

An Open-Source Platform for Head-Fixed Operant and Consummatory Behavior

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26 **Abstract:**

27 Head-fixed behavioral experiments in rodents permit unparalleled experimental control, precise
28 measurement of behavior, and concurrent modulation and measurement of neural activity. Here we
29 present OHRBETS (Open-Source Head-fixed Rodent Behavioral Experimental Training System;
30 pronounced 'Orbitz'), a low-cost, open-source ecosystem of hardware and software to flexibly pursue the
31 neural basis of a variety of motivated behaviors. Head-fixed mice tested with OHRBETS displayed
32 operant conditioning for caloric reward that replicates core behavioral phenotypes observed during freely
33 moving conditions. OHRBETS also permits for optogenetic intracranial self-stimulation under positive or
34 negative operant conditioning procedures and real-time place preference behavior, like that observed in
35 freely moving assays. In a multi-spout brief-access consumption task, mice displayed licking as a function
36 of concentration of sucrose, quinine, and sodium chloride, with licking modulated by homeostatic or
37 circadian influences. Finally, to highlight the functionality of OHRBETS, we measured mesolimbic
38 dopamine signals during the multi-spout brief-access task that display strong correlations with relative
39 solution value and magnitude of consumption. All designs, programs, and instructions are provided freely
40 online. This customizable ecosystem enables replicable operant and consummatory behaviors and can
41 be incorporated with methods to perturb and record neural dynamics *in vivo*.

42 **Impact Statement:**

43 A customizable open-source hardware and software ecosystem for conducting diverse head-fixed
44 behavioral experiments in mice.

45

46 **Introduction:**

47 Studying mouse behavior under head-fixed conditions offer many distinct advantages over freely
48 moving conditions. Head-fixation offers high degrees of behavioral control that enables consistent
49 delivery of stimuli to the animal, precise measurement of behavior, and isolation of subcomponents of
50 behavior (Bjerre & Palmer, 2020). Holding the mouse stable permits a wide range of behavioral
51 experiments that have features that are challenging or impossible to conduct reliably in the freely moving
52 condition including the delivery of somatosensory stimuli to select locations, temporally precise odor
53 delivery (Han et al., 2018), presentation of visual stimuli to fixed parts of the visual field (Krauzlis et al.,
54 2020; The International Brain Laboratory et al., 2021), temperature manipulations (Jung et al., 2022), and
55 high-resolution video recording of facial expression or paw movement (Dolensek et al., 2020; Mathis et
56 al., 2018). Eliminating or controlling physical approach behaviors also allows for isolation of both
57 appetitive and consummatory behaviors and related neuronal dynamics. By removing turning associated
58 with locomotion, head-fixed behavioral approaches offer enhanced compatibility with neuroscience
59 approaches that require tethers including optogenetics and fiber-photometry. Furthermore, head-fixation
60 is also compatible with tools for measuring and manipulating neuronal activity at the single cell level,
61 including two-photon calcium imaging and holographic optogenetics.

62 Motivated behaviors are essential for survival and can be disrupted in brain circuits, leading to
63 various diseases such as addiction and obesity. (Kenny, 2011; Rossi and Stuber, 2018; Volkow et al.,
64 2017). Motivation in animal models is often assessed and quantified using multiple tasks that attempt to
65 isolate distinct behavioral components such as appetitive and consummatory behaviors that can be the
66 product of independent or overlapping brain circuits (Panksepp, 1982; Robinson and Berridge, 1993). To
67 determine the role of brain circuits in distinct components of behavior, behavioral models with a high
68 degree of experimental control and reproducibility are paramount as they can isolate components of
69 behavior and limit variability across labs, subjects, and trials. There are a variety of approaches in freely
70 moving rodents that model individual components of motivated behavior. Motivation is often modeled
71 using operant responding on levers or nose pokes to earn a caloric reward or intracranial brain
72 stimulation. A highly controlled version of operant responding includes retractable levers and retractable
73 lick-spouts to limit access of both operant and consummatory responses, respectively. In contrast,
74 consummatory behaviors require measuring the volumetric amount of appetitive or aversive solutions. A
75 particularly useful model is the brief-access task, which consists of trial-based presentations of one of
76 multiple solutions, enabling recording of behavioral and neuronal responses to gradations of both
77 rewarding and aversive solutions within a single session (Boughter et al., 2002; Davis, 1973; Smith,
78 2001). Despite the widespread use of these procedures in freely moving animals, there has been limited
79 adaptation of these tasks for head-fixed rodents despite advantages.

80 Here, we present OHRBETS, a low-cost, open-source ecosystem of hardware and software for
81 quantifying both operant and consummatory behavior in head-fixed mice. OHRBETS features the ability
82 to precisely limit operant and consummatory behaviors during operant conditioning, replicating the
83 retractable levers and spout aspects of the freely moving condition. OHRBETS has a multi-spout design
84 that allows multiple solutions to be presented independently in a single behavioral session, enabling
85 various behavioral experiments like probabilistic reinforcement tasks and choice behavior. The platform
86 is also flexible and includes connectivity for additional customizable components. OHRBETS consists
87 largely of 3D printed and low-cost components that reduce the total cost per system and maximizes
88 reproducibility. Multiple research groups have developed models for head-fixed operant behavior with a
89 variety of operant responses (Bloem et al., 2022; Cui et al., 2017; Guo et al., 2014; Stephenson-Jones
90 et al., 2020; Vollmer et al., 2022, 2021), but many of these systems are built for a single experimental
91 procedures with minimal publicly available resources needed for consistent replication. To assist with
92 modification and reproduction of our system, we have created a GitHub repository
93 (<https://github.com/agordonfennell/OHRBETS>) that contains 3D models (also available through
94 TinkerCad), assembly instructions, wiring diagrams, behavioral programs, and scripts for analysis. The
95 OHRBETS ecosystem will allow any investigator to harness the strength of head-fixed approaches to
96 study the neurobiological underpinnings of motivation and related disease states while maintaining many
97 crucial behavioral phenotypes established in freely moving animals.

98 **Results:**

99 **OHRBETS Overview:**

100 We developed OHRBETS, a low-cost, open-source system for head-fixed behaviors in mice
101 (**Figure 1A-E, Figure 1- figure supplement 1**). Our system consists of custom 3D printed and
102 inexpensive, commercially available components bringing the total cost to around \$600 for the operant-
103 only version and around \$1,000 for the operant + multi-spout version. For head-fixation, mice are
104 implanted with a metal head-ring and are easily and quickly secured on the head-fixed system for daily
105 behavioral sessions (**Figure 1A-B**). To deliver solutions, including sucrose, we use gravity fed tubing
106 attached to a stainless-steel lick spout that is gated by a solenoid. The position of the lick spout is
107 controlled using a custom 3D printed micropositioner (Backyard Brains 2013; Hietanen et al., 2018), and
108 licks are detected using a capacitive touch sensing. To limit access to consumption, paralleling a
109 retractable lick spout from the widely used freely moving operant assay, we used a linear actuator
110 (adapted from (Buehler, 2016a)) that is controlled using a 5V micro servo for extending and retracting
111 the spout (**Figure 1D**). A 43.2 mm diameter wheel (Lego, 86652c01) coupled to a rotary encoder is
112 mounted underneath the mouse such that their forepaws' deflections left or right can serve as the operant

113 response (International Brain Laboratory 2021). To limit access to operant responding, paralleling
114 retractable levers used in the freely moving operant assay, we developed a wheel brake controlled via
115 an additional micro servo (**Figure 1E**). All behavioral components are controlled by an Arduino Mega and
116 the timing of events are relayed via serial communication and recorded using a Python program (**Figure**
117 **1C**). Our system is inexpensive and easily assembled following instructions freely available through our
118 GitHub repository (https://github.com/agordonfennell/open_stage).

119 To characterize the effectiveness of our retractable spout and wheel brake, we conducted
120 experiments to determine the timing and reliability of the hardware. We measured the linear travel of 5
121 sets of retractable spouts using high speed video recording (200 fps) during 1000, 1 cm spout
122 extensions/retractions and determined the position of the spout using DeepLabCut (Mathis et al., 2018)
123 (**Figure 1D, Figure 1- figure supplement 1B**). We found that the retractable spout follows a consistent
124 and reliable pattern with >98% of extensions reaching a terminal position within 0.3 mm of each other in
125 under 180ms of the extension command (**Figure 1A, Figure 1- figure supplement 1B A-E**). We
126 measured the braking ability of 4 sets of wheel brakes by manually rotating the wheel at different rates
127 in both directions and then programmatically engaging the brake (**Figure 1E, Figure 1- figure**
128 **supplement 1G**). The wheel brake rapidly stopped wheel rotation in 100% of trials, even with manual
129 velocities that exceed that which a mouse can produce (**Figure 1E**). Furthermore, we analyzed the
130 effectiveness of the brake to stop wheel rotation in data obtained during operant conditioning experiments
131 and found that most mouse-generated rotations ceased in under 250ms (**Figure 1- figure supplement**
132 **1**). Together, these results indicate OHRBETS produces reliable spout extension/retraction and wheel
133 braking using inexpensive micro servos and 3D printed components, and therefore will effectively limit
134 access to consummatory and operant responses during behavioral experiments.

135

136 **OHRBETS trained mice show multiple established characteristics of operant behavior observed**
137 **in freely moving animals.**

138 We developed a training procedure that permits measuring operant conditioning in head-fixed
139 mice, and we conducted a series of experiments to determine if operant behavior conducted with
140 OHRBETS reproduces behavior seen in freely moving rodents (Kliner et al., 1988; Reilly, 1999; Winger
141 and Woods, 1985). We trained head-fixed, water-restricted mice to perform operant conditioning in 3
142 stages: 1) free-access lick training, 2) retractable spout training, and 3) operant conditioning (*Methods*).
143 To measure the reproducibility of OHRBETS, all experiments were conducted using 4 independent
144 Operant-Stage assemblies (referred to as box ID, data shown in supplements).

145 We trained mice on a single session of free-access lick training to facilitate licking from the spout
146 and reduce stress associated with head-fixation (**Figure 1- figure supplement 2**). Free-access lick
147 training consisted of a 10 min session where each lick immediately triggered a delivery of ~1.5 μ L of 10%
148 sucrose which approximates free-access consumption from a standard lick spout (**Figure 1- figure**
149 **supplement 2A**). During training, 100% of mice licked for sucrose throughout the session (**Figure 1-**
150 **figure supplement 2B**). Like the standard freely moving free-access assay (Johnson, 2018; Spector et
151 al., 1998), OHRBETS trained mice licked in discrete licking bouts (**Figure 1- figure supplement 2C-D**).
152 The total number of licks as well as the licking microstructure, including licks per bout and bout duration,
153 were consistent across sex, cohort, and box ID (*t*-test or One-Way RM ANOVA n.s.; **Figure 1- figure**
154 **supplement 2E-P**), as well as across freely moving and head-fixed conditions (*t*-test n.s.; **Figure 1-**
155 **figure supplement 2Q-V**).

156 Next, mice completed 3 sessions of retractable lick spout training - building the association
157 between spout extension and the availability of reward to enhance the learning rate in subsequent
158 operant conditioning (Steinhauer et al., 1976) (**Figure 1F-J, Figure 1- figure supplement 3**). Each
159 session consisted of 60 trials of spout extension, delivery of 5 pulses of 10% sucrose (~1.5 μ L/pulse,
160 200ms inter-pulse interval), and a 5 s access period for liquid to be consumed during which a 5 kHz tone
161 was presented. Mice licked to consume sucrose delivered on most trials with a short latency between
162 spout extension and licking throughout each session (**Figure 1G, Figure 1- figure supplement 3A-E**).
163 By the third session of training, 31 out of 31 mice licked during 90% of trials (**Figure 1H**). Mice
164 demonstrated a learned association between spout extension and a simultaneous auditory tone with the
165 availability of sucrose, as they reduced their latency from spout extension to first lick across the 3
166 sessions of training (Two-Way RM ANOVA: Session^{***}, **Figure 1I**). No changes in the proportion of trials
167 with a lick or the number of licks per trial over sessions were observed (One-Way RM ANOVA: Session
168 n.s.; **Figure 1I, J**). Female mice displayed a higher lick latency in response to spout extension compared
169 to males on the first session of training (Two-Way RM ANOVA: Sex^{**}, Sex x Session^{**}; Session 1
170 HSD^{***}), but the proportion of trials with a lick and the number of licks per trial was not statistically different
171 between males and females (Two-Way RM ANOVA: Sex n.s., Sex x Session n.s.; **Figure 1- figure**
172 **supplement 3E, H, K**). There were no differences in behavior across cohorts or behavioral systems
173 (Two-Way RM ANOVA: main effects and interactions n.s.; **Figure 1- figure supplement 3F, I, G, J, M**),
174 aside from a significant interaction between cohort and session for the mean trial lick count (Two-Way
175 RM ANOVA: Cohort x Session^{**}; **Figure 1- figure supplement 3L**). These data indicate that mice rapidly
176 learn to lick for sucrose during discrete windows of access.

177 After free-access lick training and retractable spout training, water-restricted mice were operantly
178 conditioned for sucrose (**Figure 1K-N, Figure 1- figure supplement 4**). Operant conditioning consisted
179 of 6 sessions of responding for 10% sucrose under fixed-ratio schedule (1/4 rotation for session 1; 1/2

180 rotation for sessions 2-6; **Figure 1K; Figure 1- Figure Supplement 6; Methods**). To assess if mice
181 learned the operant requirement, we examined whether mice increased responding in the active direction
182 over sessions and exhibited a response bias for the active over the inactive response (Heyser et al.,
183 2000). We found that mice learned to turn the wheel to obtain 10% sucrose in as little as 1 session, as
184 25/31 mice showed greater rotation in the active direction compared to the inactive direction (**Figure 1L,**
185 **session 1**). By the 6th session of operant conditioning, 29/31 mice showed an increase in net rotation in
186 the active direction (t -test***; **Figure 1L**, data from all sessions shown in **Figure 1- figure supplement**
187 **4A**), that was the product of increased rotation in the active direction and no change in rotation in the
188 inactive direction (Two-Way RM ANOVA: Session x Direction***; **Figure 1M**). As a group, mice showed
189 significantly more rotation in the active direction compared to the inactive direction starting at the second
190 session (Two-Way RM ANOVA: Session x Direction***; Session 2-6 Active vs Inactive: HSD***; **Figure**
191 **1N**). Mice that were tested in each of the 4 boxes showed similar inter-lick intervals, trial lick counts, and
192 latency to lick (**Figure 1- figure supplement 4B-D**). When analyzing behavioral data based on sex,
193 cohort, and box ID, we found only minor differences in behavior (**Figure 1- figure supplement 4E-Y**).
194 Notably, we found that over the course of training sessions, female mice exhibit a reduced total active
195 rotation (Two-Way RM ANOVA: Sex*; **Figure 1- figure supplement 4H**), reduced total lick count (Two-
196 Way RM ANOVA: Sex*; **Figure 1- figure supplement 4Q**), and reduced bias for rotation in the active
197 direction (Two-Way RM ANOVA: Sex*; **Figure 1- figure supplement 4T**). These data indicate that mice
198 rapidly exhibit operant responding for sucrose using OHRBETS, and this behavior is consistent across
199 training history and behavioral setup with only minor differences observed a between males and females.

200 Next, we determined if OHRBETS could reproduce other behaviors that have been established
201 in freely moving rodents, including increased active responding following increased cost of reward (Kliner
202 et al., 1988; Winger and Woods, 1985) (**Figure 1O**), progressive-ratio responding with a fixed reward
203 magnitude (Reilly, 1999; Sclafani and Ackroff, 2003; Winger and Woods, 1985) (**Figure 1P**), and reversal
204 learning (Forgays and Levin, 1959; Heyser et al., 2000; Klanker et al., 2015) (**Figure 1Q**). To measure
205 the relationship between cost and active response rate, after completing 1 session with a fixed-ratio of
206 1/4 turn and 5 sessions of a fixed-ratio of 1/2 turn (**Figure 1L-N**), we increased the fixed-ratio to 1 turn
207 and measured operant responding for 4 sessions. As observed in freely moving rodents (**Figure 1- figure**
208 **supplement 5B**), when we increased the cost of reward, mice significantly increased responding in the
209 active direction but not the inactive direction (Two-Way RM ANOVA: Cost x Direction***; **Figure 1O**, all
210 sessions shown in **Figure 1- figure supplement 5A**) indicating that they show flexible response rates
211 as a function of reward cost (Kliner et al., 1988; Winger and Woods, 1985). Next, to measure the
212 motivation to seek different reward magnitudes, we tested mice over multiple sessions of progressive
213 ratio responding for sucrose of varying volumes (1, 5, 10 deliveries of ~1.5 μ L of 10% sucrose,
214 counterbalanced order). During progressive-ratio sessions, mice were tested with a linear or logarithmic

215 reinforcement schedule, where the cost for each subsequent reinforcer was higher than the last (**Figure**
216 **1P left, Methods**). Under both schedules, mice responded for rewards during progressive ratio and
217 displayed increased breakpoints for greater reward magnitude (Reilly, 1999; Sclafani and Ackroff, 2003;
218 Winger and Woods, 1985) (One-Way RM ANOVA: Number of Solenoid Openings*; **Figure 1P right**). To
219 determine if mice can learn reversals in response contingency, we trained a naïve group of mice to
220 perform operant responding with an initial rotational direction contingency for 5 sessions and then
221 switched the contingency and allowed mice to re-learn over 7 sessions. We found that mice displayed
222 reversal learning, as they reversed the terminal cumulative position (initially active - initially inactive)
223 following contingency reversal and training over 7 sessions (Heyser et al., 2000; Klanker et al., 2015)(*t*-
224 test**; **Figure 1Q**). Finally, to directly compare behavior during head-fixed and freely moving versions of
225 operant conditioning, we examined behavioral responding in the two tasks within the same mice (**Figure**
226 **figure supplement 5A, b**). We found that mice showed similar changes in response vigor with increased
227 cost of reward (**Figure 1- figure supplement 5A-C**) and similar pattern of reduction in responding over
228 the course of a session after the first 10 min (**Figure 1- figure supplement 5D**) but earned more liquid
229 in the freely moving version of the task (*t*-test***; **Figure 1- figure supplement 5E**). Together, these data
230 indicate that mice display flexible operant behavior in our head-fixed system that is sensitive to the cost
231 of reward, the magnitude of reward, and reward contingency, and produces behavior in a parallel manner
232 to freely moving operant conditioning.

233

234 **OHRBETS trained mice exhibit positive and negative operant conditioning during optogenetic** 235 **stimulation of LHA GABAergic and glutamatergic neurons**

236 After establishing that mice display operant responding for caloric rewards, we determined if they
237 would perform operant responding to obtain or avoid optogenetic stimulation of brain circuits that have
238 been previously established to be rewarding or aversive in freely moving rodents using the OHRBETS
239 system (Chen et al., 2020; Jennings et al., 2015, 2013; Rossi et al., 2019). Optogenetic stimulation allows
240 for temporally precise manipulations of genetically- and spatially- defined neuronal circuits enabling
241 greater consistency of unconditioned stimuli delivery across a multitude of experimental conditions. We
242 used optogenetic stimulation of LHA GABAergic neurons (LHA^{GABA}) as a appetitive unconditioned
243 stimulus because activation of these neurons produces positive reinforcement (Jennings et al., 2015),
244 and optogenetic stimulation of LHA Glutamatergic neurons (LHA^{Glut}) as an aversive unconditioned
245 stimulus because activation of these neurons is aversive (Chen et al., 2020; Rossi et al., 2019). To
246 selectively manipulate LHA^{GABA} and LHA^{Glut} neurons, we expressed cre-dependent channelrhodopsin-2
247 (ChR2) or cre-dependent mCherry in the LHA of *Slc32a1^{Cre}* (*Vgat-cre*) or *Slc17a7^{Cre}* (*Vglut2-cre*) mice
248 (Vong et al., 2011) and implanted bilateral optic fibers with a head-ring to facilitate head-fixation (**Figure**

249 **2A, Figure 2- figure supplement 1A, Methods).** Following incubation, mice were tested using freely
250 moving and OHRBETS intracranial self-stimulation (ICSS) in counterbalanced order.

251 Mice displayed high levels of active responses to obtain optogenetic stimulation of LHA^{GABA}
252 neurons that was consistent across freely moving and head-fixed procedures (**Figure 2D-I, Figure 2-
253 figure supplement 1B-G, Figure 2- Figure Supplement 2**). We first trained mice to nose poke (fixed-
254 ratio 1 poke) or turn a wheel (fixed-ratio 1/2 turn) to obtain optogenetic stimulation (1 s, 20 Hz, 5 ms pulse
255 duration) of LHA^{GABA} cells over 4-5 sessions (**Figure 2B, C**; training data shown in **Figure 2- figure
256 supplement 1B-G**). Next, we measured operant responses for different stimulation frequencies by
257 running mice through 5 sessions of ICSS with one of 5 stimulation frequencies (1, 5, 10, 20, 40 Hz) in
258 counterbalanced order. On the last 20 Hz self-stimulation training session, *Vgat-cre* mice expressing
259 ChR2 in the LHA (LHA^{GABA}:ChR2) displayed high levels of operant responding for the active hole or active
260 direction and displayed strong discrimination between active and inactive responses; *Vgat-cre* mice
261 expressing the mCherry control construct in the LHA (LHA^{GABA}:Control) displayed little to no responding
262 and did not discriminate between responses (Two-Way RM ANOVA: Response ID x Group** for both
263 systems; **Figure 2D, F**). In both the freely moving and head-fixed conditions, LHA^{GABA}:ChR2 mice
264 displayed greater active response rates for higher stimulation frequencies (Two-Way RM ANOVA: Group
265 x Stimulation Frequency*** for both systems; **Figure 2E, G, H**) that were positively correlated across the
266 two versions of the task (Pearson's Product Moment**, **Figure 2I**). On the contrary, LHA^{GABA}:Control mice
267 displayed no change in responding to changes in frequency and no correlation across the two
268 procedures. Similar to freely moving ICSS (Stuber et al., 2011; Witten et al., 2011), mice trained with
269 positive reinforcement rapidly ceased responding once optogenetic stimulation was withheld and then
270 resumed responding once optogenetic stimulation was reintroduced (**Figure 2L, left**). These data
271 indicate that OHRBETS can robustly elicit motivated behaviors to obtain rewarding optogenetic
272 stimulation in a similar manner to freely moving rodent behavioral paradigms.

