Turkish Psychological Articles, June 2023, *26*(51), 11-13 DOI: 10.31828/tpy1301996120230404m000053

Summary
Anticipatory Nausea and Vomiting in C57BL/6J Inbred Mice

Çınar Furkan İlhan
Middle East Technical University

Sezen Kışlal[1]
Middle East Technical University

Cancer patients experience nausea and vomiting because of emetogenic chemotherapy treatment (Hesketh, 1999). Antiemetic drugs are prescribed to alleviate these iatrogenic effects (Hesketh et al., 2017). However, if chemotherapy-induced nausea and vomiting are not effectively controlled, cancer patients may associate their feelings of illness with environmental stimuli in the hospital (Roscoe et al., 2011). As a result, 30% of cancer patients develop anticipatory nausea and vomiting (ANV) (Kamen et al., 2014). Patients who develop ANV report a low quality of life due to impaired in physical, cognitive and social functionality and they may discontinue cancer treatment (Kamen et al., 2014).

The ANV is accepted as an instance of classical conditioning (Stockhorst et al., 2007). In the terminology of classical conditioning, chemotherapy drugs are the unconditioned stimulus (US) that causes nausea and vomiting which is the unconditioned response (UR) (Stockhorst et al., 1993). As the chemotherapy sessions continue, patients associate environmental stimuli in the hospital with nausea and vomiting which causes these stimuli to become conditioned (CS) (Rodríguez, 2013). Smells, equipment, sounds, etc. in the clinic are potential CSs (Stockhorst et al., 2007). As chemotherapy sessions progress, reminders of chemotherapy treatment induce nausea (Chan et al., 2015). This phenomenon, observed in cancer patients as a result of classical conditioning, is called ANV (Rodríguez, 2013). The risk of developing ANV increases with repeated chemotherapy sessions and may persist long after the treatment (Aapro et al., 2005). Understanding the psychological and neurobiological mechanisms of ANV has substantial clinical value to develop new treatment methods.

Conditioned Context Aversion (CCA) is employed as the animal model of ANV (Cloutier et al., 2017, 2018; Limebeer and Parker, 2000). Researchers have shown that injecting illness-inducing agents in a novel environment containing various cues causes rats to develop aversion to that context. (Rodriguez et al., 2000). In this model, illness-causing agent is the US that causes the gastrointestinal stress which is the UR. The context in which illness is experienced is the CS. (Symonds and Hall, 2000). Lithium chloride (LiCl) is frequently used as an illness inducing agent (Symonds et al., 1998).

Although extensive studies have been conducted in mice to elucidate the role of specific genes and transgenes involved in the neurobiological basis of learning and memory, CCA studies have been mainly conducted on rats (Cloutier et al., 2017; Parker et al., 1984; Rodríguez et al., 2000). Only one recent study investigated the development CCA in mice (Kislal and Blizard, 2016).

In this study, we investigated CCCA learning in C57BL/6J (B6) mice, a strain that commonly used in memory and learning studies. Our aim was to evaluate whether inbred B6 mice would show conditioned aversion to a illness-paired context.

Experiment 1

The aim of our first experiment was to investigate whether inbred B6 mice would develop CCA to a context after its pairing with LiCl-induced illness.

Method

Housing

12-week-old B6 male mice weighing between 19 and 25 g were used. Mice were housed in cages with transparent walls (365 x 207 x 140 mm). The colony room was maintained on a 12-hour light/dark cycle at 24 °C +/-1. Animals were given *ad libitum* access to standard mouse chow but water restricted as described. Experiments were ethically approved by the Middle East Technical University Animal Ethics Committee.

Experimental Groups

Mice were divided into two groups according to their body weights: LiCl (n = 10) and NaCl (n = 9). During conditioning, animals in the LiCl group received injections of lithium chloride (LiCl); animals in the NaCl group received an injections of sodium chloride (NaCl).

