Effect of Diazepam on Acquisition, Retention, and Some Performance Variables in the Morris Water Maze

by

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B.Sc., University of Lethbridge, 1989

A Thesis Submitted in Partial Fulfilment of the
Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Psychology

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ABSTRACT

Diazepam is known to produce anterograde amnesia in both humans and animals. The present investigation sought to determine if this impairment is a direct result of diazepam's interference with mnemonic processes or a result of deficits in performance or retrieval. Diazepam (3 mg/kg) was administered prior to training in the Morris water maze either before or after the rats had learned the location of a submerged escape platform. Diazepam was found to impair acquisition but not retrieval of spatial information. This impairment was not due to the sedative, hypothermic or state-dependent learning effects of diazepam. In addition, there was no evidence of tolerance to the amnesic effects of diazepam over the 27 days of testing. These results replicate previous findings in the Morris water maze and provide new evidence that the deficit is primarily mnemonic in nature. Possible neural and electrophysiological substrates, the role of endogenous benzodiazepines, and the strategies used by diazepam-treated rats are discussed.

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ACKNOWLEDGEMENT

I would like to thank Ron for his logistical expertise and insight as well as his enthusiasm and support of my endeavours. This research was supported by an NSERC operating grant awarded to Dr. R. W. Skelton and a University of Victoria Fellowship awarded to R. K. McNamara.

INTRODUCTION

Since their synthesis by Hoffmann-La Roche, chlordiazepoxide (Librium®; 1955) and it's more potent analog diazepam (Valium®; 1959), have come to dominate the market for anxiolytic/sedative drugs (Blackwell, 1973). Soon after their release onto the market, benzodiazepines (BZD) were discovered to have amnesic properties (Feldman, 1963; Knight & Burgess, 1968). Since these early observations, a large literature has accumulated which effectively replicates and characterises BZD-induced memory impairment (Cole, 1986; Ghoneim & Mewaldt, 1990; Lister, 1985; Romney & Angus, 1984). The common finding among these studies is that BZDs impair acquisition while sparing short-term memory and retrieval processes. In fact, BZDs may actually produce a significant retrograde facilitation of memory (Brown, Brown, & Bowes, 1983; Ghoneim, Hinrichs, & Mewaldt, 1984; Hinrichs, Ghoneim, & Mewaldt, 1984; Mewaldt, Hinrichs, & Ghoneim, 1983; Rodrigo & Lusiardo, 1988).

Although the amnesic effects of BZDs may be considered a positive side-effect in presurgical situations, problems may arise because [1] BZDs are so widely prescribed and are typically taken while patient is engaged in his daily activities, [2] incomplete tolerance develops to the amnesic effects (Ghoneim, Mewaldt, Berie, & Hinrichs, 1981; Griffiths, McLeod, Bigelow, Liebson, & Roache, 1984; Lucki, Rickels, & Geller, 1985, 1986), [3] subjects may not be subjectively aware of their memory impairment, even after doing poorly on memory tasks (Hinrichs, Mewaldt, Ghoneim, & Berie, 1982), [4] the amnesia can last up to 14 hours after BZD administration (Ghoneim, Mewaldt, Berie, & Hinrichs, 1981), [5] the use of BZDs in a psychiatric context (i.e., to combat phobias) may hinder habituation or the acquisition of appropriate coping skills (Hafner & Marks, 1976). Therefore, it would be of practical value to determine the nature of this impairment more precisely.

It is not yet clear whether BZDs impair learning and memory processes directly or as a secondary result of performance deficits. Two alternative explanations are sedation and state-dependent learning. Sedation is a state of

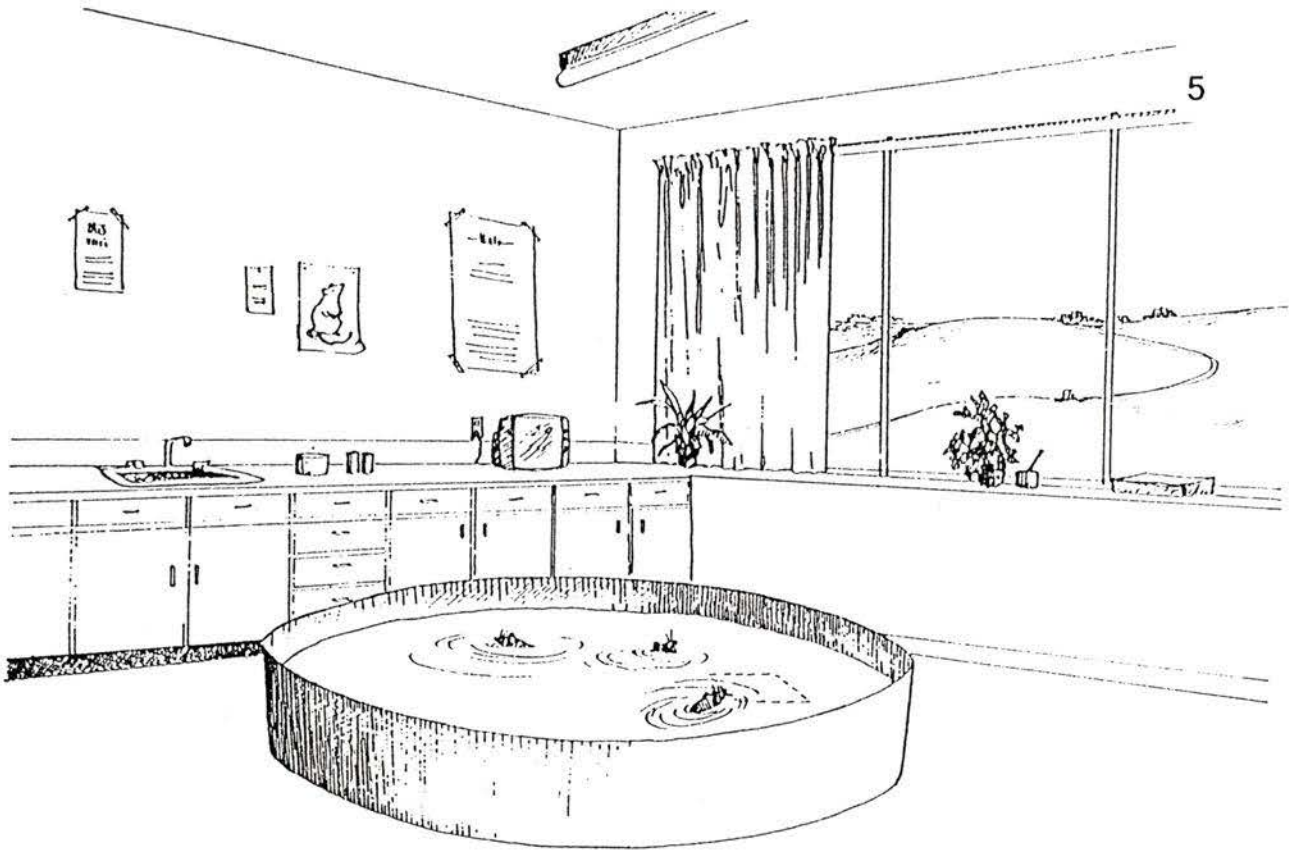
myorelaxation or drowsiness that may impair sensory and motor processes but not memory *per se*. This notion has been both supported (George & Dundee, 1977; Liljequist, Linnoila, & Mattila, 1978; Preston, Ward, Broks, Traub, & Stahl, 1989) and rejected (Desjardins, Moerschbaecher, Thompson, & Thomas, 1982; Ghoneim & Mewaldt, 1990; Pandit, Heisterkamp, & Cohen, 1976; Pomara, Stanley, Block, Guido, Stanley, Greenblatt, Newton, & Gershon, 1984; Rodrigo & Lusiardo, 1988). State-dependent learning is a phenomenon in which accurate recall only occurs when the subject is in the same drug state during both acquisition and recall (Overton, 1974). State-dependent learning may account for some of the amnesic effects of BZDs (Jensen & Poulsen, 1982; Petersen & Ghoneim, 1980), but cannot account for all of BZD's amnesic effects. For example, memory for information that is acquired and retrieved under the same drug state has been impaired whereas memory for information acquired in a non-drug state and recalled in a drug state is as good as, or better than placebo controls (Ghoneim et al., 1984; Hinrichs et al., 1984). In sum, it is not clear whether BZDs exert their amnesic effects through sedation, state-dependent learning or disruption of mnemonic processes.

