How sucrose preference is gained and lost: An in-depth analysis of drinking behavior during the sucrose preference test in mice

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Title: How sucrose preference is gained and lost: An in-depth analysis of drinking behavior during the sucrose preference test in mice

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Running title: Analyzing the sucrose preference test with lickometry

60 pages, 14 figures, 240 words in Abstract, 83 words in Significance Statement, 619 words in Introduction, 2069 words in Discussion
Abstract

The sucrose preference test (SPT) is a widely used preclinical assay for studying stress-sensitive reward behaviors and antidepressant treatments in rodents, with some face, construct, and predictive validity. However, while stress-induced loss of sucrose preference is presumed to reflect an anhedonic-like state, little detail is known about what behavioral components may influence performance in the SPT in stress-naïve or stressed rodents. We analyzed the licking microstructure of mice during the SPT to evaluate how preference is expressed and lost following chronic stress. In stress-naïve mice, preference is expressed as both longer and more numerous drinking bouts at the sucrose bottle, compared to the water bottle. We also found evidence that memory of the sucrose bottle location supports preference. Through manipulations of the caloric content of the sweetener or caloric need of the mouse, we found that energy demands and satiety signals do not affect either preference or the underlying drinking behavior. Both acute and chronic stress impaired sucrose location memory and reduced the number of drinking bouts at the sucrose bottle, the latter of which explained the loss of sucrose preference in stress susceptible mice compared to stress resilient mice. Female mice generally exhibited similar drinking behavior to male mice but may be less susceptible to chronic stress and display better memory performance than male mice, both before and after chronic stress. Our data suggests that chronic stress inhibits a sucrose preference by reducing reward seeking behavior without affecting palatability.

Significance statement: The sucrose preference test is a widely used behavioral assay of reward and hedonic state. Here we provide a detailed behavioral analysis of drinking behavior during the test in male and female mice and reveal that the overall test results depend on multiple behavioral components, including rapid feedback related to palatability, reward seeking, and memory of reward location. These behaviors were largely independent of caloric content or
sex of the mouse. Chronic stress lowered sucrose preference by reducing reward seeking, but not palatability.
Introduction

Depression is a devastating mental illness affecting 5% of the world's population yearly (Global Burden of Disease Collaborative Network, 2021). Understanding brain mechanisms underlying symptoms of depression is crucial for improving treatments. Rodent models of depressive symptoms are important in these efforts as they allow us to study and target the neural, synaptic, and genetic changes associated with depressive-like behaviors.

Many rodent behaviors have been used to study depression-relevant pathology and antidepressant efficacy. Some of these, including the commonly used forced swim and tail suspension tests or learned helplessness test, have been criticized for lack of face validity and, in recent years, their use have been discouraged in favor of behaviors with better validity, such as behaviors that probe responses to rewarding stimuli, like the sucrose preference test (SPT) (Bale et al., 2019; Reardon, 2019; Scannell et al., 2022).

In the SPT, rodents choose between drinking regular tap water or a low concentration sucrose solution. Stress-naïve rodents have a strong preference for the sucrose solution but this preference is diminished or lost following chronic stress. This stress-induced behavioral change resembles anhedonia, a core symptom of human depression. Importantly, preference for sucrose is restored by chronic (approximately 2 weeks) but not acute (24-48 hrs) administration of selective serotonin reuptake inhibitors (SSRIs) (LeGates et al., 2018), thus mimicking the delayed response of human depressive symptoms to SSRIs.

Results in the SPT are typically reported as the percentage of the total liquid consumed that was from the sucrose solution (Liu et al., 2018). It is widely assumed that loss of sucrose preference after chronic stress results from the sucrose solution being less rewarding, i.e. it reflects a change in hedonic state, particularly when accompanied by changes in other reward behaviors. Overall drinking behavior has many components, however, not all associated with
hedonic processing. Sucrose preference may be expressed through immediate responses to
the sweet taste and motivation to seek out the sweet solution. Motivation may be affected by
satiety signals or caloric need. Finally, expression of sucrose preference may be supported by
memory of the presence and location of the sucrose solution. Lickometry has been used to
study the drinking behavior of mice when presented with sweet and caloric solutions (reviewed
in Johnson, 2018), but these experiments are usually performed in stress-naïve rodents and in
contexts that do not involve a choice. While some studies have used lickometry during the SPT,
only total licking has been reported (Tye et al., 2013; Cerniauskas et al., 2019). Further, these
experiments have often been performed in water-deprived or food-deprived rodents, which
could itself affect drinking behaviors. Upon completion of the project reported here, Verharen
and colleagues (2023) published a paper performing lickometry during a sucrose preference
test with untrained mice in operant chambers under food-deprived conditions. Here they used
computational modeling to distinguish learning and hedonic component of sucrose consumption
and found that chronic stress reduced hedonic components of sucrose consumption whereas
acute stress affected the learning rate.

In parallel to the experiments of Verharen et al. (2023), we also used lickometry to dissect the
components of drinking behavior that underlie the expression of sucrose preference in stress
naïve and chronically stressed mice in their home cages under non-food deprived conditions.
We found that mice expressed sucrose preference through several distinct drinking behaviors
related to palatability, reward seeking, and reward memory. These behaviors were largely
independent of caloric content or sex of the mouse. Finally, we found that impaired reward
seeking following chronic stress seemed to largely mediate the loss of sucrose preference in
stress-susceptible mice. Our findings are generally consistent with those of Verharen and
colleagues, however, there are noteworthy differences in methodology and results that we will
expand upon in the discussion section.
Methods

Animals

All procedures were approved by the University of Maryland Baltimore Animal Use and Care Committee and were conducted in full accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Male and female C57Bl/6J mice were acquired from Jackson Laboratories. Upon delivery mice were transferred to the room where experiments were performed and housed for a week before beginning experiments. Mice were 8 weeks of age upon initiating experiments. Mice were housed on 12 hour light-dark cycle (lights on at 7 am, lights off at 7 pm) in a standard plexiglass mouse cage with ad-lib access to autoclaved chow (LabDiet, 5010) and water. The vivarium was kept at 20-23 °C. Upon initiation of experiments mice were singly housed for the duration of the experiments.

Lickometry setup and analysis

We used an inexpensive lickometry system described in Hayar et al. (2006) to record the drinking behavior of mice during the SPT. In brief, an aluminum plate was placed towards the front of the home-cage with a wire connecting the metal plate to the ground of an AD converter (Axon Instruments Digidata, Molecular Devices). Two bottles were placed at the front end of the home-cage in custom-designed and 3D-printed bottle holders. Bottles were made from 50 mL falcon tubes that were cut and had a plastic valve that was secured in the screw cap of the tube (Fig. 1-1). After filling bottles with water or sweet solutions, parafilm was used to close the top and a wire was inserted into the bottle connecting the solution to the signal input of an AD converter. When the mouse stands on the aluminum plate and drinks from the bottle, the circuit is completed, and a junction potential created between the mouse and the liquid is recorded as...
a positive deflection with an amplitude of approximately 400 mV. The setup was designed for wires to be connected between the aluminum plate/bottles and the AD converter at all times while allowing the lid to tightly close on the homecage. Thus, the setup could be kept in the homecage throughout the experiments and the same device was reused for each mouse.

Signals were digitized using pClamp software and exported for offline analysis using custom scripts in MatLab. A primary analysis was performed to detect individual licks. Recordings were loaded into MatLab using ABFLoad (Collman, 2020) (https://github.com/fcollman/abfload) and licks were detected using peak detection (prominence >80 mV, width 15-80 ms). Licks with inter-lick intervals of <50 ms were labelled as double-peaks resulting from noise and excluded.

To determine if lickometers had accurately captured the drinking behavior of mice overnight, correlations were made between the liquid consumed from each bottle and the total amount of licking at the bottle overnight. The data from an individual mouse from that night was excluded if a bottle had leaked overnight, as determined by visual inspection, or if it was manually determined that the lickometer had performed insufficiently (usually seen as detecting too few licks resulting from disrupted connection between the cage and the AD converter or due to cross-over of signals between inputs resulting from poor isolation). Lickometer performance was determined via visual identification of outliers in a correlation plot and subsequently confirmed through inspection of the raw lickometry signal. These analyses took place blinded to the contents of the bottle or the condition of the mouse. The resulting correlation between liquid consumed and the total amount of licking reveal a strong correlation ($R^2 > 0.8$, Fig. 1-2A) for all experiments, indicating that the lickometer accurately captured the drinking behavior for subsequent analyses. When estimating this correlation between experimental conditions within experiments, no difference was observed in the slope of the correlation (Fig. 1-2B).