Address for Correspondence: [1]Asst. Prof. Sezen Kışlal, Middle East Technical University, Faculty of Arts and Sciences, Department of Psychologyi Çankaya / Ankara
E-mail: sezenk@metu.edu.tr

Drug Injection

NaCl, LiCl and sucrose were purchased Sigma (St. Louis, MO, USA). LiCl was administered at a dose of 4.5 mEq/kg NaCl injections (0.9%) were used as sham treatment. All drugs were injected at a volume of 0.3 mL/kg. All injections were given intraperitoneally.

Procedure

The experiment consisted of habituation, water acclimation (WA), conditioning, recovery, taste familiarization, and recovery phases.

First, the animals were handled 3 minutes per day for the last 5 days of the 7-day habituation phase. On the last day, water restriction was started at 17:30. Then, the animals received 3 WA sessions during which standard plastic water bottles were presented only between 10:00-10:30 and 17:00-17:30. On the next day of the WA session, 30 minutes conditioning was conducted starting at 12:30. Various visual, audial, tactile, and olfactory stimuli were added to the conditioning cages and the conditioning room to create a novel context. The walls of the conditioning cages were covered with black and white tapes, and cat litter was used as bedding in the conditioning cages. In the conditioning room, 75 decibels of white noise, 60 watts of dim red light, and lemon oil scent were employed. During conditioning, each animal was placed in its conditioning cage and moved to the conditioning room. After 15 minutes, animals received injections of LiCl to induce illness or NaCl as sham treatment. Green glass bottles with stainless steel ball-bearing spouts were used to present water and sucrose. Fluid consumption was measured by weighing these bottles before and after conditioning. During the two-day recovery period, the animals were given access to water as in the WA phase in their homecages. The next day following the recovery period, the animals were given sucrose solution (0.5%) in their home cages in the colony room between 12:30-13:00 to familiarize them to the taste of the solution and water between 17.00-17.30 hours. After the next day of the taste familiarization phase, each animal was placed back in the conditioning cages and conditioning room for 30 minutes starting at 12:30 without any injection. In the first retention, half of the mice in both groups (LiCl and NaCl) were tested for water consumption and the other half for sucrose consumption. In the second retention, the mice were tested with the solution that was not used in the first retention test.

Data Analysis

Statistical analyzes were performed using GraphPad Prism (Version 9). T-test, one-way analysis of variance (ANOVA) and Tukey post-hoc tests were used to determine statistical significance for the conditioning and retention tests. Alpha level was set at $p < .05$.

Results

Independent sample t-test results showed that LiCl group (M = 0.183, SD = 0.094) drank significantly less water than NaCl group (M = 1.001, SD = 0.184) during conditioning, $t(17) = 12.36$, $p < .001$.

During retentions, independent sample t-test results showed no significant difference in water consumption among LiCl (M = 0.784, SD = 0.102) and NaCl (M = 0.843, SD = 0.109) groups, $t(17) = 1.224$, $p = .233$. Also no significant difference was observed in sucrose consumption among LiCl (M = 0.717, SD = 0.203) and NaCl (M = 0.844, SD = 0.165) groups, $t(17) = 1.487$, $p = .155$

The aim of our first experiment was to test whether B6 mice would develop CCA. During conditioning, animals injected with LiCl but not NaCl showed suppression of water consumption. However, there was no difference in both sucrose and water consumption during retention test.

Experiment 2

In our second experiment, we increased the dose of LiCl to see whether B6 mice would develop CCA with higher doses.

Housing

Housing conditions were the same as our first experiment.

Experimental Groups

Mice were divided into three groups according to their body weights: LiCl – Low Dose (n = 8), LiCl – High Dose (n = 8) and NaCl (n = 8). During conditioning, animals in the LiCl – Low Dose group was injected with 6 mEq/kg LiCl, and animals in the LiCl – High Dose group was injected with 7.5 mEq/kg LiCl and animals in the NaCl group were injected with 0.9% NaCl.

Drug Injections

LiCl was administered at 6 mEq/kg (low dose) and 7.5 mEq/kg (high dose) doses. 0.9% NaCl injections were used as sham treatment. All drugs were injected at a rate of 0.3 mL/kg.

