# TASTE AND ODOR AVERSION CONDITIONING IN MUS *MUSCULUS* AND ASSOCIATED CHANGES IN HEART RATE AND FLUID CONSUMPTION

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## ABSTRACT

The chemical senses of olfaction and taste have long been recognized as important to survival. Taste Aversion, and to some extent odor aversion conditioning is a robust phenomena. We looked at affects of aversively conditioned odor or taste had on heart rates, lick rates, and water consumption of Mus *musculus*. This involved pairing either saccharine water (taste), or Amyl-Acetate (odor) with Lithium Chloride injection induced illness. Following a single conditioning trial mice were again exposed to taste or odor stimuli. Heart rates, lick rates, and water consumption rates were compared for both pre and post conditioning, and between groups. Results indicate heart rate increases as well as lick and water consumption rate decreases for taste aversion conditioned mice. These tendencies were also seen for odor conditioned mice, but did not reach significance at the 0.05 level.

## INTRODUCTION

Olfaction and taste have long been recognized as important to most animals. Rodents are dependent on olfaction for selecting safe food, choosing appropriate mates, and for avoiding situations that could prove fatal. Rodents also depend on gustatory cues to prevent them from consuming substances that could prove deadly.

It is well recognized that animals who become ill after eating or drinking a given substance will avoid that substance in the future (Garcia and Koelling, 1965). This taste aversion condition is reproduced under laboratory conditions by encouraging the animal to drink or eat a tasty food and then making the animal ill. The animal associates the illness with the taste of what was previously consumed and avoids further contact. Although taste aversion conditioning is well established, odor aversion conditioning without the use of an associated flavor is difficult to obtain (Backer and Booth, 1989, Holder, 1991, Palmerino, Rusiniak, and Garcia, 1980, Panhuber, 1982, Rusiniak, Hankins, Garcia, and

Brett, 1979). Pairing of a variety of either odors combined with a variety of tastes, as well as odor only in preference studies has not yet provided clear evidence that single exposure conditioning for odor alone is possible (Lucas and Sclafani, 1995).

Avoidance is only one reaction to possibly threatening stimuli. Physiological reactions are also associated with such stimuli. These can include changes in heart rate, blood pressure, respiration rate, temperature, and skin conductance, which are all common indices of stress (Abdeen, Taylor, Youngblood, and Printz, 1995, Cocke and Thiessen, 1986, Hunt, Hess, and Campbell, 1997, Young and Leaton, 1994).

It is reasonable to assume that a cue, such as taste or odor, associated with a threatening event, such as Lithium Chloride (LiCl) induced illness, would, on reexposure produce some of the physiological changes commonly associated with stress. We attempted to determine if mice exposed to a taste or odor stimulus associated with LiCl induced illness would produce behavioral avoidance (decreased water consumption and decreased lick rates) as well as a physiological change associated with stress (increased heart rate).

## METHODS

### Subjects

Twenty-three naive male, Swiss, albino mice (Mus *musculus*), approximately 160 days old, were housed individually in plastic cages with stainless steel covers and maintained on a 12:12-h light:dark cycle. Mice had free access to food and free access to water except during the eight days of experimental sessions and the two weeks preceding these experimental sessions.

### Apparatus

A two piece aluminum electrical project box 15 x 10 x 10 cm was used as a conditioning chamber. The cover of the project box was used as a lid for the conditioning chamber. A standard water bottle 2.3 cm inside diameter entered the center of one 10 x 10 cm. side of the conditioning chamber. A .8 cm outside diameter nozzle which accommodated standard .4 cm inside diameter plastic aquarium air hose entered at each 10 x 10 cm side of the conditioning chamber. The air hose entering the drinking spout end of the chamber supplied either odorized or unodorized air, while the air hose at the opposite end of the chamber routed air directly to a roof mounted ventilation duct. Two plastic 1.3 cm electrical conduit clamps were mounted 2 cm apart on the bottom of the chamber. The conduit clamps secured the mouse restraining device which consisted of a 3 cm diameter syringe cover cut to approximately 10 cm in length. A whole was cut to approximately 1 cm in diameter in the other end of the syringe cover to accommodate the mouse's muzzle. A cap for the other end of the restraining device was made from a plastic tube just slightly larger in diameter than the syringe cover

and cut to about 4 cm in length. A hole was made in the closed end of the tube to accommodate the mouse's tail.

Air entering the chamber was filtered through a 100 ml flask containing either 50 ml of d $H_2O$, or 45 ml d $H_2O$ mixed with 5 ml of Amyl-Acetate (C7H14O2). An aquarium air pump supplied pressurized air at a volume of 1 liter/minute.