A total of 380 mouse x night lickometry recordings were performed. Out of these, 49 recordings (12.9%) were excluded due to bottle leaking (20 recordings), setup-to-digitizer connection...
issues (21 recordings), and software malfunction (8 recordings). Connection issues were
caused by the aluminum plate being covered by bedding, uninsulated wires causing crossed or
dissipated signals, excessive tension on wires causing dissipation of signals, or lost connection
between wire and aluminum plate. The frequency of these specific issues was not recorded.

Previous studies on licking microstructure have identified three readily distinguishable groups
based on inter-lick intervals (ILIs): **bursts**, licks with ILIs of less than 250 ms; **clusters**, licks with
ILIs between 250 and 500 ms; and **pauses**, licks with ILIs over 500 ms, during which rodents
engage in other behaviors (Davis and Smith, 1992). We created histograms of the ILIs from our
three experiments and as expected observed three distinguishable groups of licks, with ILIs (1)
<180 ms, (2) 180-320 ms, (3) >320 ms (Fig. 1A). In mice, these ILIs are faster than those in rats
and are consistent with previous reports in C57Bl/6 mice (90% of licks occur with an ILI of <160
ms) (Boughter Jr et al., 2007; St. John et al., 2017). We further observed that the large majority
of licks (>90%) belong to the first group which is consistent with previous observations (Davis
and Smith, 1992). The three categories are likely explained as the “miss” of one lick (clusters) or
2 licks (pauses) from a normal drinking burst. In order to quantify drinking behavior
independently from lickometer fidelity or fine-motor lick function, we grouped with ILIs <1000 ms
as **lick bouts**, defined as 4 or more licks with ILIs <1000 ms (Fig. 1B), as done previously and
having been shown to reflect subjective preference (Yang et al., 2020) and in some instances
showing superior sensitivity to differences in drinking behavior than either bursts or clusters
(Johnson et al., 2010).

In secondary analyses using a custom matlab script, we identified and counted the **number of
lick bouts**, the **average number of licks per bout**, and the **average frequency of lick bouts** in 30
min intervals for the duration of the recording. Another custom script was used to count the
number of times each bottle was approached after a pause of >5 mins (**return to bottle**) and the
number of times mice switched from one bottle to the other with an ILI of <60 s (**quick switch**).
Sucrose preference test

Mice were habituated to two bottles containing tap water in their homecage with free access for a full night. Their contents were weighed individually at the beginning and end to determine how much liquid had been consumed from each bottle. We used these results to determine the side preference, based on the bottle from which the mice drank the most. For the second night, mice were trained to recognize sucrose solution. A solution consisting of tap water containing sucrose (Domino Sugar, Yonkers, NY) at a concentration of 2% w/v replaced the tap water in the least preferred bottle and mice were allowed to freely drink from either bottle for one night with the volume consumed from each bottle determined as previously described. Following the training night, the position of the bottles was switched, and the sucrose solution was exchanged for a 1% sucrose solution. The amount consumed from each bottle was then measured over two nights with the position of the bottles switched between the two nights. A sucrose preference was calculated for each night. The final sucrose preference was calculated as the average of the sucrose preference over the two test nights. For subsequent SPTs with the same mice, tests with 1% sucrose solution were performed immediately without prior re-training.

In some experiments, 1% sucrose was substituted for a non-caloric sweetener (PURE, 0.1% w/v). SPT was performed while mice had ad-lib access to food. However, in some experiments, food was mildly restricted (approximately 50% of normal consumption) during the SPT night followed by normal ad-lib access to food at the conclusion of each SPT night. Mice were allowed at least 3 days of normal housing conditions between each altered SPT testing.

Multimodal stress
We used a multimodal restraint stress protocol, which has been shown to reduce sucrose preference in mice and increase the stress hormone corticosterone (Hesselgrave et al., 2021; Cole et al., 2022; Troppoli et al., 2022). Mice were restrained in tubes (Midwest Scientific, Fenton, MO or custom-designed, 3D printed) and simultaneously subjected to loud white noise, strobe lights, approximately 30° cage tilting, and predator odor (fox urine, Trap Shack Company, Neillsville, WI). This was continued for 4 hours per day (starting at 9:00 and ending at 13:00) for either 1 day (acute stress) or daily for 2-3 weeks (chronic stress). SPT was started 4 hours after the end of acute stress or 29 hours after ending the last session of chronic stress. Following chronic stress, analysis was performed separately on mice resilient to stress (>70% sucrose preference post-stress) and mice susceptible to stress (<70% sucrose preference post-stress). The 70% cut-off point for discriminating between susceptible and resilient mice as this cut-off is 2-3 standard deviations below the mean sucrose preference observed in stress-naïve mice based on historical behavioral data from our lab (not shown).

Experimental Design and Statistical Analyses

The reported data arises from 3 separate experiments. The first experiment sought to investigate through which components of drinking behavior mice expressed a preference for sucrose. 20 male mice were used for this initial experiment and data was analyzed using paired t-tests or Wilcoxon’s signed rank test for each behavioral component. Represented results are an average of data from two consecutive nights of testing. Statistical details can be found in the legend of figure 2 and figure 4. The second experiment further explored the effects of sex as well as the influence of caloric intake and acute stress on the behavioral components underlying the sucrose preference. 8 male mice and 7 female mice were first tested in a standard sucrose preference test and a 2-way repeated measures ANOVA
was performed for each behavioral component using sex and bottle content as factors each with 2 levels (male-female and water-sucrose, respectively). Statistical details are found in legend to figure 5. Subsequently mice were tested on the sucrose preference test with various manipulations as described above. An initial 3-way ANOVA was performed for each behavioral component to determine if the sex of the mice affected how each experimental manipulation modulated behavior. While licks per bout at the sucrose bottle was affected by the sex of the mouse as described in the legend to figure 6, we found no evidence of sex affecting modulation of behavioral components by different experimental manipulations. As such, data was collapsed across sexes and a 2-way repeated measures ANOVA was performed for each behavioral component using test context and bottle content as factors with 5 and 2 levels, respectively. Statistical details can be found in the legend to figure 6. Additionally, a 2-way repeated measures ANOVA was performed individually for male and female mice to test sex-specific effects again using test context and bottle content as factors with 5 and 2 levels, respectively. Statistical details can be found in Extended Data figure 6-1 for male mice and figure 6-2 for female mice. The third experiment sought to determine which behavioral components were affected by chronic stress resulting in a loss of sucrose preference. 16 male and 7 female mice were tested before and after chronic stress. Mice were subsequently divided into stress susceptible and resilient mice as described above. While the experiment was initially designed to determine if sex of the mouse affected the outcomes of chronic stress, we found that female mice were less sensitive to chronic stress in this experiment. Instead, we performed individual analyses on male resilient and susceptible mice and on male and female resilient mice. We performed a 2-way ANOVA for each behavioral component using stress and bottle content as factors each with 2 levels. This analysis was performed individually for the 8 stress-susceptible and 8 stress-resilient male mice. Statistical details can be found in the legend of figure 7. We further analyzed the data for the 8 male and 6 female stress-resilient mice using a 3-way ANOVA.
ANOVA for each behavioral component with sex, stress, and bottle content as factors each with 2 levels. Statistical details can be found in the legend of figure 8.

Statistics

Data was prepared in Excel and statistical tests were performed using Graphpad Prism. Figures were made in Graphpad Prism and MatLab. Pearson correlations were used to evaluate whether lickometers sufficiently detected drinking behavior. Normality was tested using the Anderson-Darling test. For comparisons of two groups when normality was violated, the Mann-Whitney U test was used for unpaired data and the Wilcoxon test was used for paired data, otherwise a T-test (unpaired or paired) was used. For data containing one dimension of several factors, a Friedman test was used with a Dunn's multiple comparison post-hoc test. For comparisons in 2 or 3 dimensions, a 2- or 3-way repeated measures ANOVA or mixed-effects analysis was used and post-hoc comparisons were corrected for multiple comparisons with a Šídák's correction.

