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Alcohol Impairs Learning of T-maze Task but Not Active Avoidance Task in Zebrafish

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The aim of this study is to investigate whether alcohol alters learning and memory processes pertaining to emotional and spatial factors using the active avoidance and T-maze task in zebrafish. In the active avoidance task, zebrafish were trained to escape from one compartment to another to avoid electric shocks (unconditioned stimulus) following a conditioned light signal. Acquisition of active avoidance task appeared to be normal in zebrafish that were treated with 1% alcohol for 30 min for 17 days until the end of the behavioral test, and retention ability of learned behavior, tested 2 days later, was the same as control group. In the T-maze task, the time to find a reservoir was compared. While the latency was similar during the 1st training session between control and alcohol-treated zebrafish, it was significantly longer in alcohol-treated zebrafish during retention test 24 h later. Furthermore, when alcohol was treated 30 min after 2nd session without prior treatment, zebrafish demonstrated similar retention ability compared to control. These results suggest that chronic alcohol treatment alters spatial learning of zebrafish, but not emotional learning.

Acute or chronic alcohol consumption leads to impairment of spatial learning and memory in rats (Fleming et al., 1981; Charness et al., 1989; Acheson et al., 1993; Matthews et al., 2002). Acute alcohol-exposure produces abnormality by inhibiting NMDA receptor-mediated current (Simson et al., 1991; Shummers et al., 1997), and chronic exposure has been shown to induce impairment by potentiating NMDA receptor-mediated neurotoxicity (Iorio et al., 1993). Chronic exposure also inhibits long-term potentiation in the hippocampus (Blitzer et al., 1990), the leading experimental model for the synaptic memory. Meanwhile, studies on the effects of alcohol on emotional learning and memory have produced somewhat inconsistent results. Several studies have reported the impaired acquisition and retention of passive and active avoidance tasks in association with alcohol consumption (Casamenti et al., 1993). Others, however, failed to observe any significant impairment in the alcohol-consumption group (Melis et al, 1996; Naylor et al., 2001).

Zebrafish have proved to be invaluable in the field of development and genetics, due to the possibility of convenient screening for mutant lines, transparency during the embryo stage, and their low maintenance cost. Additionally, the richness of their behavior repertoire, which allows the development of varying and complex behavioral paradigms, makes the zebrafish a suitable vertebrate model for neurobehavioral study (Fetcho and Liu, 1998; Gerlai et al., 2000; Dlugos et al., 2003). Recently, as the types of behavioral analyses that can be performed with zebrafish are extended, attempts have been made to take advantage of zebrafish to better understand the effect of alcohol on the CNS. To date, relatively simple behavior patterns and responses have been used; for examples, startle reaction, swimming behavior, light and dark preference, and pigment response (Gerlai et al., 2000).

In this study, we tested the effect of alcohol on the cognitive ability of zebrafish, using two separate learning paradigms: the active avoidance task and the T-maze task, which bring into play emotional and spatial factors, respectively.

Materials and Methods

Adult zebrafish (*Danio rerio*) were purchased from a commercial supplier, housed in recirculating aquaria (40-60 L at 28°C) with fluorescent lighting (12 hour light/dark cycle), and fed fish food daily. For the active avoidance task, the zebrafish were trained using an apparatus similar to that described by Pradel *et al.* with some minor modifications (Pradel et al., 1998). Briefly, the fish were allowed to swim freely for 20 min in a shuttle box

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separated by a barrier with an opening in the middle. The training session consisted of 40 trials that were repeated without interruption and each trial began with the illumination of a red light (3W) on one side of the shuttle box, where a fish was present. A mild electrical shock was administered to the fish after 12 sec of light illumination and terminated as soon as the fish crossed the hurdle, in order to escape to the opposite side of the shuttle box, with this process being referred to as "escape response". If the fish returned before the end of the trial, the electrical shock was delivered again. After repeated trials, the fish learned to swim from the lighted side to the dark side in response to the light, even before the application of the electrical shock, which was called "avoidance response". A learner was defined as a trained fish that satisfied the learning criterion of eight successful avoidance responses within 10 consecutive trials during a training session of 40 trials. Those fish that could not satisfy the learning criterion during the entire training session were called "non-learners". Learners were tested for retention under identical conditions 2 days later. Retention rate (RR) was calculated to quantify the ability of each learner to recall: RR=1- (trials to criterion during test/trials to criterion during training).