Pulse rates were monitored via a light sensitive athletic pulse monitor intended for humans. The monitor was clamped to the tails of the mice to obtain their heart rate measures. Lick rates were measured by a lickometer consisting of a modified touch activated, capacitance switch door knob alarm. The capacitance switch was adjusted to close at mouse body capacitance. The switch was attached to the stainless steel drinking spout. Contact with the drinking spout activated an event counter via the switch. Water consumption rates were measured using a graduated drinking tube attached to the drinking spout. An electronic, digital stop watch was used for timing all pre and post-conditioning trials.

## Procedure

Mice were given ad lib. access to food and water for 105 days. They were then placed on a 23 hr. water deprivation regime. The water deprivation regime began 7 days prior to acclimating the subjects to the apparatus.

Animals were then acclimated to the apparatus for 7 days for a period of 5 minutes each day. Following acclimation trials mice were allowed ad. lib. access to water for one hour. Acclimation trials consisted of placing mice in the restraining device and conditioning chamber with cover. The pulse rate monitor was attached to their tails. During acclimation mice were exposed to pressurized air supplied by an aquarium air pump at a rate of 1 liter/minute and filtered through a 100 ml flask of d $H_2O$.

Subjects were then randomly assigned to one of four groups receiving one of two treatment conditions for the purpose of collecting pre-conditioning data: Groups one and two were selected for presentation of air filtered through $C_7H_{14}O_2$ (Amyl-Acetate). They were also allowed access to d $H_2O$ for drinking. These mice were placed in the restraining device inside of the conditioning chamber with the chamber in place and with the heart rate monitor attached to their tails for a period of 5 minutes each day for 3 days. Pre-conditioning readings were recorded for all three measures in the following manner: heart rates were recorded once a minute for five minutes and averaged for per minute heart rate; lick rates were recorded once a minute for five minutes and averaged for per minute lick rate; water consumption rates were established by calculating the difference in the amount of water measured before the trial began and after the trial was completed. Each mouse received one hour of water each day following their time in the conditioning chamber. Group's three and four were selected for presentation of clean air and a 43% solution of saccharine drinking water. All other conditions were identical to that of group's one and two, and all readings were recorded in the same way.

On the third day of pre-conditioning within 5 minutes of removal from the conditioning chamber, mice in group's two and four recieved a single interperitoneal injection of 10 ml/kg of 0.3M LiCl. Injection occured before the mice were returned to their cages for their hour access to water. All of the mice in group one and group three (control groups) received an interperitoneal injection of 10ml/kg of physiological saline within 5 minutes of being removed from the conditioning chamber and before being returned to their cages and one hour access to water.

Post-conditioning data collection began on the day immediately following conditioning day and continued for a total of 5 days. Subjects in all groups were placed in the same condition as during pre-conditioning. Data was collected and recorded for all groups in the same manner as during pre-conditioning.

## RESULTS

Within group pre and post-conditioning means were compared using a repeated measures ANOVA . In addition, between mean differences were tested with a one way ANOVA (Table 1). All comparisons were tested at the .05 confidence level unless otherwise specified.

The within group comparisons for the paring of LiCl and saccharine water reached significance as indicated by decreased post conditioning lick rates (df = 1,46, SS = 13104.2, F = 5.98) (Figure 1) and decreased post-conditioning fluid consumption rates (df = 1,46, SS = 178.8, F = 23.15) (Figure 2). However the group which received pairing of LiCl and Amyl-Acetate odor did not demonstrate a significant decrease in lick rate or fluid consumption. Post conditioning heart rate increases were not significant for either group.

Mice that receiving an injection of saline in conjunction with the Amyl-Acetate odor also displayed significant change in post-conditioning lick rate (df = 1,38, SS = 3971.88, F = 6.91) (Figure 1) and decreased post-conditioning fluid consumption rate (df = 1,38, SS = 104.68, F = 13.34) (Figure 2). The group that received an injection of saline in conjunction with the taste of saccharine water showed a significant post-conditioning drop in fluid consumption (df = 1,46, SS = 189.41, F = 8.51) (Figure 2) level but post-conditioning decrease in lick rates did not reach significance (Figure 1). These groups did not display significant increases in post-conditioning heart.(Figure 3).