273 Mice displayed high levels of responding to avoid optogenetic stimulation of LHA^{Glut} neurons
274 under negative reinforcement during the head-fixed procedure but not the freely moving procedure. To
275 elicit negative reinforcement (responses to cease an aversive stimulus) in the head-fixed procedure, we
276 trained mice to turn a wheel to earn a 3 s pause of continuous stimulation of LHA^{Glut} neurons at 5 Hz for
277 sessions 1 - 5 and 10 Hz for session 6 - 11 (**Figure 2b**). Following training, *Vglut2-cre* mice with
278 expression of ChR2 in the LHA (LHA^{Glut}:ChR2) displayed high levels of responding in the active direction
279 and strong discrimination between the active and inactive directions, while *Vglut2-cre* mice with
280 expression of mCherry control construct in the LHA (LHA^{Glut}:Control) displayed little to no responding and
281 no discrimination (Two-Way RM ANOVA: Direction x Group*; **Figure 2J, Figure 2- Figure Supplement
282 2**). Over the course of training, LHA^{Glut}:ChR2 mice, but not LHA^{Glut}:Control mice, increased the number
283 of pauses earned (Two-Way RM ANOVA: Stimulation Frequency x Group***; **Figure 2K**). Compared to

284 LHA^{Glut}:Control, LHA^{Glut}:ChR2 mice showed substantially higher active rotation as well as a moderately
285 higher inactive rotation (**Figure 2- figure supplement 1K, M**). LHA^{Glut}:ChR2 mice acquired negative
286 reinforcement behavior at a reduced rate compared to positive reinforcement (7 sessions to acquisition
287 of negative reinforcement vs 1 session for positive reinforcement; **Figure 2K, Figure 2- figure**
288 **supplement 1F, L**). Like positive reinforcement, LHA^{Glut}:ChR2 mice that were trained on negative
289 reinforcement rapidly ceased responding when the optogenetic stimulation was removed and resumed
290 responding when optogenetic stimulation was reintroduced (**Figure 2L, right**). To compare behavior in
291 head-fixed to freely moving procedures, we trained the same mice under negative reinforcement in a
292 freely moving procedure. We found that, compared to LHA^{Glut}:Control mice, LHA^{Glut}:ChR2 mice displayed
293 suppressed amounts of active-responding, number of pauses earned, and inactive responding during the
294 freely moving condition (**Figure 2- figure supplement H-J**). The discrepancy between acquisition of
295 negative reinforcement in the head-fixed assay versus the freely moving assay could be attributed to the
296 reduced range of actions mice can make in the head-fixed assay. These results indicate that OHRBETS
297 can elicit responding under negative reinforcement using a simple stimulation procedure that is incapable
298 of producing responding in traditional freely moving conditions.

299

300 **Head-fixed mice express real-time place preference and avoidance behaviors**

301 We designed and tested a procedure analogous to real-time place testing (RTPT) (Britt et al.,
302 2012; Kravitz et al., 2012; Stamatakis and Stuber, 2012; Tye and Deisseroth, 2012) in head-fixed mice
303 (**Figure 3**). RTPT is extensively used to measure the appetitive or aversive characteristics of neuronal
304 manipulations. With the same mice utilized for operant conditioning (*Methods*), we used stimulation of
305 LHA^{GABA} neurons as a positive unconditioned stimulus and stimulation of LHA^{Glut} neurons as a negative
306 unconditioned stimulus because these two populations have been previously shown to drive real-time
307 place preference (RTPP) and real-time place avoidance (RTPA), respectively (Jennings et al., 2015; Nieh
308 et al., 2016; Rossi et al., 2019) (**Figure 3A**, for fiber placement see **Figure 3- figure supplement 1A**).
309 *Vgat-cre* and *Vglut2-cre* mice expressing mCherry were pooled after observing no statistical differences
310 in behavior between the two genotypes. In the standard version of the task, freely moving mice traverse
311 a two-chamber arena in which they receive optogenetic stimulation when the mouse is located in one of
312 the two chambers (**Figure 3B, top**). Using OHRBETS, the response wheel was divided into two halves
313 relative to the starting position of the wheel, one of which was paired with optogenetic stimulation (**Figure**
314 **3B, bottom**). To enhance the mouse's ability to determine their position on the wheel, we included a tone
315 that indicated the mouse's position in the two zones. The two chamber RTPT assay offers a distinct
316 advantage for comparing behavior across different versions of the assay because throughout the entire
317 session duration the subject is in one of two states (stimulated or not), allowing for a one-to-one

318 comparison of the amount of time stimulated over the duration of the fixed session. For both tasks, mice
319 were initially habituated without stimulation for 1 session and then underwent RTPT over 6 sessions with
320 frequency and chamber/wheel-zone pairing counterbalanced (**Figure 3C**). For the head-fixed procedure,
321 mice were initially trained without any cue indicating the wheel zone. After initial training, we paired the
322 wheel zones with tones and found that mice exhibited more obvious RTPP/RTPA (**Figure 3- figure**
323 **supplement 1D**), so in subsequent sessions these zone cues were added to the task design. Using this
324 approach, we measured the similarity in RTPT behavior with a range of rewarding and aversive
325 stimulation magnitudes across freely moving and head-fixed procedures.

326 Mice expressed similar behaviors in the RTPT assay during freely moving and head-fixed
327 procedures (**Figure 3D-F, Figure 3- figure supplement 1**). Specifically, mice expressing mCherry
328 (LHA:Control mice) did not show preference nor aversion for the stimulation paired chamber/zone across
329 all stimulation frequencies in both the freely moving and head-fixed procedures and did not show
330 correlations across the two assays (Two-Way RM ANOVA: Stimulation Frequency n.s.; **Figure 3D-F**).
331 On the contrary, LHA^{GABA}:ChR2 mice showed strong place preference while LHA^{Glut}:ChR2 mice showed
332 strong place aversion for the paired chamber/zone with higher stimulation frequencies compared to lower
333 stimulation frequencies (Two-Way RM ANOVA: Stimulation Frequency*** for both groups; **Figure 3D, E**).
334 There was no statistical difference between the amount of time in the paired chamber/zone in the freely
335 moving and head-fixed versions of the task (Two-Way RM ANOVA: System n.s. for both groups,
336 Stimulation Frequency x System n.s. for both groups; **Figure 3D, E**). Furthermore, the time in the paired
337 chamber/zone was correlated across freely moving and head-fixed procedures for LHA^{GABA}:ChR2 and
338 LHA^{Glut}:ChR2 mice, but not LHA:Control mice (Pearson's Product moment: * for LHA^{GABA}:ChR2, ** for
339 LHA^{Glut}:ChR2; **Figure 3F**). Examining the correlation of behavior between the two assays at different
340 stimulation frequencies in individual mice (**Figure 3- figure supplement 1B**) revealed that, compared to
341 LHA:Control mice, LHA^{GABA}:ChR2 mice and LHA^{Glut}:ChR2 mice showed a greater Pearson's Product
342 Moment R and regression slope estimate (**Figure 3- figure supplement 1C, left-mid**). Furthermore,
343 LHA^{Glut}:ChR2 mice showed lower regression *p*-values compared to mCherry controls while the
344 LHA^{GABA}:ChR2 mice displayed a trend towards lower *p*-values (**Figure 3- figure supplement 1C, right**).
345 Together these results indicate that OHRBETS elicits RTPT behavior similar to freely moving procedures
346 and provides a useful experimental approach for measuring the valence of stimuli.

347

348 **OHRBETS trained mice display consummatory behaviors dependent on the concentration of**
349 **appetitive and aversive solutions**

350 Exposure to appetitive and aversive taste solutions provides an approach to measure neuronal
351 correlates of appetitive and aversive events in addition to operant responding. Within-session
352 consumption of unpredictable tastants allows for measuring a range of behavioral and neuronal
353 responses to gradations in solution valence. We adapted OHRBETS to include a retractable, radial multi-
354 spout consisting of 5 spouts (**Figure 4A, Figure 4- Figure Supplement 3**). Using this system, we
355 delivered up to 5 solutions with different concentrations in the same session with a task design adapted
356 from the Davis Rig (Davis, 1973; Smith, 2001). Each behavioral session consisted of 100 trials with 3 s
357 of free-access consumption separated by 5 – 10 s inter-trial intervals during which all spouts retracted
358 (**Figure 4B**). The 5 solutions were delivered in pseudorandom order such that each solution was
359 delivered 2 times every 10 trials. To control for modest spout effects (**Figure 4- figure supplement M-
360 O**), we conducted the experiment counterbalanced over 5 sessions such that each spout was paired with
361 each concentration (**Figure 4C, J, Q**). Using this approach, we measured within-session consumption of
362 gradations in concentration of an appetitive solution (sucrose) and two aversive solutions (quinine and
363 hypertonic sodium chloride (NaCl)).

364 Prior to behavioral training, mice were water-restricted to 80-90% baseline bodyweight (Guo et
365 al., 2014). However, during behavioral sessions, multiple mice were able to consume enough fluid to
366 maintain weight above 90% baseline body weight. Separate groups of mice were used for sucrose,
367 quinine, and sodium chloride solution sets to control for training history. All groups of mice were initially
368 conditioned on free-access licking in 1 - 2 sessions and then conditioned with the multi-spout procedure
369 for 3 - 7 sessions prior to 5 sessions of counterbalanced spout pairing (summarized in **Figure 4**). The
370 licks measured using this approach approximate consumption, as total number of licks during each
371 session is strongly correlated with weight in fluid consumed during the session (**Figure 4- figure
372 supplement 1A**). Using this approach, we successfully elicited a range of consumption responses for
373 each solution set.

374 Mice displayed gradations in licking for different concentrations of sucrose, quinine, and sodium
375 chloride (**Figure 4C-W**). For each solution set, licking bouts during the access period (representative
376 session depicted in **Figure 4D, K, R**, mean binned lick rate across all trials depicted in **Figure 4E, L, S**)
377 displayed inter-lick intervals similar to freely moving consumption (**Figure 4- figure supplement 1C**).
378 Mice licking for gradations of sucrose (**Figure 4C-I**) showed a modest range of licking behavior where
379 trials with higher concentrations of sucrose elicited a greater number of licks (One-Way RM ANOVA:
380 Concentration***; **Figure 4F, H**) and longer time spent licking during the trial (**Figure 4G**). Mice licking
381 for gradations of quinine (**Figure 4J-P, Figure 4-figure supplement 2**) showed a modest range of licking
382 behavior where trials with higher concentrations of quinine elicited a lower number of licks (Loney and
383 Meyer, 2018) (One-Way RM ANOVA: Concentration***; **Figure 4M, O**) and shorter time spent licking
384 during the trial (**Figure 4N**). Mice licking for gradations of NaCl (**Figure 4Q-W, Figure 4- Figure**

385 **Supplement 3**) showed a large range of licking behavior where trials with higher concentrations of NaCl
386 elicited a lower number of licks (One-Way RM ANOVA: Concentration***; **Figure 4T, V**) and shorter time
387 spent licking during the trial (**Figure 4U**). Each solution set produced unique time-courses of licking
388 behavior over the course of the session (**Figure 4H, Figure 4- figure supplement 1D**). Mice in the
389 sucrose set started with high licking rates and showed a gradual satiation that resulted in decreased
390 licking across all concentrations (**Figure 4I, Figure 4- figure supplement 1D**); mice in the quinine set
391 started with high licking rates but rapidly dropped by around trial 40 across all concentrations (**Figure 4P,**
392 **Figure 4- figure supplement 1D**); and mice in the NaCl set showed only a minor reduction in licking
393 across all concentrations throughout the session (**Figure 4W, Figure 4- figure supplement 1D**).
394 Comparing the total number of licks per session across the three sets of solutions revealed that mice
395 displayed the highest number of licks during the sucrose set, then NaCl, then quinine (**Figure 4- figure**
396 **supplement 1E**). Comparing task engagement using the proportion of trials with licking across the three
397 sets of solutions, mice in the quinine set showed substantially lower proportion of trials with licks
398 compared to mice in the sets for sucrose or NaCl (**Figure 4- figure supplement 1F**). Mice displayed little
399 to no relationship between the number of licks in the session and weight of the mouse or amount of fluid
400 consumed/provided on the previous session (**Figure 4- figure supplement 1G-J**). We also found that
401 older mice displayed higher lick rates for sucrose (**Figure 4- figure supplement 1K-L**). Finally, we found
402 no sex differences in task performance, except a lower proportion of trials with licking in female mice
403 (**Figure 4- figure supplement 4**). Altogether, these data indicate that OHRBETS successfully elicits a
404 range of consumption behavior for differential concentrations of appetitive and aversive solutions.

405 Given that mice showed a smaller range of licking for gradations in quinine compared to NaCl,
406 we further investigated licking behavior with additional sets of 1:4 serial dilutions of quinine with higher
407 concentrations (starting concentration: Low = 1 mM (**Figure 4 J-P**), Med = 5 mM, High = 10 mM) (**Figure**
408 **4- figure supplement 2**). Each quinine set produced a modest range of licking behavior with less licking
409 for higher concentrations of quinine (**Figure 4- figure supplement 2A-D**). Mice displayed a lower total
410 licking in the High set compared to the Med and Low sets (**Figure 4- figure supplement 2E**), and mice
411 in all sets showed similar task engagement as indicated by proportion of trials with licking (**Figure 4-**
412 **figure supplement 2F**). Mice in all sets abruptly stopped licking part-way through the session (**Figure**
413 **4- figure supplement 2C**). Overall, each quinine set was capable of producing a range of licking behavior
414 but failed to support licking throughout the entirety of the behavioral session.

415

416 **Homeostatic demand shifts within-session consumption of gradients of sucrose and NaCl**

417 To determine if OHRBETS multi-spout assay could detect shifts in consumption behavior
418 following behavioral challenges, we measured consumption of a gradient of sucrose concentrations
419 across homeostatic demand states. We trained mice in the multi-spout brief-access task for 5 sessions
420 under water-restriction, then 5 sessions under food-restriction, and ending with 5 sessions under no
421 restriction (*ad-libitum*) (**Figure 5A**). We observed strong effects of restriction state on consumption
422 behavior across sucrose concentrations (**Figure 5B-D, Figure 5-figure supplement 1A-E**). Most
423 notably, mice showed a substantially larger range of licking behavior under food-restriction compared to
424 water-restriction and *ad-libitum* (Two-Way RM ANOVA: Concentration x State***; **Figure 5B**). Mice
425 showed vastly different levels of total number of licks with the greatest number of licks for all
426 concentrations under water-restriction, then food-restriction, then *ad-libitum* (Two-Way RM ANOVA:
427 State***; **Figure 5B, C**). Mice also displayed differences in licking rate throughout the session (Two-Way
428 RM ANOVA: State***, Trial Bin x State***; **Figure 5C, D**). The minor scaling in licking across sucrose
429 concentrations under water-restriction compared to food-restriction could indicate that the water
430 component of the solutions is strongly appetitive under water-restriction. Using OHRBETS, we measured
431 changes in the relative consumption of concentrations of sucrose across homeostatic demand states that
432 closely parallels the effect of homeostatic demand on sucrose consumption described in freely moving
433 rodents (Glendinning et al., 2002; Smith et al., 1992; Spector et al., 1998).

434 To determine if our head-fixed multi-spout assay could detect shifts in consumption of NaCl, we
435 measured consumption of a gradient of NaCl concentrations across sodium demand states. We first
436 trained mice under water-restriction (**Figure 4**) before allowing mice to return to *ad-libitum* water. Next,
437 we manipulated sodium appetite using furosemide injections followed by access to sodium depleted chow
438 (sodium-deplete) or standard chow (sodium-replete) and then measured consumption of a gradient of
439 NaCl concentrations in our multi-spout assay over 2 sessions (counterbalanced order of sodium appetite
440 state) (**Figure 5E**). Mice displayed greater licking under the sodium-deplete state compared to the
441 sodium-replete state (Two-Way RM ANOVA: State***, Concentration x State**; **Figure 5F-H, Figure 5-**
442 **figure supplement 1F-J**). Specifically, mice when sodium-deplete showed higher levels of licking for
443 both water and 0.25M NaCl. Mice displayed more licking throughout the session when sodium-deplete,
444 indicating a heightened demand (Two-Way RM ANOVA: State*; **Figure 5G-H**). The increased licking for
445 water when sodium-deplete can potentially be attributed to higher levels of thirst, as previously described
446 (Jalowiec, 1974). Together, these results indicate that mice show a range of consummatory behaviors
447 that are sensitive to homeostatic demand and that OHRBETS offers a platform for assessing shifts in
448 consummatory drive in a reliable fashion in head-fixed mice.

449

450 **Light/dark cycle shifts within session consumption of gradients of sucrose**

451 To characterize behavior across the circadian light/dark cycle, we measured consumption of a
452 gradient of sucrose concentrations under food-restriction during the dark cycle or light cycle in separate
453 groups of mice (**Figure 6A**). During 2 sessions of free-access consumption, mice tested in the dark cycle
454 consumed significantly more 10% sucrose compared to mice tested in the light cycle (Two-Way RM
455 ANOVA: Cycle^{***}; **Figure 6B**) (Bainier et al., 2017; Smith, 2000; Tönissaar et al., 2006). Across 8
456 sessions of the multi-spout assay, mice tested in the dark cycle licked more compared to mice tested in
457 the light cycle (Cycle^{**}; **Figure 6C left**); however, over sessions 4 - 8 there was no effect of light cycle
458 on licking (*t*-test *P*=0.099; **Figure 6C right**). Despite similar overall licking in the multi-spout assay, we
459 found that experiments conducted during the light and dark cycle resulted in distinct licking across
460 sucrose concentrations (Concentration x Cycle^{***}, **Figure 6E**) (Bainier et al., 2017; Tönissaar et al.,
461 2006). Furthermore, compared to mice tested in the light cycle, mice tested during the dark cycle showed
462 higher levels of consumption early in the session (Time x Cycle^{***}; **Figure 6F, G**). Together, these results
463 indicate that the light/dark cycle affects sucrose consumption and testing mice in the light cycle leads to
464 pronounced reductions in consumption in early training sessions.

465

466 **Comparing the reproducibility of the multi-spout brief-access task across independent** 467 **laboratories**

468 To determine if our system produces quantitatively similar consumption across labs, we compared
469 behavior of food-restricted mice tested in the dark cycle trained on the multi-spout brief-access to a
470 gradient of sucrose concentrations obtained with our head-fixed system across independent labs and
471 geographic locations (**Figure 6- figure supplement 1**; data collected in the Stuber lab is shown in **Figure**
472 **5**, and data collected in the Roitman lab is shown in **Figure 6**). We observed qualitative differences in
473 the binned licking rate over the 3 s access period (**Figure 6- figure supplement 1B**), with higher licking
474 rate in mice tested in the Roitman lab near the onset of the access-period. We also found that mice tested
475 in the Roitman lab exhibited a small, but significant, reduction in inter-lick intervals compared to the Stuber
476 lab (**Figure 6- figure supplement 1**). However, despite these nominal differences, there were no
477 statistical differences in the mean licking for each concentration of sucrose across labs (**Figure 6- figure**
478 **supplement 1D**). These data indicate that our system produces similar consumption behavior when run
479 in different labs, geographic locations, and experimenters.

480

481 **OHRBETS combined with fiber photometry to assess ventral striatal dopamine dynamics to** 482 **multiple concentrations of rewarding and aversive solutions.**

483 To demonstrate the utility of the multi-spout assay run on OHRBETS, we performed simultaneous
484 dual fiber-photometry in the mesolimbic dopamine system during the multi-spout assay. The activity of
485 ventral tegmental area dopamine neurons and the release of dopamine in the nucleus accumbens are
486 well known to scale with relative reward value such that the most rewarding stimuli produces increases
487 in dopamine release and the least rewarding stimuli produces modest decreases in dopamine release
488 (Eshel et al., 2015; Hajnal et al., 2004; Tobler et al., 2005). We used multi-spout brief-access to a gradient
489 of an appetitive solution (sucrose) and an aversive solution (NaCl) to elicit a range of consummatory
490 responses (**Figure 4, 5**) while simultaneously recording dopamine dynamics in the medial nucleus
491 accumbens shell (NAcShM) and lateral nucleus accumbens shell (NAcShL) (**Figure 7A**, placements
492 shown in **Figure 7- Figure Supplement 6**). To record dopamine dynamics in the NAc, we expressed the
493 dopamine sensor GRAB-DA (GRAB-DA1h (Sun et al., 2018) or GRAB-DA2m (Sun et al., 2020)) in the
494 NAcShM and NAcShL (counterbalanced hemispheres across mice) of wild-type mice and implanted
495 bilateral optic fibers with a head-ring to facilitate head-fixation (**Figure 7A, Methods**). Mice were tested
496 with multi-spout access to a gradient of sucrose concentrations under water-restriction and food-
497 restriction, in counterbalanced order, and then a gradient of NaCl concentrations under water-restriction
498 (**Figure 7B**). Across each stage of the task, mice exhibited scaling in licking behavior that replicated data
499 shown in **Figure 4** and **Figure 5** (**Figure 7-figure supplement 1**). During the multi-spout assay, we
500 observed dynamics in dopamine signals in both the NAcShM and NAcShL during the consumption
501 access period (**Figure 7C-O**). During consumption of sucrose under food-restriction, where we observe
502 a large range in licking across concentrations of sucrose (**Figure 7-figure supplement 1, left**), we
503 measured strong scaling of GRAB-DA fluorescence in the NAcShL and moderate scaling in the NAcShM
504 (representative mouse **Figure 7D**; mean fluorescence **Figure 7E**; mean fluorescence during access
505 **Figure 7F**, Two-Way RM ANOVA: Solution x Region***; CDF shown in **Figure 7-figure supplement**
506 **2A**). Specifically, we observed significantly higher responses in the NAcShL compared to the NAcShM
507 at higher concentrations of sucrose (10, 20, and 30% HSD*). On a trial-by-trial basis, we observed a
508 correlation between the amount of licking on a trial and GRAB-DA fluorescence (**Figure 7G**; CDF shown
509 in **Figure 7-figure supplement 2B**). During consumption of sucrose under water-restriction, where we
510 observe high levels of licking but minimal range across concentrations of sucrose (**Figure 7-figure**
511 **supplement 1, mid**), we measured moderate scaling of GRAB-DA fluorescence in the NAcShL and little
512 scaling in the NAcShM (representative mouse **Figure 7H**; mean fluorescence **Figure 7I**; mean
513 fluorescence during access **Figure 7J**, Two-Way RM ANOVA: Solution x Region***; CDF shown in
514 **Figure 7-figure supplement 2C**). Specifically, mice displayed significantly higher GRAB-DA responses
515 in the NAcShL compared to the NAcShM at higher concentrations of sucrose (**Figure 7F**). Like dynamics
516 during food-restriction, GRAB-DA fluorescence was positively correlated with licking within the trial
517 (**Figure 7K**, CDF shown in **Figure 7-figure supplement 2D**). During consumption of the aversive tastant
518 (NaCl) under water-restriction, where we observed a large range of licking across concentrations of NaCl

519 **(Figure 7-figure supplement 1, right)**, we measured strong scaling of GRAB-DA fluorescence in both
520 the NAcShL and NAcShM (representative mouse **Figure 7L**, mean fluorescence **Figure 7M**; mean
521 fluorescence during access **Figure 7N**, Two-Way RM ANOVA: Solution x Region***, CDF shown in
522 **Figure 7-figure supplement 2E**). Despite the interaction between solution and region of the NAc, there
523 was a significantly higher GRAB-DA fluorescence in the NAcShL only during 0.25M NaCl. Like other
524 stages of the task, we observed a clear correlation between GRAB-DA fluorescence and licking during
525 the trial (**Figure 7O**, CDF shown in **Figure 7-figure supplement 2F**). Taking advantage of the head-
526 fixed preparation, we were able to record the activity of the NAcShL and NAcShM simultaneously and
527 found a strong correlation in GRAB-DA fluorescence in the two regions across each stage of the task
528 **(Figure 7-figure supplement 3)**.