Sex as a biological variable

To determine if the sex of the mice influenced drinking behavior during the SPT, we used both male and female C57Bl/6 mice. In cases where the sexes have been combined, a prior comparison revealed that there was no significant difference between sexes. Further, in figures where the sexes of the mice have been combined, symbol shapes can be used to identify individual values from female mice (downward-pointing triangle) and male mice (upward-pointing triangle). Male and female mice were stored in separate, but adjacent, rooms and females were tested one day after the males.
Code/Software

All MatLab analysis scripts and 3D designs are available on GitHub (github.com/AndreasBWulff/SPT_Lickometry & github.com/AndreasBWulff/RestraintTube)
Results

How is sucrose preference expressed in stress-naive male mice?

What aspects of drinking behavior account for a preference in stress-naive mice? Twenty male C57Bl/6 mice were singly housed and underwent the SPT as described above while drinking behavior was recorded (Fig. 2A).

When only water was present in the home-cage, mice exhibited a preference for one bottle as observed in the volume consumed (Fig. 2C, p<0.0001). Mice produced more licks (Fig. 2D, p<0.0001) and lick bouts (Fig. 2E, p<0.0001) at the preferred water bottle. This indicates that when mice have a preference for one bottle over the other, even without sucrose available, preference is expressed, in part, as a greater number of lick bouts at the preferred bottle. Mice exhibited a strong preference for the 1% sucrose over two nights (Fig. 2H, p<0.0001). As with the side preference, mice expressed their sucrose preference by licking more at the sucrose bottle (Fig. 2I, p<0.0001) and more lick bouts at the sucrose bottle (Fig. 2J p<0.0001).

Interestingly, their total fluid consumption was comparable under both conditions, indicating that they regulate their total intake, regardless of the presence of the sucrose reward (Fig. 2B). Mice thus appear to be motivated to seek out the sucrose bottle when they initiate drinking. Because the preference remains when the bottle location is switched, preference is not an artifact of ease of access or habitual approach to the bottles.

We next analyzed drinking behavior within a bout. No difference was seen in the average number of licks within a bout between bottles when sucrose is absent (Fig. 2F), despite the presence of a side preference. When sucrose was present, in contrast, mice exhibited significantly more licks per bout at the sucrose bottle than the water bottle (Fig. 2K, p<0.0001).

More licks per bout for sucrose solution suggests that the mice receive an acute reward
feedback while they are drinking, presumably gustatory, that causes them to continue their
drinking for a longer time. This rewarding feedback is absent in the case of a side preference.
There was no difference in the frequency of licks during lick bouts in the presence or absence of
sucrose (Fig. 2G,L), indicating that the acute reward feedback does not affect licking motor
function.

The SPT is usually performed over 2 nights with the sucrose bottle location switched between
night 1 and night 2. However, some studies test only one night, switching the location of the
bottle midway during the night and others test for only one to four hours. We thus analyzed the
time course of drinking behavior in the SPT during the night (Fig. 3). When the lights turn off, we
observed a sharp increase in drinking, which peaked about one hour later, followed by a slow
decline. A second spike in drinking was observed around 8 hours after the first peak, 2-3 hours
prior to lights on. Although mice exhibited a preference for sucrose during the entirety of the
session, the difference between the drinking at the sucrose and water bottle was somewhat
weaker in the beginning of the session and during periods of sparse drinking. This difference
was more pronounced on night 2 than night 1. This indicates that mice can distinguish between
the two bottles in <2 hrs, but that they continue to learn the presence and location of the
sucrose bottle and improve their performance over the next 4-5 hrs. They also identify the new
location of the sucrose bottle quickly on night 2.

A memory component to the expression of sucrose preference

The development of sucrose preference within and between nights suggests that learning and
memory may be involved in the expression of sucrose preference. To examine this, we used
two different measurements. First, we measured how often mice switched from drinking from
one bottle to the other within a 60 second window. We surmised that if mice were aware of the
presence of sucrose but did not remember the location of the bottle, then they would realize the
error upon drinking from the water bottle and quickly switch to the sucrose bottle (Fig. 4A). If so, then we predict that there should be more quick switches from water to sucrose than from sucrose to water. Indeed, we found that mice exhibited more quick switches from water to sucrose than vice versa (Fig. 4B, p<0.01), although the difference was small. We further found that this difference in quick switches was not present on the first night of testing (Fig. 4H) but emerged on the second night of testing (Fig. 4J), perhaps as the mice approach the location where the sucrose was located on the night prior and realize their error. However, there was no significant difference in the number of quick switches during the first two and last two hours of testing (Fig. 4D,F).

We next measured the number of times mice returned to either the sucrose or water bottle following a >5 min pause from drinking. If mice remember the location of the sucrose solution, then we predicted that they would be more likely to return directly to the sucrose bottle when they start drinking again (Fig. 4A). Indeed, we found that mice were significantly more likely to return to the sucrose bottle than the water bottle following a >5 min pause (Fig. 4C, p<0.0001), consistent with the contribution of some working memory of the sucrose location. This difference was observed on night 1 (Fig. 4I, p<0.05) and more strongly pronounced on night 2 (Fig. 4K, p<0.0001). No significant difference in returns to the sucrose or water bottles was observed during the first 2 hours of testing (Fig. 4E), but mice returned significantly more often to the sucrose bottle during the last two hours of testing (Fig. 4G). This suggests that the memory of the sucrose location develops during the testing session.

Together these data indicate that in stress-naïve mice, sucrose preference is expressed through three factors: 1) Reward seeking, resulting in more drinking bouts from the sucrose solution; 2) an acute reward feedback, causing longer drinking bouts of the sucrose solution; and 3) a working memory of the location of sucrose bottle.
Drinking behavior may be influenced by a variety of other environmental and biological factors. We next investigated how drinking behavior is affected by the sex of the mouse, caloric intake, and acute stress.

We first compared male and female mice on the standard SPT. Male and female mice produced licks that can be divided using the same ILI criteria but a slight shift of the peak towards shorter intervals was observed for the lick pauses in females (Fig. 1). We did, however, not observe any sex difference in the sucrose preference and both male and female mice showed a strong preference for the sucrose solution in volume consumed (Fig. 5A, p<0.0001) and total licks (Fig. 5B, p<0.0001). Both male and female mice initiated more lick bouts at the sucrose bottle than the water bottle (Fig. 5C, p<0.001) and both exhibit more licks per bout at the sucrose bottle than water bottle (Fig. 5D, p<0.01). There was also no sex difference in the intra-bout lick frequency (data not shown). These data suggest that male and female mice express their sucrose preference via similar drinking behaviors. It should be noted that the females were smaller than the males (Fig. 2-1) and thus consume more sucrose per bodyweight than males.

We next examined whether male and female mice exhibited comparable performance on memory measurements. A 2-way ANOVA found that mice performed significantly more quick switches from water to sucrose than sucrose to water, largely resulting from the performance of male mice (Fig 5E, p<0.01), although we did not observe significant sex or sex-switch interaction effects. We also found that both male and female mice returned significantly more to the sucrose bottle than water bottle (Fig. 5F, p<0.0001) but a 2-way ANOVA also revealed a significant sex-bottle interaction which a post-hoc test revealed to be caused by the females returning more often to the sucrose bottle than the male mice (Fig 5F, p<0.05). During the first two hours of testing, we further observed a trending sex-bottle interaction (p = 0.058). A Post-hoc test revealed that female mice returned significantly more to the sucrose bottle than the
water bottle (Fig. 5G, p<0.001), which was not observed in the males. However, during the last two hours of testing both male and female mice returned more to the sucrose bottle than the water bottle (Fig. 5H, p<0.0001), with no effect of sex being observed. Thus, while male and female mice both form a working memory of the sucrose location, these data indicate that female mice may develop this memory faster than the male mice.

Caloric intake is known to influence drinking behavior, with post-ingestive inhibition being known to influence the number of lick bouts at the sucrose bottle (Davis, 1973; Yang et al., 2020). Further, while acute stress does not affect sucrose preference, it may still change the behaviors underlying that preference. To investigate how caloric intake and stress affect drinking behavior, after the standard SPT, we intermittently performed versions of the SPT that were manipulated to (1) replace the 1% sucrose with 0.1% sucralose, a non-caloric sweet tastant, (2) subject the mice to acute multimodal stress on the day prior to the SPT, and (3) subject the mice to a mild food restriction during the SPT (Fig. 6A). Following these manipulated tests, we repeated a standard SPT to determine if there were any longer-term changes to drinking behavior over time. We observed a significant increase in the expressed preference for the sweetener over water when 1% sucrose was replaced with 0.1% sucralose (Fig. 6B, p<0.001; Fig. 6C, p<0.01). This resulted from more licks and more lick bouts at the sucralose bottle (Fig. 6D, p<0.01; Fig. 6E, p<0.01), but no difference in licks per bout (Fig. 6F). In contrast, acute stress and mild food restriction did not significantly affect sucrose preference (Fig. 6B), although the was a small, but significant decrease in consumed sucrose (Fig. 6C, p<0.01) and number of lick bouts at the sucrose bottle (Fig 6E; p<0.01). Interestingly, acute stress also increased licks per bout at the sucrose bottle (Fig. 6F, p<0.05).