The between groups comparisons of all groups did not reach significance but comparisons indicate significantly lower lick rates for odor and saline than for both taste and odor paired with saline (df = 1,53, SS = 5415.24, F = 6.44) (Figure 5). Odor and saline also had a lower fluid consumption rate than taste and saline although this difference did not quite reach significance (df = 1,53, SS = 106.22, F = 3) (Figure 5). Lick rates for Amyl-Acetate and LiCl when compared with saline and LiCl approached but did not reach significance (df = 1,58, SS = 8242.16, F = 3.44) (Figure 6). All other comparisons for groups 2 and 4 failed to reach the .05 criterion level.

## DISCUSSION

Mice exposed to a taste, (in this case saccharine water), and made ill with Lithium Chloride induced illness, show a decrease in willingness to consume that taste. Lick rates and fluid consumption rates decreased. These results mirrored those of Garcia et al. (1965). Mice exposed to the odor of Amyl-Acetate and then made ill do not show the same tendency to avoid the non-tasty fluids served in conjunction with that odor. Lucas et al, (1995) speculated it may be difficult to aversively condition an odor without a corresponding taste.

What was unusual in this experiment was the lack of significant increases in heart rate measures for either the group exposed to taste and illness or the group exposed to odor and illness. Heart rate increase is a common indicator of stress (Abdeen et al., 1995, Young et al., 1994). Forced exposure to an odor or taste that previously caused illness should have caused a stressful situation. The fact that heart rate changes don't appear to be a good indicator of stress in this case probably has something to do with experimental design. It is quite possible that a single exposure to the aversive stimuli was simply not enough to produce the desired result.

Animals in control groups also showed a significant decrease in willingness to consume fluids after conditioning. The pain of the saline injection alone could have caused this result. However saline injected control groups for other taste conditioning studies do no support this speculation.

The animals in groups that were given saccharine water consumed more fluid than those animals in groups given distilled drinking water. This difference in lick rates and fluid consumption rates between groups had more to do with taste than because of any other variable.

If odor conditioning is not so robust a phenomenon as taste conditioning it may require more than a single exposure. If heart rate is a reasonable measure of stress on danger, it should have increased upon re-exposure to the aversively conditioned stimulus. This occurred for the saccharine and LiCl pairing unfortunately it was also seen for the control groups. Heart rate actually decreased for the odor - LiCl pairing. This may reflect a requirement for repeated pairing of odor stimuli and illness for odor aversion conditioning (figure 3).

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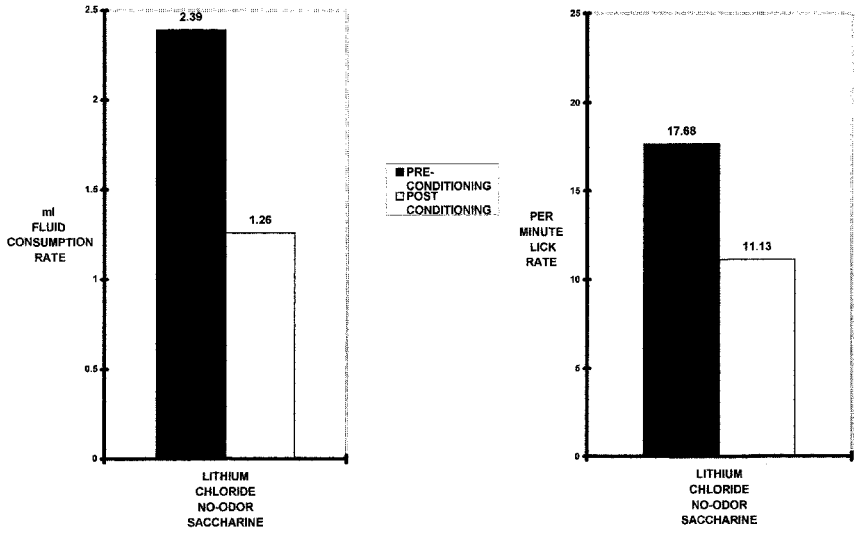


Figure 1. Comparison of pre and post-conditioning mean fluid consumption rates and mean lick rates for group 4.