529 Interestingly, the NAcShM and NAcShL show a differential range of GRAB-DA fluorescence
530 across each stage of the task. The NAcShM shows a disproportionately higher range of GRAB-DA
531 fluorescence during multi-spout consumption of NaCl compared to the other stages of the task (**Figure**
532 **7-figure supplement 4B**, Two-Way RM ANOVA: Stage x Brain Region***). The higher range of
533 dopamine release in the NAcShM during consumption of a range of aversive solutions compared to
534 appetitive solutions could indicate a specialized role for the NAcShM in mediating behavioral responses
535 to aversive stimuli as previously described for footshock conditioning (Jong et al., 2018). OHRBETS
536 allowed us to isolate consumption behavior in response to a range of rewarding and aversive solutions
537 while performing dual site fiber photometry and revealed robust dopamine responses that scales with
538 solution value and consumption. Furthermore, these data indicate that OHRBETS is highly compatible
539 with neural recording and manipulation techniques that would be challenging with freely moving
540 behavioral designs.

541 **Discussion:**

542 OHRBETS is a customizable, inexpensive system for head-fixed behavior in mice that enables a
543 variety of behavioral experiments, including operant conditioning, real-time place testing, and multi-
544 solution brief-access consumption, accurately replicating behaviors in freely moving. These data
545 demonstrate that a diverse set of operant and consummatory behaviors are compatible with head-fixed
546 procedures run with a single hardware setup and will serve as a resource for future investigations into
547 these behaviors using neuroscience approaches that rely on head-fixation.

548 Behavior measures within our head-fixed adaptations of freely moving operant assays reproduce
549 many important phenotypes originally characterized in freely moving behavior. Mice rapidly learn operant
550 responding for sucrose and then flexibly express responding as a function of reward-cost and reward-
551 size (Kliner et al., 1988; Reilly, 1999; Sclafani and Ackroff, 2003; Winger and Woods, 1985). Using

552 optogenetic stimulation, which offers tighter control over the precise magnitude and timing of appetitive
553 and aversive states, mice exhibited quantitatively similar positive ICSS, RTPP, and RTPA behavior with
554 our head-fixed and freely moving approaches. The ability to conduct ICSS and RTPT with a single setup
555 is particularly useful in measures of valence-related neural circuits, but these results also imply that the
556 head-fixed RTPT procedure could be used to test the appetitive and aversive quality of other stimuli that
557 are challenging to test in freely moving conditions including discrete somatosensory stimuli. Taken
558 together, our results establish that our behavioral system produces robust, reproducible operant behavior
559 consistent with the commonly employed freely moving counterparts.

560 In addition to the operant conditioning experiments, our system can facilitate multi-solution brief-
561 access experiments for studying consummatory behavior. In our task, mice show consumption of a
562 gradient of sucrose, quinine, and NaCl concentrations that closely matches behavior with the freely
563 moving version of the task (Corbit and Luschei, 1969; Coss et al., 2022; Garcia et al., 2021; Glendinning
564 et al., 2002; John et al., 1994; Loney and Meyer, 2018; Smith et al., 1992; Villavicencio et al., 2018).
565 Licking increased monotonically with increased concentrations of sucrose across all homeostatic states
566 (Garcia et al., 2021; Glendinning et al., 2002; Smith et al., 1992; Spector et al., 1998). However,
567 homeostatic demand states produced pronounced differences in the range of consumption behavior
568 across sucrose concentration, as food-restriction produced a substantially larger range of licking behavior
569 compared to water-restriction. One unexpected finding was that mice showed vastly different behavior
570 when licking for the aversive tastants quinine and hypertonic NaCl. When licking for quinine, mice abruptly
571 ceased consumption for all concentrations mid-way through the session. On the other hand, when licking
572 for NaCl, mice continue to consume large amounts of low concentrations of NaCl throughout the entire
573 session. These results may be explained by an additive effect of quinine that builds in aversion over trials
574 and results in a lingering bitter taste (Leach and Noble, 1986) that attenuates motivation to initiate
575 consumption. During the NaCl sessions, NaCl may stimulate thirst (Kraly et al., 1995; O'Kelly, 1954;
576 Stricker et al., 2002) resulting in enhanced motivation to consume water. Thus, the multi-spout brief
577 access task with gradients of NaCl can be a uniquely advantageous approach for eliciting a high number
578 of strongly aversive events while continuing to engage behavior. Changes in task design could improve
579 performance during the quinine task, such as including water rinse trials between each quinine trial
580 (Loney and Meyer, 2018). In addition to using a gradient of solution concentrations, any number of
581 combinations of tastants could be used to study a whole host of behavioral phenomena including innate
582 and conditioned consumption behaviors.

583 Using our multi-spout brief-access task in conjunction with GRAB-DA fiber-photometry, we
584 observed dopamine dynamics that positively correlated with relative solution value and consumption.
585 Previous studies have revealed that dopamine release in the ventral striatum (Hajnal et al., 2004) and
586 dopamine neuron activity scales with reward magnitude (Eshel et al., 2015; Tobler et al., 2005). We found

587 that dopamine release in these subregions' scales with the relative value of the solution being consumed
588 and the amount of concurrent consumption and is strongly influenced by solutions present in a session
589 and the mouse's homeostatic demand state. Most interestingly, we found that the dopamine release in
590 the NAcShM has a much larger amplitude during multi-spout consumption of a gradient of NaCl
591 concentrations than during consumption of a gradient of sucrose concentrations. This result implies that
592 dopamine release in the NAcShM tracks value, and the range of values during the multi-spout
593 consumption of gradients of NaCl is greater than the range of values during multi-spout consumption of
594 gradients of sucrose. Alternatively, this result could indicate a specific role of dopamine release in the
595 NAcShM that corresponds to shaping behavior or learning in the face of aversive events (Jong et al.,
596 2018). By conducting these experiments using OHRBETS, we removed approach behaviors that occur
597 prior to consumption and isolated neuronal responses specifically during consumption (Chen et al., 2022)
598 without interference of activity ramps observed in freely moving behavioral designs (Howe et al., 2013).
599 Future experiments are necessary to reveal the specific contribution of licking, taste, and value to wide-
600 spread dopaminergic signals and how these signals causally influence ongoing consumption or learning.

601 Eliminating locomotion improves compatibility with many standard neuroscience approaches
602 including optogenetics, fiber-photometry, electrophysiology, and calcium imaging. To prevent twisting of
603 tethers, each of these approaches require a commutator in freely moving conditions, but with head-
604 fixation the need for a commutator is eliminated. This facilitates multiplexed experiments with
605 simultaneous use of multiple approaches that each rely on independent tethers without the risk of
606 weighing down the animal, tangling, or twisting to the point of affecting task performance. For fiber-
607 photometry, fixing the animal dramatically reduces motion artifacts, thereby reducing the need for an
608 isosbestic to correct for motion (**Figure 7- Figure Supplement 5**). This opens the ability to conduct
609 experiments with fluorescence biosensors without known isosbestic points or without true isosbestic
610 points. Recent advances in optical imaging have opened up new approaches in freely moving animals,
611 but the cutting edge of optical technologies will typically start with tabletop microscopes. Using head-
612 fixed models permits users to embrace cutting edge imaging technologies without waiting for further
613 advances to bring the technology into freely moving animals. The use of OHRBETS allows for enhanced
614 compatibility with a variety of neuroscience technologies and will enable novel, multiplexed experiments
615 that would be difficult or impossible to conduct in freely moving animals.

616 While head-fixed experiments offer many advantages, they come with important caveats,
617 limitations, and experimental design considerations. Head-fixation can be acutely stressful to mice and
618 causes increased levels of circulating stress markers (Juczewski et al., 2020), which could impair learning
619 and interact with other manipulations. The advantage of limiting the range of behaviors a subject can
620 display comes at the cost of reduced naturalistic character, which can impair behavior and related
621 neuronal activity (Aghajani et al., 2015; Aronov and Tank, 2014). Furthermore, isolation of components

622 of behavior provides powerful insight into the neuronal mechanisms that underlie the particular
623 component of behavior but may impair insight into how the related neuronal circuits function during more
624 complex behaviors and contexts. Even in the presence of these caveats, extensive research conducted
625 in head-fixed non-human primates has made vast progress in a multitude of areas of neuroscience
626 (Mirenowicz & Schultz, 1996; Parker & Newsome, 1998; Schultz et al., 1997) including appetitive and
627 consummatory behaviors (Bromberg-Martin et al., 2010; Haber & Knutson, 2010). The greatest insights
628 into the neuronal mechanisms of behavior will come from a mixture of both naturalistic behaviors and
629 highly controlled behaviors facilitated by head-fixed behaviors made possible with OHRBETS.

630 The OHRBETS ecosystem presented here was designed to be scalable, flexible, and compatible
631 with external hardware. By using low-cost, open-source, and 3D printed components and publishing
632 extensive instructions for assembly, our system is affordable and scalable across labs of all sizes and
633 budgets. Despite the use of low-cost and 3D printed components, our system is remarkably consistent
634 and reliable across hundreds of behavioral sessions. Our hardware and software are modular, as all
635 hardware components can be easily swapped, and all behavioral programs are written to produce data
636 with a uniform format. Using different combinations of components will facilitate conducting a wide variety
637 of behavioral experiments including all the experiments presented in this manuscript and many more. By
638 using an Arduino Mega case as a microprocessor mounted within a 3D printed enclosure, one can
639 integrate many different forms of connectivity to interface with external hardware. In the online models,
640 we have options for communication via BNC, Cat6, and DB25 that can be easily combined to suit the
641 user's needs. Altogether, OHRBETS is a complete platform for diverse behavioral experiments in head-
642 fixed animals that can be easily adapted by the broader scientific community to conduct an even wider
643 range of procedures that are compatible with monitoring and manipulating neural dynamics *in vivo*.

644

645 **Materials and Methods:**

646 **Instructions for Assembling the OHRBETS**

647 Detailed part list, 3D models, electronic wiring diagrams, behavioral programs, and instructions
648 for assembling are available publicly on our GitHub repository
649 (<https://github.com/agordonfennell/OHRBETS>).

650 **Hardware**

651 3D printed components designed and available via the web-based cad software TinkerCAD
652 (Autodesk) and printed using a filament printer (Ultimaker S3) using PLA or resin printer (Form3) using
653 Clear Resin. We also tested components built by the online printing service CraftCloud and found that
654 they work similarly to ones printed in the lab. The micropositioner design was based on one created by
655 Backyard Brains (Backyard Brains, 2013) and the retractable spout design was based on one created by
656 an independent designer (Buehler, 2016b).

657 All behavioral hardware was controlled using an Arduino Mega 2560 REV3 (Arduino). The timing
658 of events was recorded via serial communication from the Arduino to the computer (PC, running
659 Windows10) by USB. Lick spouts were made by smoothing 23 gauge blunt fill needles using a Dremel
660 with a sanding disk. Liquid delivery was controlled by solenoids (Parker) gated by the Arduino, using a
661 24V transistor. The retractable spout, radial spout, and wheel brake utilized micro servos (Tower Pro
662 SG92R). Licks on each spout were detected individually using a capacitive touch sensor (Adafruit
663 MPR121) attached to each metal spout. Importantly, the baseline capacitance of each sensor was kept
664 to a minimum and touch thresholds were reduced from standard values (see GitHub for detailed
665 instructions). Micropositioners were assembled from 3D printed components, Super Glue (Loctite Super
666 Glue ULTRA Liquid Control), screws, and nuts.

667 **Hardware Validation**

668 We measured the consistency of the retractable spout extension latency and terminal positions
669 using video recording. We recorded 1,000 extension/retractions in 5 separate retractable spouts using a
670 high-speed video camera (Basler, acA800-510um, 200 fps). We then estimated the position of the spout
671 using DeepLabCut (Mathis et al., 2018) and analyzed the position of the spout relative to the mean
672 terminal position of the spout over time. We measured the consistency of the wheel brake latency using
673 experimenter and mouse rotation. We recorded 1,770 wheel rotations produced by an experimenter and
674 measured the effect of braking using 4 separate head-fixed systems. We computed the binned rotational
675 velocity by taking the mean instantaneous velocity within 25ms time bins (**Figure 1E, Figure 1-Figure**

676 **Supplement 1 H**). We also assessed the rotation following brake engagement with all brake events
677 during all operant data included in **Figure 1**.

678 **Software**

679 All behavioral programs were written in the Arduino language and executed on the Arduino Mega
680 during the behavioral session. The timing of hardware and behavioral events were sent from the Arduino
681 and recorded on a PC computer (Windows 10) via serial communication or through a fiber-photometry
682 console via TTL communication. Fiber-photometry data was collected using Synapse (Tucker Davis
683 Technologies). Data processing, statistical analysis, and data visualization was performed using custom
684 scripts in Python (version 3.7) and R (version 4.0.4). All behavioral programs and pre-processing scripts
685 used to produce the data in this manuscript are freely available through our GitHub
686 (https://github.com/agordonfennell/open_stage).

687 **Animals**

688 All behavioral procedures were pre-approved by University of Washington or University of Illinois
689 at Chicago Animal Care and Use Committees. A mixture of wild-type and transgenic mice on a C57BL/6J
690 background were used for experiments throughout the paper. All mice were bred in the lab from mouse
691 lines obtained from Jackson Laboratory aside from 16 wild-type mice obtained directly from Jackson
692 Laboratory. No differences were observed across transgenic lines so all data was pooled. Mice used in
693 fiber-photometry and optogenetic experiments were singly housed to prevent damage to the optical fibers
694 while all other mice were group housed. Mice were kept on a reverse 12h light/dark cycle and behavioral
695 experiments were conducted within the dark-cycle unless otherwise noted.

696 **Surgeries**

697 Mice (>P55) were anesthetized using isoflurane (5% induction, 1.5-2% maintenance), shaved
698 using electric clippers, injected with analgesic (carprofen, 10 mg/kg, s.c.), and then mounted in a
699 stereotaxic frame (Kopf) with heat support. Skin overlying the skull was injected with a local anesthetic
700 (lidocaine, 2%, s.c.) and then sterilized using ethanol and betadine. Next, an incision was made using a
701 scalpel, and the skull was cleared of tissue and scored using the sharp point of a scalpel. The skull was
702 leveled, 2 burr holes were drilled in the lateral portion of the occipital bone, and 2 micro screws were
703 turned into the bone. We then coated the bottom of a stainless-steel head-ring (custom machined, see
704 GitHub for design) with Super Glue, placed it onto the skull of the mouse, and then encased the head-
705 ring and skull screws with dental cement making sure the underside of the ring remained intact. After the
706 dental cement had time to fully dry, the mouse was removed from the stereotaxic frame and allowed to

707 recover with heat support before being returned to their home cage. Mice were allowed to recover for at
708 least 1 week prior to dietary restriction.

709 Mice used for optogenetic or fiber-photometry experiments underwent the same procedure as
710 above with the addition of a viral injection and fiber implantation. Following implantation of skull screws,
711 we drilled a burr hole overlaying the brain region target. We then lowered a glass injection pipette into
712 the target brain region and injected the virus at a rate of 1nL/s using a Nanoject III (Drummond), waited
713 5 min for diffusion, and then slowly retracted the pipette. For optogenetic experiments, we injected 300nL
714 of AAV5-EF1a-DIO-hChR2(H134R)-eYFP (titer: 3.2e12) or AAV5-Ef1a-DIO-mCherry (titer: 3.3e12), and
715 for fiber-photometry experiments, we injected 400nL of AAV9-hSyn-GRAB-DA1h (titer: 2.7e13) or AAV9-
716 hSyn-GRAB-DA2m (titer: 2.4e13). The following stereotaxic coordinates (relative to bregma) were used
717 for injection targets: LHA (0° angle; AP: -1.3 mm; ML: +/- 1.1 mm; DV: -5.2 mm), NAc medial shell (10°
718 angle; AP: 1.7 mm; ML: +/- 1.5 mm; DV: -4.8 mm), and NAc lateral shell (10° angle; AP: 1.7 mm; ML: +/-
719 2.5 mm; DV: -4.6 mm). Next, we lowered a 200 µm optic fiber for optogenetic experiments (1.25mm
720 ferrule, 6mm fiber length, RWD) or a 400 µm optic fiber for fiber-photometry experiments (2.5 mm ferrule,
721 6 mm fiber length, MFC_400/470-0.37_6mm_MF2.5_FLT, Doric) 0.2 mm dorsal to the injection site and
722 then encased the fiber extending from the brain, metal ferrule, and head-ring with Super Glue and dental
723 cement. Mice were allowed to recover for at least 2 weeks prior to behavior or dietary restriction.

724 **Behavior**

725 **Habituation to Head-Fixation and Free-Access Lick Training**

726 Prior to head-fixed behavior, mice were habituated to the experimenter and head-fixation stage
727 over 4 sessions. In the first session, mice were brought into the behavioral room and allowed to explore
728 the head-fixed apparatus to become acquainted with the sights, smells, and sounds of the behavioral
729 box. On the second session, mice were brought into the behavior room and scruffed twice. In the third
730 session, mice were brought into the behavior room, scruffed twice, and then gently had their rear end
731 and hind paws placed in a 50 mL conical twice. After each habituation session, the mouse was
732 immediately provided food or water depending on their deprivation status. Mice undergoing head-fixed
733 operant conditioning for sucrose or head-fixed multi-spout consumption were habituated to head fixation
734 and trained to lick for sucrose in a single 10 min session. During this session, mice were given free-
735 access to water (mice used for quinine and NaCl multi-spout experiments Figure 4,5) or 10% sucrose
736 (mice used for all other experiments). Free-access was approximated using closed-loop delivery of a
737 pulse of fluid (~1.5 µL) each time the mouse licked the spout. During the training session, mice were
738 head-fixed, and the spout was brought forward to gently touch the mouse's mouth to encourage licking

739 before being moved to be positioned ~2-3 mm in front of the mouse's mouth where it remained throughout
740 the session.

741 **Head-fixed Operant Conditioning for Sucrose**

742 Retractable spout training consisted of 3 daily sessions of 60 trials with 5 s access periods
743 separated by 20-40 s inter-trial intervals. During each access period, an auditory tone (5 kHz) was played
744 and 5 pulses of ~1.5 μ L sucrose were delivered with a 200ms inter pulse interval. We delivered pulses
745 of sucrose to encourage licking in bouts and to minimize the chance that a large droplet of sucrose would
746 fall.

747 Operant conditioning training consisted of 6 30 min sessions of initial training, 4 sessions of
748 increased fixed-ratio, and then 5-6 sessions of progressive-ratio. Throughout operant conditioning, one
749 direction of rotation was assigned as the active direction and the opposite direction was assigned as
750 inactive (counterbalanced across mice). Rotation in the active direction earned sucrose delivery. Each
751 sucrose delivery consisted of wheel brake engagement, followed by spout extension and 5 pulses of ~1.5
752 μ L of 10% sucrose with an inter pulse interval of 200ms. During the 3 s access period, the spout remained
753 extended, a 5 kHz auditory tone was presented, and the brake was left engaged. Rotation in the inactive
754 direction led to wheel brake engagement for the same length of time as the total brake time with active
755 rotation, but the spout did not extend, and sucrose was not delivered. During initial training, the fixed-
756 ratio of reward was 1/4 turn in the first session and 1/2 turn during the next 5 sessions. During increased
757 cost sessions, the fixed-ratio was increased to 1 turn. A total of 3 mice that underwent initial training were
758 removed from progressive ratio training, 2 for not learning the task and 1 that lost their headcap during
759 behavior. During progressive ratio, the wheel turn cost of reward was increased either semilogarithmic
760 (0.25, 0.5, 0.81, 1.21, 1.71, 2.3, 3.1, 4.1, etc., approximating (Richardson and Roberts, 1996) or linearly
761 by 0.5 rotation (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, etc.) each time a reward was earned. The session duration
762 was 1h, or 15 min without earning a reinforcer, whichever comes first.

763 Reversal training was performed in a naive cohort of mice using an identical procedure to initial
764 operant conditioning training, except in session 6 the direction of the wheel rotation that was reinforced
765 was inverted (right turn reinforced \rightarrow left turn reinforced). Following reversal, the mice were trained on the
766 task for an additional 7 sessions of operant conditioning.

767 **Optogenetic Experiments**

768 *Vgat-cre* and *Vglut2-cre* mice underwent surgery for experiments with optogenetics outlined
769 above. After at least 4 weeks of recovery, mice were trained on head-fixed and freely moving versions of
770 RTPT and ICSS experiments in series. All mice were trained on RTPT prior to ICSS, but the order of
771 head-fixed and freely moving versions were counterbalanced across mice.

772 Freely moving RTPT consisted of 1 session of habituation and 6 sessions of RTPT with different
773 stimulation frequencies. During habituation, mice were scruffed, attached to an optic fiber, and allowed
774 to explore the RTPT chamber for 10 min. The RTPT chamber was a two-chamber apparatus (50 × 50 ×
775 25 cm black plexiglass) with two identical compartments. Over the next 6 sessions, mice underwent daily
776 20 min RTPT sessions with stimulation paired with one of the two compartments. The position of each
777 mouse was tracked in real time using Ethovision (Noldus) and when the mouse's center point was
778 detected in one of the two compartments it triggered continuous laser stimulation (5ms pulses, ~10mW
779 power, frequencies: 0, 1, 5, 10, 20, 40 Hz). To prevent associations between stimulation and chambers
780 in the RTPT chamber, the stimulation frequency and compartment paired with laser stimulation were
781 counterbalanced across sessions.