We also confirmed that mice were significantly more likely to switch from the water to the sweet bottle than vice versa (Fig. 6G, p<0.0001) and this was independent of the context of the test. However, we found that replacing sucrose with sucralose resulted in more returns to the sweet
bottle and fewer returns to the water bottle following a >5 min pause from drinking (Fig. 6H, p<0.05) indicating that mice developed a stronger working memory of the location of the sucralose bottle than of the sucrose bottle. Additionally, acute stress significantly decreased the number of returns to the sucrose bottle (p<0.001) while not affecting the number of returns to the water bottle suggesting that stress may acutely impair working reward location memory.

However, after acute stress, mice still returned significantly more to the sucrose bottle than the water bottle indicating that working reward location memory was not entirely abolished.

Male and female mice exhibited similar drinking behaviors and were affected similarly by metabolic changes and acute stress and were for these reasons combined for the analyses described above (Fig. 6). However, sex-specific analyses can be found in Extended Data figures for male (Fig. 6-1) and female (Fig. 6-2) mice.

Both male and female mice exhibited a similar pattern of drinking over night with a spike in drinking towards the beginning and the end of the night (Fig. 6-3). However, this appeared to be more pronounced in the males than the females (Fig. 6-3C-D). Replacing sucrose with sucralose did not affect the drinking pattern appreciably (Fig. 6-3E-F) but acute stress resulted in increased drinking at the beginning of the night, compared to unstressed mice and compared to the end of the night (Fig. 6-3G-H). This front-loading of drinking was also observed when mice were food-restricted (Fig. 6-3I-J) but is not seen when retested on the standard SPT (Fig. 6-3K-L). The dip in drinking observed around 3 a.m. was also more pronounced in the food-restricted mice, perhaps as they ran out of chow.

Overall, these results indicate that neither caloric content nor acute stress had major effects on sucrose preference.

*Effects of chronic stress on drinking behavior*
Since sucrose preference appears to be expressed through several drinking behaviors favoring the sucrose solution, we next set out to determine what aspects of the drinking behavior are affected after chronic stress to result in a loss of sucrose preference.

We tested a third cohort of male and female mice on a standard SPT before and after being subjected to chronic multimodal stress (CMMS) (Fig. 7A). In this experiment, CMMS significantly reduced sucrose preference (Fig. 7B, p<0.0001), and increased total fluid consumption (Fig. 7C). There was a significant effect of sex (p<0.05) and a trending sex-stress interaction (p=0.058) on sucrose preference, with female mice somewhat less sensitive to chronic stress than male mice (Fig. 8A). Because we wished to determine the changes underlying loss of sucrose preference, we divided mice into stress susceptible or stress resilient based on whether they had <70% or >70% sucrose preference following chronic stress (Hesselgrave et al., 2021; Troppoli et al., 2022). Of 16 male mice, 8 were susceptible, and of 7 female mice, only 1 was susceptible. We thus decided to use only male mice for analysis of differences between resilient and susceptible mice to avoid possible interference of sex differences.

Consistent with the loss of sucrose preference, susceptible mice consumed more water following chronic stress than during baseline (Fig. 7D, p<0.01). Resilient mice also showed a small, but significant increase in their consumption of water after stress (Fig. 7J, p<0.05). Decreased sucrose preference following chronic stress in susceptible mice resulted from more licks (p<0.01) and lick bouts (p<0.05) at the water bottle and fewer lick bouts at the sucrose bottle (p<0.05) than at baseline (Fig. 7E,F). Susceptible mice showed significantly more licks per bout at the sucrose bottle compared to the water bottle both before (p<0.05) and after chronic stress (p<0.001) and, while a significant effect of stress was found (p<0.0001), this effect was observed for both bottles (Fig. 7G). Neither chronic stress nor bottle content affected 20
the intra-bout lick frequency (data not shown). Chronic stress thus increased the number of licks per bout at both the water bottle and sucrose bottle.

Resilient mice increased licks at both the water bottle (Fig. 7K, p<0.05) and sucrose bottle (p<0.01) and while they increased the number of lick bouts initiated at the water bottle after stress (Fig. 7L) they continued to initiate more lick bouts at the sucrose bottle than water bottle after stress (p<0.001) which was not observed in the stress susceptible mice (Fig. 7F). Similar to susceptible mice, resilient mice produced more licks per bout at the sucrose bottle than water bottle both before (Fig. 7M, p<0.01) and after stress (p<0.01), with stress increasing licks per bout independently of bottle content (P<0.05).

The biggest effect of chronic stress was on the return to the sucrose bottle. At baseline, both susceptible and resilient mice returned to the sucrose bottle significantly more often than to the water bottle after a pause (Fig. 7I,O, p<0.0001). After chronic stress, susceptible mice show no difference in number of returns to either bottle (Fig. 7I) and, while resilient mice return more to the sucrose bottle than water bottle after stress (Fig. 7O, p<0.05), they return significantly more to the water bottle (p<0.01) and significantly less to the sucrose bottle (p<0.001) suggesting some impairment to their reward location memory.

Resilient mice continued to make significantly more quick switches from the water bottle to the sucrose bottle after chronic stress (Fig. 7N, p<0.001). At baseline, the susceptible mice did not exhibit a difference in the number of quick switches between the water bottle and the sucrose bottle (Fig. 7H, N), unlike the previous cohorts, making it impossible to detect an effect of chronic stress.

Taken together, chronic stress reduced the initiation of lick bouts at the sucrose bottle, without affecting the duration of the lick bouts once initiated in susceptible but not resilient male mice.

Memory performance was also impaired by chronic stress in both resilient and susceptible mice,
as indicated by the failure to return directly to the sucrose bottle. Resilient mice appeared to compensate for this loss of reward location memory by switching quickly from the water bottle to the sucrose bottle, but not susceptible mice.

We examined sex differences in the drinking behavior following stress in resilient male and female mice before and after chronic stress. Female stress resilient mice exhibited significant increase in sucrose consumption following chronic stress (Fig 8B, p<0.01) which was not observed in male mice resulting in female mice consuming more sucrose than male mice following stress (Fig 8B, p<0.05). However, no sex difference was observed in licks, lick bouts and licks per bout (Fig 8C,D,E) with both male and female mice exhibiting significant increase in licks and licks per bout across bottles after stress (Fig 8C,E, p<0.001). Male resilient mice exhibited more water-to-sucrose than sucrose-to-water quick switches (Fig. 8G, p<0.0001) and a loss of preferential return to the sucrose bottle following a >5 min pause from drinking (Fig. 8H) after chronic stress which was not observed in female mice.

Thus, female and male resilient mice exhibited comparable reward seeking behavior in their initiation of lick bouts and palatability response in the form of licks per bout. Male resilient mice, however, exhibited impaired reward location memory that was not observed in female mice. Male resilient mice, but not females, compensated for this impairment by switching quickly to the sucrose bottle when erroneously approaching the water bottle first.
**Discussion**

We used lickometry to study drinking behavior in mice during the SPT to understand how sucrose preference is expressed and lost following chronic stress.

**Sucrose preference is mediated by reward responsiveness, seeking, and memory**

We found that sucrose preference is expressed via three separable behavioral components. We observed an acute reward response to the palatable sucrose solution, evidenced as longer drinking bouts for sucrose than water. The longer drinking bouts suggest rapid positive feedback within the millisecond-second time range of the individual bout. Additionally, mice displayed reward seeking, resulting in a greater number of drinking bouts at the sucrose bottle (Fig. 2).

Mice form memories of specific reward locations (Gauthier and Tank, 2018). We observed a memory component to the SPT in two measures. After training, mice were more likely to approach the sucrose bottle first, showing they remembered its location (Fig. 4). They also updated this location rapidly after bottles were switched. Female mice exhibited better reward location memory performance than males (Fig. 5), with females developing a reward location memory faster than males in the first hours of the SPT (Fig. 5).