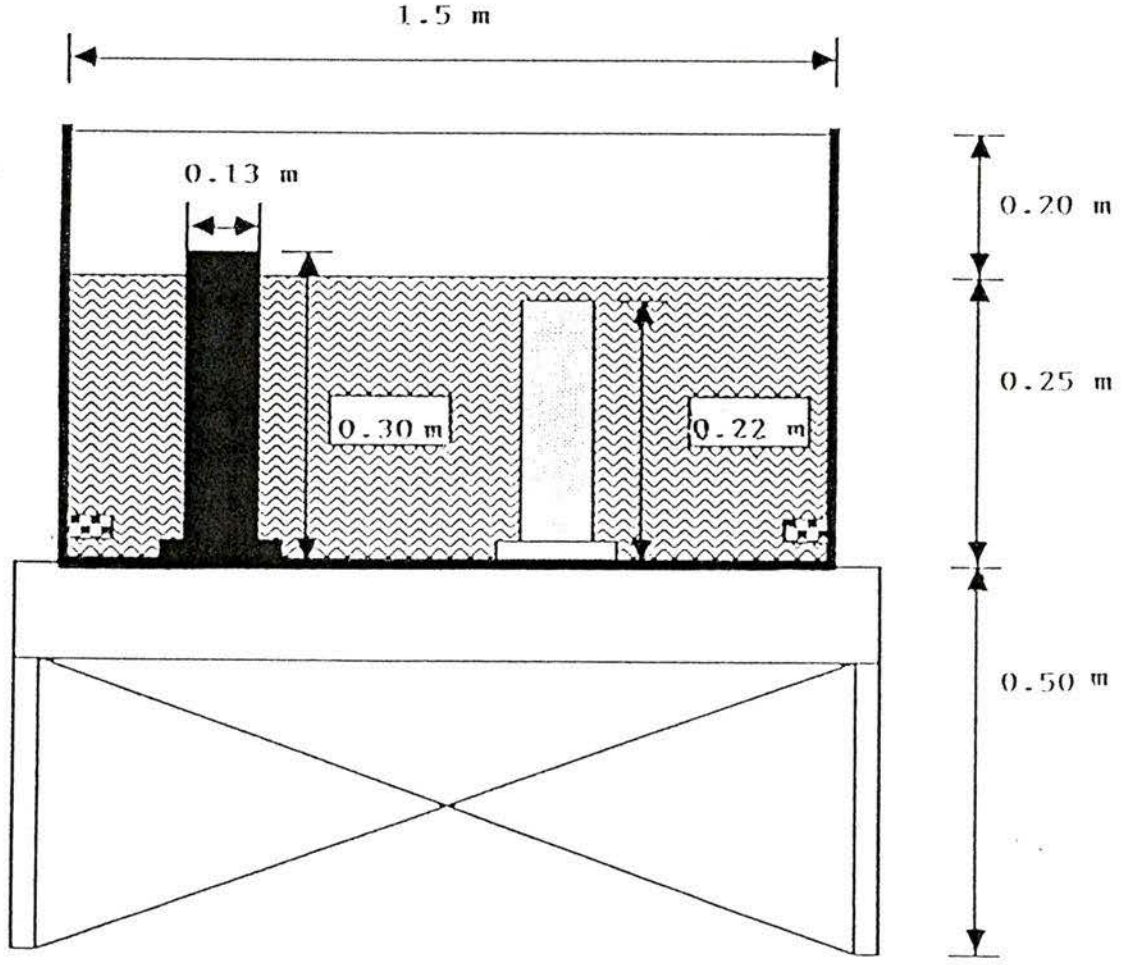
One approach to this problem would be to study the effects of BZDs in animals. BZDs have been found to impair learning and memory in animals (Cole, 1986; Dantzer, 1977; Thiebot, 1985), but many of these investigations failed to dissociate learning impairments from performance deficits. For example, BZDs may impair the acquisition of a passive avoidance task but this cannot be unambiguously attributed to impairments of learning and memory because BZDs increase pain thresholds (Houser & Pare, 1973; Wuster, Duka, & Herz, 1980) thereby attenuating the significance of the shock (Thiebot, 1985). Alternatively, the impairment of passive-avoidance may occur through state-dependent learning processes (Patel, Ciofalo, & Lorio, 1979). BZDs also impair successive discriminations, but, again, this cannot be unambiguously attributed to memory deficits. In this paradigm, BZD-treated rats overrespond during the waiting phase (errors of commission; Cole, 1986), an effect which has been attributed to the disinhibitory properties of BZDs rather than impairments of learning and

memory *per se* (Cole, 1990). In a third popular paradigm, the appetitively motivated radial arm maze (Olton & Samuelson, 1976), BZDs have inconsistent effects, both impairing performance (Hodges & Green, 1986; Willner & Birbeck, 1986) or having no effect (Hiraga & Iwasaki, 1984). In sum, the nature of BZD-induced memory impairment has not been clearly delineated in either animals or man.

One paradigm which may help to resolve this controversy is the Morris water maze (Fig. 1; Morris, 1981; Morris, 1984). In this task, rats are required to locate a submerged platform in a pool filled with opaque water by using extra-maze cue configurations (Sutherland & Rudy, 1989). This task appears well suited to the examination of BZD-induced amnesia because several different measures of *learning* and *performance* can be measured simultaneously over long periods of testing. Previous investigations have revealed that both chlordiazepoxide (McNaughton & Morris, 1987) and diazepam (McNamara & Whishaw, 1990) impair place-learning in the Morris water maze. However, whether this deficit is the result of mnemonic or performance impairments has not been determined. Regarding the myorelaxant effects of diazepam, McNaughton and Morris (1987) found that chlordiazepoxide-treated rats swam slower than controls but also found that chlordiazepoxide-treated rats swam faster than controls when the escape platform was removed from the pool. This leaves ambiguous whether the place-learning deficit was related to myorelaxation. Further, because the Morris water maze uses cold water (18-26^{o}C ±1) to motivate the animal, it is possible that this impairment was due to BZD-induced hypothermia, rather than a direct effect of BZDs on memory processes (Riccio & Richardson, 1984; Zarrindast & Dibayan, 1989). Additionally, it is possible that that the observed place-learning deficit resulted from impairments of retention, recall or sedation. The present study sought to determine the significance of hypothermia, perception/attention, myorelaxation, and retention deficits in relation to the amnesic effects of diazepam. Finally, it seemed worthwhile to examine the state-dependent learning phenomenon in the present paradigm as well as tolerance development to the amnesic effects of diazepam.

Figure 1: Illustration of the Morris water maze. Only one platform was present during a given training session. (top illustration adapted from "Plasticity in the Neocortex: Mechanisms Underlying Recovery From Early Brain Damage" by Kolb, B., and Whishaw, I. Q., 1989, Progress in Neurobiology, 32, p. 242. © 1989 by Pergamon Press. Adapted with permission.).




1.5 m
0.13 m
0.20 m
0.25 m
0.30 m
0.22 m
0.50 m

METHOD

Animals

Twenty-five Long Evans male rats (Charles-River, Quebec) weighing 400-550 kg served as subjects. They were housed individually in the animal vivarium where lights were maintained on a 12:12 hr light-dark cycle. All tests were conducted during the light portion of the cycle. Food and water were available ad libitum.

Apparatus

The Morris water maze consisted of a circular pool (diameter: 150 cm, height: 45 cm), with a featureless white inner surface. The pool was filled to a height of 25 cm with 22°C (± 1°C) water, in which 1500 ml of powered skim milk was dissolved. The hidden escape platform was a clear Plexiglas stand (13 X 13 cm) submerged 3 cm below the water surface so that it was invisible at water level. The visible platform was a black stand (13 X 13 cm) that protruded 5 cm above the surface of the water.

Drugs and Group Assignment

At the beginning of the experiment, rats were randomly divided into five treatment groups. The first group (Diazepam; Hoffmann-La Roche; n = 5) was given diazepam throughout both acquisition and reversal phases (see procedure). The second group (Saline; n = 5) received an equivalent volume of saline for both phases of testing and served as the placebo control. The third group (diazepam-saline, DS; n = 5) received diazepam for the first phase (acquisition) and saline for the second phase (platform reversal). The fourth group (saline-diazepam, SD; n = 5) received saline first and then diazepam. The fifth group (Switch; n = 5) received saline until they acquired the platform location, at which point they were switched to diazepam (3 mg/kg) for the remainder of the acquisition phase, and also for the reversal phase. Diazepam (Hoffman-La Roche Inc.) was always injected IP in a dose

of 3 mg/kg; saline (0.9%) was delivered in an equivalent volume of 0.6 ml/kg. All injections were administered in the pool room 20 min prior to testing. Control experiments have found that equivalent volumes of the commercial diazepam vehicle (propylene glycol, ethanol, buffer) does not have any significant effects on maze performance (unpublished observations).

Procedure

The experiment was divided into an initial acquisition phase (I) which consisted of training all groups to acquisition criterion, plus a probe test, a cue test, retraining and a drug reversal probe; and a reversal phase (II) which included training all groups to criterion performance with a reversed platform location plus a second probe test.

Phase I: Initial acquisition. During initial acquisition, the hidden escape platform was located in the centre of the northwest quadrant. All groups were given four trials each day and tested until an acquisition criterion was reached (mean group distance under 250 cm over two consecutive days). For each trial the rat was placed in the water facing the pool wall at one of four randomly determined starting locations (north, south, east, or west pole). During each trial, the rat's swim path, drawn on a map of the pool and measured with a map-reading device, and escape latency, measured with a stopwatch to a tenth of a second, were recorded. Once the rat located the platform, it was permitted to remain on it for 15 seconds, and the occurrence of a rear was recorded. The rat was removed from the pool if it did not locate the platform within 60 seconds. The rat was placed on the platform for 15 sec at the end of the final trial if it did not locate the platform at least once on the previous four trials. After each trial, the rat was returned to a waiting cage positioned 90 cm under a 250 W brooding lamp (for warmth) and allowed to remain there for the 5 min intertrial interval. The rat's core body temperature was measured rectally three times daily: prior to drug administration (pre-drug), thirty minutes after drug administration (pre-swim), and immediately following the last trial (post-swim). Pre-swim temperature change (T_c) was calculated relative to the pre-drug temperature;

post-swim temperature change (T_c) was calculated relative to the pre-swim temperature.

After acquisition was complete, a probe trial was given to assess the strength and accuracy of initial acquisition. Rats were required to swim in the pool without the escape platform for 60 sec. All rats were released from the same starting location and the distance spent in each quadrant was recorded. Following this single trial, a cue task was given to assess any sensorimotor deficits induced by diazepam. Rats were required to navigate to a visible platform located in a different quadrant on each trial; swim path lengths and escape latencies were recorded.

For the next two days, rats were retrained to the original platform location to compensate for interference produced by the probe and cue tests. This consisted of eight additional trials (4 trials/day) with the hidden platform replaced in the old location. After reacquisition, the DS and SD groups were given the appropriate drug reversal and a second probe trial was given.

Phase II: Platform reversal. To assess the effects of drug reversal on acquisition, all rats were required to learn the location of the hidden platform placed in the quadrant diagonally opposite to the previous location. To assess proactive interference, the distance spent in the old quadrant was also measured. After each group had reached the acquisition criterion (mean group distance under 250 cm over 2 consecutive days), a final probe trial was given.