The T-maze task was slightly modified from that described by Darland and Dowling (2001). The zebrafish was trained to find a favorable reservoir in the T-maze that was made of white non-transparent acryl. It consisted of one long-arm (55 cm) and two opposite short-arms (35 cm) located at one end of the long-arm. One of the short-arms had a reservoir (24 cm square) that was 5 cm deeper than everywhere else, with sand and artificial weed hidden at the bottom. Most of the fish stayed in the reservoir once they had entered it. The fish were given 3 training sessions at 0 h, 3 h, and 24 h. Before starting the first session, the fish was allowed to explore the maze freely for 5 min. At the start of a trial, the fish was placed at the end of the long arm, and thereafter the latency to reach the reservoir was recorded. At 0 h, two trials were performed with a 5 min interval, and their average latency was referred to as the control. At both 3 h and 24 h, one trial was performed. During each trial, the fish were required to find the reservoir within 5 min and stay for 30 sec in the reservoir in order for them to be classified as "learners". Those fish that failed to do so were removed from the maze and classified as non-learners. After the 3rd training session, a probe trial was carried out in which the entrance to the reservoir was closed and the time to spend in each divided area was monitored for 5 min. The maze was divided into 3 areas: the reservoir area (RA), the non-reservoir area (NR), and the center (C). C was from the starting point to the end of the long arm, NR was from the beginning of the short arm to the wall, and RA was from the beginning of the short arm to the door of the reservoir. For the alcohol studies, the fish were transferred into a 10-liter tank filled with 1.0% ethanol and held there for 30 min once a day for 17 days.

Results

In the active avoidance task, 35 out of 50 trained control fish were classified as learners, among which 10 fish (20%) satisfied the learning criterion within 12 trials, and these were not included in further analyses due to the possibility that these fish responded to the conditioned stimulus (light) without establishing the associated relation with the unconditioned stimulus (electrical shock). In the remainder of the learners (n=27), the average number of trials necessary to satisfy the learning criterion was 24.7. The learners were tested for their level of retention under identical conditions 2 days after training. Control learners were able to satisfy the learning criterion within 14.2 trials (retention rate=0.43) (Fig. 1A), showing a high degree of

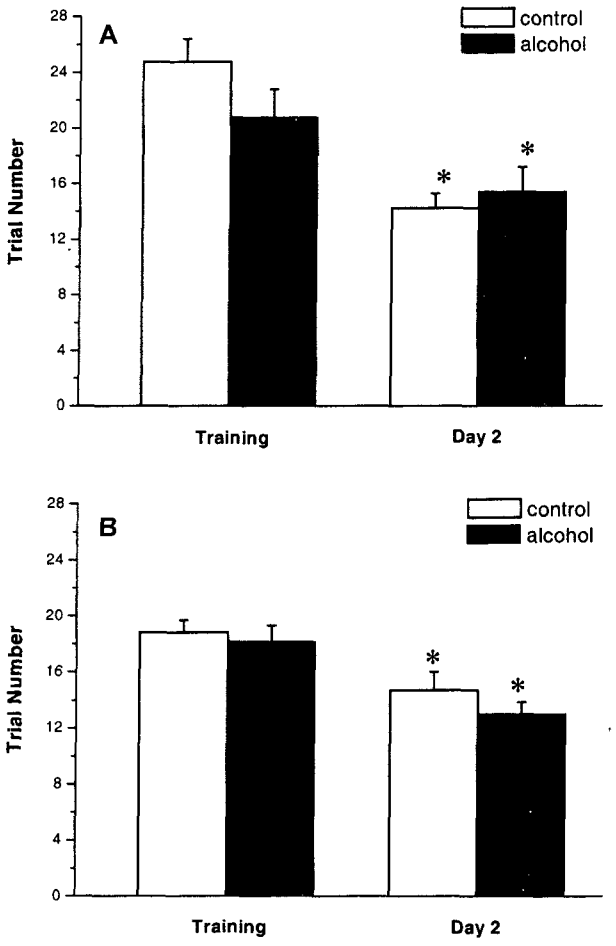

Fig. 1. Effects of chronic alcohol treatment on the active avoidance task. A, Comparisons of acquisition and retention of avoidance responses in control (n=27; RR=0.43) and alcohol-treated fish (n=15; RR=0.26). B, Comparisons of control (n=17; RR=0.22) and 1% alcohol-treated fish (n=13; RR=0.30) which satisfied the learning criterion between trial numbers 13 and 30. Error bars represent±SEM. *$P<0.05$ between training and day 2 in each corresponding group.

