Nicotine-alcohol induced differential feeding behaviour

MK Ali, D Ragoobirsingh*

Abstract

Introduction
Co-addiction of nicotine and alcohol is a worldwide problem. However, impact of nicotine–alcohol co-addiction on the feeding behaviour has not yet been characterised. This study discusses nicotine–alcohol induced differential feeding behaviour.

Materials and methods
Healthy adult Zebrafish were subjected to mild doses of addictive drugs (alcohol 1%v/v, nicotine 1 mg/l, mix alcohol 1%v/v and nicotine 1 mg/l) for 20 minutes. The treated fish were then subjected to two types of tank conditions: (A) simple non-partition feeding tank where the food was visible and (B) T-maze partition feeding tank where the food was hidden in the food arm of the T-tank. Both the control and drug exposed fish were trained to adapt to this feeding arrangement for six days before the actual experiment started. After six days of training, the feeding behaviour was recorded for 5 minutes immediately after the release of both untreated and treated fish in the feeding tanks. The levels of appetite were determined by counting the number of accumulative strike to the food particles in 5 minutes from the CCD camera footage.

Results
Analysis of feeding behaviour using Zebrafish (Danio rerio) as an animal model, showed that alcohol exposed Zebra fish exhibits intense and aggressive feeding while nicotine exposed Zebrafish have the opposite effect when subjected to conditions where food was easily visible as well as hidden which required food searching activity. Co-exposure of nicotine and alcohol to adult Zebrafish produced feeding behaviour completely different from that when exposed to either nicotine or alcohol alone. Feeding was more aggressive soon after the exposure when the food was easily accessible. But feeding was greatly reduced if the co-exposed fish was either subjected to food searching activity or allowed to recover (10–30 minutes) from drug insults.

Conclusion
Therefore, suggestion of using nicotine as a means of treating alcohol-dependent weight gain will be counterproductive if the food is easily accessible.

Introduction
Abuse of drugs particularly alcohol and nicotine are worldwide problems. Both drugs were reported to activate a common final neural pathway of the dopaminergic system that mediate the pleasurable feelings of reward. However, each drug of abuse under two conditions where food was easily accessible. But feeding was greatly reduced if the co-exposed fish was either subjected to food searching activity or allowed to recover (10–30 minutes) from drug insults.

Co-administration of alcohol and nicotine on food intake under conditions of easy accessibility as well as food searching activity is not yet well characterised. Recently, Zebrafish have been used increasingly as an animal model to quantify drug-induced changes in the behaviours such as hyperactivity and anxiety and associative learning and memory. Therefore, we have evaluated the impact on food intake in Zebrafish after co-administration of alcohol and nicotine under two conditions i.e. when the food was easily visible and when feeding involved food searching activity.

Materials and methods
The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed. Animal care was in accordance with the institution guidelines.

Healthy adult Zebrafish were subjected to mild doses of addictive drugs (alcohol 1%v/v, nicotine 1 mg/l, mix alcohol 1%v/v and nicotine 1 mg/l) for 20 minutes. The treated fish were then subjected to two types of tank conditions where food was easily accessible as well as hidden which required food searching activity.

In one study, it was reported that co-administration of alcohol and nicotine also reduced appetite. This might be due to a much greater memory impairment by co-administration of alcohol and nicotine than by alcohol alone. Under such conditions, food intake associated with food searching activities was likely to be affected. But a recent report on the ability of alcohol to overshadow the effect of nicotine suggests that food intake under the condition of co-administration of alcohol and nicotine seems similar to that observed with administration of alcohol alone. However, impact of co-administration of alcohol and nicotine on food intake under conditions of easy accessibility is not yet well characterised.
conditions: (A) simple non-partition feeding tank where the food was visible and (B) T-maze partition feeding tank where the food was hidden in the food arm of the T-tank. Both tanks were fitted with thermo cool sheets attached with dry food particles, using non-toxic adhesive glue. The feeding tanks were filled with aquarium water, until the water level touch the food particles. A CCD camera was fixed away from the side of the tank but in parallel to the thermo cool sheet so that any activity of feeding could be readily recorded. In case of simple non-partition feeding tank, the fish were released in the immediate vicinity of food particles. On the other hand, in case of T-maze partition feeding tank, fish were released at a fix distance away from the hidden food particles in the food arm of the T-tank. Both the control and drug exposed fish were trained to adapt to this feeding arrangement for six days before the actual experiment started. After six days of training, the feeding behaviour was recorded for 5 minutes immediately after the release of both untreated and treated fish in the feeding tanks. The level of appetite were determined by counting the number of accumulative strike to the food particles in 5 minutes (NASFP-5M) from the CCD camera footage.