Data Analysis

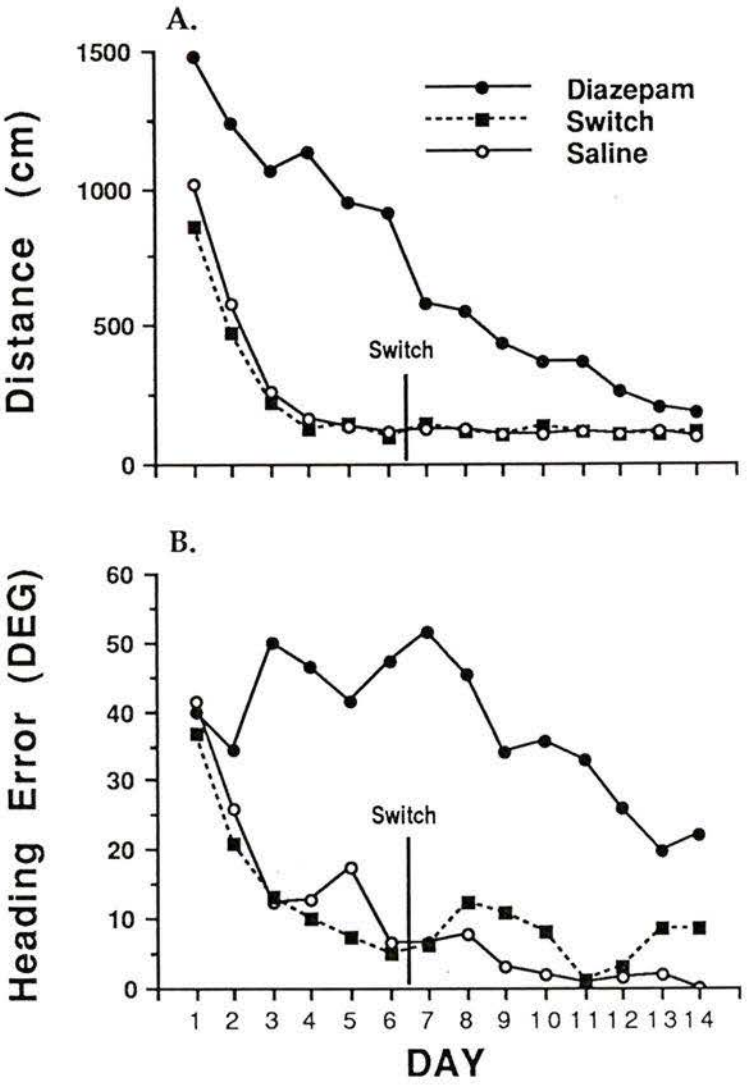
Over both phases of testing, group differences in escape latency, swim path length, heading error (degrees deviation from a straight line between the start location and the platform over the first 12 cm of the swim path), swim speed, T_c, and the probability of rearing at least once while on the platform were assessed using analysis of variance (ANOVA) with repeated measures. Pairwise comparisons were assessed using Tukey's (HSD) method (Cliff, 1987). In every case the acceptable level for statistical significance was $P<0.05$.

RESULTS

Phase I: Initial acquisition. The distance required by the Diazepam, Saline, and Switch groups to locate the submerged escape platform over the 14 d of testing is shown in Figure 2A. The Saline and Switch groups rapidly acquired the platform location, reaching asymptotic performance by the fourth day of testing. The Diazepam group was impaired on all three measures of performance. An overall ANOVA on swim path lengths revealed a significant Group effect, $F(2,97)=207.4$, $p<0.001$, Day effect, $F(13,1261)=34.9$, $p<0.001$, and Group X Day interaction, $F(26,1261)=4.7$, $p<0.001$. Escape latencies showed a similar pattern to swim path lengths (data not shown). An ANOVA on escape latencies revealed a significant Group effect, $F(2,97)=172.3$, $p<0.001$, Day effect, $F(13,1261)=53.9$, $p<0.001$, and Group X Day interaction, $F(26,1261)=8.4$, $p<0.001$. Heading errors provided an even clearer picture of diazepam's effects (see Fig. 2B) and an ANOVA on this measure revealed a significant Group effect, $F(2,97)=68.0$, $p<0.001$, Day effect, $F(13,1261)=10.6$, $p<0.001$, and Group X Day interaction, $F(26,1261)=3.1$, $p<0.001$. Post hoc comparisons revealed that the Diazepam group had longer swim paths ($P<0.01$), longer escape latencies ($P<0.01$) and greater heading errors ($P<0.01$) than the Saline group. Perhaps the most significant observation was that diazepam did not impair performance of a previously learned escape response. When the Switch group was switched from saline to diazepam on Day 7, they showed no increase in path length or any other impairment of place-learning (Fig. 2A).

Diazepam also reduced swim speed (Fig. 3) and T_c (Fig. 4) . An overall ANOVA on swim speed revealed a significant Group effect, $F(2,97)=42.7$, $p<0.001$, Day effect, $F(13,1261)=4.6$, $p<0.001$, and Group X Day interaction, $F(26,1261)=2.5$, $p<0.001$. An overall ANOVA on pre-swim T_c (Fig. 4A) revealed a significant Group effect, $F(2,22)=14.3$, $p<0.001$, Day effect, $F(13,286)=7.0$, $p<0.001$, and Group X Day interaction, $F(26,286)=4.3$, $p<0.001$. For post-swim T_c (Fig. 4B), a significant Group effect, $F(2,22)=5.8$, $p<0.01$, Day effect, $F(13,286)=22.3$, $p<0.001$, and Group X Day interaction, $F(26,286)=1.7$, $p<0.01$,

Figure 2: Effects of diazepam on (A) the distance taken to locate the escape platform and (B) heading errors. Note that the preadministration of diazepam resulted in greater distances required to find the platform as well as larger heading errors. Also when the Switch group was *switched* from saline to diazepam on day 7, neither the distance nor heading errors increased substantially.


A.
1500
1000
500
0
Distance (cm)
Diazepam
Switch
Saline
Switch
B.
60
50
40
30
20
10
0
Heading Error (DEG)
Switch
1 2 3 4 5 6 7 8 9 10 11 12 13 14
DAY

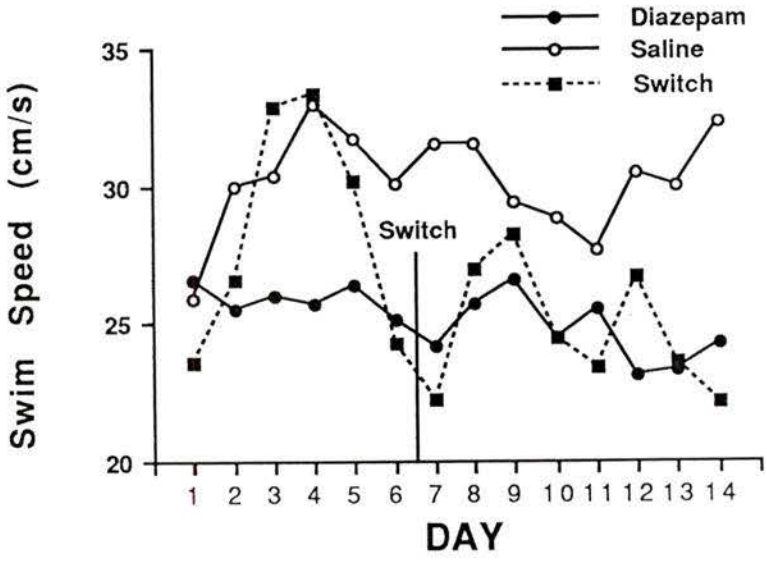
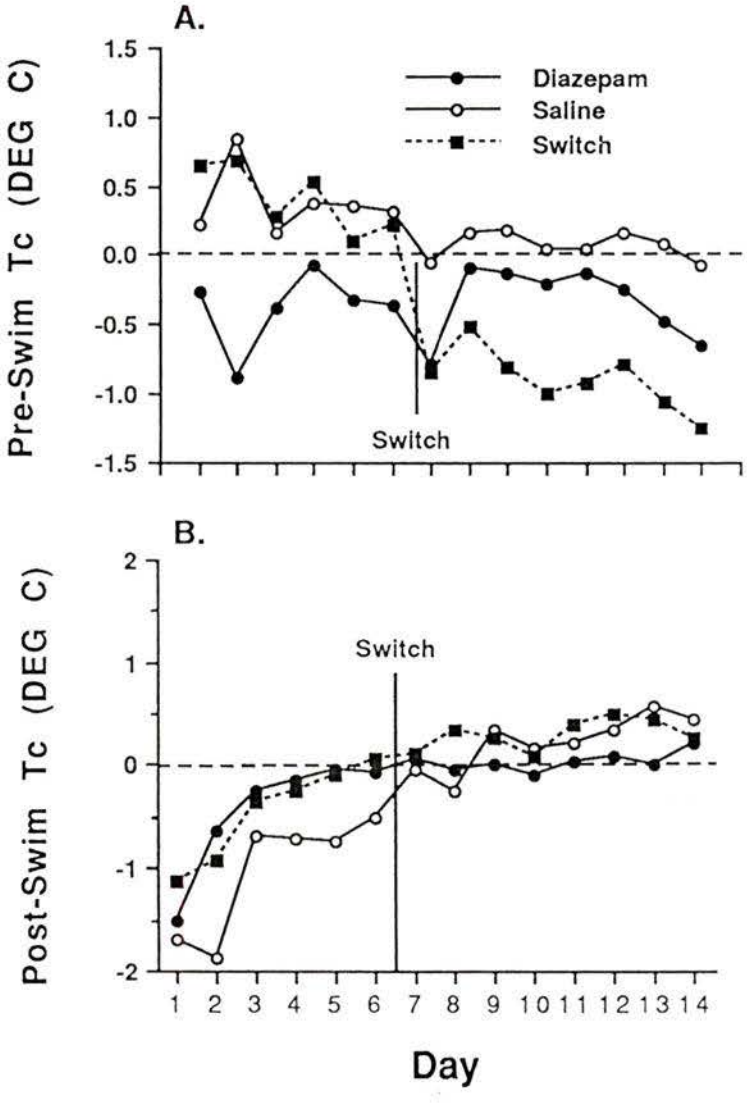


Figure 3: Effects of diazepam on swim speed. Note the consistently slower swim speeds of the Diazepam group, and the sustained swim speed reduction resulting from switching the Switch group from saline to diazepam.

Figure 4: Effects of diazepam on (A) pre-swim T_c (pre-swim - pre-drug) and (B) post-swim T_c (post-swim - pre-swim) during initial acquisition. Note: [1] the consistently lower pre-swim T_c over the course of testing, [2] when the Switch group is switched to diazepam on day 7, the pre-swim T_c is reduced and [3] all three groups show increases in post-swim T_c over the course of testing (B).