retention ability. Among 34 alcohol-treated fish, 17 fish (50%) satisfied the learning criterion after more than 12 trials, 15 fish (44%) learned the task within 12 trials, and two were unable to learn the task during the entire session. The alcohol-treated learners satisfied the learning criterion (20.7 trials, n=15) faster than the control fish did, although the difference was not statistically significant. However, when tested for retention, the alcohol-treated learners required more trials to satisfy the criterion than the control learners (average trials=15.4; retention rate= 0.26) (Fig. 1A). We tested whether this lower retention might be caused by the fast initial acquisition in the alcohol-treated fish. In order to accomplish this, only the alcohol-treated (n=13) and control fish (n=17), which required between 13 and 30 trials to satisfy the learning criterion, were included in comparison of their levels of retention. Under these conditions, the average number of trials required for acquisition of the task appeared similar in both groups (18.8 and 18.2 trials for the control and alcohol-treated fish, respectively) and the degree of retention was not significantly different (14.7 and 13.1 trials for the control and alcohol-treated fish, and retention rates are 0.22 and 0.30, respectively) (Fig. 1B), suggesting that alcohol impairs neither acquisition nor retention in the active avoidance task.

In the T-maze task, two out of 20 control fish (10%) failed to find the reservoir, six (30%) were fast learners finding the reservoir within 15 sec, and thus were not included in further analysis. For the rest of the control fish (60%), the latency in finding the reservoir declined progressively in successive training trials. The latencies were 70.3±22.7, 42.9±16.4, and 18.2±3.7 sec at 0 h, 3 h and 24 h, respectively (n=12) (Fig. 2A). Among the 28 alcohol-treated fish, two fish never found the reservoir,