782 Head-fixed RTPT consisted of 3 sessions of habituation and 12 sessions of RTPT with different
783 stimulation frequencies (6 sessions without and 6 sessions with a tone indicating the mouse's position).
784 During habituation, mice were habituated to head-fixation as outlined above but without sucrose provided.
785 Over the next 12 sessions, mice underwent daily 20 min RTPT sessions with stimulation paired to one
786 half of the wheel. Throughout RTPT, mice were head-fixed, an optic fiber was connected and covered
787 using blackout tape, and the start of the session was indicated when the wheel brake was disengaged.
788 At the start of the session, the starting wheel position was set in the unpaired zone adjacent to the paired
789 zone. The wheel rotation was tracked by recording the rotation of the wheel relative to the starting position
790 (64 positions/1 rotation) and when the mouse's position was detected in one of the two zones it triggered
791 continuous laser stimulation (5ms pulses, ~10mW power, frequencies: 0, 1, 5, 10, 20, 40 Hz). During
792 sessions 1-6 of RTPT, there were no extraneous cues indicating which zone the mouse was located in.
793 During sessions 7-12 of RTPT, there were tone cues (5 and 10 kHz) that indicated if the mouse was in
794 the paired or unpaired zones of the wheel. To prevent learned associations between stimulation and
795 zones over multiple sessions, we counterbalanced the following factors across sessions: the stimulation
796 frequency, side of the wheel paired with laser stimulation, and the tone paired with laser stimulation.

797 Freely moving operant conditioning for optogenetic stimulation with positive reinforcement
798 consisted of 1 session of habituation, 4 sessions of ICSS training, and 6 sessions of ICSS with different
799 stimulation frequencies. During habituation, mice were scruffed, attached to an optic fiber, and allowed
800 to explore the ICSS chamber (MED Associates) for 20 min. The ICSS chamber contained 2 nose pokes
801 with a light cue located inside and a light cue located above each nose poke, as well as an auditory tone

802 generator. Time stamps of hardware and behavioral events were recorded using MED associates. During
803 daily 20 min ICSS sessions, 1 nose poke into the active nose poke triggered 1 s of laser stimulation and
804 concurrent illumination of the active nose poke light cues and 5 kHz auditory tone. Nose pokes during
805 the 1 s stimulation period were recorded but did not result in an additional stimulation. Nose pokes in the
806 inactive hole were recorded but had no programmed consequence. To train mice to respond for laser
807 stimulation, mice were run through 5 sessions of ICSS training with 20 Hz stimulation. To measure the
808 operant response rates across stimulation frequencies, mice were run through an additional 6 sessions
809 of ICSS with different stimulation frequencies (5ms pulses, ~10mW power, frequencies: 0, 1, 5, 10, 20,
810 40 Hz).

811 Head-fixed operant conditioning for optogenetic stimulation with positive reinforcement consisted
812 of 5 sessions of ICSS training and 6 sessions of ICSS with different stimulation frequencies. During daily
813 20 min ICSS sessions, wheel rotation in the active direction triggered 1 s of laser stimulation and
814 concurrent 5 kHz auditory tone. The wheel brake was disengaged throughout the behavioral session.
815 Rotation during the 1 s stimulation period was recorded but did not count towards additional stimulation.
816 Rotation in the inactive direction was recorded but had no programmed consequence. Mice were trained
817 over 5 sessions of ICSS for 20 Hz stimulation with a fixed-ratio of 1/4 turn on session 1 and fixed-ratio of
818 1/2 turn on sessions 2-5. To measure the operant response rates across stimulation frequencies, mice
819 were tested over an additional 6 sessions of ICSS with different stimulation frequencies (5ms pulses,
820 ~10mW power, frequencies: 0, 1, 5, 10, 20, 40 Hz) and a fixed-ratio of 1/2 turn.

821 Freely moving operant conditioning for optogenetic stimulation with negative reinforcement
822 consisted of 1 session of habituation and 3 sessions of ICSS training. The habituation and behavioral
823 hardware were identical to the freely moving operant conditioning for optogenetic stimulation with positive
824 reinforcement described above. During daily 20 min ICSS sessions, continuous stimulation was turned
825 on at the start of the session and 1 nose poke into the active nose poke paused laser stimulation for 3 s
826 and triggered concurrent illumination of the active nose poke light cues and 5 kHz auditory tone. Nose
827 pokes during the 3 s pause period were recorded but did not result in an additional pause. Nose pokes
828 in the inactive hole were recorded but had no programmed consequence.

829 Head-fixed operant conditioning for optogenetic stimulation with negative reinforcement consisted
830 of 11 sessions of ICSS training. During daily 20 min ICSS sessions, laser stimulation was turned on at
831 the start of the session and wheel rotation in the active direction paused laser stimulation for 3 s and
832 triggered a concurrent 5 kHz auditory tone. The wheel brake was disengaged throughout the behavioral
833 session. Rotation during the 3 s pause period was recorded but did not count towards additional pause.
834 Rotation in the inactive direction was recorded but had no programmed consequence. Mice were run

835 through 5 sessions of ICSS training for 5 Hz stimulation and then 6 sessions for 10 Hz stimulation with a
836 fixed-ratio of 1/4 turn in session 1 and fixed-ratio of 1/2 in sessions 2-11.

837 **Head-fixed Multi-Spout Consumption**

838 Mice were habituated to head-fixation as outlined above, and then ran through 1-3 sessions of
839 spout training (see *Habituation to Head-Fixation*). The multi-spout assay consisted of daily sessions with
840 100 trials of 3 s access to 1 of 5 different solutions (pseudorandom order with 2 presentations of each
841 solution per every 10 trials), each session with an inter-trial interval of 5-10 s sampled from a uniform
842 distribution. Licks were detected using a capacitive touch sensor and triggered solution delivery via
843 solenoid opening. The duration of opening for each solenoid was calibrated before each experiment to
844 deliver approximately 1.5 μ L per solenoid opening by weighing the weight of water produced with 100
845 solenoid openings. To control for a spout effect, the pairing of solutions to spouts was counterbalanced
846 such that each spout was paired with each solution over every 5 sessions. Mice were trained for a
847 minimum of 3 sessions prior to the 5 consecutive sessions that are averaged together and used for
848 analysis. Mice in experiments with quinine or NaCl were initially trained in the multi-spout assay with
849 water on all 5 spouts for 3 sessions prior to introducing quinine or NaCl solutions. Mice in quinine
850 experiment were tested with the low quinine dilution set (1 mM, 1:4 serial dilution) for 8 sessions, high
851 (10 mM, 1:4 serial dilution) for 3 sessions, and med (5 mM, 1:4 serial dilution) for 3 sessions in series.

852 **Homeostatic Demand Multi-Spout Experiments**

853 For experiments with alterations in the homeostatic demand for sucrose solution, mice were
854 trained on the multi-spout assay under 3 homeostatic states in series (water-restricted, food-restricted,
855 then *ad-libitum*). First, mice under water-restriction were trained in the multi-spout assay for different
856 concentrations of sucrose over 8 sessions. Mice were then removed from water-restriction and
857 maintained on food-restriction for 1 week prior to being run through the multi-spout assay for 5 sessions.
858 Finally, mice were removed from all restrictions and maintained with *ad-libitum* access to food and water
859 for 3 sessions prior to being run through the multi-spout assay for 8 sessions. The final 5 sessions from
860 each homeostatic demand state were used for analysis. Mice that went through the fiber-photometry
861 recording experiment were run through the same procedure except the order of water-restriction and
862 food-restriction was counterbalanced across mice, and they were run for 3 sessions of free-access spout
863 training.

864 For experiments with alterations in the homeostatic demand for sodium chloride, mice were
865 trained under water-restriction and were then run under two homeostatic states in counterbalanced order
866 (sodium-deplete, sodium-replete). First, mice under water-restriction were trained in the multi-spout
867 assay with different concentrations of sodium chloride over 10 sessions. Mice were removed from water-

868 restriction and given *ad-libitum* access to water for 48h prior to manipulations of sodium demand. To
869 generate sodium demand, we used 2 injections of diuretic furosemide (50mg/kg) over 2 days (Jarvie and
870 Palmiter, 2017). Mice were weighed, injected with furosemide, and then placed into a clean cage with
871 bedding for 2 hours before being weighed again to confirm diuretic effect (~5% weight loss). Mice were
872 then returned to a clean home cage with *ad-libitum* access water and sodium free chow (Envigo,
873 TD.90228) (sodium-deplete) or a novel sodium-balanced chow (Envigo, TD.90229) (sodium-replete).
874 Mice underwent the same procedure a second time 24h later and then were tested for behavior after an
875 additional 24h. Mice were tested in the multi-spout assay under either sodium-deplete or sodium-replete
876 states in a single session. Following 48h of *ad-libitum* access to water and standard laboratory chow,
877 mice went through the furosemide treatment and behavioral testing again with the opposite homeostatic
878 state.

879 **Fiber-Photometry**

880 Wild-type mice underwent surgery for expression of dopamine sensors and fiber implantation as
881 outlined above (see *Surgeries*) before undergoing multi-spout consumption of sucrose under different
882 homeostatic demand states (see *Homeostatic Demand Multi-Spout Experiments*). We recorded
883 dopamine dynamics in the NAc medial shell and lateral shell simultaneously during behavior in the multi-
884 spout assay over 3 consecutive sessions in each homeostatic demand state. After head-fixation, we
885 connected to the mouse's fiber implant, patch cables (Doric, 400 μ m, 0.37NA, 2.5 mm stainless steel
886 ferrules) coupled to a 6-port mini cube (Doric, FMC6_IE(400-410)_E1(460-490)_F1(500-540)_E2(555-
887 570)_F2(580-680)_S) that was coupled to an integrated fiber-photometry system (Tucker-Davis
888 Technologies, RZ10X). We delivered 405 nm and 465 nm light sinusoidal modulated at 211 Hz and 331
889 Hz, respectively. The average power for each wavelength was calibrated to 30 μ W using a power meter
890 (Lux integrated with the RZ10x) prior to the experiment. The fluorescent emission produced by 405 nm
891 and 465 nm excitation were collected using the same fiber used to deliver light and were measured on a
892 photodetector (Lux) and demodulated during recording. The timing of behavioral events were recorded
893 via TTL communication to the fiber-photometry system.

894 Fiber-photometry was analyzed using custom Python and R scripts that are freely available
895 through our GitHub (https://github.com/agordonfennell/open_stage). A custom Python script was used to
896 convert raw data into tidy format and then an assortment of custom R functions were used to process the
897 fiber-photometry signals. The decay in signal throughout the session for the 405 and 465 channels were
898 corrected by fitting and subtracting a 3rd degree polynomial to each raw signal. We then normalized the
899 signals by computing z-scores using the mean and standard deviation of the entire session. Using the
900 onset of each access period, we created perievent time histograms with time relative to access onset
901 and then resampled signals to 20 samples per second. We used the 405 signal to assess movement

902 artifacts but did not observe any abrupt changes in fluorescence that typically indicate such artifacts
903 (**Figure 7- Figure Supplement 5**). The 405 signal was not used to correct the 465 signal because we
904 observed simultaneous opposing signals in the 405 and 465 signal that may be attributed to the fact that
905 405 is not an ideal isosbestic signal for GRAB-DA. The 465 signal in perievent time histograms was
906 shifted based on the mean signal during the 3 s prior to the onset of the access period. To summarize
907 the dopamine signal during access to each solution, we computed the average and peak signal during
908 the access period.

909 **Histology**

910 We conducted *post hoc* histology to determine the location of viral expression and optical fiber
911 locations for mice in optogenetic and fiber-photometry experiments. At the conclusion of the experiment,
912 mice were deeply anesthetized and transcardially perfused with 20 mL 1x PBS and 20 mL 4%
913 paraformaldehyde (PFA). Heads were removed and post-fixed in 4% PFA for 24h, brains were removed
914 and post-fixed for an additional 24h, and then brains were transferred to 30% sucrose until they sank.
915 Brains were frozen at -20°C and sectioned at 40 µm on a cryostat (Leica). Every other brain section was
916 collected in 1x PBS and then mounted on a glass slide. Slides were cover-slipped using the mounting
917 medium Fluoroshield with DAPI for visualizing cell nuclei. Sections that contained the bottom of the optic
918 fiber were imaged with epifluorescence at 5x magnification (Zeiss, ApoTome2; Zen Blue Edition). The
919 location of optic fibers was determined by mapping the position of the fiber using a mouse brain histology
920 atlas (Paxinos and Franklin, 2001). The position of fibers was overlaid onto a vector image of the
921 corresponding atlas section using Illustrator (Adobe).

922 **Statistical Analysis and Visualization**

923 Results with repeated measures design were analyzed using a repeated measure analysis of
924 variance (rmANOVA) using the afex package (0.28.1) in R. We computed *post hoc* comparisons using
925 Tukey's Honest Significant Difference (HSD) using the emmeans package (1.6.0) in R. Results with two
926 variables were analyzed using a two-sided unpaired *t*-test using base R. Correlations were computed
927 using Pearson's product moment correlation coefficient using base R. For all statistics, significance was
928 set at P values less than 0.05. Details for all statistical results presented in the paper can be found in the
929 stats table included with this manuscript.

930 Data was visualized using the ggplot2 (3.3.3) package in R. Combined plots were assembled
931 using patchwork (1.1.1). Color scales were produced using pals (1.7) or viridis (0.6.2). 3D renderings of
932 the head-fixed hardware were produced using Fusion 360 (Autodesk). Plot components were assembled
933 and further edited using Illustrator (Adobe).

934 **Acknowledgements**

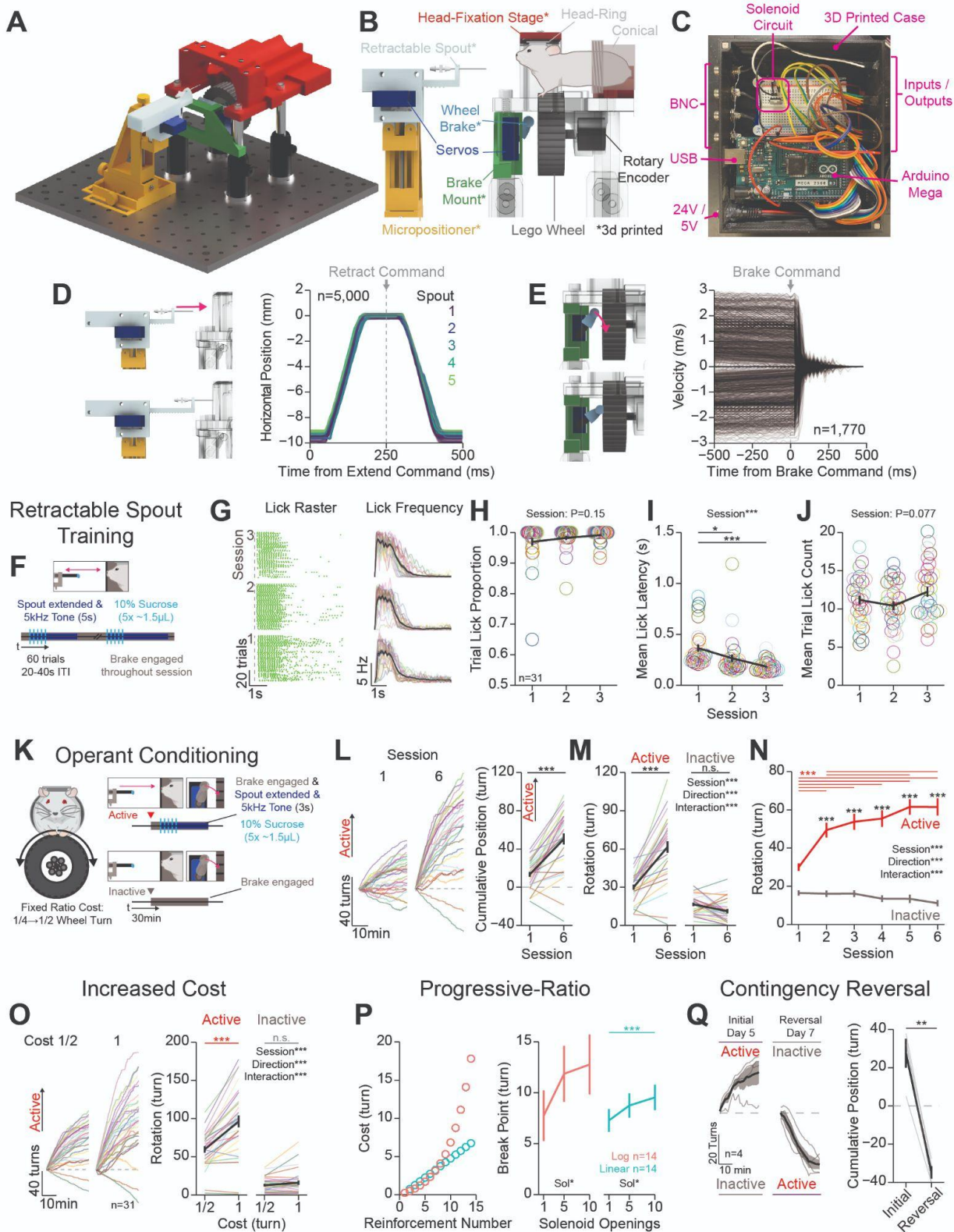
935 A.G.F was supported by F32 DA054719, T32 DA7278-27, R01 DA038168, R21 DA050868, and
936 P30 DA048736. G.D.S was supported by R37 DA032750, R01 DA038168, R21 DA050868, and P30
937 DA048736. M.R.B. and R.G. were supported by R37 DA03339, P50MH119467 (project 4), and P30
938 DA048736. M.F.R. and P.B. were supported by R21 DA050868.

939 We would like to thank Dr. Vijay Namboodiri and Madelyn Hjort for providing initial training to
940 A.G.F. in the Arduino language. Lydia Gordon-Fennell for creating vector illustrations of head-fixed mice
941 in figure diagrams and editing the manuscript. Barbara Benowitz for editing the manuscript. Dr. Spencer
942 Smith for providing initial designs for metal head-rings and head-fixation stage. Dr. Nick Steinmetz for
943 providing insights into the behavior of mice during head-fixed behavior with wheel turning as an operant
944 response.

945

946 **Figures**

947 **Figure 1**



948

949 **Figure 1: Mice Rapidly Learn Head-Fixed Operant Conditioning for Sucrose and Display Operant**
 950 **Behaviors Established in Freely moving Experiments: (A-E) System overview. (A) 3D rendering of**

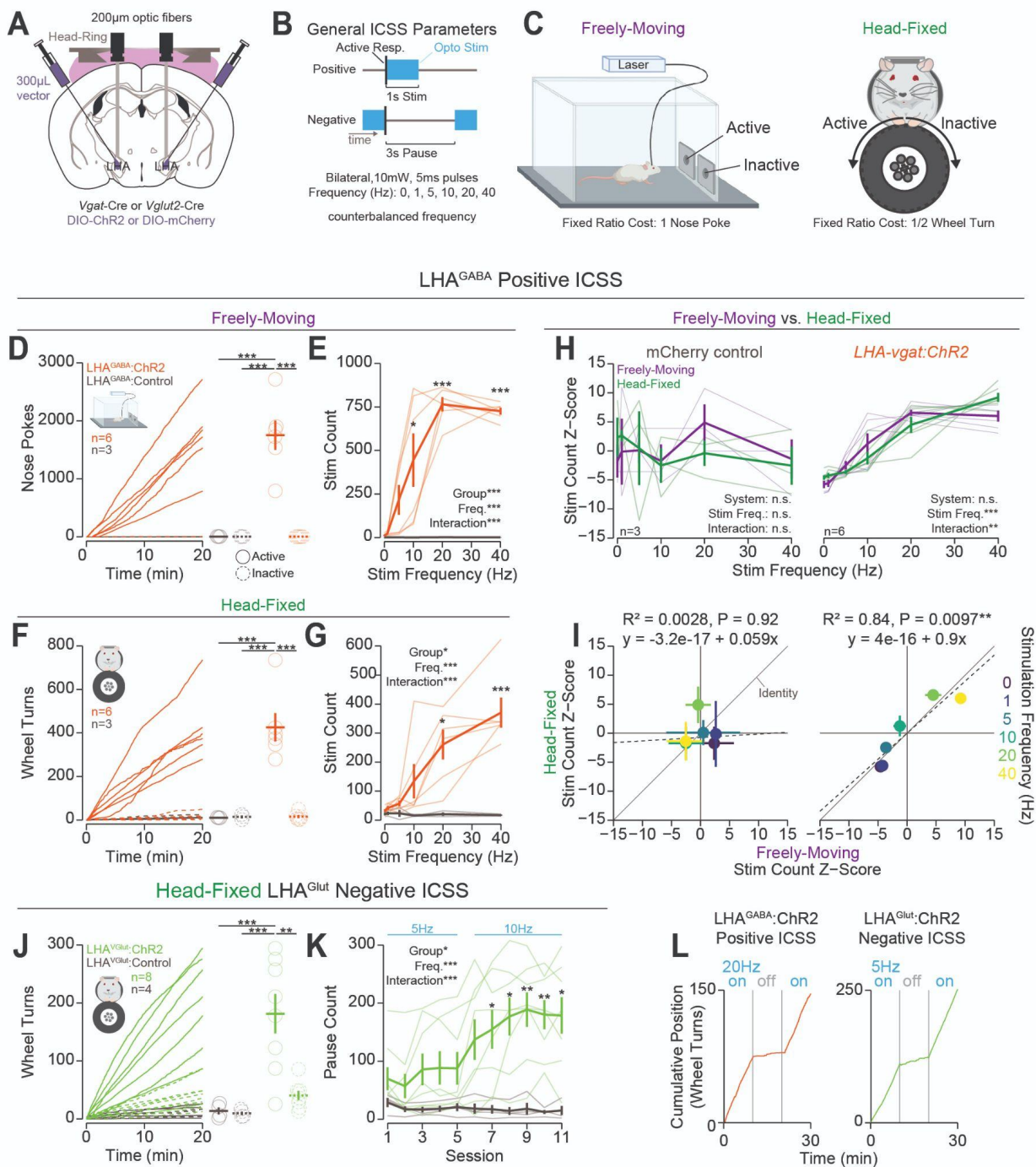
951 our open-source, low-cost, head-fixed system (Open-Source). B) Cartoon depicting the critical
952 components of our system (* indicates 3D printed components). C) Image of the Arduino based
953 microprocessor and custom enclosure used for controlling hardware and recording events. D)
954 Validation of our 3D printed retractable spout powered by a low-cost micro servo. left: 3D rendering of
955 the linear travel of the spout; right: horizontal position of the spout tip determined using DeepLabCut
956 over time during 1,000 extension/retractions with 5 unique retractable spout units. E) Validation of our
957 3D printed wheel brake powered by a low-cost micro servo. 3D rendering of the rotational travel of the
958 wheel brake (left); binned rotational velocity of the wheel produced by manual rotation before and after
959 the brake is engaged (right). F) Cartoon depicting the task design for retractable spout training. G)
960 Licking behavior throughout retractable spout training; lick raster for a representative mouse with each
961 lick represented as a tick (left); mean binned frequency of licks (right). (H-J) Summary of behavior
962 throughout retractable spout training: proportion of trials with at least 1 lick (H); Mean latency from
963 spout extension command to first lick on trials with a lick (I); mean number of licks within each **5 s**
964 access period (J). K) Cartoon depicting the task design for operant conditioning. L) Cumulative position
965 of the wheel throughout the session (left) and at the conclusion of the session (right) on the first and
966 sixth session of training (Positive direction indicate rotation in the active direction; session 1 vs session
967 6 *t*-test***). (M-N) Total rotation of the wheel throughout a session broken down based on direction on
968 the first and sixth session of training (M) and across training sessions (N). O) Cumulative position of the
969 wheel throughout the last session (left), and the mean total rotation of the wheel in the last 3 sessions
970 of fixed-ratio 1/2 turn and 1 turn. P) Progressive-ratio schedule of reinforcement (left) and break points
971 across different reward magnitudes set by the number of solenoid openings (One-Way RM ANOVA*).
972 Q) Cumulative position of the wheel throughout the session (left) and at the conclusion of the session
973 (right) on the last session of initial training and reversal training (*t*-test; initial vs. reversal: *t*-test**).
974 *(Unless otherwise noted, effects listed on plots indicate statistical significance for Two-Way RM ANOVA*
975 *effects; Multi color lines and rings depict individual mice; Black lines depict mean across mice; Black*
976 *asterisks above horizontal bars in (N) and (P) indicate significant differences in active rotation across*
977 *sessions, while black asterisks above means indicate significant differences between active and*
978 *inactive rotation within a session; see stats table for details).*