We also observed more switches from water to sucrose than vice versa. An increase in switches between bottles may also be reflective of enhanced exploratory behavior of male mice but, if so, then switches should be evenly distributed between the two switch directions (i.e. even number of switches from water to sucrose and from sucrose to water). We observed a greater number of switches from water to sucrose, suggesting that when mice approach the less rewarding water bottle, they remember that the other bottle has sucrose and quickly switch to the sucrose bottle; a form of error correction (Fig. 4). This effect was small, but significant, suggesting that these error corrections were rare. Males exhibited more error correction than females (Fig. 5).
The temporal resolution of lickometry provides a better understanding of the pattern of drinking and the time when sucrose preference is optimally expressed which should be considered when designing experiments. While sucrose preference is evident on the first night, it is only strongly expressed after several hours of testing. Additionally, mice developed a preference for one bottle even when both contained water. Performing the SPT over shorter periods of time, in a single test only, and without switching the location of the bottle may lead to less accurate measurement of sucrose preference.

*Caloric content does not appreciably affect drinking behavior during standard SPT*

The behavioral components underlying the SPT could be driven by the sweetness of the solution or its caloric content. Gut-brain signaling can be responsible for a preference for sucrose over artificial sweeteners (Buchanan et al., 2022). Moreover, increasing sucrose concentrations above 3-10% results in a decline in drinking bouts due to post-ingestive inhibitory feedback. Food restriction increases the number of lick bouts without affecting the number of licks per bout (Davis, 1973; Davis and Smith, 1992; Spector et al., 1998; Yang et al., 2020). On the other hand, pairing non-caloric sweetener with intragastric infusion of carbohydrates results in increased consumption and licks per bout, while reducing number of bouts via post-ingestive positive feedback (reviewed in Sclafani, 2001).

We found that mild food restriction did not affect the number or duration of lick bouts in the SPT (Fig. 6). This may be due to the low concentration of sucrose used (1%), which is less than that observed to induce gastric feedback (Davis and Smith, 1992; Spector et al., 1998; Sclafani, 2001). The food-restriction used in this experiment is very mild assuming that if caloric content influenced consumption during the standard SPT, we should be in a sensitive range where small changes to caloric need could be observed in the drinking behavior. It is likely that a more severe food-restriction would affect drinking behavior. Thus, demand for caloric intake seems to drive sucrose consumption only in a more food-deprived state.
Replacing sucrose with a ten-fold lower concentration of sucralose (Buchanan et al., 2022), revealed a small, but significant, enhancement of preference for sucralose compared to 1% sucrose, expressed as an increase in the number of total licks and lick bouts (Fig. 6). Sucralose substitution also enhanced reward location memory performance, with mice returning less to the water bottle and more to the sucralose bottle (Fig. 6). The enhanced preference and memory performance may be caused by a relief of inhibitory feedback from the gut that would otherwise occur when mice consume sucrose. However, it may also be reflective of an enhanced perceived sweetness of the sucralose solution compared to the sucrose solution. While mice prefer a 20x higher sucrose solution over sucralose (Buchanan et al., 2022), other studies suggest that the preference threshold for sucralose is about 100x higher than that of sucrose when compared to water (Bachmanov et al., 2001). Overall, the relatively small differences between sucrose and sucralose further suggests that caloric intake plays only a minor role in the SPT.

Stress impairs reward memory and reduces reward seeking resulting in an anhedonic-like phenotype

The SPT is widely used to test for stress-induced anhedonia. We therefore tested how acute and chronic stress affected drinking behavior during the SPT. Interestingly, while acute stress did not significantly affect overall sucrose preference, it did produce a range of effects on drinking behavior. While acute stress reduced the number of lick bouts specifically at the sucrose bottle, it also increased the number of licks per bout at both the sucrose and water bottle (Fig. 6). Acute stress further reduced the number of times mice returned directly to the sucrose bottle, without affecting returns to the water bottle, resulting in an overall reduced reward location memory performance (Fig. 6). Finally, acutely stressed mice drink more at the beginning of the dark phase and less drinking throughout the rest of the night (Fig. 6-3). This contrasts with stress-naive mice, which exhibit a smaller spike in drinking during the first few
hours of the dark phase and a second spike in drinking of approximately the same size during the last few hours (Fig. 3, Fig. 6-3). Perhaps stressed mice seek to rehydrate themselves by initiating a few but long lick bouts in the beginning of the night followed by less drinking while they rest. Interestingly, these results conflict with data from rats, where acute foot-shock stress did not significantly affect cluster size or cluster number but did reduce intra-cluster lick frequency (Mitra et al., 2016). This discrepancy may be due to species differences, different stressors, different test time (16 hours vs 1 hour), or different sucrose concentrations (1% vs 10%).

Finally, comparison of susceptible and resilient male mice after chronic stress revealed that loss of sucrose preference was due to a reduced number of lick bouts at the sucrose bottle, indicative of impaired reward seeking (Fig. 7). Susceptible mice continued to make more licks per bout at the sucrose bottle compared to the water bottle, suggesting that they still found the sucrose solution palatable. Memory impairments induced by chronic stress may also contribute to the reduction in sucrose preference, but both resilient and susceptible males exhibited impaired performance. The detrimental effects of chronic stress on motivation have been well established using methods such as the forced-ratio or progressive-ratio lever pressing task (Kleen et al., 2006; Hollon et al., 2015). Given the low and equal effort required to drink from the two bottles in the SPT, it might be assumed that loss of sucrose preference is because sucrose is no longer perceived as palatable. Our results suggest, however, that the palatability of sucrose remains intact following chronic stress and that the loss of sucrose preference results from reduced reward seeking.

Female mice were less susceptible to chronic stress than male mice in our experiment (Fig. 8). Producing stress-induced depressive-like behaviors in female mice has proven challenging (Autry et al., 2009; Borrow et al., 2019; Cao et al., 2021), and often requires use of different stressors than for males (Hodes et al., 2015; Harris et al., 2018). Resilient females may be
better protected against stress-induced cognitive impairments because, unlike males, they retained reward location memory (Fig. 8). Other rodent studies have also suggested that females are better protected against stress-induced cognitive impairments than males (Luine, 2002; Bowman et al., 2003).

Our results are generally similar to the recent study of Verharen et al. (2023) which used a similar approach, along with computation modeling, to tease apart behavioral components of expressed sucrose preference in naïve and stressed states. Similar to our findings, they reported that sucrose preference was expressed through both increased lick bouts (called choices in their paper) and licks per bout, as well as in computed hedonia and learning rate parameters. However, some key differences between their study and ours are worth noting. First, they performed most sucrose preference tests while mice were in a food-deprived state, whereas we did not use food deprivation. Acute food deprivation may influence a wide range of behaviors including hedonic, metabolic, and cognitive performance. When chow was present in their study, sucrose preference was greatly reduced largely due to a reduction in number of lick bouts. Their observation is consistent with other lickometry studies showing that severe food-restriction activates gut-brain circuits to drive sucrose intake (Davis and Smith, 1992; Spector et al., 1998; Sclafani, 2001). Another difference is that Verharen and colleagues modelled the role of learning in sucrose preference, and found that learning was unaffected by chronic stress. In contrast, we used a more direct approach to estimate reward memory by counting returns to the sucrose bottle and observed significant memory impairment after chronic stress. Finally, their study found that chronic mild stress reduced sucrose preference by reducing both number of lick bouts and licks per bout and a general reduction in number of licks. However, here we found that CMMS increased consumption while decreasing lick bouts and memory performance but found no change in licks per bout. We further expanded on this finding by dividing stressed mice into susceptible and resilient and showing that while both susceptible and resilient mice had
reduced memory performance, only susceptible mice showed a decrease in licks per bout. These discrepancies may be explained by differences in hunger state, differences in test lengths and number of test sessions and/or differences in lick bout detection parameters. Behavior-specific effects underlying stress-induced loss of sucrose preference points to anatomical loci of interest for future studies in animal models of depression.

The SPT is considered one of the better measures of depression-relevant reward behavior. It holds some construct validity because loss of sucrose preference can be induced by chronic stress, a major risk factor for human depression. It has some face validity as the loss of sucrose preference represents a murine anhedonic-like state, resembling this core symptom of human depression. And it has some predictive validity because selective serotonin reuptake inhibitors are able to restore sucrose preference in chronically stressed mice when administered chronically, but not acutely (e.g. LeGates et al., 2018).