A.
Pre-Swim Tc (DEG C)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Diazepam
Saline
Switch
Switch
B.
Post-Swim Tc (DEG C)
2
1
0
-1
-2
Switch
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day

was revealed. Post hoc analysis showed that the Diazepam group swam slower ($P<0.01$) and had lower pre-swim ($P<0.01$), but not post-swim, T_c measures relative to the Saline group. Thus, diazepam lowered body temperature, but did not significantly exacerbate the hypothermia produced by swimming in the pool. In the Switch group, diazepam treatment did result in a sustained reduction of swim speeds ($P<0.05$)(Fig. 3) and pre-swim T_cs ($P<0.01$)(Fig. 4). Oddly, the Switch group's swim speed was erratic and decreased on the day before the group was switched to diazepam. The cause of this anomaly is unknown.

The diazepam-induced impairment of acquisition, but not retention, was confirmed in the probe trial that immediately followed acquisition (Fig. 5). Here the Saline and Switch groups ($P<0.01$), but not the Diazepam group, demonstrated a preference for the correct quadrant, with distances in the correct quadrant being significantly above chance levels. Swim speeds did not differ between groups during the probe trial (Fig. 5B). Surprisingly, only the Saline group showed reductions in post-swim T_c during the probe trial (Fig 5C). Figure 6 shows the actual swim paths taken during the probe trial by rats closest to their group mean. These paths reveal the typical pattern of their respective groups; saline-treated rats showed a concentrated search in the correct quadrant; diazepam-treated rats swam all over the pool in gradual loops; rats switched from saline to diazepam concentrated their search in the correct quadrant, but tended to have more gradual turns than rats treated with saline.

None of the groups were impaired when required to navigate to the black visible platform, $F(2,97)=1,73$, $p=0.18$, (Fig. 7). This result suggests that the diazepam-treated animal can learn to swim to a single visual cue to escape the cold water. Further, diazepam treated rats can coordinate their behaviour to reach and climb onto the platform.

During the drug reversal probe trial (Fig. 8), the Saline group showed a significant reduction of the distance spent in the correct quadrant relative to the previous probe trial ($P<0.01$). This suggests that the previous probe trial

Figure 5: Effects of diazepam on (A) the distance spent in the correct quadrant, (B) swim speed, and (C) post-swim T_c (post-swim - pre-swim) during the first probe trial. Note that both the Saline and Switch groups, but not the Diazepam group, demonstrated an 'above chance' preference for the quadrant that previously contained the escape platform. Also none of the group's swim speeds differed during the probe trial. Finally, only the Saline group's post-swim T_c was reduced. Data expressed as mean ± S.E.M.. * $P<0.01$ compared to chance level (25%) in (A) and to Saline T_c in (C).

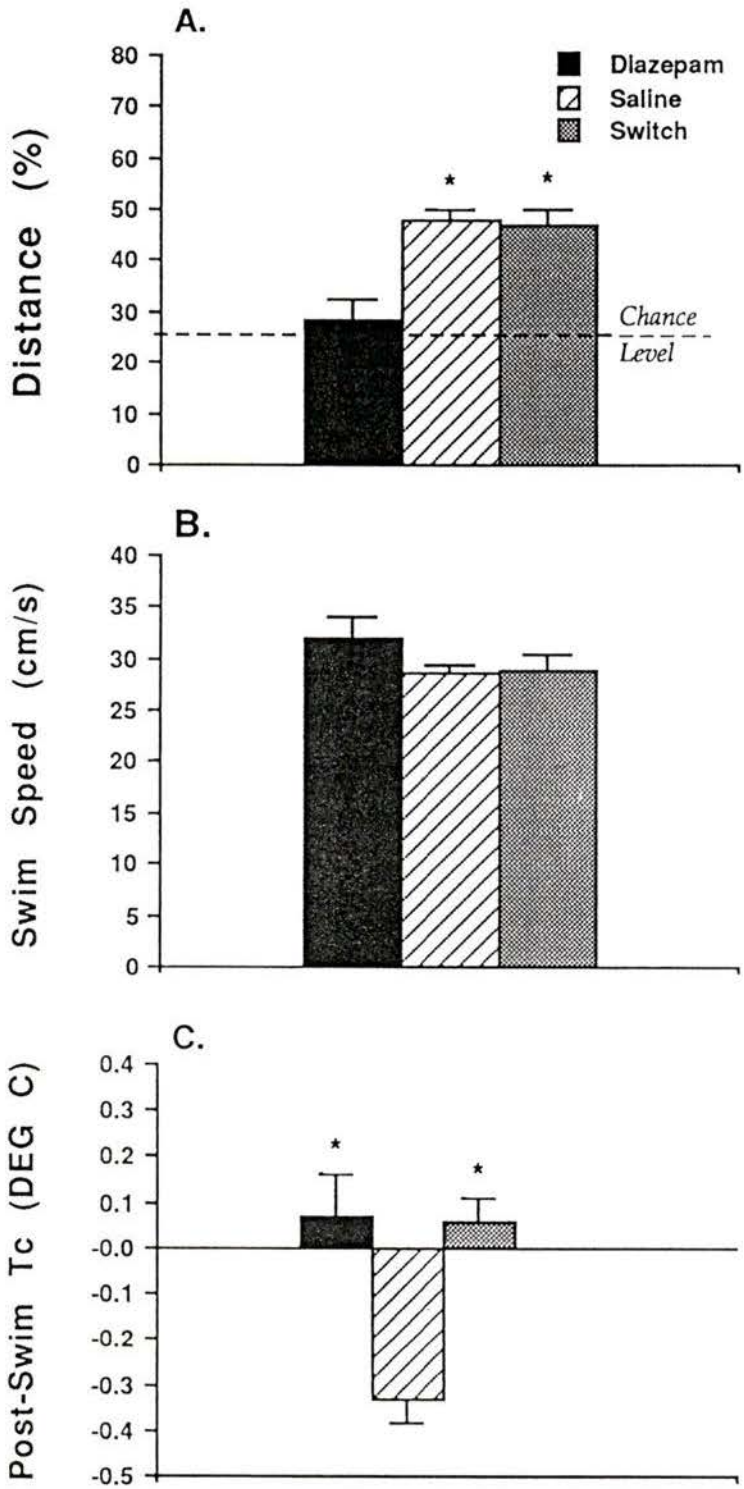
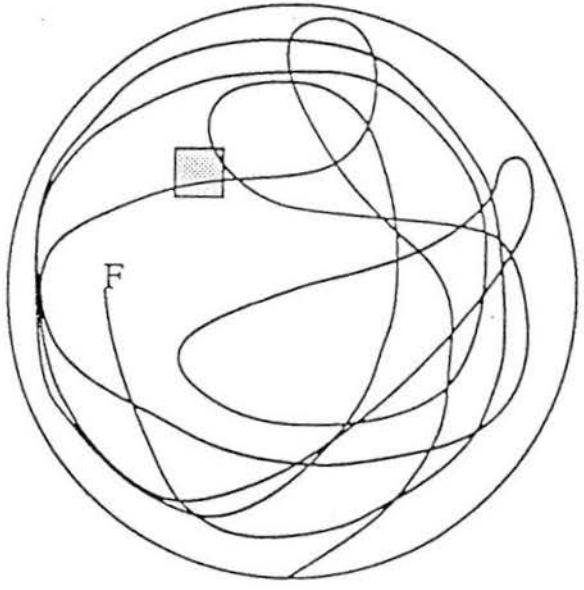
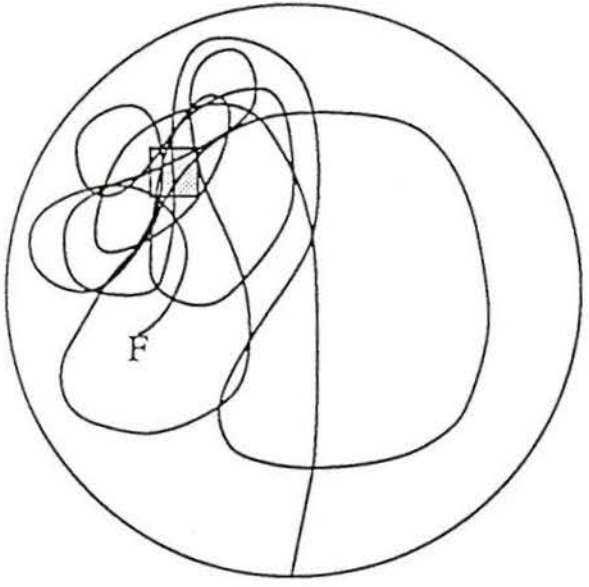

A.
Distance (%)
80
70
60
50
40
30
20
10
0
Diazepam
Saline
Switch
*
*
Chance
Level
B.
Swim Speed (cm/s)
40
35
30
25
20
15
10
5
0
C.
Post-Swim Tc (DEG C)
0.4
0.3
0.2
0.1
-0.0
-0.1
-0.2
-0.3
-0.4
-0.5
*
*

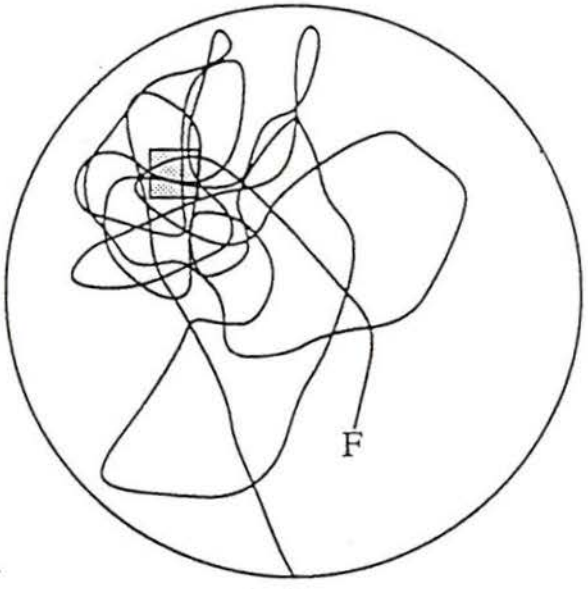
Figure 6: Swim paths during the first probe trial from rats closest to their group mean. Note that the Switch group rat, despite having an elongated and circuitous swim path, still spent the majority of time in the correct quadrant. The 'F' denotes where the rat was removed from the pool after the trial was finished.