**Results**

Table 1 describes the evaluation of NASFP-5M when the food was easily visible. Release of well-trained control fish near the vicinity of attached food particles of feeding tanks produced NASFP-5M of 9 ± 2, N = 15. The NASFP-5M produced by immediate release of trained fish treated with alcohol was not significantly different, 10 ± 2, N = 15, from that of the control. However, when the alcohol treated fish were allowed to recover from treatment for 10, 20 and 30 minutes and then released into the feeding tanks, the NASFP-5M of 13 ± 2, N = 15; 12 ± 2, N = 15 and 12 ± 2, N = 15, respectively, were recorded. These were much higher than the control NASFP-5M (9 ± 2, N = 15). Thus our results agree with the increased food appetite as reported earlier but such increases only take place during the recovery periods after alcohol insult. In contrast, the NASFP-5M produced by immediate release of trained fish treated with nicotine was greatly reduced, 4 ± 1, N = 15, compared to that of the control, NASFP-5M (9 ± 2, N = 15). Releasing nicotine treated fish into the feeding tanks after allowing recovery from nicotine insult for 10, 20 and 30 minutes increased the NASFP-5M (5 ± 2, N = 15; 6 ± 1, N = 15 and 8 ± 2, N = 15, respectively). These, however, are still lower than that of the control, NASFP-5M (9 ± 2, N = 15). Our results were once again in good agreement with the earlier observation of the anti-appetite effect of nicotine. Fish co-exposed to alcohol and nicotine, surprisingly produced a much greater NASFP-5M (14 ± 2, N = 15) when released into the feeding tanks near the vicinity of food particles compared to the control, NASFP-5M (9 ± 2, N = 15). The co-exposed fish also showed aggressive swimming activities near the vicinity of the food particles compared to the control, alcohol and nicotine treated fish. It has been suggested that alcohol could overshadow the nicotine effects. But higher NASFP-5M in alcohol and nicotine co-treated fish compared to the alcohol alone treated fish clearly indicates that not only the alcohol is able to overshadow anti-appetite effect of nicotine, but it also induces aggressive behaviour in combined alcohol and nicotine treated fish. Since the co-treated fish show induced aggressive behaviour, their release near the vicinity of food particles seems to be contributing more in increasing NASFP-5M which may or may be associated with drug induced appetite level. However, release of co-treated fish near the vicinity after 10, 20 and 30 minutes of recovery from the drug insult, showed a continuous decline in NASFP-5M (13 ± 1, N = 15; 9 ± 2, N = 15 and 6 ± 2, N = 15). After 20 and 30 minutes of recovery, the

<table>
<thead>
<tr>
<th></th>
<th>Total strike score from 15 individual fish</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
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<tr>
<td>Alcohol (Alc)</td>
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</tr>
<tr>
<td></td>
<td>10 minutes</td>
<td>150</td>
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<td></td>
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<td>190</td>
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<td></td>
<td>30 minutes</td>
<td>187</td>
</tr>
<tr>
<td>Nicotine (Nic)</td>
<td>Immediate</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>10 minutes</td>
<td>58</td>
</tr>
<tr>
<td></td>
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<td>79</td>
</tr>
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<td></td>
<td>30 minutes</td>
<td>85</td>
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<td>Alc + Nic</td>
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<td>113</td>
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observed NASFP-5M was nearly the same as the NASFP-5M produced by the nicotine treated fish of the same recovery periods. Therefore, our results clearly suggest under easy accessible feeding conditions, alcohol- and alcohol-nicotine co-treated fish produced higher uptake of foods while the nicotine treated have lower food uptake compared to the control treated. Alcohol treated fish maintained higher uptake of food during the recovery periods of 30 minutes compared to the control while alcohol–nicotine co-treated fish showed rapid and higher food uptake during the initial recovery periods of 0–10 minutes but declined rapidly with increasing recovery periods.

In order to assess the memory-dependent food uptake, we have also recorded the NASFP-5M soon after the release of the control fish at a fixed distance away from the hidden food particles in the food arm of T-tanks. In the first two days of recording, the NASFP-5M were greatly reduced as fish take too long to reach the food arm of T-tanks. The NASFP-5M, however, increased sharply in the third day. Beyond five days, the NASFP-5M was more or less similar (± 1, N = 15), but less compared to the NASFP-5M observed when the food was easily visible and accessible (± 2, N = 15). This difference was due to the time taken in finding the hidden food particles in the T-tanks. The time differences were initially very large in the first two days of training, but beyond five days of training the time difference was small and remained relatively stable. The results demonstrated that Zebrafish follow a strong memory cue during the food hunts. However, placing the well-trained control fish that was for the first time pre-exposed to the alcohol, nicotine or combination of both resulted in a very low NASFP-5M to the hidden food particles in the food arm of T-tanks. This result was probably due to both physical and memory impairments induced by the first time exposure to addictive drugs. Therefore, we compared the differences in the NASFP-5M to hidden food particles 5 minutes in the food arm of T-tank between control and drug exposed fish only after five days of T-tank feeding training. The NASFP-5M results were compared in two conditions, immediately after drugs treatment and after 30 minutes recovery from drugs treatments as shown in Table 2. The NASFP-5M to the hidden food particles of T-tanks for alcohol treated fish that was released immediately after drug exposure was much smaller (± 1, N = 15) compared to the control, NASFP-5M (± 1, N = 15). This lower value of NASFP-5M was because of a delay in getting to the hidden food area by alcohol treated fish. However, alcohol treated fish that were released after 30 minutes of recovery from the alcohol exposure produced a relatively higher NASFP-5M to the hidden food particles of T-tanks (± 2, N = 15) compared to the control (± 1, N = 15). Nicotine exposed fish, in contrast, show aggressive and faster swimming activities, therefore they reached the hidden food area within the first minutes of its release. This is much faster than the control. However, they only produced NASFP-5M of ± 1, N = 15, which is much smaller compared to the control, NASFP-5M (± 1, N = 15).