One limitation of the SPT is that the brain regions and circuits involved in expressing a preference and how these systems are impaired following chronic stress are not known. This may be a result of the pleiotropy of behavioral components underlying this test. Our results suggest more precise regional targets for the study of stress-induced loss of sucrose preference. The hippocampus encodes the location of rewards (Gauthier and Tank, 2018; Sosa and Giocomo, 2021). Excitatory inputs from hippocampus to nucleus accumbens enhance subjective palatability in the form of increased licks per bout (Yang et al., 2020). Here, we found that reward location memory was impaired in both resilient and susceptible male mice following chronic stress. Reward location memory is supported by hippocampus-nucleus accumbens projections and their synapses onto dopamine D1 receptor expressing neurons are weakened following chronic stress (LeGates et al., 2018), suggesting a possible circuit explanation for changes in memory for the location of the sucrose bottle. Dopamine activating D1 receptors is a key factor in the initiating of lick bouts (reviewed in Johnson, 2018). D1 receptor antagonists...
reduce the number of lick bouts without affecting licks per bout for sucrose (D'Aquila, 2010), similar to the behavior observed here after chronic stress. Additionally, the orbitofrontal cortex contains neurons that predict the initiation of a lick bout and their disruption affects the number of lick bouts initiated (Gutierrez et al., 2006). These systems are possible targets for better understanding the neurobiological mechanisms of stress-induced anhedonia.

Conclusion

Our analysis of drinking behavior during the SPT in mice revealed that acute hedonic responsivity, motivational salience, and reward location memory all contribute to the expression of sucrose preference, with little influence of caloric content. Male and female mice exhibited similar drinking behavior, although females exhibited reduced hedonic responsivity and better memory performance than males. Chronic stress did not affect palatability, but impaired location memory. Stress-induced impairment of reward seeking distinguished stress susceptible from stress resilient mice. These results inform our understanding of the behavioral and neurobiological mechanisms underlying stress-induced anhedonic-like behavior as measured using the SPT.

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Figure 1. Licks can be grouped into bursts, clusters, and bouts based on inter-lick interval.

A) Histograms of the interlick-interval (ILI) reveal three distinct groups of licks that are comparable among all three cohorts and across sexes. The large majority of licks have ILIs < 180 ms as delimited by the first dotted line. The second group have ILIs between 180 ms and 320 ms located between the first and second dotted line. The last group of licks have ILIs between 320 ms and approximately 1000 ms. These three different groups can be defined as bursts, clusters, and bouts respectively. B) A representative example of a raster plot from a mouse during the sucrose preference is shown below the graphs. Each vertical line represents a lick at either the water bottle (blue) or sucrose bottle (green). Horizontal lines above the raster plot indicates whether the licks based on their ILI can be considered to belong to a burst (red), cluster (orange), or bout (purple).
Figure 2. Sucrose preference is expressed via increased number of licks, lick bouts and licks per bout

A) Mice were habituated with two bottles containing water prior to testing mice with one bottle containing sucrose over two nights. The location of the bottle was switched between the two nights and drinking behavior was averaged over the two nights. C-E) When sucrose is absent mice still drink more from one preferred bottle (purple) than another (blue) which can be observed in the volume consumed (C, t = 7.22, p<0.0001), total licks (D, W = 132, p <0.0001) and number of lick bouts (E, t = 5.86, p<0.0001). F-G) No difference is seen between preferred and unpreferred water bottle in licks per bout (F, t = 1.92, p = 0.07) or intra-bout lick frequency (G, t = 0.29, p = 0.78). H-L) When sucrose is present, mice drink more from the sucrose bottle (green) than water bottle (blue) resulting in a robust expression of sucrose preference (gray). This is seen in volume consumed (H, W = 210, p<0.0001), total licks (I, W = 208, p<0.0001), number of lick bouts (J, t = 10.33, p<0.0001), and licks per bout (K, t = 9.05, p<0.0001) but not intra-bout lick frequency (L, t = 1.82, p = 0.09).
Figure 3. Temporal analysis of drinking reveals circadian-like pattern of drinking during the SPT.

Line graph shows average drinking of water (blue) and 1% sucrose (green) during the two sessions of SPT (light color band indicates standard error). The raster plots below show the individual licks (vertical lines) for each individual animal in rows. Upon entering the dark phase (grey) at 7:00 PM, a spike in drinking is observed which slowly decline resulting in a sustained pause from drinking from 1-3 AM before a second spike in drinking is observed at 4:00 AM.

N=19 mice for night 1, N=20 mice for night 2.
Figure 4. Evidence of error correcting and sucrose location memory in drinking behavior.

A) To examine the influence of memory during the SPT, we measured the number of quick switches (<60s) from water to sucrose as an estimate of error detection, and the number of returns to the sucrose bottle following a >5min pause from drinking as an estimate of sucrose location memory. B-C) Averaged over the two nights of testing, mice performed more quick switches from water to sucrose than from sucrose to water (B, W = -111, p = 0.006) and returned more to the sucrose bottle than the water bottle following a >5 min pause from drinking (C, t = 7.90, p<0.0001). D-G) Averaged over the two nights, during the first two hours of testing, mice did not differ in number of quick switches between bottles (D, W = -7, p = 0.77) or in number of returns to either bottle following a pause from drinking (E, W = 35, p = 0.195) and during the last two hours of testing mice did not differ in number of quick switches between bottles (F, W = -2, p = 0.94) but did return more to the sucrose bottle than water bottle following a pause from drinking (G, W = 210, p < 0.0001). H-K) On night 1, mice did not show difference in number of quick switches between bottles (H, W = -30, p = 0.38) but did return more to the sucrose bottle than water bottle (I, W = 124, p = 0.011). On night 2, mice performed more quick switches from water to sucrose than from sucrose to water (J, W = -124, p = 0.0004) and returned more often to the sucrose bottle than the water bottle following a pause from drinking (K, W = 210, p < 0.0001).
Figure 5. Male and female exhibit comparable drinking-behavior during SPT with 1% sucrose.

Both sexes drink more 1% sucrose than water as seen in volume consumed (A, F(1,13) = 421.3, p < 0.0001; males t = 15.95, p < 0.0001; females t = 13.18, p < 0.0001), total licks (B, F(1,13) = 379.8, p < 0.0001; males t = 14.20, p < 0.0001; females t = 13.40, p < 0.0001), lick bouts (C, F(1,13) = 120.5, p < 0.0001; males t = 7.70, p < 0.0001; females t = 7.83, p < 0.0001), and licks per bout (D, F(1, 13) = 68.71, p < 0.0001; males t = 6.42, p < 0.0001; females t = 5.35, p = 0.0003). Mice performed more quick switches (<60 s) from water to sucrose than from sucrose to water largely driven by performance in male mice (E, F(1,13) = 21.23, p = 0.0005; males t = 4.47, p = 0.0013; females t = 2.125, p = 0.104). Sex significantly affected the ratio with which mice returned to the sucrose bottle and water bottle following a >5 min pause from drinking (F, F(1,13) = 4.76, p = 0.048) with both male and female mice returning significantly more to the sucrose bottle than the water bottle (males t = 15.88, p < 0.0001; females t = 17.84, p < 0.0001) but females returning more to the sucrose bottle than males (t = 2.64, p = 0.028). During the first two hours of the night we observed a trending effect of sex on the ratio with which mice returned to the sucrose and water bottle (G, F(1, 13) = 4.341, p = 0.0575) with female mice returning significantly more to the sucrose bottle than water bottle which is not observed for male mice (males t = 1.87, p = 0.16; females t = 4.60, p = 0.0010). During the last two hours of testing mice return more to the sucrose bottle than water bottle following a pause from drinking (H, F(1, 13) = 125.8, p < 0.0001; males t = 6.89, p < 0.0001; females t = 8.91, p < 0.0001).
Figure 6. Little effect of caloric content but wide-ranging effects of acute stress on drinking behavior in male and female mice.

A) Following a standard SPT mice underwent three preference tests to measure effects of caloric content and acute stress on drinking behavior. First, 1% sucrose was replaced with 0.1% non-caloric sucralose. Second, sucrose preference was tested on nights following acute multimodal stress. Third, sucrose preference was tested while food was restricted to approximately 50% of normal consumption. Finally, mice were re-tested on the standard SPT.