Diazepam



Switch



Saline

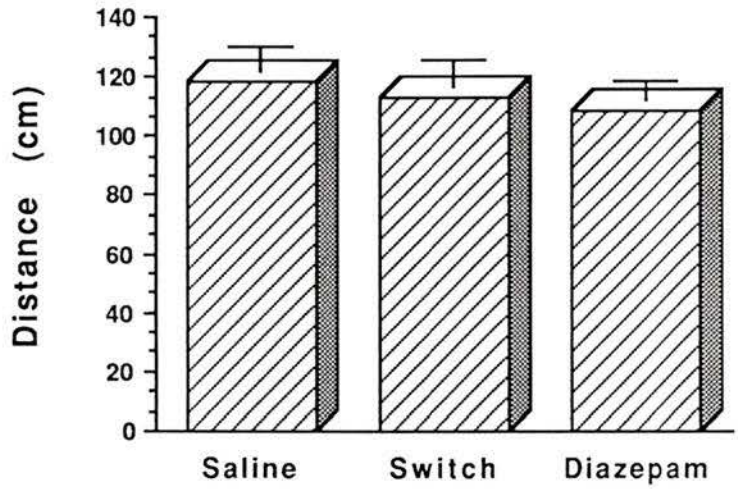


Figure 7: The distance taken by each treatment group when required to navigate to a visible platform. Note that none of the treatment groups differ in their ability to reach the platform.

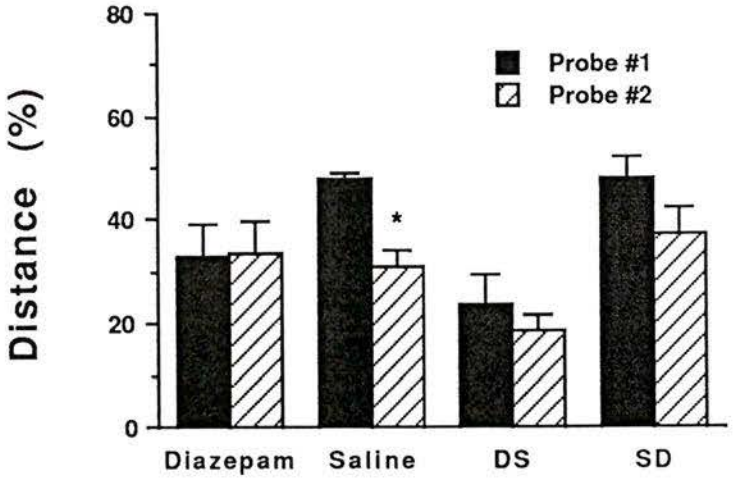


Figure 8: The percentage distance spent in the correct quadrant during the first probe (Probe #1) trial and the drug reversal probe (Probe #2). Note that only the Saline group (unexpectedly) demonstrated a significant reduction in the duration spent in the correct quadrant during the second probe trial. *$P<0.01$ compared to probe #1.

and the cue task disrupted retention of the original platform location and that no conclusions can be made regarding the state-dependent learning phenomenon from this test.

Phase II: Platform reversal. The swim path lengths and heading errors for the reversal phase are shown in Figure 9. Once again, diazepam significantly impaired the rats' ability to locate the hidden platform. An overall ANOVA on swim path lengths revealed a significant Group effect, $F(4,95)=19.0$, $p<0.001$, Day effect, $F(7,665)=22.9$, $p<0.001$, and Group X Day interaction, $F(28,665)=1.8$, $p<0.01$. Once again escape latencies showed a similar pattern to swim path lengths (data not shown), and an ANOVA revealed a significant Group effect, $F(4,95)=23.1$, $p<0.001$, Day effect, $F(7,665)=25.1$, $p<0.001$, and Group X Day interaction, $F(28, 665)=2.7$, $p<0.001$. Further, a significant Group effect, $F(4,95)=33.3$, $p<0.001$, and Day effect, $F(7,665)=5.8$, $p<0.001$, but not a significant Group X Day interaction, $F(28,665)=0.7$, $p=0.90$, were found for heading errors. Post hoc analysis revealed that only those groups receiving diazepam (SD, Diazepam, Switch) demonstrated impaired acquisition, showing longer escape latencies ($P<0.01$), longer swim paths ($P<0.01$), and greater heading errors ($P<0.01$) relative to the Saline group. The DS group behaved like the Saline group and was significantly better than the diazepam-treated groups ($P<0.01$). The probe trial (Fig. 10) confirmed these findings with the groups treated with diazepam (Diazepam, SD, Switch), but not saline (Saline, DS), failing to show a preference for the correct quadrant ($P<0.01$). During the probe trial, none of the groups differed on swim speeds or post-swim T_c (Fig. 10 B, C). Interestingly, the DS group showed no evidence of residual impairment from previous diazepam treatment, spending more time in the correct quadrant than any other group. Also, there was no evidence of tolerance to the amnesic effects of diazepam. This was revealed during the platform reversal phase (see Fig. 9A) where the Diazepam group (chronic; 18 d) was as impaired as the SD (acute) group on escape latency, swim path length, and heading error.

The percentage of distance spent by each group in the previously correct quadrant is illustrated in Figure 11. All of the groups except for the DS group

Figure 9: An illustration of (A) the distance required to locate the hidden escape platform and (B) heading errors during reversal acquisition. Note that those groups receiving diazepam (SD, Diazepam, Switch) have greater distances and heading errors.

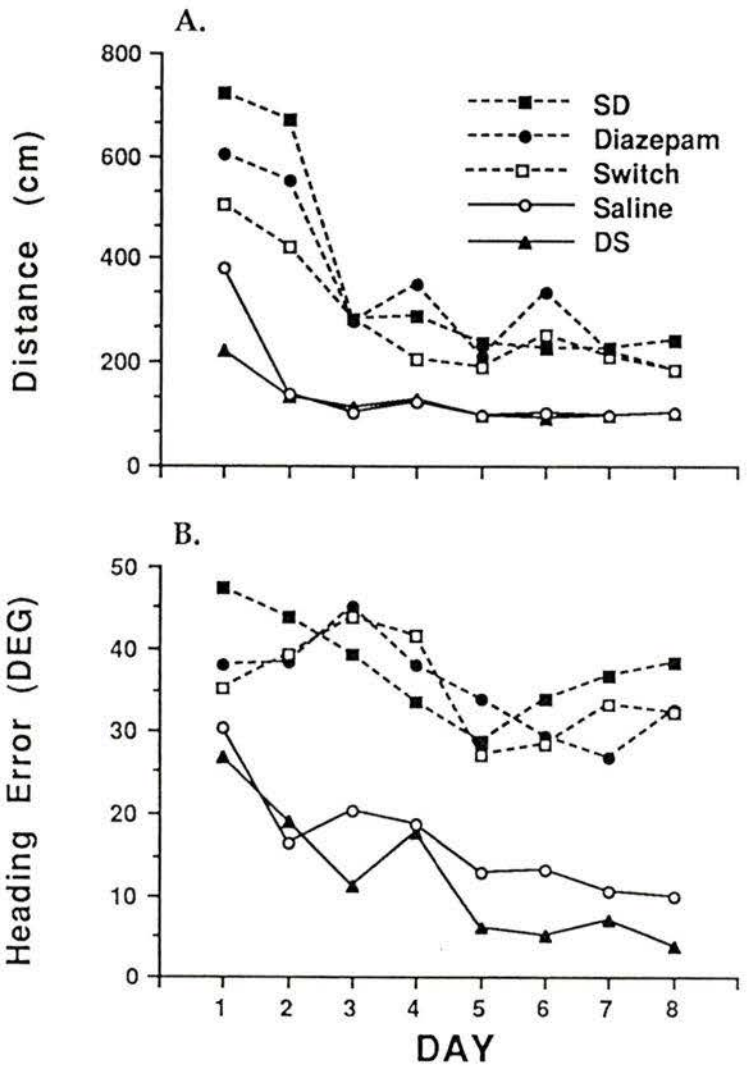
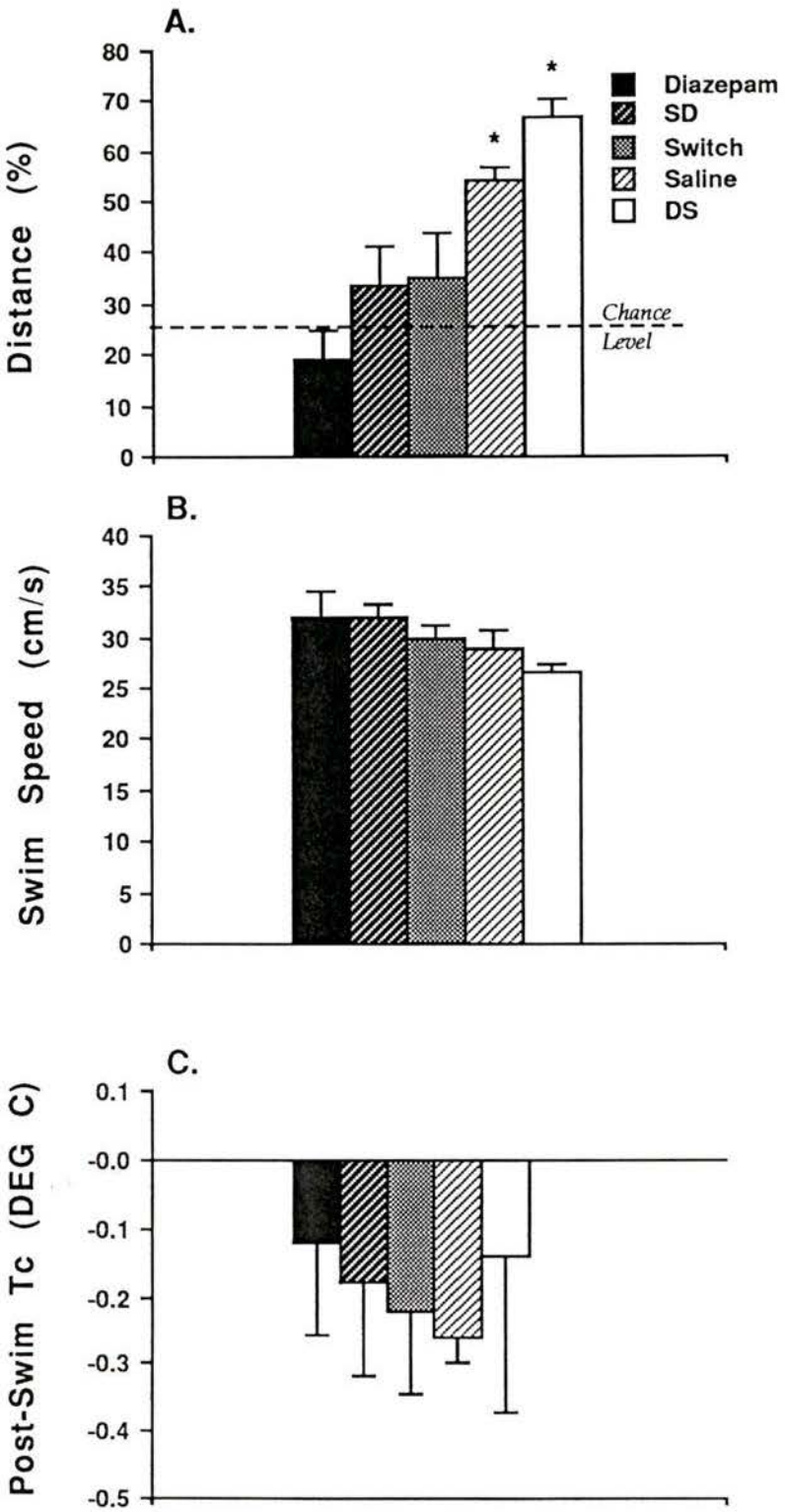