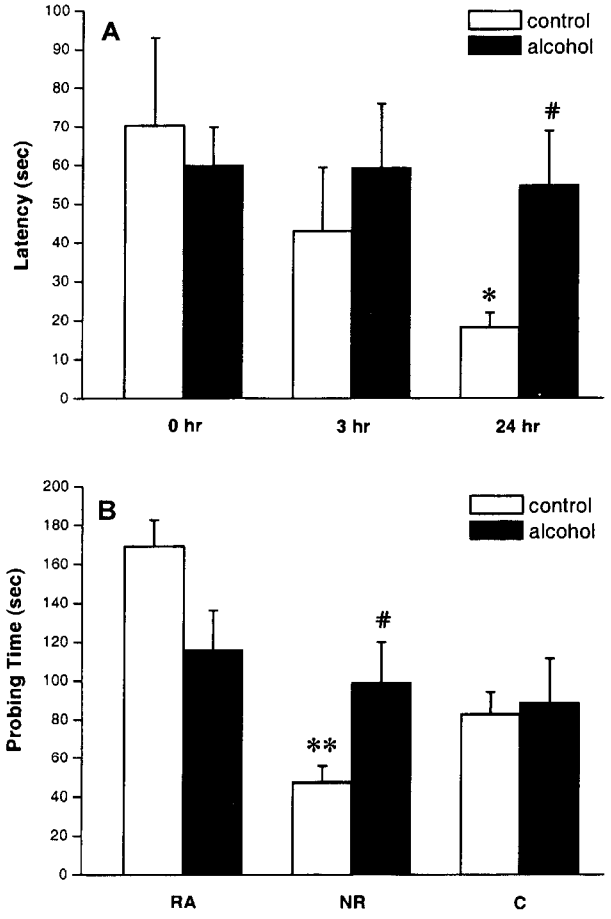

Fig. 2. Effects of chronic alcohol treatment on the acquisition of T-maze task. A, Reduced latency to reach a reservoir with trials tested at 0, 3, and 24 h in control fish (n=12) (open square). No change in latency was observed in successive trials in the alcohol-treated fish (n=13) (closed square). $^{*}P<0.05$ between 0 h and 24 h in the control fish; $^{\#}P<0.05$ between the control and alcohol-treated fish at 24 h. B, Time spent in three divided areas during probe trial for control fish and alcohol-treated fish. RA; reservoir area, C; center, NR; non-reservoir area. $^{*}P<0.001$ between RA and NR of control fish; $^{\#}P<0.05$ between control and alcohol-treated fish at NR. Error bars represent±SEM. All the tasks were analyzed using a two-way ANOVA followed by two-tailed t-Test.

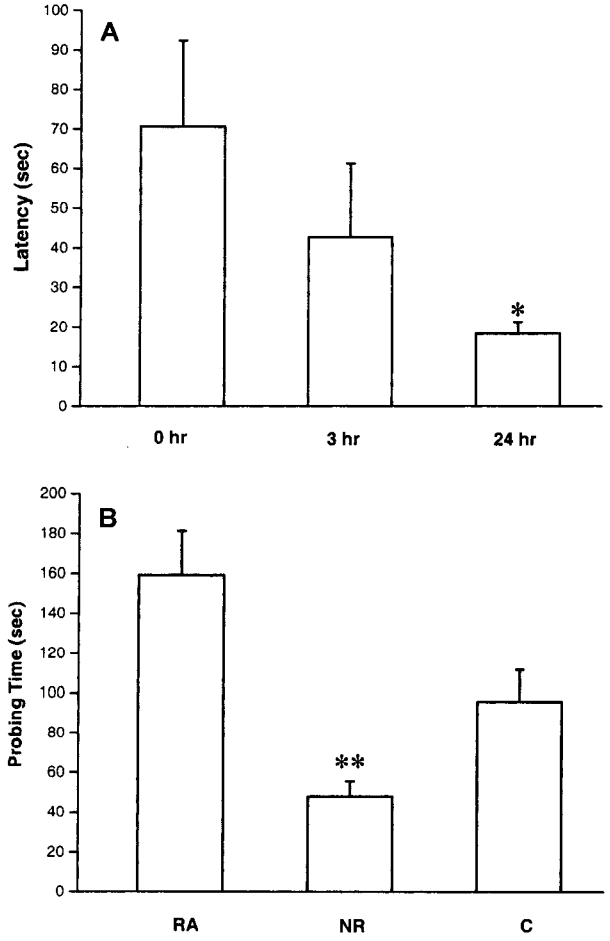

Fig. 3. Effects of acute alcohol treatment on the retention ability of T-maze task. A, Reduced latency to reach a reservoir with trials tested at 0, 3, 24 h (n=11). $^{*}P<0.05$ between 0 h and 24 h for the control fish. B, Time spent in three divided areas during probe trial for acute alcohol treated fish (n=11). RA=reservoir area, C=center, NR=non-reservoir area. $^{**}P<0.001$ between RA and NR for the control fish. Error bars represent±SEM.

and 11 fish (39%) were fast learners. In the alcohol-treated group, the fish displayed better acquisition performance in the T-maze task at 0 h (59.8±10.0 sec) than the control fish, although this difference was not statistically significant. In subsequent trials carried out at 3 h and 24 h, however, the fish treated with alcohol took longer to find the reservoir than the control fish (59.2± 16.7 and 54.7±14.1 sec at 3 h and 24 h, respectively; n=13). In fact, the alcohol-treated fish never improved their performance significantly during the course of the trials. This learning deficit became most apparent at 24 h (Fig. 2A). To further clarify the difference between the two groups, a probe trial was carried out following the trials at 24 h. The control fish spent most of their time in the reservoir area (RA). This tendency became obscure in the alcohol-treated fish (Fig. 2B), suggesting that the alcohol-treated fish failed to learn the location of the reservoir in the course of their training, and thus did not acquire the spatial memory that could be used during the probe trial. Since the zebrafish were subjected to alcohol consumption at a fixed time (8 p.m., for 30 min) until the end of the behavior test, we further tested whether the impairment of the retention ability of the 3rd trial might be partially caused by the alcohol treatment before the final test, rather than by chronic consumption. The fish were treated with 1% alcohol for 30 min after the 2nd trial without prior exposure and then tested for retention ability at 24 h (70.6±21.8, 42.7±18.5, and 18.5±2.8 sec at 0 h, 3 h, and 24 h, respectively; n=11). No difference in latency between the control and alcohol-treated fish was observed, which was subsequently confirmed by a probe trial (Fig. 3).