979

980

981

982 **Figure 2**



983

984 **Head-fixed Operant Conditioning to Obtain Stimulation of LHA^{GABA} Neurons or Avoid Stimulation**
 985 **of LHA^{Glut} Neurons:** A) Approach, placements depicted in Figure 2- figure supplement 1A. B) Diagram
 986 of the experimental approach for positive reinforcement in LHA^{GABA} mice and negative reinforcement in
 987 LHA^{Glut} mice. C) Cartoon depicting the freely moving (left) and head-fixed (right) versions of the operant
 988 task. D) Cumulative (left) and total (right) nose pokes under positive reinforcement for 40 Hz stimulation
 989 in LHA^{GABA}:ChR2 (red) and LHA^{GABA}:Control (grey) mice. E) Total number of stimulations earned under

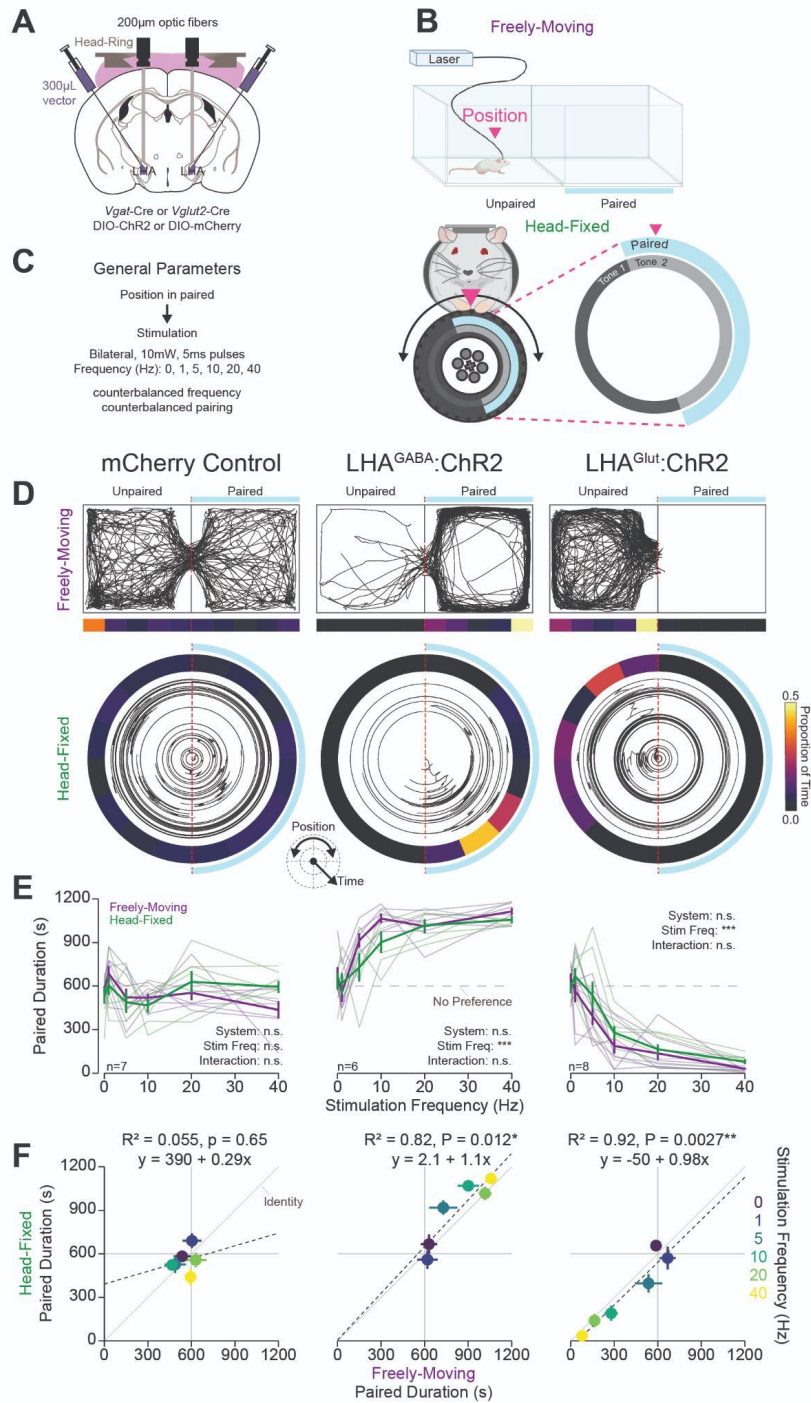
990 positive reinforcement for multiple stimulation frequencies (1 frequency/session). (F, G) same as (D, E)
991 except during the head-fixed version of the task. H) Comparisons of the z-score of the total number of
992 stimulations across frequencies in freely moving (purple) and head-fixed (green). Z-scores were
993 calculated for each mouse x system independently. No significant *post hoc* differences when comparing
994 systems at the same stimulation frequency. I) Correlation of the z-score of the total number of stimulations
995 in the freely moving and head-fixed version of the task. J) Cumulative rotation over a session under
996 negative reinforcement for 5 Hz and 10 Hz stimulation in LHA^{Glut}:ChR2 (lime green) and LHA^{Glut}:Control
997 (grey) mice. K) Total pause count across all training sessions (frequency schedule indicated with blue
998 text above the plot; *asterisks above means indicate significant differences determined by Bonferroni*
999 *adjusted t-test*). L) Cumulative position over a 30 min session with the laser turned off from 10-20 min.
1000 (*Unless otherwise noted, Effects listed on plots indicate statistical significance for Two-Way RM ANOVA*
1001 *effects; Faded lines and rings depict individual mice; asterisks above means indicate significant*
1002 *differences determined by HSD between stim count at a corresponding stim frequency or pause count at*
1003 *a corresponding session; asterisks above horizontal lines indicate significant difference determined by*
1004 *HSD between means indicated by line; see stats table for details*).

1005

1006

1007

1008 **Figure 3**



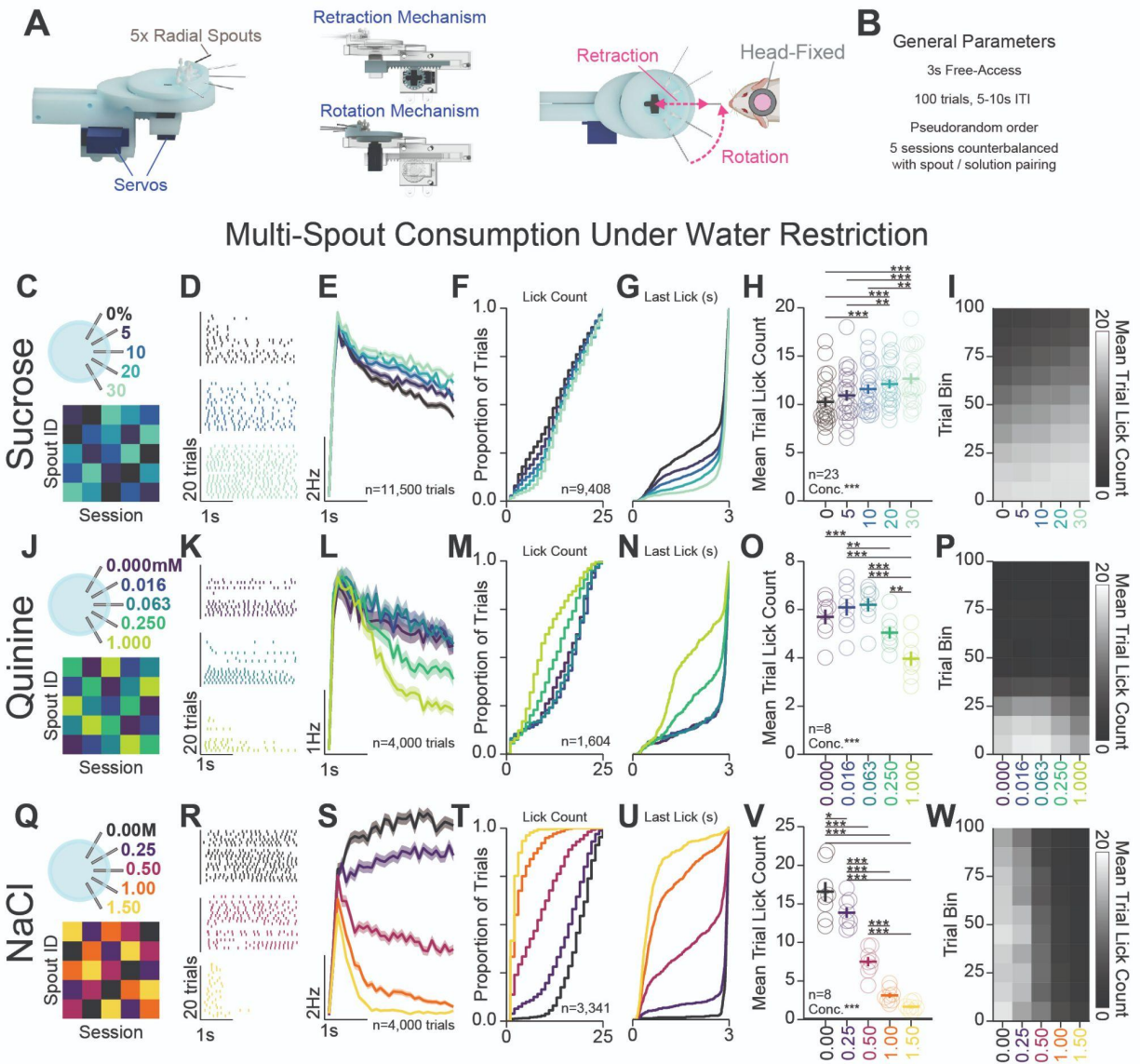
1009

1010 **Head-fixed Real-Time Place Preference and Aversion Associated with Stimulation of LHA**
 1011 **Subpopulations Mirrors Freely moving Behavior:** A) Approach, placements depicted in Figure 2-
 1012 figure supplement 1A. B) Cartoon depicting the freely moving and head-fixed versions of the operant
 1013 task. In the head-fixed task, the mouse's position was determined relative to the position of the wheel
 1014 and the mouse could rotate the wheel to navigate through the paired and unpaired zones. C) Task design.
 1015 (D-F) Behavior during the RTPT task; left column contains data from mCherry controls (both

1016 LHA^{GABA}:Control and LHA^{Glut}:Control), middle contains LHA^{GABA}:ChR2, right contains LHA^{Glut}:ChR2. D)
1017 Representative traces of the mouse's position in the 2 chamber arena in freely moving RTPT (top) and
1018 the position of the wheel over time in head-fixed RTPT (bottom). The right side of the arena or wheel was
1019 paired with optogenetic stimulation as indicated by the blue bar/arc. The proportion of time in binned
1020 areas of the arena or wheel are shown in the heat maps under or surrounding the traces (color scale
1021 represents the proportion of time in each position bin). E) Amount of time spent in the paired zone during
1022 a 20 min (1200 s) session for varying frequencies; values above 600 s are indicative of preference, values
1023 below are indicative of avoidance. Colors represent the version of the task as indicated in the left column.
1024 F) Correlation between the mean time spent in the paired zone across mice (5 values) during freely
1025 moving (abscissa) and the head-fixed (ordinate) versions of RTPT at different stimulation frequencies
1026 (colors represent stimulation frequency; error bars represent SEM). (In (E) asterisks depict Two-Way RM
1027 ANOVA effects, no HSD differences between systems were detected at corresponding stimulation
1028 frequencies; see stats table for details).

1029

1030 **Figure 4**



1031

1032 **Head-Fixed Consumption of Gradients of Rewarding and Aversive Solutions During Brief Access:**

1033 A) 3D rendering of the multi-spout unit that retracts and rotates to allow brief access periods to 1 of 5 lick
 1034 spouts to the head-fixed mouse. B) Task design. (C-I) Multi-spout consumption of a gradient of
 1035 concentrations of sucrose data. C) Procedure: mice received 5 sessions of 5x multi-spout
 1036 counterbalanced to have each solution of each spout once. Colors represent concentrations of solution
 1037 as defined in the label adjacent to the multi-spout cartoon. D) Lick raster of a representative mouse
 1038 depicting the licks for water, medium concentration, and high concentration during the 3 s access period.
 1039 E) Mean binned lick rate for all mice for each concentration. (F-G) Cumulative distribution of the number
 1040 of licks in trials with a lick (F) and the time of the last lick within each licking bout (G). H) The mean
 1041 number of licks per trial for each concentration. I) The mean number of licks for each concentration per

1042 trial binned by 10 trials over the course of the session. (J-P) same as (C-I), but for data from multi-spout
1043 consumption of a gradient of concentrations of quinine. (Q-W) same as (C-I), but for data from multi-
1044 spout consumption of a gradient of concentrations of NaCl. (*Main effects listed on plots are results of*
1045 *One-Way RM ANOVA; asterisks depict HSD comparisons between concentrations indicated by*
1046 *horizontal line; Faded lines depict individual mice; see stats table for details).*

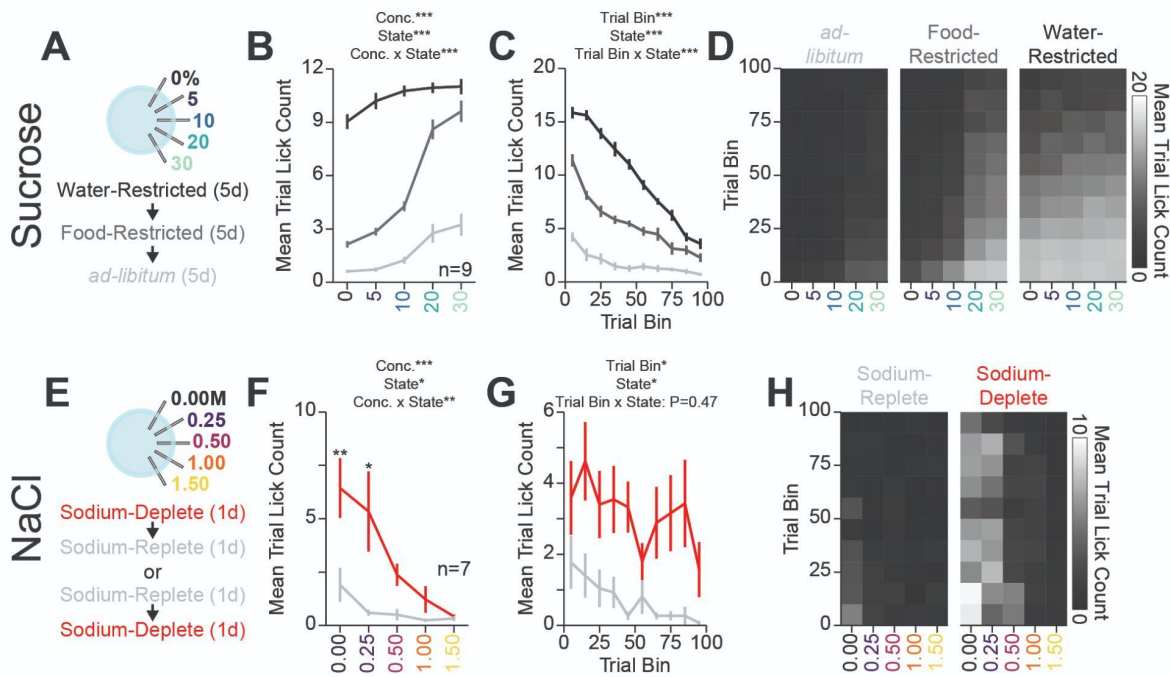
1047

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1049

1050 **Figure 5**

Multi-Spout Consumption Across Homeostatic Demand



1051

1052 **Homeostatic Demand Shifts Within Session Consumption of Gradients of Sucrose and NaCl:** A) Procedure: Mice ran sequentially through water-restriction, food-restriction, and *ad-libitum* states. During each state, mice received 5 sessions of multi-spout counterbalanced to have each concentration of sucrose on each spout once. B) The mean number of licks per trial for each concentration of sucrose in the *ad-libitum* (light gray), food-restricted (dark gray), and water-restricted (black) states (*HSD*: every mean is significantly different from every other, except 30% sucrose consumption under food and water-restriction). C) Mean trial lick count across all concentrations of sucrose in bins of 10 trials across the session for each homeostatic state. D) The mean number of licks for each concentration of sucrose per trial binned by 10 trials over the course of the session for each homeostatic state. E) Procedure: In sodium replete or sodium deplete states in counterbalanced order, mice received 1 session of multi-spout with a gradient of concentrations of NaCl. The pairing of solution concentrations and spouts remained consistent. F) The mean number of licks per trial for each concentration of NaCl in the sodium replete (gray) and deplete (red) states. G) Mean trial lick count across all concentrations of NaCl in bins of 10 trials across the session for each homeostatic state. H) The mean number of licks for each concentration of NaCl per trial binned by 10 trials over the course of the session for each homeostatic state. (*Main*

1067 *effects listed on plots are results of Two-Way RM ANOVA; asterisks indicate differences between*
1068 *homeostatic demand state at a corresponding concentration; see stats table for details).*

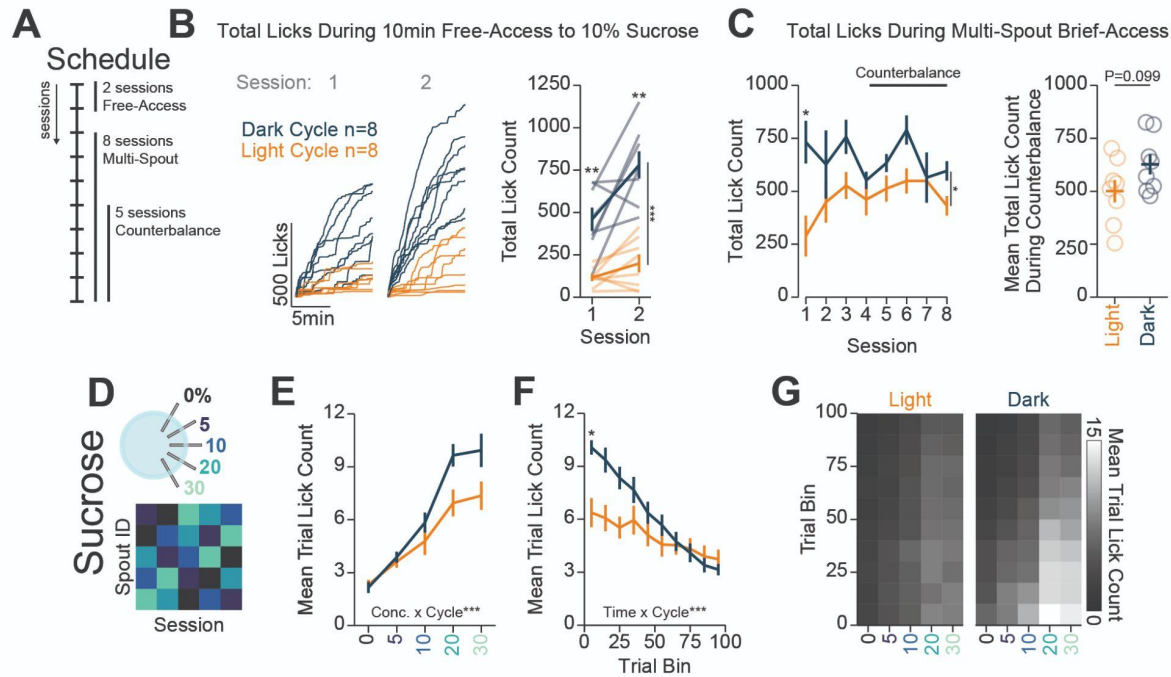
1069

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1071

1072 **Figure 6**

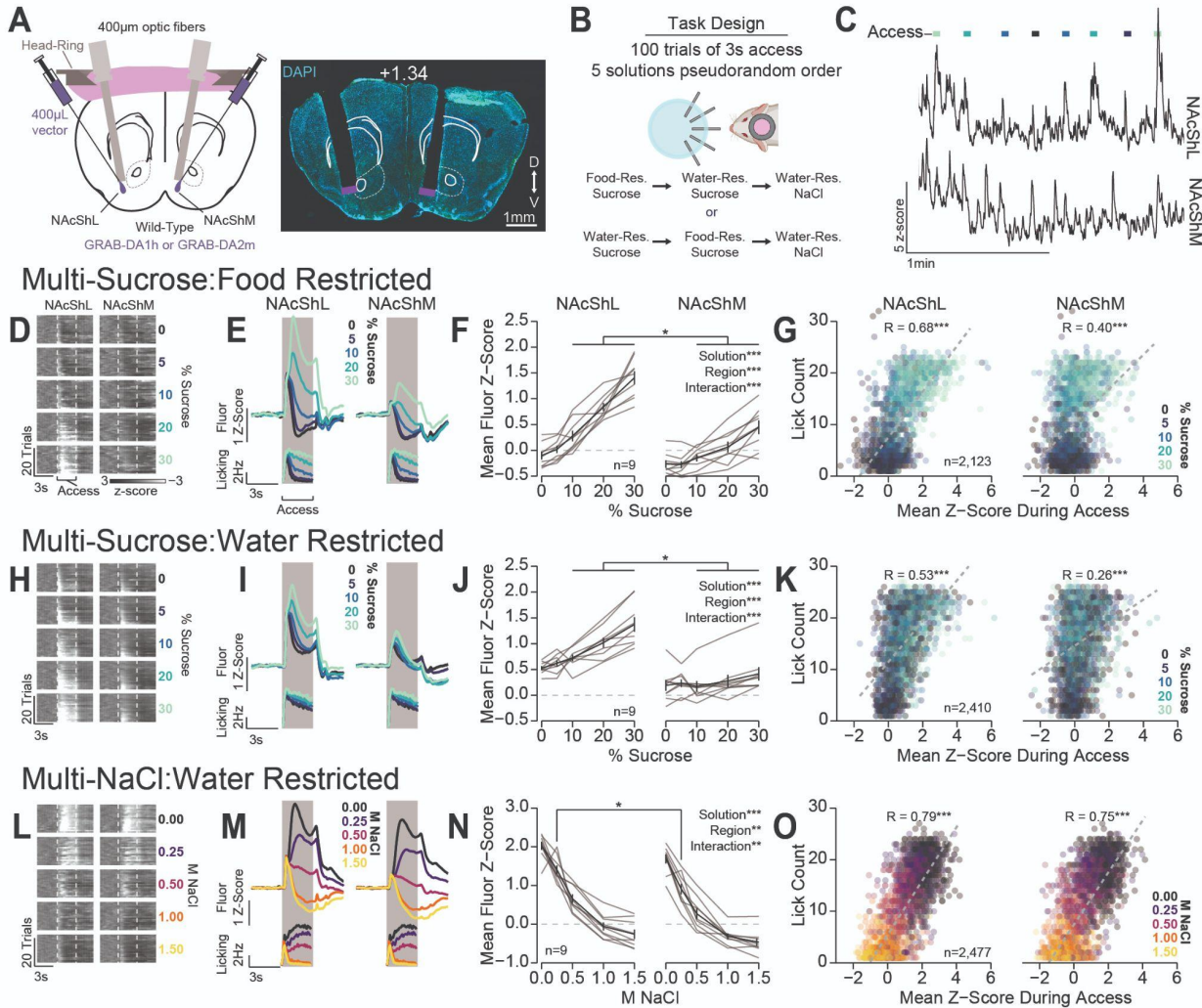
Multi-Spout Consumption Across Light/Dark Cycle