A.
800
600
400
200
0
Distance (cm)
SD
Diazepam
Switch
Saline
DS
B.
50
40
30
20
10
0
Heading Error (DEG)
1
2
3
4
5
6
7
8
DAY

Figure 10: The percentage distance in the correct quadrant (A), swim speed (B), and post-swim T_c (pre-swim - post-swim)(C) during the final probe trial. Note that the saline treated groups (Saline and DS), but not the groups receiving diazepam (Diazepam, SD, Switch), demonstrate a preference for the correct quadrant, despite comparable swim speeds and post-swim T_cs. *P<0.01 compared to chance level (25%).


A.
Distance (%)
80
70
60
50
40
30
20
10
0
*
*
Diazepam
SD
Switch
Saline
DS
Chance
Level
B.
Swim Speed (cm/s)
40
35
30
25
20
15
10
5
0
C.
Post-Swim Tc (DEG C)
0.1
-0.0
-0.1
-0.2
-0.3
-0.4
-0.5

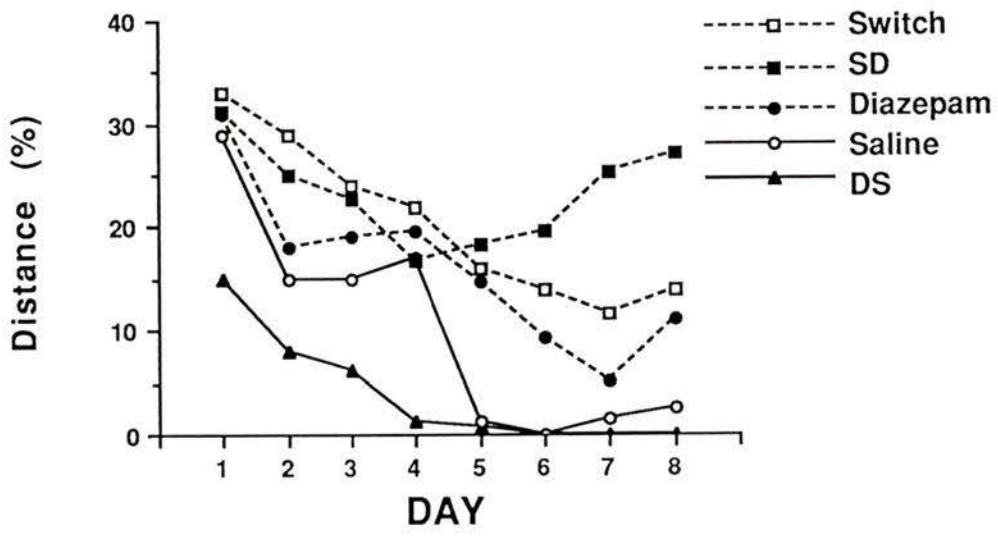


Figure 11: An illustration of the percentage distance spent in the previously correct quadrant during the reversal phase. Note that the DS group demonstrated little proactive interference while the SD group remained at chance levels throughout testing.

displayed proactive interference during the initial testing with the reversed location. All of the groups except for the SD group showed a gradual decline in the distance spent in the previously correct quadrant. Analysis of variance revealed a significant Group effect, $F(4,20)=27.7$, $p<0.001$, Day effect, $F(7,140)=15.4$, $p<0.001$, but not a significant Group X Day interaction, $F(28,140)=1.2$, $p=0.30$. Post hoc analysis revealed that relative to the Saline group, the Switch and SD groups spent a greater percentage of distance in the old quadrant ($P<0.01$) while the DS group spent a lower percentage of distance in the old quadrant ($P<0.05$). The greatest perseveration to the previously correct quadrant was shown by the SD group, which had been originally trained under saline and then reversed to diazepam. This is opposite to the predictions of the state-dependent learning hypothesis.

Performance variables were also affected by diazepam during the reversal phase. Diazepam-treated rats again swam slower under diazepam relative to the saline-treated controls. The DS group showed a marginal reduction of swim speed relative to controls. Analysis of swim speed data (Fig. 12) revealed a significant Group effect, $F(4,95)=23.9$, $p<0.001$, Day effect, $F(7,665)=2.8$, $p<0.01$, but not a significant Group X Day interaction, $F(28,665)=1.3$, $p=0.10$. Those groups receiving diazepam (Diazepam, SD, Switch), as well as the DS group ($P<0.05$), had slower swim speeds relative to the Saline group ($P<0.01$). Although there were no group difference in post-swim T_cs, diazepam-treated rats showed reductions of pre-swim T_c compared to saline controls (Fig. 13). The DS group's pre-swim T_c did not differ from saline-treated controls. Analysis of pre-swim T_c data (Fig. 13A) revealed a significant Group effect, $F(4,20)=37.8$, $p<0.001$, Day effect, $F(7,140)=2.6$, $p<0.01$, and Group X Day interaction, $F(28,140)=1.6$, $p<0.05$. However, analysis of post-swim T_c data (Fig. 13A) did not reveal a significant Group effect, $F(4,20)=1.6$, $p=0.21$, although there was a significant Day effect, $F(7,140)=1.6$, $p<0.05$, and Group X Day interaction, $F(28,140)=1.6$, $p<0.05$. For the pre-swim T_c measure, only those groups treated with diazepam (Diazepam, Switch, SD) had lower temperatures relative to the Saline group ($P<0.01$). Analysis of the probability of rearing while on the platform failed to reveal a significant Group effect, $F(1,8)=0.7$, $p=0.42$, Day effect, $F(2,16)=2.5$,

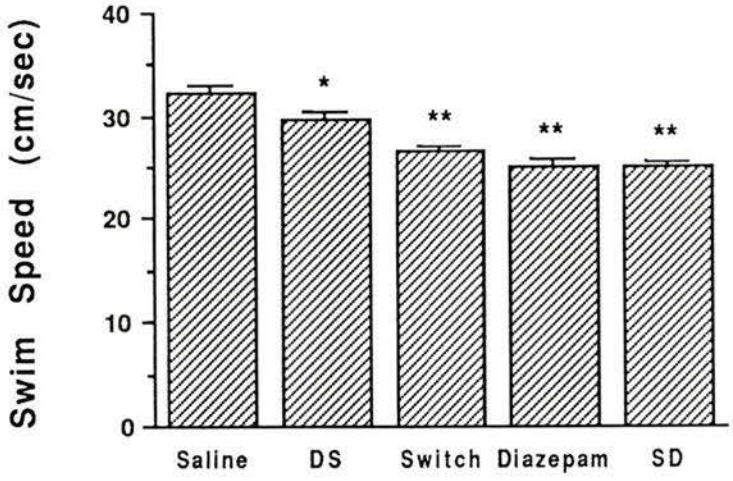
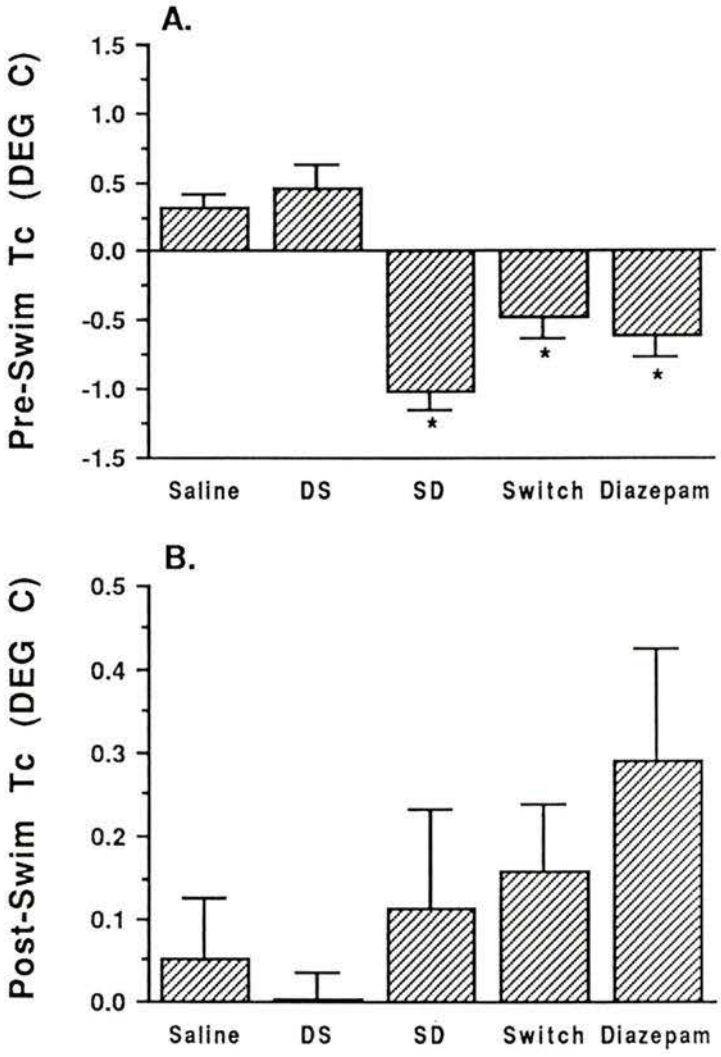


Figure 12: Effects of diazepam on swim speed during reversal acquisition. *$P<0.05$, **$P<0.01$ compared to the Saline group. Data presented as mean ± S.E.M.