Discussion

Our results indicated that alcohol impaired the learning of the T-maze task, but not of the active avoidance task, while not affecting the initial acquisition in the zebrafish. The differing effects of alcohol on the T-maze and active avoidance tasks suggest the existence of two separate pathways processing emotional and spatial learning in the zebrafish. According to an ablation study in goldfish, which belong to the teleost species as do the zebrafish, two separate brain areas of the telencephalon, which are responsible for these learning processes, have been independently identified: dorsomedial lesions caused impairment of emotional learning, whereas dorsolateral lesions impaired temporal and spatial learning (Portavellia et al., 2002). Since we used conditioned stimulus (light) temporally overlapped with unconditioned stimulus (electrical shock) in the active avoidance task, in which emotional factors are mainly processed and neither temporal nor spatial factors are involved, the involvement of the dorsolateral telencephalon is unlikely. Also, in our preliminary experiments, the rank orders of acquisition of emotional and spatial tasks in the fish were not correlated with each other, suggesting that each task is processed through parallel pathways (data not shown). It can therefore be concluded that, under the present conditions, alcohol selectively impairs the function of the neural substrate underlying spatial learning. Furthermore, since the alcohol-treated fish performed the task as well as the control fish during the training session in our study, the inability of the alcohol-treated fish to learn the T-maze task was unlikely to be due to the potential impairment of performance.

A number of studies using the rat have produced concordant results, indicating that spatial learning and memory are impaired by either acute or chronic consumption of alcohol, mainly because of alcohol-induced dysfunction of the hippocampus. To date, the most plausible mechanisms underlying the effect of alcohol on brain function are understood to involve the regulation of the major neurotransmission systems, especially in the hippocampus. Alcohol is known to potentiate $GABA_A$ receptor and block NMDA receptor-mediated responses (Simson et al., 1991; Nakagawa and Iwasaki, 1995; Shimizu et al., 1998), and this effect is, in turn, responsible for the suppression of the hippocampal function. Activation of NMDA receptors is critically required in the early stage of various types of learning, which initiates a cascade leading to consolidation of learning. The presence of glutamatergic and GABAergic neurons has been demonstrated immunohistochemically in the zebrafish brain (Edwards and Michel, 2002) and also electrophysiologically in the goldfish brain (Fucile et al., 1999). In addition, injection of MK-801, an NMDA receptor antagonist, into the brain prevented the learning of the active avoidance task in goldfish (Xu et al., 2001). Whether alcohol can modify the physiological function of NMDA receptors in the zebrafish brain remains to be studied.

Meanwhile, a limited number of studies have been done regarding the effect of alcohol on emotional learning, and the results of these studies seem to be contradictory. For example, when acutely treated before training, alcohol impaired retention of the passive avoidance task, yet when treated immediately after training, it failed to impair retention in the rat (Naylor et al., 2001), suggesting alcohol produces anterograde amnesia in the passive avoidance task. Since there was no comparable study on the active avoidance task, it is premature to generalize this anterograde amnesia effect of alcohol. In fact, the acquisition of the active avoidance task was not impaired following the termination of chronic treatment of alcohol in rats (Melis et al., 1996), which is consistent with the present finding that alcohol does not alter the learning ability of zebrafish in the active avoidance task other than the initial facilitation of acquisition performance.

In conclusion, our findings show that chronic treatment of alcohol impairs learning of the T-maze task but not the

active avoidance task in zebrafish, suggesting more susceptibility of spatial learning to alcohol. In addition, this study raises the possibility that the advantages of zebrafish, such as their low maintenance cost, easy handling, and diverse genetic lines, would make them a useful model for studying the effect of addictive drugs on the brain.

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