This drop in the NASFP-5M to hidden food particles was also partly because nicotine exposed fish had more or less equal accessibility to both non-food and food arms of T-tanks whereas control fish were mostly confined to the food arm of T-tanks during our recording periods. Lower scoring of NASFP-5M to hidden food particles, clearly suggest that the anti-appetite properties of nicotine over-shadow nicotine induced enhanced memory-dependent performance reported earlier. Nicotine–ethanol co-exposed fish that were released immediately after the exposure, on the other hand, mostly confined to the released area during the 5 minutes of recording periods. However, the co-treated fish that reached the hidden food area produced higher NASFP-5M. Therefore, the NASFP-5M to the hidden food particles in the food arm of T-tanks, obtained from 15 independent recording was (± 3, N = 15). These results did not indicate any role of memory-dependent feeding patterns, but pointed more towards the aggressive feeding behaviour when the food was encountered accidentally. Even after 30 minutes recovery of nicotine–alcohol exposed fish, the NASFP-5M to the hidden food particles in the food arm of T-tanks was not enhanced. The results were more or less similar to the observed NASFP-5M produced by immediate release of nicotine treated fish in

<table>
<thead>
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<th>Table 2</th>
<th>Effect of withdrawal from addictive drugs on the NASFP-5M when the food was hidden</th>
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<tbody>
<tr>
<td></td>
<td>Total strike score from 15 individual fish</td>
</tr>
<tr>
<td>Control</td>
<td>97</td>
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<tr>
<td>Alcohol (Alc)</td>
<td>58</td>
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<tr>
<td>30 minutes</td>
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<tr>
<td>Nicotine (Nic)</td>
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<td>30 minutes</td>
<td>86</td>
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<tr>
<td>Alc + Nic</td>
<td>51</td>
</tr>
<tr>
<td>30 minutes</td>
<td>41</td>
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the T-tank. However, unlike nicotine treated fish, after 30 minutes nicotine–alcohol exposed fish arrived late in the hidden food area and confined mostly in these areas but produced a fewer NASFP-5M.

Discussion

It has been suggested that in co-exposure of nicotine and alcohol, the alcohol effect overshadows the nicotinic effect\(^\text{10,11,24}\), but our results clearly suggest that co-exposure resulted into different types of behaviour towards foods that were not the characteristics of alcohol and nicotine induced behaviour. Lack of food searching activity; in the nicotine–alcohol co-exposed fish but aggressive feeding activity when the food was easily accessible clearly suggest that nicotine–alcohol exposed fish possess much greater memory impairment associated with aggressive behaviour. Therefore, our results were in good agreement with earlier findings\(^\text{11,25}\). However, lack of aggressive feeding after 30 minutes withdrawal from the co-exposure when the food was easily accessible as well as even after locating hidden food in case of T-tank, suggest that aggressive behaviour was of short duration. In this case, nicotine effect seems to overshadow the alcohol effect. This reasoning seems to be in good agreement with earlier reports which showed that the effect of nicotine was greatly suppressed in presence of alcohol but the nicotinic effect reappeared with the increasing alcohol concentration. Feeding was greatly reduced if the co-treated fish was either subjected to food searching activity or allowed to recover (10–30 minutes) from drug insults. Therefore, suggestion of using nicotine as a means of treating alcohol-dependent weight gain will be counterproductive if the food is easily accessible.

Conclusion

Exposure of adult Zebrafish to alcohol produced more aggressive feeding compared to the control when subjected to conditions where food was easily available (visible) as well as hidden which required food searching activity. Feeding activity remained stable even after 30 minutes withdrawal from the drugs. Nicotine exposed Zebrafish produced opposite effects. Co-exposure of nicotine and alcohol to adult Zebrafish produced feeding behaviour completely different from that of Zebrafish exposed to either nicotine or alcohol alone. Feeding was more aggressive soon after the exposure when the food was easily accessible. But feeding was greatly reduced if the co-treated fish was either subjected to food searching activity or allowed to recover (10–30 minutes) from drug insults. Therefore, suggestion of using nicotine as a means of treating alcohol-dependent weight gain will be counterproductive if the food is easily accessible.

Acknowledgement

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References

17. Sisson M, Gerlai R. Associative learning performance is impaired in zebrafish (Danio rerio) by the NMDA-R antagonist.
Research study


