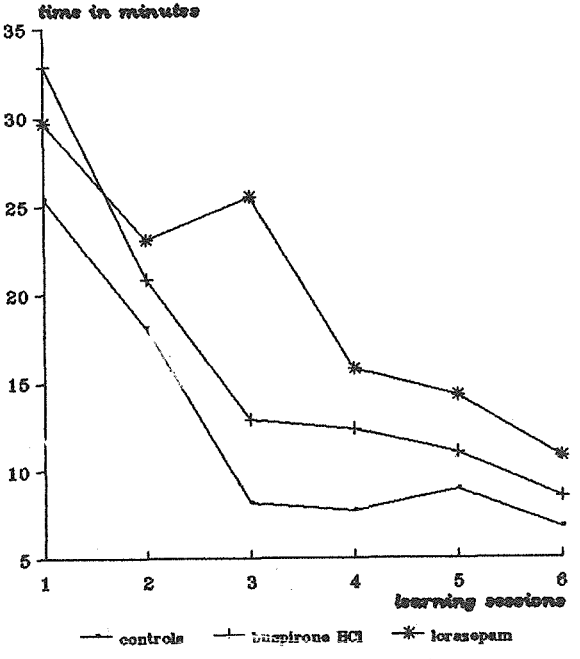


*Figure 5. Total time in maze (Part II of experiment)*

**Behavioural data:** Lorazepam-treated mice were more sedated than buspirone hydrochloride treated mice, the latter being more active and more resistant to handling in the isolation cage. However, in general, the overall activity of both drug-treated groups was less than that of the controls (the latter spending more time exploring the isolation cage, an activity being similar to that observed in part I), suggesting that both drugs produced sedation although not to the same extent.

The behaviour of the animals in the maze did not differ significantly. All the groups recognised the waiting compartment and the maze quite effectively. The motor activity in the maze was also similar although the lorazepam treated mice were more 'confused' and 'frustrated', visiting the blind chambers more frequently than the controls or buspirone-treated mice.

## Discussion

The results support the findings by other investigators (Brown et al, 1982; Lister and File, 1984) that lorazepam impairs performance in the acquisition (anterograde amnesia) but not in the recall of information. In fact recall of information learned in part I of the experiment parallelled that of the controls, but the acquisition of new information in part II of the experiment was significantly impaired. In terms of the dichotomous system proposed by Waugh and Norman (1965) and Atkinson and Shiffrin (1968), this data can be interpreted to suggest that lorazepam impaired the transfer of material from short-term memory to long-term memory without impairing previously consolidated information.

On the other hand, buspirone hydrochloride did not affect either the acquisition or retrieval of information. These results do not support those obtained by Rowan et al (1990), the latter observing an impairment of performance of passive avoidance and spatial learning tasks in rats. This divergence may result from the fact that in the experiment performed by Rown et al (1990), the doses of buspirone hydrochloride varied from 0.5 to 2.0 mg/kg (given intraperitoneally), whereas in this experiment a much lower dose of approximately 0.143 mg/kg body weight, equivalent to a 10 mg dose in a 70 kg adult, was used. Therefore, although in this experiment, buspirone hydrochloride was not found to affect memory performance, it does not imply that amnesic effects due to the drug are not possible at higher doses. The aim of this experiment was to investigate the amnesic effect at doses which are usually administered in humans.

An important observation was that anterograde amnesia produced by lorazepam was not due to the sedation characteristics of the drug although conclusive results on this issue are difficult to obtain (Curran 1986). The motor activity of lorazepam-treated mice in the maze was similar to the controls, indicating, at least, that the motivational factor driving the animals towards the correct exit was not impaired. Any other physiological mechanisms which might be affected by sedation in such a way as to reduce memory performance, but not observable by the naked eye pose serious limitations in concluding on the extent to which sedation might effect performance. Since the mechanism of action of lorazepam, but not buspirone hydrochloride, is mediated via the benzodiazepine receptor, these results suggested that anterograde amnesia produced by benzodiazepines (manifested by lorazepam in this experiment) may be due to an interaction of these drugs with their respective receptors. However, the relationship between the amnesic

effects of benzodiazepines and occupancy of the benzodiazepine receptor deserve further investigation.