Figure 13: Effects of diazepam on (A) pre-swim T_c (pre-swim - pre-drug) and (B) post-swim T_c (post-swim - pre-swim) during reversal acquisition. Note the lower pre-swim T_cs, but not post-swim T_cs, of the diazepam-treated rats. *P<0.01 compared to the Saline group. Data presented as mean ± S.E.M.


A.
Pre-Swim Tc (DEG C)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
*
*
*
Saline
DS
SD
Switch
Diazepam
B.
Post-Swim Tc (DEG C)
0.5
0.4
0.3
0.2
0.1
0.0
Saline
DS
SD
Switch
Diazepam

p=0.15, or Group x Day interaction, F(2,16)=0.524, p=0.63. However, it might be noted that both the Saline and Diazepam groups showed a dishabituationof rearing when the platform was reversed, but only the Saline group showed a full habituation of rearing by the final day of the reversal phase.

DISCUSSION

The present study found that diazepam-treated rats have severe place-learning deficits as revealed by longer swim paths, slower escape latencies and larger heading errors relative to saline-treated controls. Diazepam-treated rats also had slower swim speeds and lower core body temperatures throughout acquisition. The group switched from saline to diazepam demonstrated total savings, despite reductions of core body temperature and swim speed. Diazepam did not impair acquisition of a visible platform task. When the location of the submerged platform was reversed, only those rats receiving diazepam demonstrated impaired acquisition. There was no evidence of tolerance to the diazepam-induced amnesia over the 27 days of testing. The present impairment of acquisition replicates previous findings in the Morris water maze (McNamara & Whishaw, 1990; McNaughton & Morris, 1987) and further suggests that this deficit is primarily mnemonic, specific to encoding processes and non-tolerating. The following discussion will focus on each of these principle findings individually and in relation to some current themes in the BZD literature.

Diazepam has sedative properties which have two consequences: attentional/perceptual impairments and myorelaxation. In the present study, the place-learning deficit cannot be attributed to the myorelaxation effects of diazepam for three reasons: [1] the Diazepam group swam consistently slower than the Saline group, even as the group reached criterion levels, [2] the Switch group did not show impaired maze performance in spite of a reduction in swim speed, and [3] the Diazepam group did not swim slower during the probe trials. These results suggest that the place-learning deficit produced by diazepam cannot be attributed to the myorelaxation side-effects.

BZD-induced memory impairments have been attributed to deficits of attention/perception in both humans (Preston et al., 1989) and animals (Willner & Birbeck, 1986). In the present study, for example, the Diazepam

group's acquisition impairment may have been the result of blurred vision resulting from diazepam (e.g., Gilman, Goodman & Gilman, 1980). However, the Switch group, while receiving diazepam, was still able to navigate to the hidden platform. Because the distance taken to locate the platform did not increase when diazepam was administered, it is likely that the Switch group continued to use the same, efficient 'place' strategy (Sutherland & Dyck, 1984). This finding would suggest that the diazepam-treated rat can use, perceive and attend to spatial cues to locate the platform. However, because the Switch group was already familiar with relevant spatial cues, it is possible that they were better able to use those cues once their vision was obscured by diazepam. Thus, it is possible that diazepam impaired acquisition by impairing the rat's perception of ambient room cues. Visual discrimination tests are presently being designed to analyze the perceptual capabilities of diazepam-treated rats.

As mentioned, it is possible that the BZD-induced acquisition impairment may be attributed to hypothermia. Several studies have demonstrated that diazepam can induce hypothermia (present results; Clark & Lipton, 1981; Wiechman & Spratto, 1982; Zarrindast & Dibayan, 1989). Further, hypothermia alone can induce both retrograde (Riccio, Hodges, & Randall, 1968) and anterograde amnesia (Richardson, Riccio, & Morilak, 1983), and, cold water is used to motivate the rat in the Morris water maze. Therefore, the combination of both drug- and environmentally-induced hypothermia might be expected to impair memory processes. However, three results from the present study suggest that the diazepam-induced impairment of place-learning was independent of hypothermia: [1] the pre-swim hypothermia remained consistently low as acquisition progressed, [2] the Diazepam group's body temperature did not decrease during swimming more than that of controls, and [3] the Diazepam group was impaired but not hypothermic on all three probe trials. Further, the average change in body temperature for the groups receiving diazepam was -0.35°C (±0.1) prior to swimming and -0.21°C (±0.2) after swimming. These drops in body temperature are not as severe as those previously found to induce amnesia, which typically exceed 5°C below normothermia (Riccio & Richardson,

1984). These results suggest that hypothermia did not produce the observed anterograde amnesia.

As mentioned, previously reported amnesic effects of BZD have been attributed to state-dependent learning (Patel et al., 1979), but such was not the case in the present investigation. Here, rats given diazepam during both acquisition and retrieval (Diazepam group) were impaired whereas rats trained under saline and switched to diazepam (Switch group) were not. Furthermore, the group which displayed the most proactive interference in the reversal phase was the one that had been trained under saline and then reversed to diazepam. These results are opposite to what would have been predicted by the state-dependent learning hypothesis (Overton, 1974).

Diazepam also reduces indices of anxiety (Geller, Kulak, & Seifter, 1962) and produces analgesia (Houser & Pare, 1973). Anxiety has typically been viewed as an impediment to learning and memory (e.g., Eysenck, 1979). In contrast, the present results suggest that anxiety is necessary for spatial learning. It was noted incidentally that saline-treated, but not diazepam-treated, rats consistently vocalized when handled, suggesting that the diazepam-treated rats were less 'emotional' (Archer, 1973). However, diazepam did not impair acquisition of the visible platform task, suggesting that this task does not require anxiety for accurate performance and that an anxious state is not a requirement for new, cue-based learning to occur. With the hidden platform task, it was clear that anxiety was not required for performance of the task, only for its acquisition. The relationship between anxiety and memory awaits a more systematic enquiry.

It is possible that diazepam may have reduced the rate of acquisition by attenuating the aversiveness of the cold water used for motivation. In the present study, the Diazepam group swam slower over the course of testing, which could suggest reduced motivation. BZDs have been found to increase opioid activity (Wuster et al., 1980), pain thresholds (Houser & Pare, 1973), and the occurrence of punished behaviour (Geller et al., 1962). Further, naloxone, an opioid antagonist, has been found to facilitate acquisition in

the Morris water maze (Decker, Introini-Collison, & McGaugh, 1989). However, BZDs have been found to impair place-learning in the Morris water maze when 19°C (McNamara & Whishaw, 1990), 22°C (present study), and 26°C (McNaughton & Morris, 1987) water is used as well as in appetitively motivated spatial memory tasks (Willner & Birbeck, 1986). Further, naloxone does not antagonize the place-learning deficits induced by diazepam (unpublished observations). These results would suggest that the place-learning deficit induced by diazepam is reliant on neither the aversive motivation nor diazepam-induced increases of opioid activity.

Complete tolerance does not appear to develop to the amnesic effects of BZDs in humans (Ghoneim et al., 1981; Griffiths et al., 1984; Lucki et al., 1986) and did not appear to develop in the present study. The Diazepam group, after receiving diazepam daily for 18 consecutive days, and the SD group (acute), demonstrated equally impaired acquisition during the platform reversal phase. However, it is possible that tolerance may have developed with longer treatment (i.e., >27 d). Unpublished preliminary investigations have revealed that some tolerance does develop to the amnesic effects of diazepam after chronic administration (3 mg/kg for 30 d in home cage). This tolerance was not total, however, and suggests that diazepam-induced amnesia is relatively resistant to tolerance, unlike its sedative (File, 1981) and anxiolytic (Treit, 1985) actions, which appear to show tolerance within 3-5 d and 10 d, respectively. Nevertheless, it is unlikely that the gradual improvement in maze performance was the result of tolerance development to the amnesic properties of the drug since acquisition was still impaired on the first day of reversal testing.

It was found that the Diazepam group eventually reached the acquisition criterion, suggesting that the rats learned the location of the escape platform, albeit at a slower rate. Therefore, it is possible that the diazepam-induced anterograde amnesia was only partial. This interpretation seems unlikely given the impaired performance observed in the probe trial, conducted after the diazepam-treated rats had apparently acquired the platform location. This deficit in performance cannot be attributed to non-mnemonic factors,

since the Switch group was not impaired. A more likely explanation is that diazepam produced a total anterograde amnesia and the rats adopted alternative strategies to locate the platform (Sutherland & Dyck, 1984). This may include a 'taxis' strategy, such as swimming towards or away from a single cue, or a 'praxis' strategy, such as swimming in a particular pattern (e.g., sequence of loops). Support for the latter strategy comes from the finding that diazepam-treated rats typically swam in a circular pattern until eventually bumping into the platform. Indeed, this 'praxis' strategy was also demonstrated during the first probe trial (see Fig. 6). These findings suggest that diazepam produced a severe and persisting anterograde amnesia which necessitated the adoption of a response-based search strategy.

Long-term memory is commonly divided into a procedural and declarative component (Cohen & Squire, 1980). Declarative memories can be explicitly retrieved (i.e., memory of where you left your bicycle) while procedural memories can only be expressed by performance (i.e., memory of how to ride your bicycle). This distinction has been adapted to the Morris water maze, with locale (place) memory being analogous to declarative memory and taxis or praxis memories being analogous to procedural memory (Squire, 1987). For example, a declarative memory might consist of "the platform is located in that area of the pool" and a procedural memory might consist of "the platform is located X distance from the pool wall". The finding that diazepam-treated rats could locate the platform by acquiring a response-based search strategy suggests that diazepam spares procedural memory formation while selectively interfering with the formation of declarative memories. This is consistent with human patients with amnesia of an organic origin (Squire, 1987) as well as subjects treated with diazepam (Ghoneim & Mewaldt, 1990) and further emphasises the validity of this distinction.