1073

1074 **Light/Dark Cycle Shifts Within-Session Consumption of Gradients of Sucrose:** A) Schedule for
 1075 behavioral sessions. B) Licking behavior during two sessions of free-access licking for 10% sucrose
 1076 displayed as cumulative licking (left) and total lick count during the session (right). Mice ran in the dark-
 1077 cycle licked more than mice ran in the light-cycle (Two-Way RM ANOVA: Cycle***). C) Total licking
 1078 behavior during 8 sessions of multi-spout brief-access to a gradient of sucrose concentration (left) and
 1079 mean over 5 counterbalance sessions (right). Mice ran in the dark-cycle licked more than mice in the
 1080 light-cycle over all 8 sessions (Two-Way RM ANOVA: Cycle*), but not over the 5 counterbalanced
 1081 sessions (*t*-test: $P=0.099$). D) Procedure: mice were trained in 5 sessions of 5x sucrose multi-spout
 1082 counterbalanced to have each solution of each spout once. E) The mean number of licks per trial for
 1083 each concentration of sucrose for mice ran in the dark-cycle (blue) and mice ran in the light-cycle (orange)
 1084 (Two-Way RM ANOVA: Concentration x Cycle***). F) Mean trial lick count across all concentrations of
 1085 sucrose in bins of 10 trial across the session (Two-Way RM ANOVA: Time x Cycle***). G) The mean
 1086 number of licks for each concentration of sucrose per trial binned by 10 trials over the course of the
 1087 session. (Asterisks above means indicate differences between mice tested in each cycle during the same
 1088 session).

1089 **Figure 7**



1090

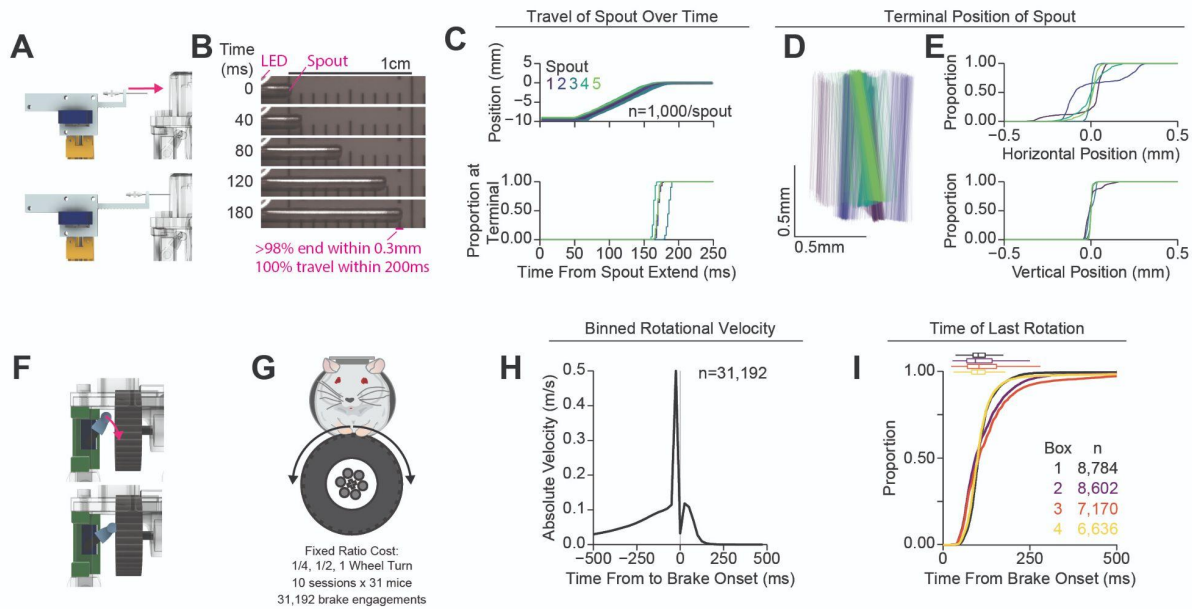
1091 **Differential Dopamine Dynamics During Multi-Spout Consumption Behavior:** A) Approach for
 1092 simultaneously recording dopamine dynamics in the NAcShL and NAcShM (left), and representative
 1093 placements of optic fibers overlaying the NAcSh (White numerical value indicates AP position relative to
 1094 bregma). B) Task design and schedule of experiment. C) Representative trace of simultaneous GRAB-
 1095 DA fluorescence in the NAcShM and NAcShL during multi-spout access to sucrose under food-restriction
 1096 (lines on top indicate access periods, color indicates sucrose concentration). (D-G) Dopamine dynamics
 1097 during multi-sucrose under food-restriction: D) Representative heat map of GRAB-DA fluorescence over
 1098 time during each trial sorted by sucrose concentration (trials averaged over 3 sessions of recording). E)
 1099 Perievent time histograms of GRAB-DA fluorescence (top) and licks (bottom). F) Mean fluorescence z-
 1100 score during access period indicating strong scaling in the NAcShL (left) and weak scaling in the NAcShM
 1101 (right). G) On individual trials, the mean z-score during access correlates with licking in both the NAcShL
 1102 (left), and NAcShM (right) (color depicts the solution concentration). (H-K) Same as D-G for dopamine
 1103 dynamics during multi-sucrose under water-restriction. (L-O) Same as D-G for dopamine dynamics during

1104 multi-NaCl under water-restriction. N) Strong scaling in NAcShL (left) and weak scaling in the NAcShM
1105 (right). (*Asterisks indicate differences between brain regions at the same solution concentration; Two-*
1106 *Way RM ANOVA effects indicated in F, J, N; correlations indicated in G, K, O; see stats table for details*).

1107

1108

1109 **Figure 1- Figure Supplement 1**



1110

1111 **Validation of Retractable Spout and Wheel Brake:** A) Cartoon of validation of the retractable spout.

1112 B) Representative still frames from video data recording the position of the spout during 1,000

1113 extension/retractions in 5 different retractable spout assemblies. An LED was used to indicate the onset

1114 of the extension command (top left of frame). C) Horizontal position of the spout during extension (top)

1115 and cumulative distribution of latencies to reach 98% of the final spout position (bottom). D) Terminal

1116 position of the spout on all recorded extensions drawn from tracking the top and bottom corners of the

1117 spout (color indicates the identity of the spout assembly, lines represent the tip of the spout in 2D space

1118 for each trial). E) Cumulative distribution of the horizontal (top) and vertical (bottom) position of the

1119 spout at the terminal location relative to the mean position at the terminal location, indicating incredibly

1120 consistent vertical positioning of the spout and highly consistent horizontal positioning of the spout. F)

1121 Cartoon of validation of the wheel brake. G) Cartoon indicating that data from fixed-ratio self-

1122 administration of 10% sucrose was used for (H-I) (n=31,192 wheel brake engagements). H) Mean

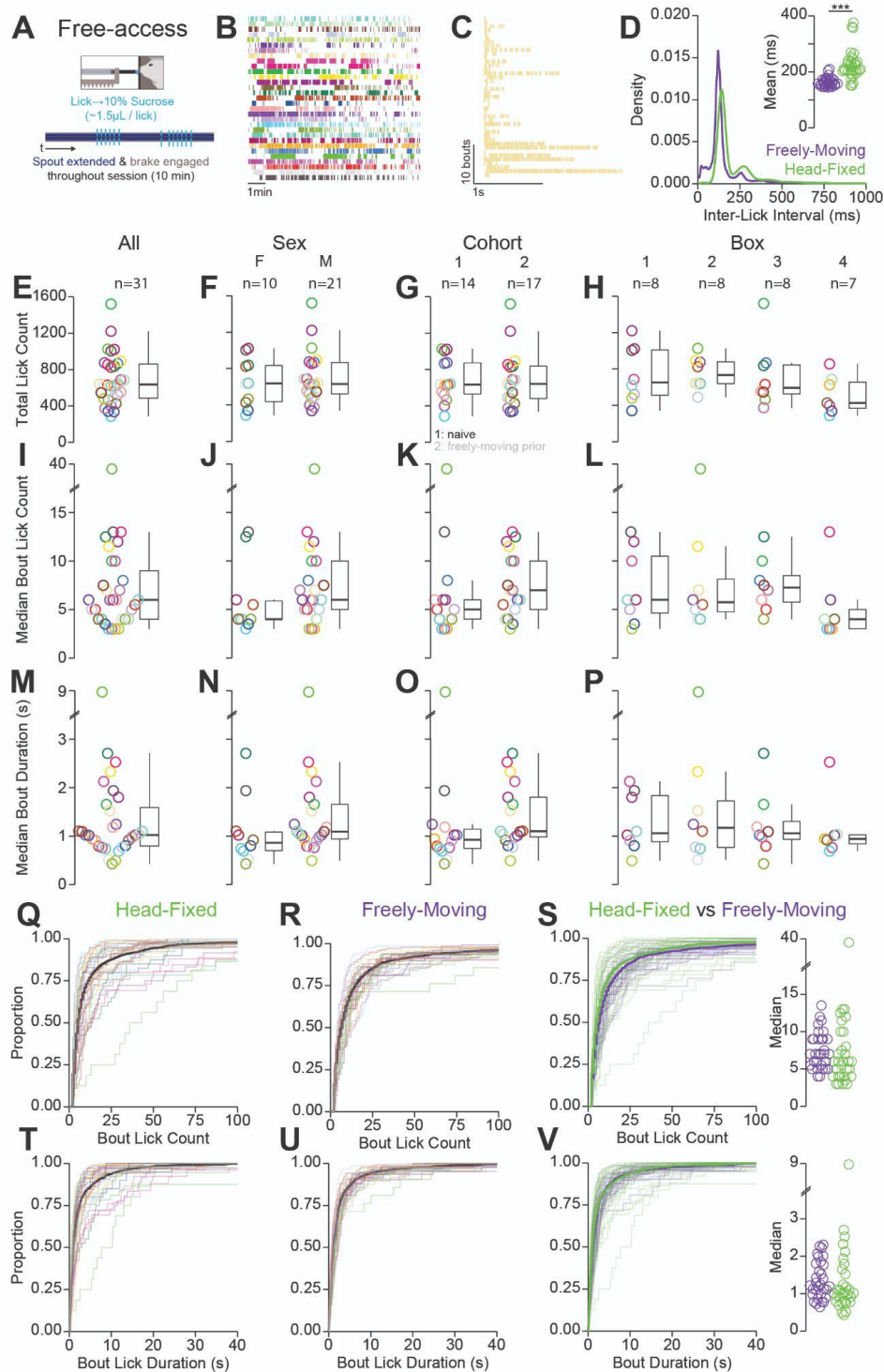
1123 absolute velocity during 25ms bins (SEM is not visible behind the line). I) Cumulative distribution of the

1124 time of last detected rotation across every trial for each of the 4 behavioral setups (box and whisker

1125 plots indicate the median and interquartile range).

1126

1127 **Figure 1- Figure Supplement 2**



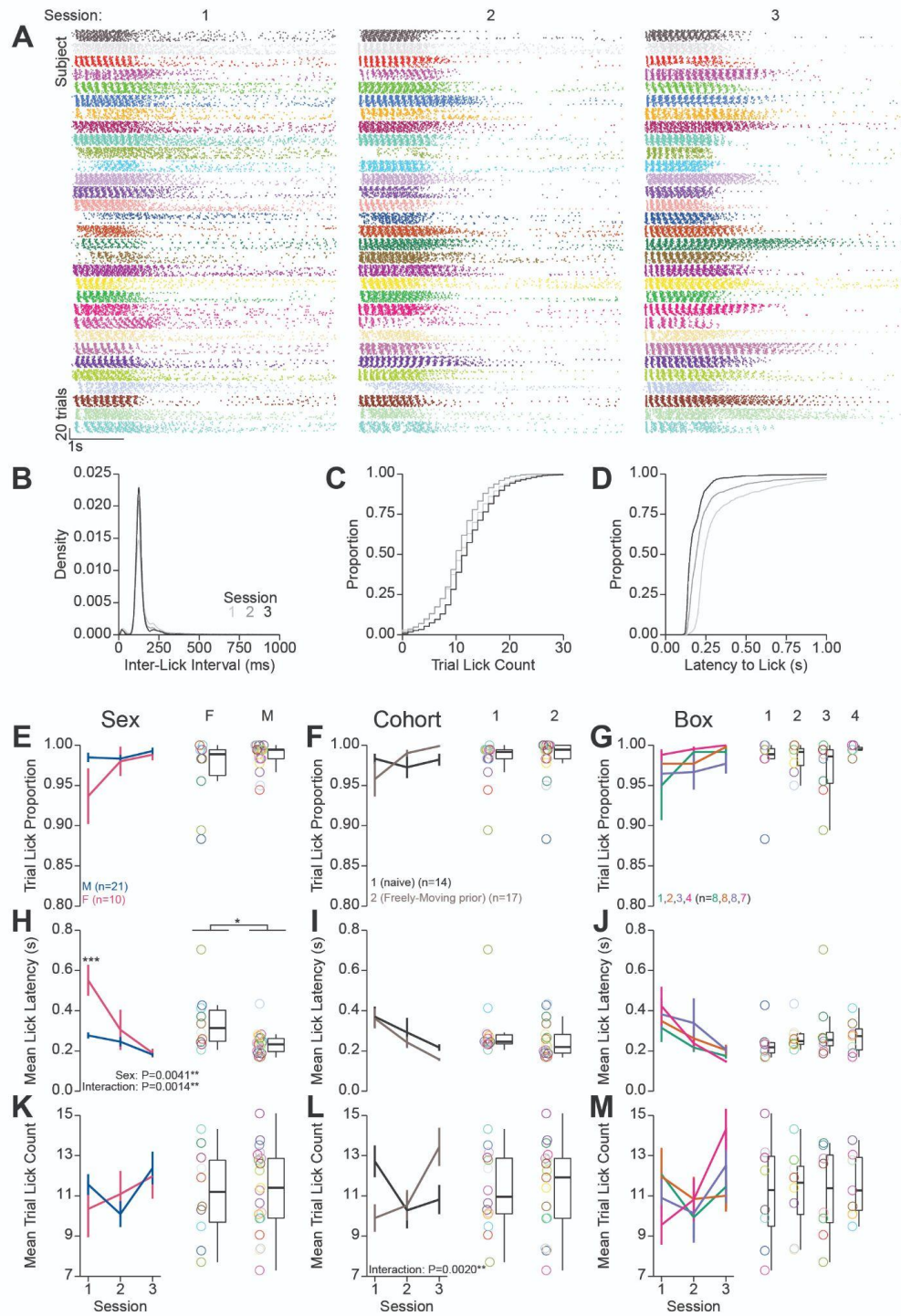
1128

1129 **Quantification of Behavior During Head-Fixed Spout Training:** A) Cartoon depicting the task design
 1130 for head-fixed, free-access consumption of sucrose (free-access lick training). B) Raster of all licks
 1131 recorded in a single 10 min session for the 31 mice in the experiment (color indicates the mouse identity
 1132 and is consistent with other plots depicting color-coded single mouse data). C) Raster of all licking bouts

1133 for a representative mouse. D) Density plot of inter-lick intervals in the head-fixed (green) and freely
1134 moving (purple) versions of free-access consumption. Inset shows the median inter-lick interval for all 31
1135 mice in both versions of the task (t -test: freely moving vs. head-fixed***). (E-P) Summaries of licking
1136 behavior during free-access in the head-fixed version of the task depicting the total lick count (E-H),
1137 median lick count per licking bout (I-L), and median duration per licking bout (M-P). Each row contains
1138 the same data from 31 mice divided by sex, cohort (1: naïve, 2: freely moving prior), and behavioral box
1139 number. No statistically significant differences in free-access licking behavior were observed. (Q-S)
1140 Cumulative distribution of the number of licks per bout in the head-fixed (Q) and freely moving (R)
1141 versions of the task. S) Data from (Q) and (R) overlaid and color coded based on the version of the
1142 task (left) and the median values for each mouse in both versions of the task (right). (T-V) Same as (Q-
1143 S) but for the duration of licking bouts (*rings and faded lines depict individual mice; no statistical*
1144 *differences for any comparison; see stats table for details*).

1145

1146 **Figure 1- Figure Supplement 3**



1147

1148 **Quantification of Behavior During Head-Fixed Retractable Spout Training:** Further quantification of
 1149 behavior during retractable spout training corresponding to data shown in Figure 1G-J. A) Lick raster of
 1150 all licks recorded across all trials across 3 sessions for the 31 mice in the experiment (color indicates the
 1151 mouse identity and is consistent with other plots depicting color coded single mouse data). B) Density of
 1152 inter-lick intervals. C) Cumulative distributions of number of licks per trial. D) Cumulative distribution of

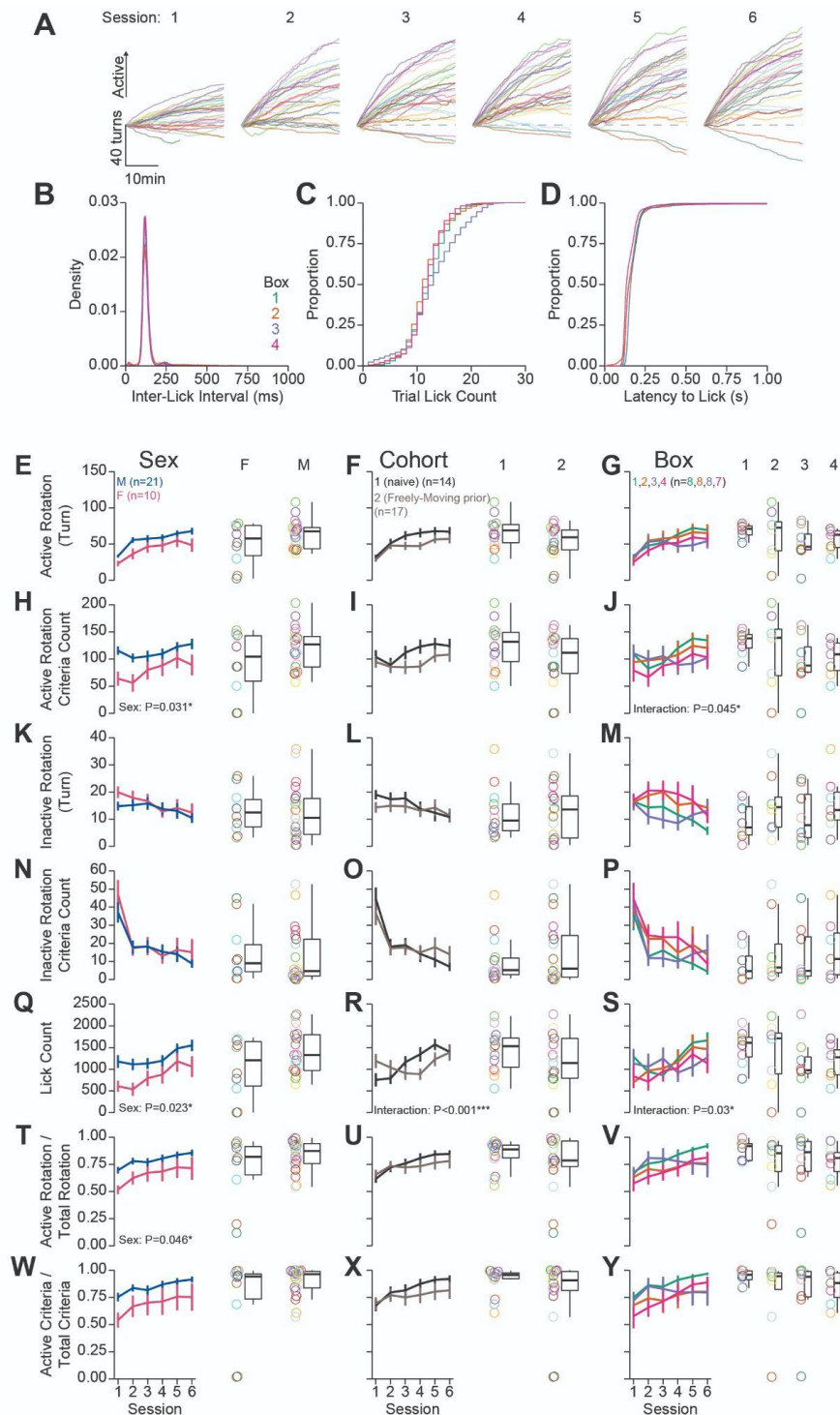
1153 latency from spout extension command to first lick on trials with at least one lick. (E-M) Summaries of
1154 behavior during retractable spout training in each session (left) and averaged across sessions (right)
1155 depicting the proportion of trials with a lick (D-G), mean latency from spout extension to first lick (H-J),
1156 and mean number of licks per trial (K-M). Each row contains the same data from 31 mice divided by sex,
1157 cohort, and behavioral box. (*Color in (B-D) represents the session number; rings depict individual mice;*
1158 *effects listed on plots indicate statistical significance for significant Two-Way RM ANOVA effects;*
1159 *asterisks above means indicate significant differences determined with HSD across group within the*
1160 *same session; asterisks above box and whisker plots indicate significant differences determined with a*
1161 *t-test or One-Way ANOVA; see stats table for details).*