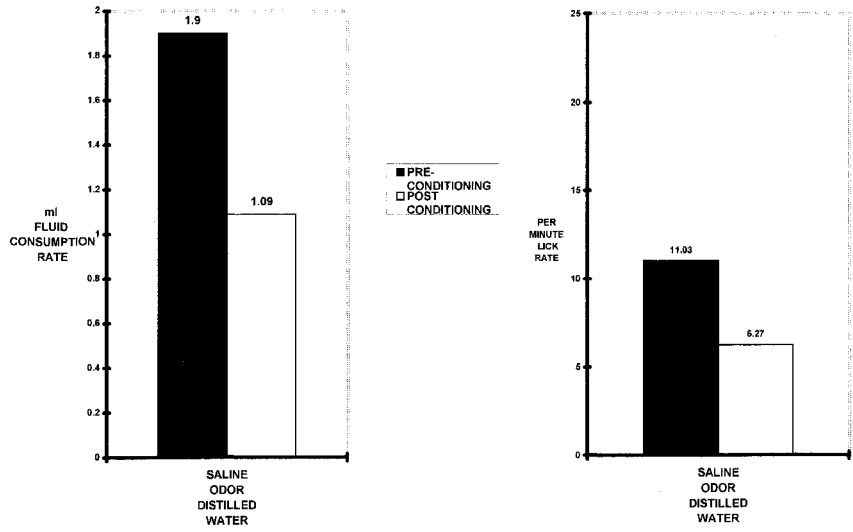


Figure 2. Comparison of pre and post-conditioning mean fluid consumption rates and mean lick rates for group 1.

**TABLE 1.** Pre and post-conditioning data comparison and comparison fo between group data.

| | | PRE-CONDITIONING $\bar{X}$ | POST-CONDITIONING $\bar{X}$ | SS | df | F |
|---|---|---|---|---|---|---|
| PER MINUTE HEART RATE | GROUP ONE | 109.28 | 135.08 | 907465.8 | 1,38 | 0.87 |
| | GROUP TWO | 122.31 | 102.65 | 723645.52 | 1,46 | 1.45 |
| | GROUP THREE | 113.57 | 144.18 | 1133624.96 | 1,46 | 1.75 |
| | GROUP FOUR | 118.09 | 155.22 | 1498785.64 | 1,46 | 1.36 |
| PER MINUTE LICK RATE | GROUP ONE | 11.03 | 6.27 | 3971.88 | 1,38 | 6.91 |
| | GROUP TWO | 10.18 | 7.89 | 5439.68 | 1,46 | 1.58 |
| | GROUP THREE | 11.14 | 10.05 | 6508.52 | 1,46 | 0.5 |
| | GROUP FOUR | 17.68 | 11.13 | 13104.2 | 1,46 | 5.98 |
| FLUID CONSUMPTION RATEml | GROUP ONE | 1.9 | 1.09 | 104.68 | 1,38 | 13.34 |
| | GROUP TWO | 1.51 | 1.28 | 108.11 | 1,46 | 1.64 |
| | GROUP THREE | 2.22 | 1.38 | 189.41 | 1,46 | 8.51 |
| | GROUP FOUR | 2.39 | 1.26 | 178.8 | 1,46 | 23.15 |

| | GROUP ONE $\bar{X}$ | GROUP TWO $\bar{X}$ | SS | df | F |
|---|---|---|---|---|---|
| PER MINUTE HEART RATE | 135.08 | 102.65 | 1118180.28 | 1,53 | 2.18 |
| PER MINUTE LICK RATE | 6.27 | 7.89 | 4894.4 | 1,53 | 0.93 |
| FLUID CONSUMPTION RATE ml | 1.09 | 1.28 | 97.8 | 1,53 | 1.28 |
| | GROUP THREE $\bar{X}$ | GROUP FOUR $\bar{X}$ | SS | df | F |
| PER MINUTE HEART RATE | 144.18 | 155.22 | 2014253.76 | 1,58 | 0.16 |
| PER MINUTE LICK RATE | 10.05 | 11.13 | 8763 | 1,58 | 0.51 |
| FLUID CONSUMPTION RATE ml | 1.38 | 1.26 | 138.84 | 1,58 | 0.33 |
| | GROUP ONE $\bar{X}$ | GROUP THREE $\bar{X}$ | SS | df | F |
| PER MINUTE HEART RATE | 135.08 | 144.18 | 1530733.52 | 1,53 | 0.13 |
| PER MINUTE LICK RATE | 6.27 | 10.05 | 5415.24 | 1,53 | 6.44 |
| FLUID CONSUMPTION RATE ml | 1.09 | 1.38 | 106.22 | 1,53 | 3 |
| | GROUP TWO $\bar{X}$ | GROUP FOUR $\bar{X}$ | SS | df | F |
| PER MINUTE HEART RATE | 102.65 | 155.22 | 10654379.72 | 1,58 | 0.25 |
| PER MINUTE LICK RATE | 7.89 | 11.13 | 8242.16 | 1,58 | 3.44 |
| FLUID CONSUMPTION RATE ml | 1.28 | 1.26 | 130.1 | 1,58 | 0 |