The neural substrate(s) mediating BZD-induced memory impairment is not known. The hippocampal formation, however, is a primary candidate. For example, it has been demonstrated that successful performance in the Morris water maze requires an intact hippocampal formation (Morris et al.,

1982; Okaichi, 1987; Sutherland et al., 1982, 1983; Sutherland & McDonald, 1990; Whishaw, 1987) and lesions of this structure produce deficits comparable to those found with diazepam-treated rats. That is, both hippocampal-lesioned and diazepam-treated rats demonstrate: [1] a severe place-learning deficit when required to use spatial cues to locate the platform, but are unimpaired when required to navigate to a single, visible cue (Morris et al., 1982), [2] adoption of a taxis strategy (Morris et al., 1982), [3] little preference for the correct quadrant when the platform is removed from the pool during a probe trial (Morris et al., 1982), [4] a failure to show a complete habituation of rearing (Sutherland et al., 1983), and [5] significant savings if the lesion or BZD treatment is given after the task has been acquired (Harley, 1979; Jarrard, 1978; Kubie, Dayyani, Sutherland, & Muller, 1989; Lister, 1985; McNaughton, 1985; Milner, 1966; Sutherland, 1985; Vachon, Kitsikis, & Roberge, 1982, 1984; Winocur, 1980).

The congruent deficits demonstrated by both hippocampal-lesioned and diazepam-treated rats suggests that diazepam impairs place-learning in the Morris water maze by interfering with hippocampal functioning, a notion proposed by Gray (1983). This notion is corroborated by the fact that [1] the hippocampus has a high density of BZD receptors (Manchon, Kopp, Bobillier, & Miachon, 1985; Supavilai & Karobath, 1980; Young & Kuhar, 1979) and [2] hippocampal excitability is depressed by BZD administration (Ademec, McNaughton, Racine, & Livingston, 1981; Albertson & Joy, 1989; Lee, Dunwiddie, & Hoffer, 1979; Olds & Olds, 1969; Rock & Taylor, 1986; Tsuchiya & Fukushima, 1978; Wolf & Haas, 1977). Because the induction of long-term potentiation (LTP) requires postsynaptic firing during tetanization (e.g., Scharfman & Sarvey, 1985), it is not surprising that diazepam is found to impede post-tetanic potentiation (Clement-Cormier, Defrance, Divakaran, Stanley, Taber, & Marchand, 1980; Mathews & Connor, 1976). Because LTP appears to be necessary for new learning to occur in the Morris water maze (Morris, Anderson, Lynch, & Baudry, 1986), diazepam may impair acquisition by impeding the development of LTP. According to one theory, hippocampal LTP serves to facilitate the transfer of information from a short-term to a long-term store located outside the hippocampus (Squire,

Cohen, & Nadel., 1984). The fact that in the present study diazepam did not impair retention of previously acquired material but did impair new learning is consistent with this notion.

The induction LTP in the hippocampus requires the coactivation of a number of afferent fibers, a phenomenon termed 'cooperativity' (McNaughton, Douglas & Goddard, 1978). For example, high-frequency stimulation of both medial and lateral perforant paths induces LTP while independent stimulation of either pathway fails or is less effective (McNaughton et al., 1978). Further, the cellular response elicited by stimulation of either perforant path can be blocked for as long as 100 msec by prior stimulation of the other, a phenomenon referred to as recurrent inhibition (e.g., McNaughton & Barnes, 1977). Recurrent inhibition is GABA (γ-aminobutyric acid)-mediated and its duration is extended by diazepam by at least 400 percent (Adamec et al., 1981). However, the cellular response elicited by stimulation of either perforant path alone is unaltered by diazepam (Matthews & Connor, 1976). Thus, prolonged recurrent inhibition may account for the fact that diazepam-treated rats are impaired when two or more cues must become associated in a spatiotemporal/Hebbian manner for successful performance, as with the hidden platform task, while still able to learn to navigate to a single cue, as with a taxis strategy or visible platform task (Sutherland & Rudy, 1989). In the Morris water maze, the diazepam-treated rat may be able to perceive, acquire and navigate to a single spatial cue, but when the rat is required to conjoin two or more cues it is impaired because one stimulus would recurrently inhibit further activation by other stimuli, thereby preventing their conjunction. Normally, the two stimuli are able to elicit a conjunctive response within the temporal window (i.e., 100~200 msec) and an association would be formed between them. Perhaps it is this successful conjunction that elicits a cascade of biochemical events that lead to increased cellular efficacy and ultimately to consolidation.

Interestingly, in aged rats hippocampal LTP develops slowly and decays quickly (Barnes & McNaughton, 1985) suggesting prolonged or exaggerated

inhibition. It is well known that aged rats (Barnes, 1988) and humans (Sharps & Gollin, 1987) have poor spatial memory abilities. If this impairment is due to an age-related increase in the inhibitory processes in the hippocampus, then the administration of a drug that potentiates GABA-mediated inhibition in the hippocampus, such as the BZDs (Ticku, 1983), would be expected to produce greater deficits in the aged. In fact this is what is found when elderly rats (Komiskey, Cook, Lin, & Hayton, 1981) and humans (Nikaido, Ellinwood, Heatherly, & Gupta, 1990; Pamora et al., 1984) are treated with BZDs. These findings suggest that too much hippocampal inhibition, produced by age-related processes or the administration of BZDs, has deleterious effects on memory and perhaps BZD-induced anterograde amnesia represents a reversible animal model of senile dementia.

Conversely, the loss of tonic inhibition in the hippocampus can be equally deleterious to learning and memory. For example, LTP saturation or kindling of the hippocampus reduce GABA-mediated inhibition (Burnham, 1989; Kapur & Lothman, 1989; Maru, Ashida, & Tatsuno, 1989) and produces spatial learning impairments (personal communication with R. J. Sutherland; McNaughton, Barnes, Rao, Baldwin, & Rasmussen, 1986). As well, both seizure patients (Wood, Hare, Glaeser, Ballenger, & Post, 1979) and patients with senile dementia of the Alzheimer's type (Zimmer, Teelken, Trieling, Weber, Weihmayr, & Lauter, 1984) have marked reductions of GABA in their cerebrospinal fluid and suffer from memory impairments (Crapper-McLachlan, Dalton, Galin, Schlotterer, & Daicar, 1985; Gallassi, Morreale, Lorusso, Pazzaglia, & Lugaresi, 1988). Further, there are also significant reductions of GABA receptors in the hippocampus of Alzheimer's patients (Chu, Penney, & Young, 1987; Hardy, Cowburn, Barton, Reynolds, Dodd, Wester, O'Carroll, Lofdahl, & Winblad, 1987). These findings suggest that GABA-mediated inhibition is important for efficient memory functioning. Given the similarity between spatial memory deficits of rats with hippocampal lesions and the cognitive disturbances experienced by Alzheimer's patients (Kesner, Adelstein, & Crutcher, 1989), the present experimental model may prove useful for investigating the biochemical pathology underlying age- and disease-related memory

disorders.

Overall, it would appear that the BZDs have an important role in memory modulation. Whether this represents an artificial, exogenously induced phenomenon or a reflection of a functional, endogenous process is not yet clear. Low concentrations of endogenous BZD (diazepam and its primary metabolite N-desmethyldiazepam), have been detected in human brain (Unseld, Fisher, Rothemund, & Klotz, 1990) and the brain and adrenals of rats (Wildmann, Mohler, Vetter, Ranadaler, Schmidt, & Maurer, 1987). It is tempting to speculate that this BZD functions as an endogenous anxiolytic. Perhaps during extreme states of anxiety or stress, large concentrations are released from the adrenal glands with the dual effect of alleviating anxiety and preventing the storage of the anxiogenic episode. Such a scenario would be maladaptive to the organism, except, perhaps, at the time of giving birth. This would serve to reduce fear at the time of giving birth as well as prevent the formation of 'painful' memories which might preempt future reproduction. Interestingly, a BZD-like substance has also been detected in human mothers' milk (Klotz, 1990) and may serve to pacify the child, but may also contribute to infantile amnesia. However, these endogenous BZD-like substances are only detectable in trace amounts in man and rat and may arise from plant sources (Wildmann, Vetter, Ranadaler, Schmidt, Maurer, & Mohler, 1988).

Conversely, a polypeptide, referred to as a diazepam-binding inhibitor (DBI), has been identified in both human and rat brain, particularly in the hippocampus and cerebellum (Alho, Costa, Ferrero, Fujimoto, Cosenza-Murphy, & Guidotti, 1985). This DBI behaves like ß-carboline, a BZD receptor 'inverse-agonist' (a substance that produces effects in the opposite [inverse] direction of the receptors agonist; e.g., proconvulsant vs. anticonvulsant), and has been found to enhance memory (Venault, Chapouthier, Prado de Carvalho, Simiand, Morre, Dodd, & Rossier, 1986). The functional significance of these endogenous substances presents an intriguing challenge for future BZD/memory research.

In summary, the present study found that diazepam produces a reversible and tolerance-resistant anterograde amnesia in the Morris water maze. This impairment does not appear to be the result of diazepam-induced myorelaxation, perceptual/attentional impairments, hypothermia, retention/retrieval impairments, or state-dependent learning. Further, diazepam-treated rats appear to adopt non-mnemonic strategies to compensate for their impairment. Because the nature of the diazepam-induced deficits found in the Morris water maze resemble those displayed by rats with hippocampal lesions, and because BZDs depress hippocampal functioning, it seems possible that the hippocampus may mediate the observed diazepam-induced anterograde amnesia. Overall, these findings replicate prior human and animal investigations and suggest that BZDs play an important role in memory modulation and consolidation. These results, along with previous findings (McNamara & Whishaw, 1990; McNaughton & Morris, 1987), suggest that the Morris water maze can serve as a useful model for dissociating the learning and performance effects of pharmaceuticals as well as an important tool for testing potential non-amnesic, anxiolytic agents. Finally, delineating the role of GABA-mediated recurrent inhibition and endogenous BZDs in learning and memory processes may prove to be a worthwhile endeavour for both theoretical and therapeutic purposes.

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Effect of Diazepam on Acquisition, Retention, and Some Performance Variables in the Morris Water Maze

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Aug. 23 1990