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1165 **Figure 1- Figure Supplement 4**



1166

1167 **Quantification of Behavior During Head-Fixed Operant Conditioning for Sucrose:** Further
 1168 quantification of behavior during operant training corresponding to data shown in Figure 1L-N. A)
 1169 Cumulative position of the wheel for all mice across all sessions. B) Density of inter-lick intervals. C)
 1170 Cumulative distributions of number of licks per trial. D) Cumulative distributions of latency from spout

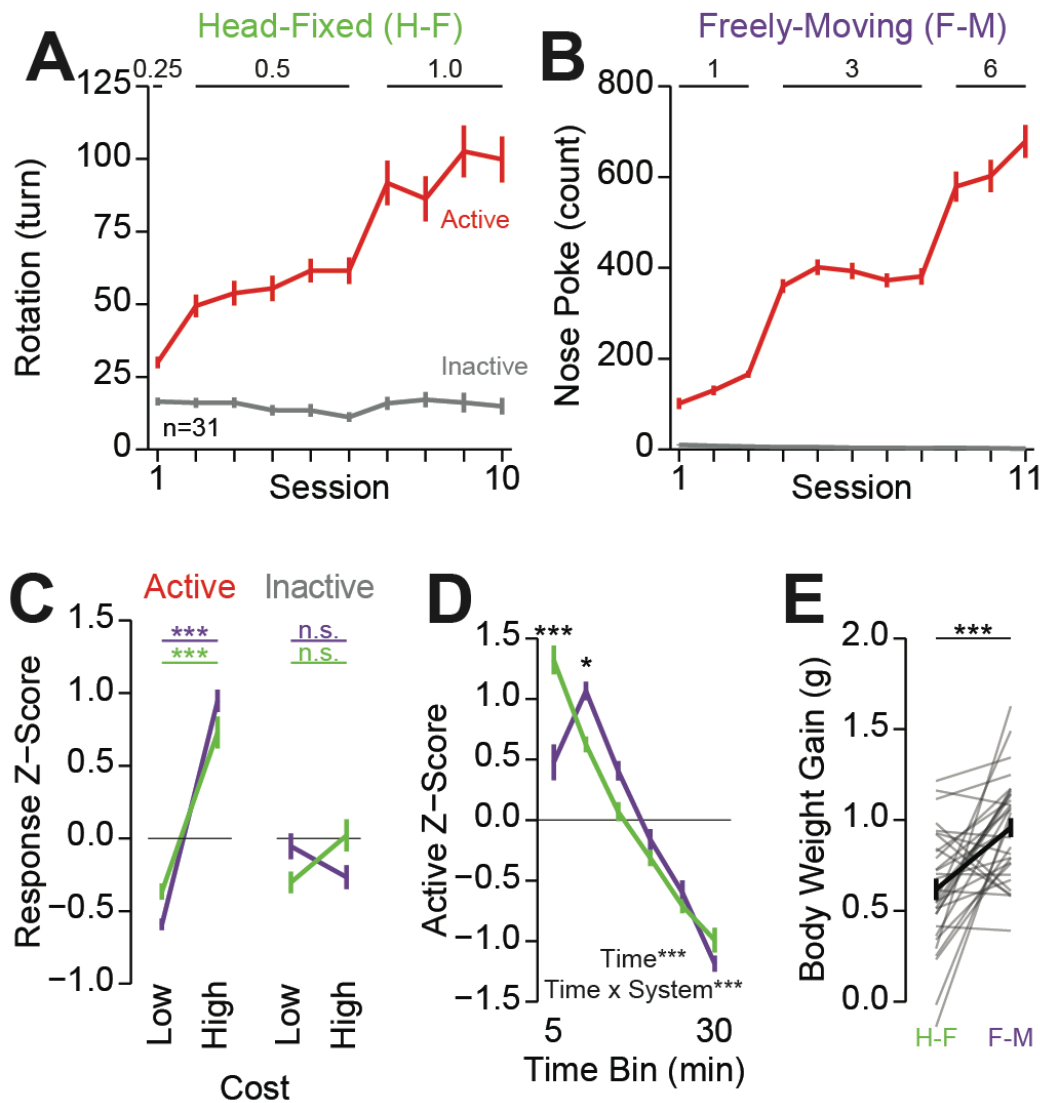
1171 extension to first lick. (E-Y) Summaries of behavior during operant training depicting numerous metrics
1172 across each row. Each row contains the same data from 31 mice divided by sex, cohort, and behavioral
1173 box number. Line graphs depict the mean and SEM for each session of training, the box and whisker
1174 plots with individual mice plotted as rings depicts the *mean over the last 3 sessions*. (Color in (B-D)
1175 represents the box number; rings and faded lines depict individual mice; Effects listed on plots indicate
1176 statistical significance for significant Two-Way RM ANOVA effects; asterisks above means indicate
1177 significant differences determined with HSD across group within the same session; asterisks above box
1178 and whisker plots indicate significant differences determined with a t-test or One-Way ANOVA; see stats
1179 table for details).

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1183 **Figure 1- Figure Supplement 5**



1184

1185 **Comparison of Head-Fixed and Freely moving Versions of Operant Conditioning:** A) The total
 1186 rotation of the wheel across all sessions of operant conditioning in the head-fixed version of the task
 1187 (numbers on the top of the plot indicate the cost of reward in wheel turns; data in the last 3 sessions of
 1188 0.5 and 1.0 correspond to data presented in Figure 1N). B) Total nose poke count across all sessions of
 1189 operant conditioning in the freely moving version of the task (numbers on top of the plot indicate the cost
 1190 of reward in nose pokes). C) Mean z-score across the last 3 sessions of responding during the low (0.5
 1191 turn/3 nose pokes) and high (1.0 turn/5 nose pokes) cost sessions (color of the line indicates the system;
 1192 horizontal lines indicate HSD comparisons within a response; no significant difference between systems
 1193 at any cost for a particular response (e.g. head-fixed active low vs freely moving active low)). D) Mean z-
 1194 score of the active response during 5 min bins within the last 3 sessions of high cost sessions (asterisk
 1195 above means indicates significant HSD comparison across systems at a given time bin). E) Total body

1196 weight gain (liquid consumption) during the head-fixed and freely moving versions of the task (*t*-test:
1197 Head-fixed vs. freely moving***). (See stats table for details).

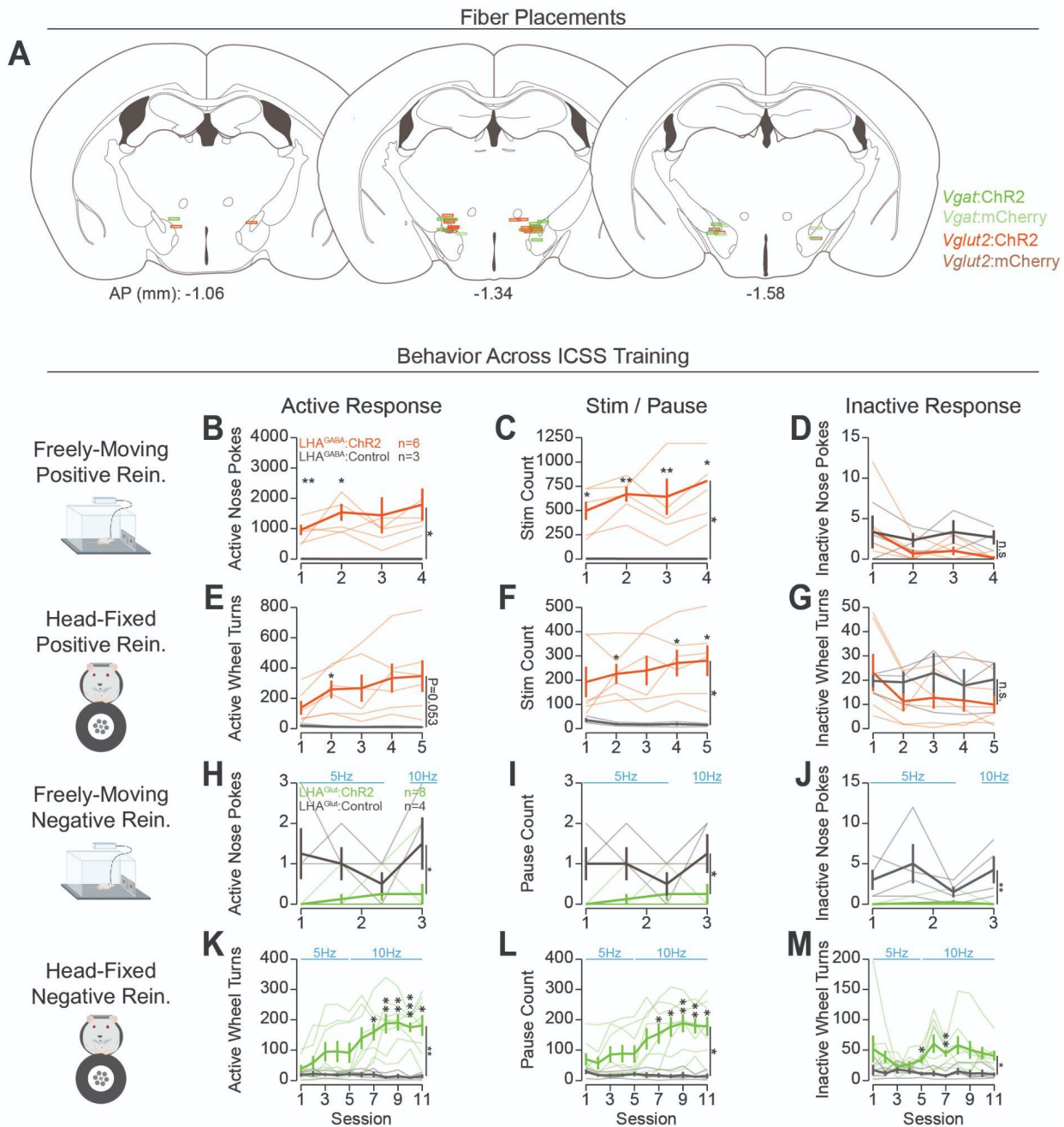
1198 **Figure 1- Figure Supplement 6**

1199

1200 **Video of Operant Responding for 10% Sucrose:** Video of operant responding for 10% sucrose under
1201 a fixed-ratio (FR) of 1/2 turn.

1202

1203 **Figure 2- Figure Supplement 1**



1204

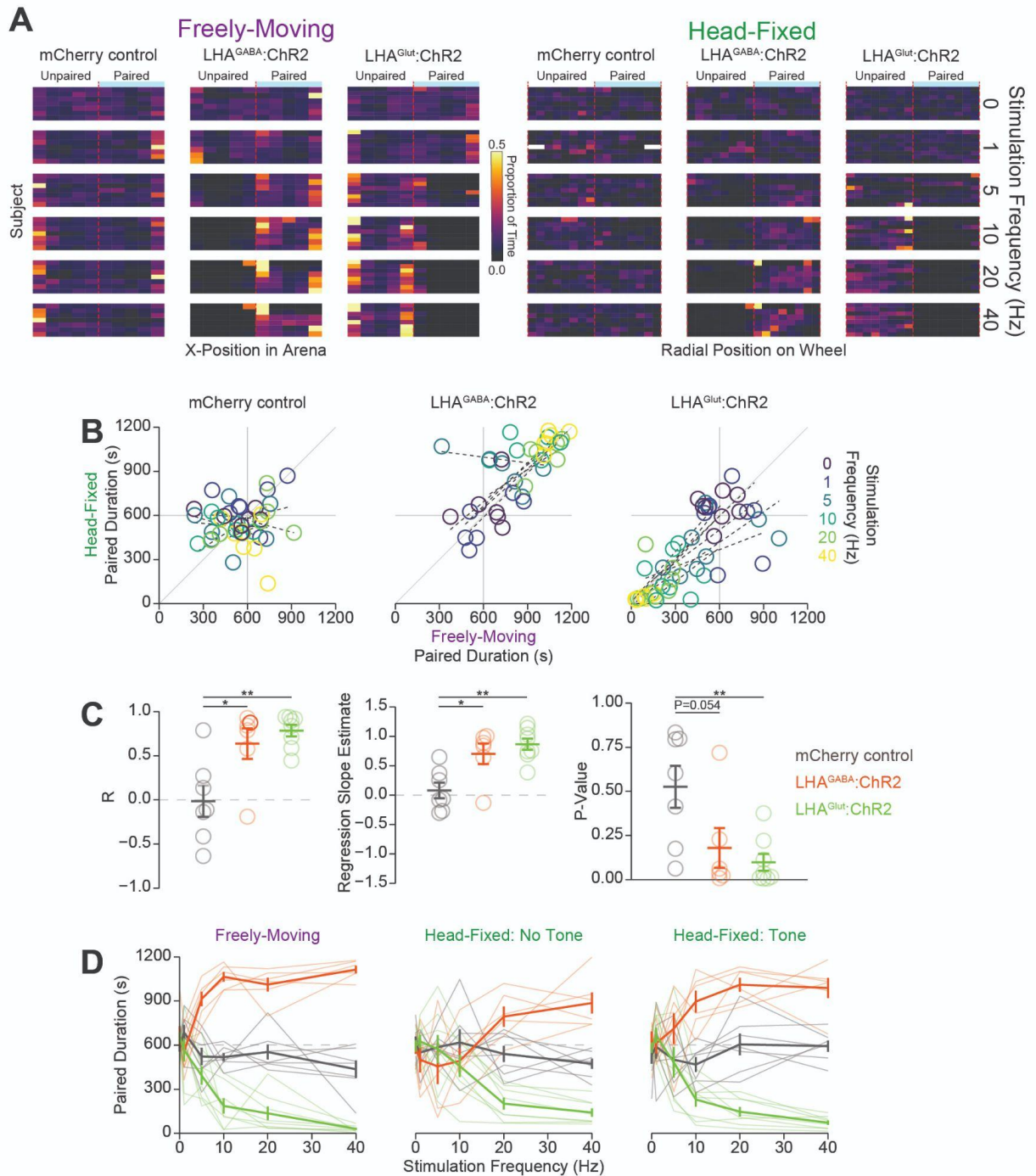
1205 **Placement of Optic Fibers and Training Data for Operant Conditioning to Obtain or Avoid**
 1206 **Optogenetic Stimulation:** A) Histological locations relative to bregma of the tip of optic fibers targeting
 1207 the LHA. Colors indicate the experimental group of the corresponding mouse. (B-M) Training data across
 1208 training for each stage of the task with counts for active responses (left column), stimulations or pauses
 1209 (mid column), and inactive responses (right column). (B-D) Behavior during freely moving positive
 1210 reinforcement. (E-G) Behavior during head-fixed positive reinforcement. (H-J) Behavior during freely
 1211 moving negative reinforcement. (K-M) Behavior during head-fixed negative reinforcement. (Asterisks

1212 *depict Two-Way RM ANOVA main effects (vertical lines) or Bonferroni adjusted t-test comparisons*
1213 *between a group at a corresponding session (over means); Faded lines depict individual mice; see stats*
1214 *table for details).*

1215 **Figure 2- Figure Supplement 2**

1216 **Video of Head-fixed Operant Conditioning to Obtain Stimulation of LHAGABA Neurons or Avoid**
1217 **Stimulation of LHAGlut Neurons:** Videos showing responding for optogenetic stimulation of LHA^{GABA}
1218 neurons under a positive reinforcement schedule (left) and responding for optogenetic stimulation of
1219 LHA^{Glut} neurons under a negative reinforcement schedule (right). The LED near the center of the frame
1220 indicates when the optogenetic stimulation is turned on under positive reinforcement or when the
1221 optogenetic stimulation is turned off under negative reinforcement.

1222 **Figure 3- Figure Supplement 1**

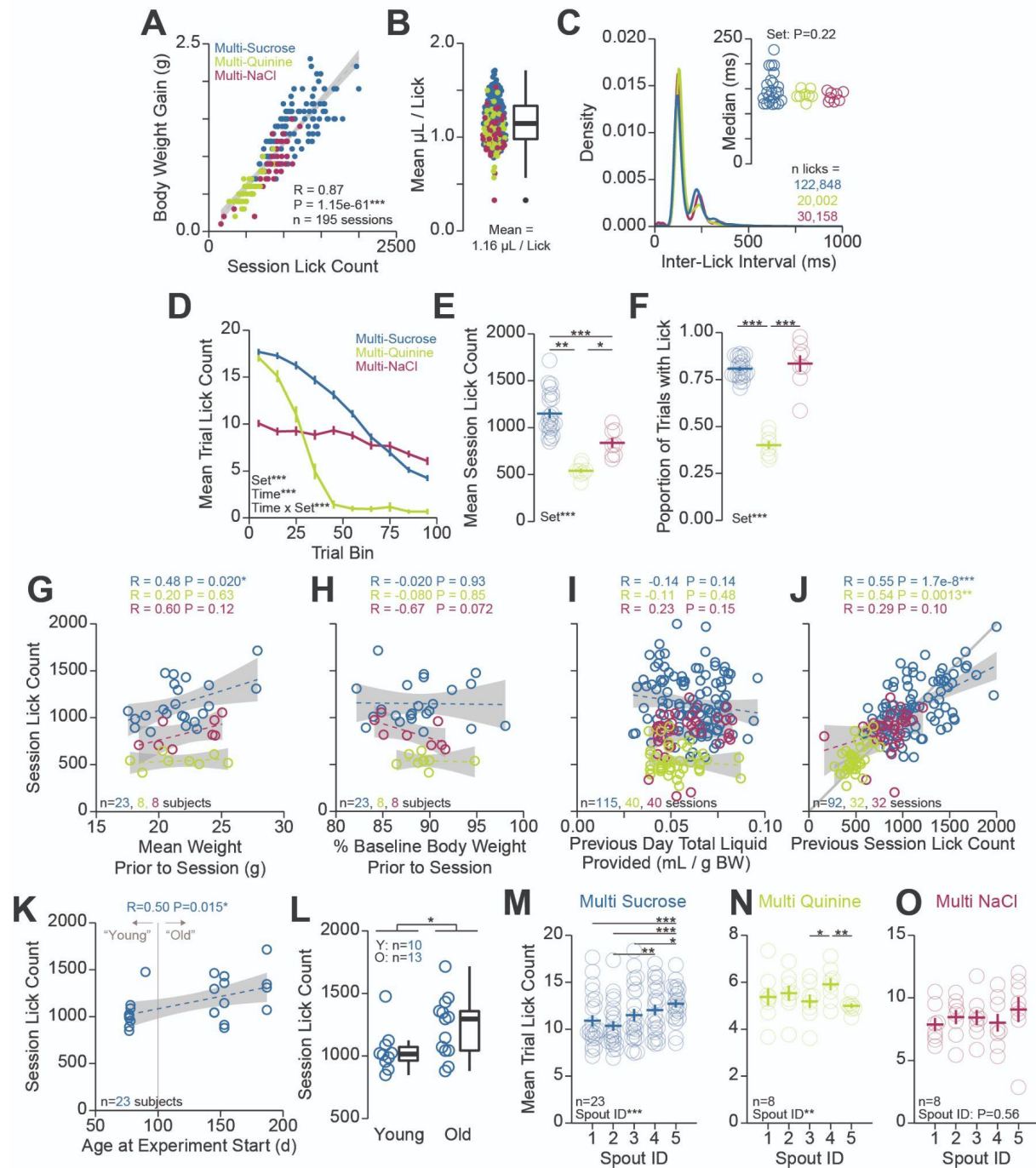


1223

1224 **Quantification of Head-fixed and Freely moving Real-Time Place Testing:** A) Heat map showing the
 1225 binned x-position in the freely moving version of the task (left 3 columns) or radial position in the head-
 1226 fixed version of the task (right 3 columns) of all mice across all stimulation frequencies (indicated by label
 1227 on right). Color represents the proportion of time spent in the binned position. The paired side was
 1228 counterbalanced across mice and sessions and is set to the right side for display purposes only. B)

1229 Correlation between the time spent in the paired zone during the freely moving (abscissa) or the head-
1230 fixed (ordinate) at different stimulation frequencies represented as different colors for individual mice. C)
1231 Summary statistics of correlations shown in (B) showing: R (left), estimated slope (middle), and P-value
1232 (right). D) Amount of time spent in the paired zone across the freely moving (left), head-fixed without a
1233 tone indicating the area the mouse was located in (middle), and head-fixed with a tone indicating the area
1234 the mouse was located in (right). (*Colors in (C-D) indicate the experimental group as indicated by the*
1235 *colors in the right side of C; asterisks depict HSD comparisons between groups as indicated by horizontal*
1236 *lines*).