B) Mice exhibited an increased preference for 0.1% sucralose than 1% sucrose over water (B, Friedman test p < 0.0001, Z = 3.94, p = 0.0003) but neither acute stress nor food-restriction affected sucrose preference. C-D) Experimental manipulations also affected the ratio of sucrose vs water drinking as seen in volume consumed (C, F(3.197, 43.15) = 33.63, p < 0.0001) and total licks (D, F(2.774, 37.45) = 12.53, p < 0.0001) with mice consuming more sweetener and less water when sucrose is replaced with sucralose (C, water t = 4.52, p = 0.0023; sweetener t = 4.91, p = 0.0011), consumed less sucrose when subjected to acute stress or mild food restriction (C, stress t = 3.71, p = 0.0093; food t = 3.69, p = 0.0097), exhibited more licks at the water bottle upon retesting under standard conditions (D, t = 4.92, p = 0.0009), and more licks at the sweet bottle when sucrose was replaced with sucralose (D, t = 4.82, p = 0.0013). E)

Experimental manipulations also affected the number of lick bouts mice initiated at the water or sweet bottle (E, F(2.969, 40.08) = 23.44, p < 0.0001) producing an increased number of lick bouts at the water bottle upon retesting under standard conditions (E, t = 4.31, p = 0.0029) increasing lick bouts at the sweet bottle when replacing sucrose with sucralose (E, t = 4.14, p = 0.0046) and reducing lick bouts at the sucrose bottle after acute stress (E, t = 3.95, p = 0.0058). F) Across all experimental manipulations, female mice produced fewer licks per bout than male mice at the sweet bottle (Not shown, F(1, 13) = 6.69, p = 0.023) but experimental manipulations affected licks per bout at the water or sweet bottle independently of sex (F(2.14, 24.66) = 3.699, p = 0.037) reducing licks per bout at the water bottle when replacing sucrose with sucralose (F, t
increasing licks per bout at the sucrose bottle following acute stress (F, t = 3.11, p = 0.036), increasing licks per bout at the water bottle upon retesting under standard conditions (F, t = 3.09, p = 0.032). G) Across all experimental manipulations, mice performed more quick switches (<60s) from water to sucrose than sucrose to water (G, F(1, 14) = 35.99, p < 0.0001). H) Experimental manipulations affected memory performance (H, F(2.864, 38.67) = 18.94, p < 0.0001) where replacing sucrose with sucralose resulted in reduced returns to water and increased returns to sucralose following a >5 min pause (H, water t = 3.05, p = 0.037; sucralose t = 3.06, p = 0.036), acute stress reduced returns to the sucrose bottle (H, t = 5.44, p = 0.0003), and upon retesting under standard conditions we observed an increased number of returns to the water bottle (H, t = 3.47, p = 0.015).
Figure 7. Chronic stress spares hedonic valuation but impairs motivational salience and memory performance in male mice.

A) Sucrose preference was measured at baseline and following CMMS in male mice. B) CMMS reduced sucrose preference (B, W = -134, p < 0.0001) and a 70% sucrose preference was used to distinguish stress-susceptible mice from stress-resilient mice. C) CMMS increased total volume consumed (C, t = 5.145, p = 0.0001). D-I) Chronic stress selectively affects drinking behavior in stress susceptible mice. D) Chronic stress affected the preferential consumption of sucrose (F(1, 7) = 32.22, p = 0.0008) by significantly increasing water volume consumed (t = 6.95, p = 0.0013). However, susceptible mice consumed significantly more sucrose than water both at baseline (D, t = 9.86, p = 0.0001) and after chronic stress (D, t = 3.99, p = 0.031). E) Chronic stress impaired the preferential licking at the sucrose bottle (F(1, 7) = 15.47, p = 0.0057) as susceptible mice licked significantly more at the sucrose bottle than water bottle at baseline (t = 8.78, p = 0.0003) but not after chronic stress (t = 2.46, p = 0.24), due to a significant increase in licking at the water bottle (t = 5.95, p = 0.0034). F) Chronic stress impaired the preferential initiation of lick bouts at the sucrose bottle (F(1, 7) = 18.72, p = 0.0035) as susceptible mice initiated significantly more lick bouts at the sucrose bottle than water bottle at baseline (t = 7.47, p = 0.0008) but not after chronic stress (t = 0.34, p > 0.99). This resulted from increased lick bouts at the water bottle (t = 3.71, p = 0.045) and fewer lick bouts at the sucrose bottle (t = 4.03, p = 0.03) after chronic stress. G) Susceptible mice produced more licks per bout at the sucrose bottle than the water bottle both at baseline (t = 3.56, p = 0.032) and after chronic stress (t = 8.60, p = 0.0001). Chronic stress significantly increased licks per bout independent of bottle content (F(1,7) = 37.76, p = 0.0005). H) Susceptible mice did not exhibit preferential quick-switching either at baseline or following chronic stress (F(1, 7) = 2.53, p = 0.16). I) Stress impaired sucrose location memory performance (F(1,7) = 28.94, p = 0.0010) as susceptible mice returned more often to the sucrose bottle than water bottle following a pause.
from drinking at baseline (t = 7.36, p = 0.0009) but not after chronic stress (t = 0.25, p > 0.99).

Following chronic stress, mice returned more often to the water bottle (t = 3.56, p = 0.054) and less often to the sucrose bottle (t = 4.05, p = 0.029). J-O) Chronic stress selectively affects memory performance in stress resilient mice. J-M) Resilient mice consume more sucrose than water (J, baseline t = 12.98, p < 0.0001; stress t = 19.12, p < 0.0001) and produce more licks (K, baseline t = 11.14, p < 0.0001; stress t = 13.74, p < 0.0001), lick bouts (L, baseline t = 8.31, p = 0.0004; stress t = 7.64, p = 0.0007), and licks per bout (M, baseline t = 5.78, p = 0.0024; stress t = 5.74, p = 0.0014) at the sucrose bottle than water bottle both at baseline and following chronic stress. Chronic stress increases consumption of water (J, baseline t = 4.36, p = 0.02), licks at both water and sucrose bottle (K, water t = 4.65, p = 0.014; sucrose t = 5.30, p = 0.0067), and lick bouts at the water bottle (L, t = 4.34, p = 0.020) and increased licks per bout independent of bottle content (F(1,7) = 10.90, p = 0.013). N) Chronic stress significantly affected error correction performance in stress-resilient mice (F(1,7) = 27.94, p = 0.0011) with mice performing more quick switches from water to sucrose than sucrose to water following chronic stress (t = 5.74, p = 0.0042) but not at baseline (t = 3.33, p = 0.074). Chronic stress results in more water-to-sucrose quick-switches (t = 5.52, p = 0.0053). O) Chronic stress significantly impaired sucrose location memory performance in stress resilient mice (F(1,7) = 71.16, p < 0.0001). After a pause from drinking, resilient mice directly return to the sucrose bottle more frequently than the water bottle both at baseline (t = 14.84, p < 0.0001) and following chronic stress (t = 3.86, p = 0.037). However, chronic stress results both in more returns to the water bottle (t = 5.8, p = 0.0040) and fewer returns to the sucrose bottle (t = 7.38, p = 0.0009).
Figure 8. Sex differences in drinking behavior after chronic stress.

A) Both stress and sex affected sucrose preference in mice (A, stress F(1, 21) = 24.25, p < 0.0001; sex F(1, 21) = 6.75, p = 0.017) with males exhibiting a significant reduction in sucrose preference that was not observed in females (males t = 6.28, p < 0.0001; females t = 1.75, p = 0.18). B-H) Comparison of resilient males and resilient females. B) Sex influenced the effect of stress on the differential consumption of water and sucrose (B, F(1, 12) = 6.21, p = 0.028) with resilient male and female mice both consuming more sucrose than water at baseline (males t = 7.91, p < 0.0001; females t = 7.36, p < 0.0001) and after stress (males t = 7.39, p < 0.0001; females t = 10.00, p < 0.0001) but females consuming more sucrose after stress than at baseline (t = 5.72, p = 0.0011) and more than stressed males (t = 3.69, p = 0.014). C) Stress affected total licking independently of sex or sucrose (C, F(1, 12) = 37.19, p < 0.0001) with resilient males and females producing more licks at the sucrose bottle than at the water bottle at baseline (males t = 8.36, p < 0.0001; females t = 7.77, p < 0.0001) and after stress (males t = 9.24, p < 0.0001; females t = 9.53, p < 0.0001). D) Stress affected the differential number of lick bouts produced at the water and sucrose bottle (D, F(1, 12) = 12.11, p = 0.0045) but resilient males and females produced more lick bouts at the sucrose bottle than water bottle at baseline (males t = 8.21, p < 0.0001; females t = 8.08, p < 0.0001) and after stress (males t = 4.53, p = 0.0016; females t = 6.499, p < 0.0001). E) Stress affected licks per bout independently of sex or sucrose (E, F(1, 12) = 19.12, p = 0.0009) and resilient males and females produced more licks per bout at the sucrose bottle than at the water bottle at baseline (males t = 4.78, p = 0.0004; females t = 4.65, p = 0.0005) and after stress (males t = 6.32, p < 0.0001; females t = 5.86, p < 0.0001). F) Stress differentially affected intra-bout lick frequency at the water and sucrose bottle driven specifically by an increase in intra-bout lick frequency at the water bottle in female mice (F, F(1, 10) = 16.20, p = 0.0024, t = 4.47, p = 0.023). G) Sex influenced how stress affected quick switching (<60s) between water and sucrose bottle (G, F(1, 12) = 7.03, p = 0.021) with
only stressed male mice exhibiting more quick switches from water to sucrose than sucrose to water ($t = 7.59, p < 0.0001$). H) Both sex and stress affected memory performance as measured by returns to sucrose bottle after a pause of >5 min (stress $F(1, 12) = 31.95, p = 0.0001$; sex $F(1, 12) = 7.34, p = 0.019$) with resilient males and females returning more to sucrose than water at baseline (males $t = 9.64, p < 0.0001$; females $t = 9.796, p < 0.0001$) but only females returning more to sucrose than water after stress ($t = 6.095, p < 0.0001$).
Extended Data Figure 1-1. Lickometry setup