## References


Atkinson, R.C. and Shiffrin, R.M. Human memory: A proposed system and its control processes. In Gardner, J.M. (edt.) Readings in human memory, 1968; pp 9-24. Methuen & Co. Ltd., 1976.

Brown, J. et al. A comparison between transient amnesias induced by two drugs (diazepam and lorazepam) and amnesia of organic origin. Neuropsychologia 1982; 20(1): 55-70.

Curran, H.V. Tranquillising memories: A review of the effects of benzodiazepines on human memory. Biological Psychology 1986; 23: 179-213.

Eysenck, M.W. Models of memory: Information processing. In Hindmarch I and Ott H. (eds.) Benzodiazepine receptor ligands, memory and information processing 1988; pp. 3-11. Berlin, Springer-Verlag.

Izquierdo, I., Pereira, M.E. and Medina J.H. Benzodiazepine receptor ligand influences on acquisition: Suggestion of an endogenous modulatory mechanism mediated by benzodiazepine receptors. Behavioural and Neural Biology 1990; 54: 27-41.

Jensen R.A. et al. Benzodiazepines alter acquisition and retention of inhibitory avoidance response in mice. Psychopharmacology 1979; 64: 125-126.

Lister, R.G. and File, S.E. The nature of lorazepam-induced amnesia. Psychopharmacology 1984; 83: 183-187.

Lister, R.G. The amnesic action of benzodiazepines in man. Neuroscience Biobehavioural Reviews 1985, 9(1): 1-7.

Lister R.G. et al. Clinical relevance of the effects of benzodiazepines on learning and memory. In Hindmarch, I and Ott, H. (eds.) Benzodiazepines receptor ligands, memory and information processing, 1988; pp. 117-127. Berlin, Springer-Verlag.

McKay A.C. and Dundee, J.W. Effect of oral benzodiazepines on memory. British Journal of Anaesthesiology 1980; 52: 1247-1257.

Nabeshima, T. et al. Effects of benzodiazepines on passive avoidance response and latent learning in mice: Relationship to benzodiazepines receptors and the cholinergic neuronal system. The Journal of Pharmacology and Experimental Therapeutics 1990; 225 (2): 789-794.

O'Boyle, C.A. Benzodiazepine-induced amnesia and the anaesthetic practice: A review. In Hindmarch, I. and Ott, H. (eds.) Benzodiazepine receptor ligands, memory and information processing 1988; pp. 146-165. Berlin, Springer-Verlag.

Roth, T. et al. Benzodiazepines and memory. British Journal of Clinical Pharmacology 1984; 18: 455-495.

Rowan, M.J., Cullen, W.K. and Moulton, B. Buspirone impairment of passive avoidance and spatial learning tasks in the rat. Psychopharmacology 1990; 100: 393-398.

Skolnick, P., Paul, S.M. and Weissman, B.A. Pre-clinical pharmacology of buspirone hydorchloride. In Miller, R.R. (ed.). Evaluations of new psychotherapeutic drugs 1981-1984: Pharmacotherapy monograph series no 3. Pharmacotherapy Publications Inc. 1984.

Soubrie, P., Simon, P. and Boissier, J.R. An amnesic effect of benzodiazepines in rats? Experientia (Basel) 1976; 32: 359-360.

Thiebot, M.H. Some evidence for amnesic-like effects of benzodiazepines in animals. Neuroscience and Behavioural Reviews 1985; 9: 95-100.

Waugh, N.C. and Norman, D.A. Primary memory. In Gardener, J.M. (ed.). Readings in human memory 1965; pp. 7-24. Methuen and Co. Ltd. 1976.