Procedure

The procedure was the same as that used in the first experiment.

Data Analysis

Data analysis was the same as our first experiment.

Results

One-way ANOVA test results showed that there was a significant difference in water consumption among the three groups during conditioning. Tukey post-hoc test results showed that NaCl (M = 1.085, SD = 0.252) group had lower water intake than both LiCl – Low Dose (M = 0.202, SD = 0.084; $p < .001$) and LiCl – High Dose (M = 0.208, SD = 0.059; $p < .001$) groups. No significant difference was observed among the LiCl – Low Dose group and the LiCl – High Dose group ($p = .998$).

During retention, one-way ANOVA results showed no significant difference in water consumption, $F(2, 21) = 1.227$, $p = .313$ among the three groups. Also, there was no significant difference in sucrose consumption among the three groups, $F(2, 21) = 0.199$, $p = .821$.

The aim of our second experiment was to test whether B6 mice would develop CCA with higher doses of LiCl. During retention tests, the three groups displayed similar water and sucrose consumption.

Discussion

The present study was conducted to investigate whether inbred B6 mice would develop CCA against a novel context. In the first experiment, mice were injected with LiCl at a dose of 4.5 mEq/kg to induce illness. In the second experiment, LiCl dose was increased to 6 mEq/kg and 7.5 mEq/kg. Control animals were injected with saline. During retention tests, water and sucrose consumptions were used as the index of CCA. In both experiments, there was no significant difference in sucrose and water consumption of animals injected with either LiCl or NaCl.

Previous studies have shown that fluid consumption of rats decreases when they are re-exposed to the environment where they received LiCl injections (Rodríguez, 2013). These findings also replicated in outbred mice (Kislal and Blizard, 2016, 2018). However, in our study, no significant reduction in fluid consumption was observed even when LiCl were administered in high doses. One reason for the lack of aversion-induced suppression of fluid consumption may be that a single conditioning trial was insufficient for inbred mice to develop CCA. Previously, one study found that rats did not develop aversion after a single conditioning trial (Parker et al., 1984). However, our previous studies with outbred mice reveal that a single conditioning trial is sufficient for animals to develop CCA (Kislal and Blizard, 2016, 2018). Future studies should investigate whether inbred mice will develop CCA with multiple conditioning trial.

Another reason for the lack of aversion in inbred mice may be related to neuronal processes required for such learning to occur. Studies have shown that B6 mice show high performance in hippocampus- but not amygdala-related task (D'hooge et al., 2001; Risinger and Cunningham, 2000). However, the neural substrate for CCA learning remain elusive. Further research is needed to identify brain regions involved in CCA learning.

Researchers also measured other behaviors to investigate the development of CCA. Limebeer et al. (2006), recorded orofacial and somatic responses of animals in an environment where they received LiCl and NaCl injections (Limebeer et al., 2006). Rats were found to exhibit a conditioned gaping reaction when they are re-exposed to the reinforced context (Limebeer et al., 2006). It has been suggested that this reflex is an indicator of CCA learning. In another study, forepaw movement and gaping reflex were employed to investigate CCA (Parker et al., 1984). It was found that animals showed more frequent foot movements and gaping reflexes in the environment paired with LiCl (Parker et al., 1984). Other behavioral indicators, such as body washing, scratching, and rearing were also used to measure CCA (Doobay et al., 2021). Another explanation of our findings may be that B6 mice did develop CCA, but fluid consumption was insufficient to detect CCA learning. The aforementioned behavioral indicators of aversion learning have not been studied in mice. Future studies should investigate whether some behavioral indicators can be used instead of fluid consumption to detect CCA learning.

Conclusion

No suppression of fluid consumption was observed when inbred mice were re-exposed to the environment where they experienced illness. The reason for this observation may be that inbred mice is not sensitive to develop CCA with our procedural design. It is also possible that inbred mice did develop CCA, but our response measure was not suitable to detect the development of aversion learning. Future studies may offer insights into why inbred mice differ from rats and inbred mice in CCA learning.