Image depicts the in-cage component of the lickometry setup used to record drinking behavior of mice during the sucrose preference test. Bottles were made from 50 mL falcon tubes with the ends cut off and plastic sippers inserted into the screw lid. A custom-designed 3D-printed bottle holder was used to secure the bottles to the side of the mouse cages in a way that allowed the cages to be completely closed during the testing. An aluminum plate was placed at the bottom of the mouse cages under the bottles with a wire connected to the ground of the digitizer. A wire was connected from the inside of each bottle to the signal inputs of a digitizer. Additionally, cages contained standard bedding not depicted in the image.
Extended Data Figure 1-2. Capacitive lickometer accurately captures the drinking behavior of mice during the sucrose preference across sex and behavioral setting.

Correlations between total licks and volume consumed were performed for all included recording sessions. A) In all cohorts this correlation was highly significant (p<0.0001) with an $R^2 = 0.83$ in cohort 1, $R^2 = 0.85$ in cohort 2, and $R^2 = 0.90$ in cohort 3. B) The slope of this correlation was not significantly different between different experimental sessions either for (D) cohort 1 ($F(2, 228) = 1.55$, $p = 0.21$), (E) cohort 2 ($F(7, 578) = 1.890$, $p=0.07$), or (F) cohort 3 ($F(3, 656) = 2.041$, $p=0.11$).
Extended Data Figure 2-1. Bodyweight was greater for male than female mice throughout the experiments. In cohort 1, male mice weighed 23.13 +/- 1.944 g (mean +/- SD). In cohort 2, male mice weighed 24.148 +/- 2.265 g at the beginning of testing and 26.585 +/- 2.391 g at the end whereas female mice weighed only 18.436 +/- 1.465 g at beginning of testing and 20.303 +/- 0.916 g at the end of testing (mean +/- SD). In cohort 3, male mice increased bodyweight up to 24.705 +/- 1.717 g before stress whereas females increased weight up to 19.260 +/- 1.297 g (denoted by dotted lines). CMMS dampened further increases in bodyweight and ended with a bodyweight of 25.029 +/- 1.461 g for males and 19.523 +/- 1.054 g for females.
Extended Data Figure 6-1. Non-caloric sweetener preferential consumption and acute stress impairs memory performance in male mice.

A) Following a standard SPT male mice underwent three preference tests to measure effects of caloric content and acute stress on drinking behavior. First, 1% sucrose was replaced with 0.1% non-caloric sucralose. Second, sucrose preference was tested on nights following acute multimodal stress. Third, sucrose preference was tested while food was restricted to approximately 50% of normal consumption. Finally, mice were re-tested on the standard SPT.

B) Mice exhibited an increased preference for 0.1% sucralose than 1% sucrose over water (B, Friedman test p = 0.0041, Z = 2.874, p = 0.016) but neither acute stress nor food-restriction affected sucrose preference. C-D) Experimental manipulations also affected the ratio of sucrose vs water drinking as seen in volume consumed (C, F(2.736, 17.78) = 10.46, p = 0.0004) and total licks (D, F(2.662, 17.30) = 6.038, p = 0.0065) with mice exhibiting more licks at the sweet bottle when sucrose was replaced with sucralose (D, t = 4.63, p = 0.014). E) Experimental manipulations also affected the number of lick bouts mice initiated at the water or sweet bottle (E, F(2.068, 13.44) = 9.556, p < 0.0025) although a post-hoc analysis found only a trending increase in lick bouts at the sweet bottle when replacing sucrose with sucralose (E, t = 3.44, p = 0.054). F) Male mice consistently exhibited more licks per bout at the sweet bottle compared to the water bottle (F(1, 7) = 57.92, p = 0.0001) which was not found to be significantly affected by experimental manipulations (F(1.817, 10.45) = 2.57, p = 0.13). G) Across all experimental manipulations, mice performed more quick switches (<60s) from water to sucrose than sucrose to water (G, F(1, 7) = 14.20, p = 0.0070). H) Experimental manipulations affected memory performance (H, F(2.416, 15.70) = 7.235, p = 0.0043) where replacing sucrose with sucralose resulted in reduced returns to water following a >5 min pause (H, t = 3.97, p = 0.029), and acute stress reduced returns to the sucrose bottle (H, t = 3.34, p = 0.049).
Extended Data Figure 6-2. Non-caloric sweetener increases preferential consumption and acute stress impairs memory performance in female mice.

A) Following a standard SPT female mice underwent three preference tests to measure effects of caloric content and acute stress on drinking behavior. First, 1% sucrose was replaced with 0.1% non-caloric sucralose. Second, sucrose preference was tested on nights following acute multimodal stress. Third, sucrose preference was tested while food was restricted to approximately 50% of normal consumption. Finally, mice were re-tested on the standard SPT.

B) Mice exhibited an increased preference for 0.1% sucralose than 1% sucrose over water (B, Friedman test $p = 0.0045$, $Z = 2.70$, $p = 0.027$) but neither acute stress nor food-restriction affected sucrose preference. C-D) Experimental manipulations also affected the ratio of sucrose vs water drinking as seen in volume consumed (C, $F(2.206, 13.24) = 19.66$, $p < 0.0001$) and total licks (D, $F(1.891, 11.34) = 6.277$, $p = 0.016$) with mice consuming more sweetener and when sucrose is replaced with sucralose (C, $t = 4.12$, $p = 0.025$) and exhibiting more licks at the water bottle upon retesting under standard conditions (D, $t = 3.93$, $p = 0.030$). E) Experimental manipulations also affected the number of lick bouts mice initiated at the water or sweet bottle (E, $F(2.574, 15.44) = 13.42$, $p = 0.0002$) although a post-hoc analysis found no significant differences in lick bouts produced across the experimental manipulations. F) Mice consistently produced more licks per bout at the sweet bottle compared to the water bottle ($F(1, 6) = 105.6$, $p < 0.0001$) and this was not significantly affected by experimental manipulation ($F(1.979, 11.38) = 2.414$, $p = 0.13$). G) Across all experimental manipulations, mice performed more quick switches (<60s) from water to sucrose than sucrose to water (G, $F(1, 6) = 24.12$, $p = 0.0027$). H) Experimental manipulations affected memory performance (H, $F(2.240, 13.44) = 7.35$, $p = 0.0059$) where acute stress reduced returns to the sucrose bottle (H, $t = 4.32$, $p = 0.0197$).
Extended Data Figure 6-3. Circadian pattern of drinking is more robust in males than females and is disrupted by acute stress.

Pattern of drinking during standard sucrose preference (A-B), when sucrose is replaced with sacrolose (C-D), after mice have been subjected to acute stress (E-F), while mice are undergoing mild food restriction (G-H), and upon retesting under standard conditions (I-J) in male (left column) and female (right column) mice.
A) Water - Water

C) Water - Water

E) Water - Water

G) Water - Water

B) Water - 1% Sucrose

H) Water - 1% Sucrose

I) Water - 1% Sucrose

J) Water - 1% Sucrose

D) Water - 1% Sucrose

F) Water - 1% Sucrose

K) Water - 1% Sucrose

L) Water - 1% Sucrose