1237 **Figure 4- Figure Supplement 1**



1238

1239 **Quantification of Head-Fixed Consumption During Brief Access:** A) Correlation between the number
 1240 of licks and body weight gain within each session. B) Estimated volume consumed per lick produced by
 1241 dividing the body weight gain by the number of licks within the session. C) Density plot of inter-lick
 1242 intervals within each session set. Inset shows the median inter-lick interval. D) Mean trial lick count across
 1243 all concentrations in bins of 10 trials across the session for each solution set. E) Mean total number of
 1244 licks for sessions for each solution set. F) Proportion of trials with at least 1 lick for each solution set. (G-

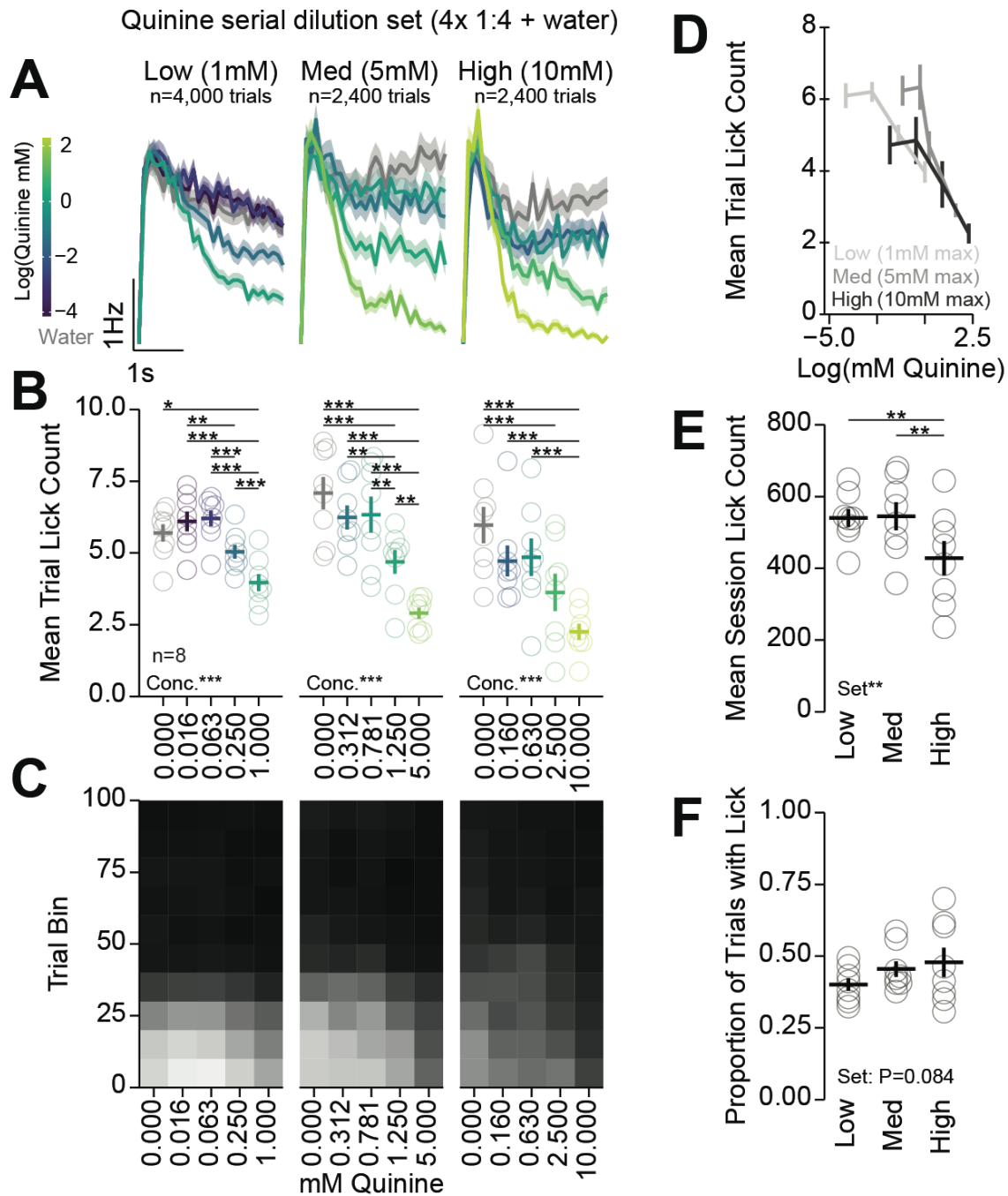
1245 J) Correlations between different factors (abscissa) and session lick count (ordinate): G) mean weight
1246 prior to the session, H) percent baseline body weight prior to the session, I) total liquid consumed +
1247 provided to the mouse on the session prior to the session, J) the lick count on the previous session. K)
1248 Correlation between the age of the mouse at the experiment start (abscissa) and session lick count
1249 (ordinate) during the multi-spout consumption of a gradient of sucrose concentrations (mice used in multi-
1250 spout consumption of a gradient of NaCl concentrations and quinine concentrations did not have enough
1251 variance in age to conduct this analysis). L) Mean session lick count in mice defined as young (<100d at
1252 start of experiment) and old (>100d at start of experiment) (*t*-test: Young vs. Old*). M) Mean trial lick
1253 count across spouts for data presented in Figure 4. Although there were spout effects observed,
1254 counterbalancing solution and spout pairings across sessions controls for these effects. (*Main effects*
1255 *listed on plots are results of One-Way ANOVA (C-F) or One-Way RM ANOVA (M-O); asterisks depict*
1256 *HSD comparisons between solution sets indicated by horizontal lines; Faded lines and rings depict*
1257 *individual mice; see stats table for details).*

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1261 **Figure 4- Figure Supplement 2**



1262

1263 **Multi-Spout Consumption of Different Gradients of Concentrations of Quinine:** Analysis of 3
 1264 gradients of concentrations of quinine. Each concentration set had water and 4 concentrations of quinine
 1265 with a 1:4 serial dilution starting at 1 mM (low), 5 mM (med), and 10 mM (high). (A-C) left, middle, and
 1266 right columns depict data from low, med, and high concentration sets of quinine. A) Mean binned lick rate
 1267 for all mice for each concentration. B) The mean number of licks per trial for each concentration. C) The

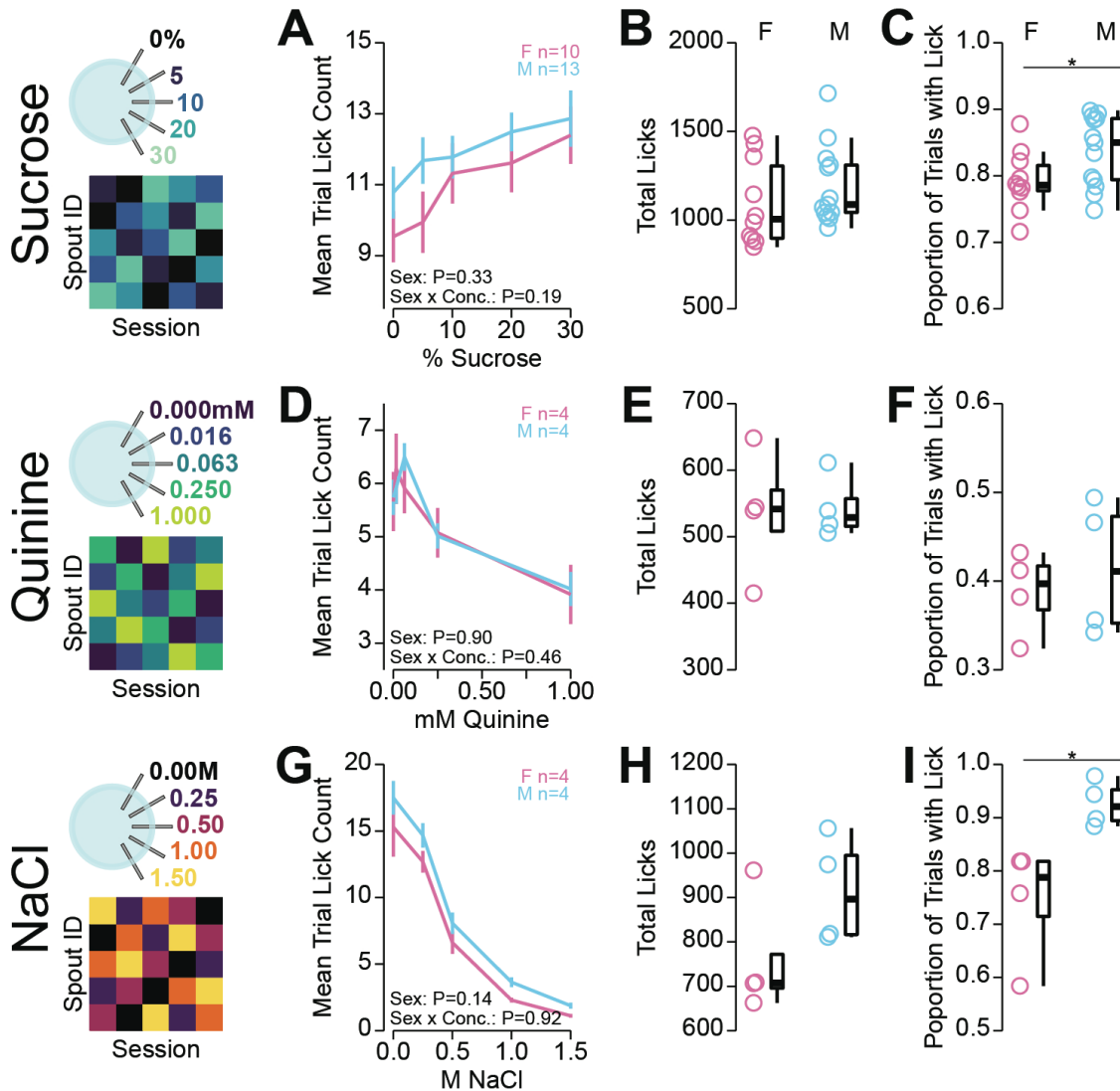
1268 mean number of licks for each concentration per trial binned by 10 trials over the course of the session.
1269 D) The mean number of licks per trial for quinine concentrations greater than 0 shown on a log scale of
1270 quinine concentration on the abscissa. E) Mean session lick count for each concentration set. F)
1271 Proportion of trials with a lick for each concentration set. (*Main effects listed on plots are results of One-*
1272 *Way RM ANOVA; asterisks depict HSD comparisons indicated by horizontal lines; Faded rings depict*
1273 *individual mice; see stats table for details).*

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1277 **Figure 4- Figure Supplement 3**



1278

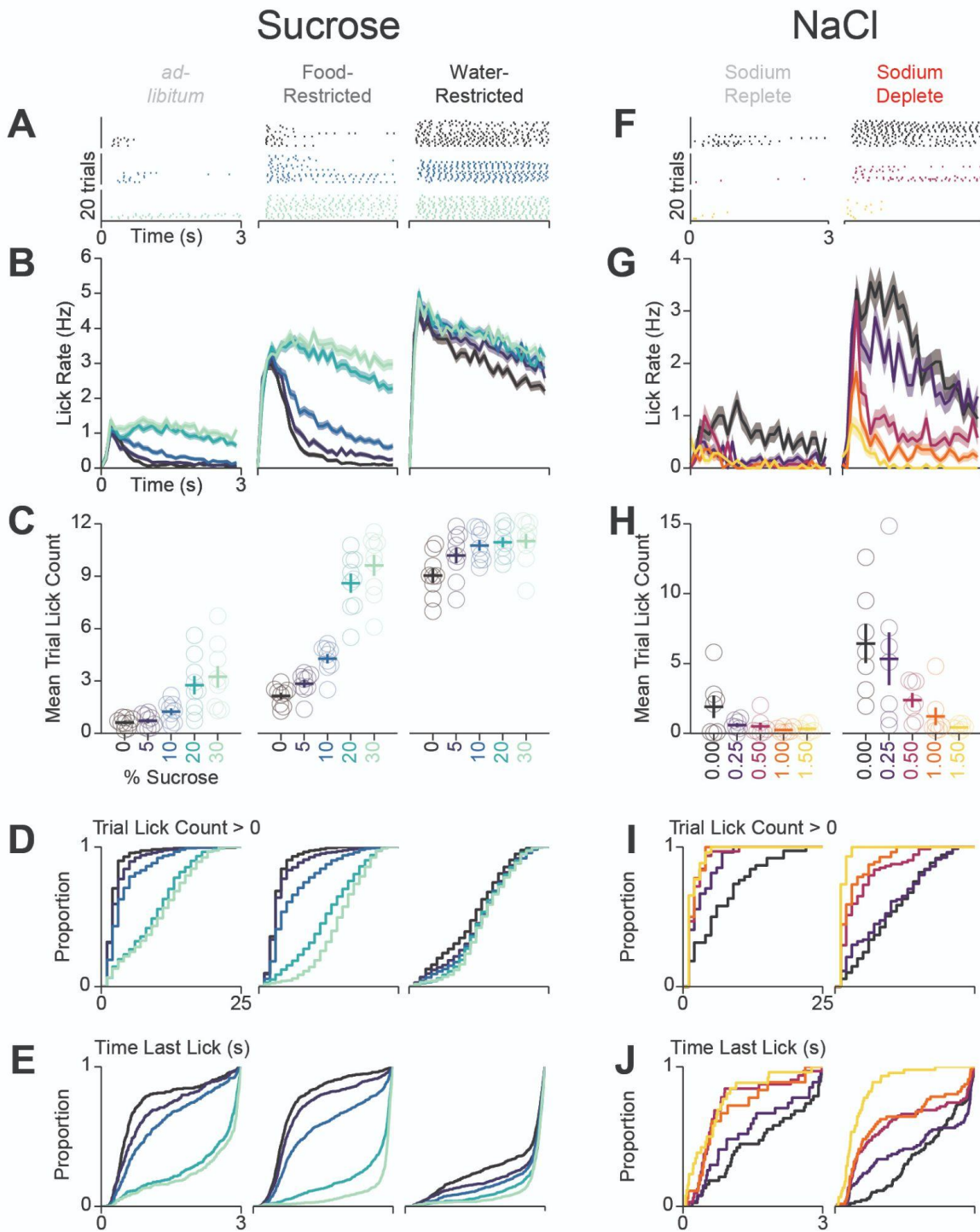
1279 **Sex differences in Head-Fixed Multi-Spout Consumption Behavior:** Investigation of potential sex
 1280 effects on behavior in the multi-spout brief-access assay shown in Figure 4. (A-B) Consumption of a
 1281 gradient of concentrations of sucrose. A) The mean number of licks per trial for each concentration. B)
 1282 Mean session lick count. C) Proportion of trials with a lick. (D-F) same as (A-B), but for consumption of a
 1283 gradient of concentrations of quinine. (G-I) same as (A-B), but for consumption of a gradient of
 1284 concentrations of NaCl. (Main effects listed on plots are results of Two-Way RM ANOVA; no differences
 1285 between sexes at a corresponding concentration; asterisks indicate sex differences determined by t-test;
 1286 see stats table for details).

1287 **Figure 4- Figure Supplement 3**

1288 **Video of Consumption Behavior in the Multi-NaCl Assay Under Water-Restriction:** Video shows
1289 licking behavior during the first 25 trials of the multi-spout assay for gradients of NaCl concentrations
1290 under water-restriction. Each video depicts a single 3 s trial played back at half-speed. Videos are
1291 organized to display trials from top to bottom (earlier trials on the top), and NaCl concentration from left
1292 to right (lower concentrations on the left). However, concentrations were provided in pseudorandom
1293 order.

1294

1295 **Figure 5- Figure Supplement 1**

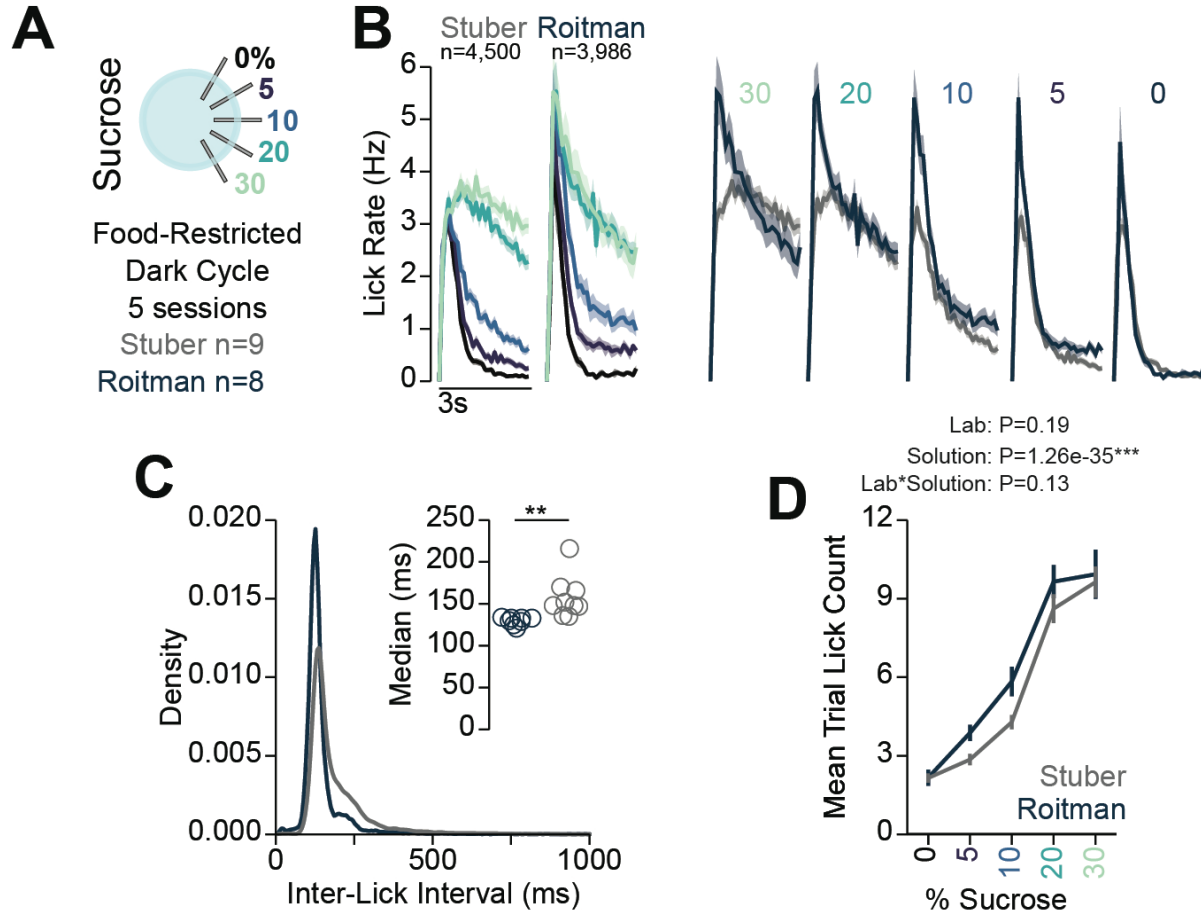


1296

1297 **Behavioral Details for Differences in Consumption Across Homeostatic Demand:** Sets of columns
 1298 containing data from mice in Figure 5 undergoing multi-spout consumption of a gradient of concentrations
 1299 of sucrose (left 3 columns) or NaCl (right 2 columns) across homeostatic demands. A) Lick raster of a
 1300 representative mouse depicting the licks for water, medium concentration, and high concentration during

1301 the 3 s access period. B) Mean binned lick rate for all mice for each concentration. C) The mean number
1302 of licks per trial for each concentration (same data as Figure 5 but with single mice displayed). (D-E)
1303 Cumulative distribution of the number of licks in trials with a lick (D) and the time of the last lick within
1304 each licking bout (E). (F-J) same as (A-E), but for consumption of a gradient of concentrations of NaCl.
1305

1306 **Figure 6- Figure Supplement 1**

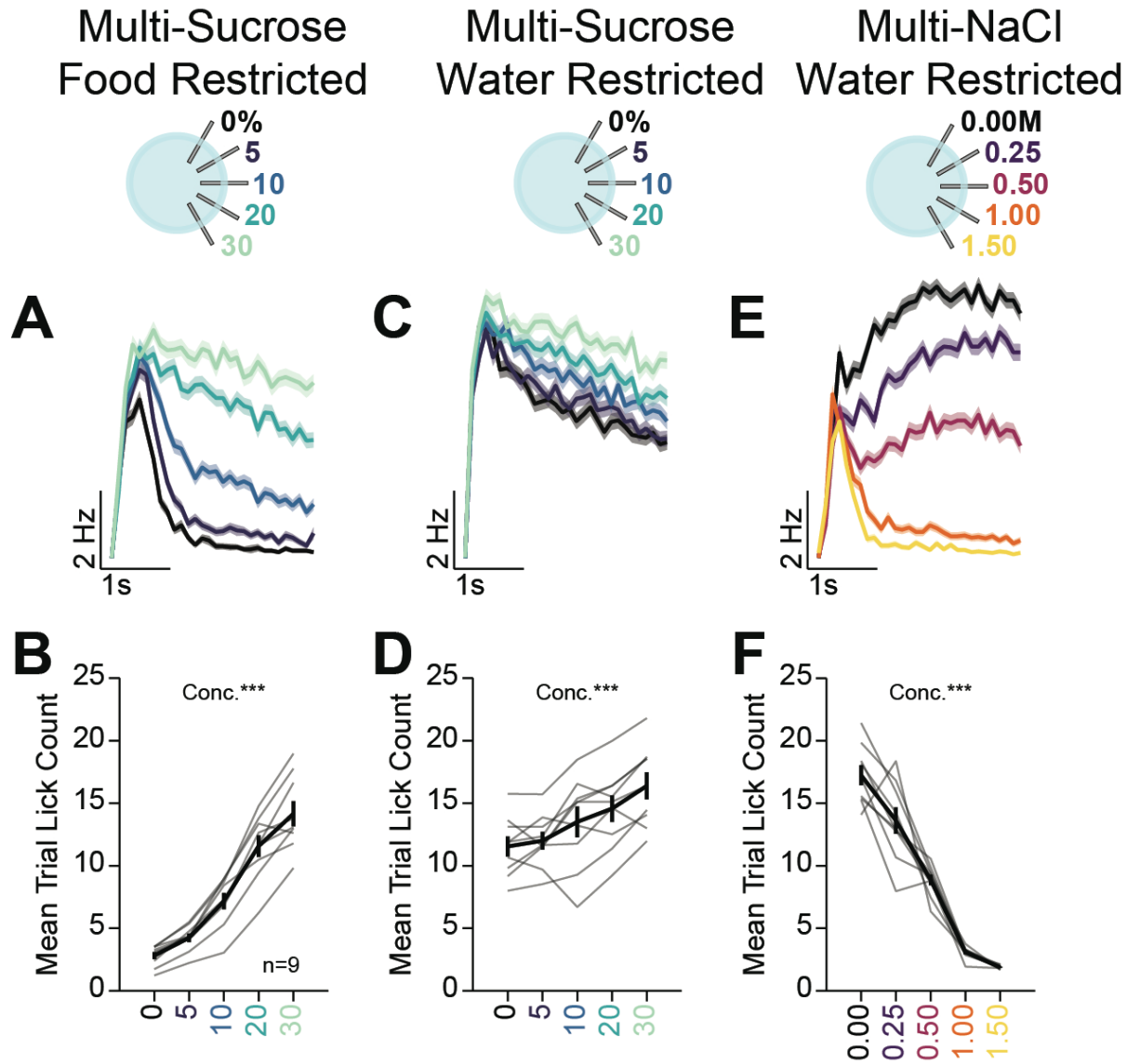


1307

1308 **Comparison of Multi-Spout Behavior Across Labs:** A) Data included in figure: comparison between
1309 mice that were food-restricted and ran through multi-spout brief-access to a gradient of sucrose
1310 concentrations in the dark-cycle in the Stuber lab (data shown in Figure 5) and the Roitman lab (data
1311 shown in Figure 6). B) Mean binned lick rate for all mice for each concentration indicated by color with
1312 facets for lab (left) and for each lab indicated by color with facets for sucrose concentration (right). C)
1313 Density plot of inter-lick intervals for mice ran in the Stuber and Roitman labs indicating a higher density
1314 of low inter-lick-intervals in mice ran in the Roitman lab compared to the Stuber lab. Inset shows the
1315 median inter-lick interval (*t*-test**). D) The mean number of licks per trial for each concentration of sucrose
1316 for mice ran in the Stuber and Roitman labs indicating no effect of lab (Two-Way RM ANOVA stats listed
1317 on plot).

1318

1319 **Figure 7- Figure Supplement 1**



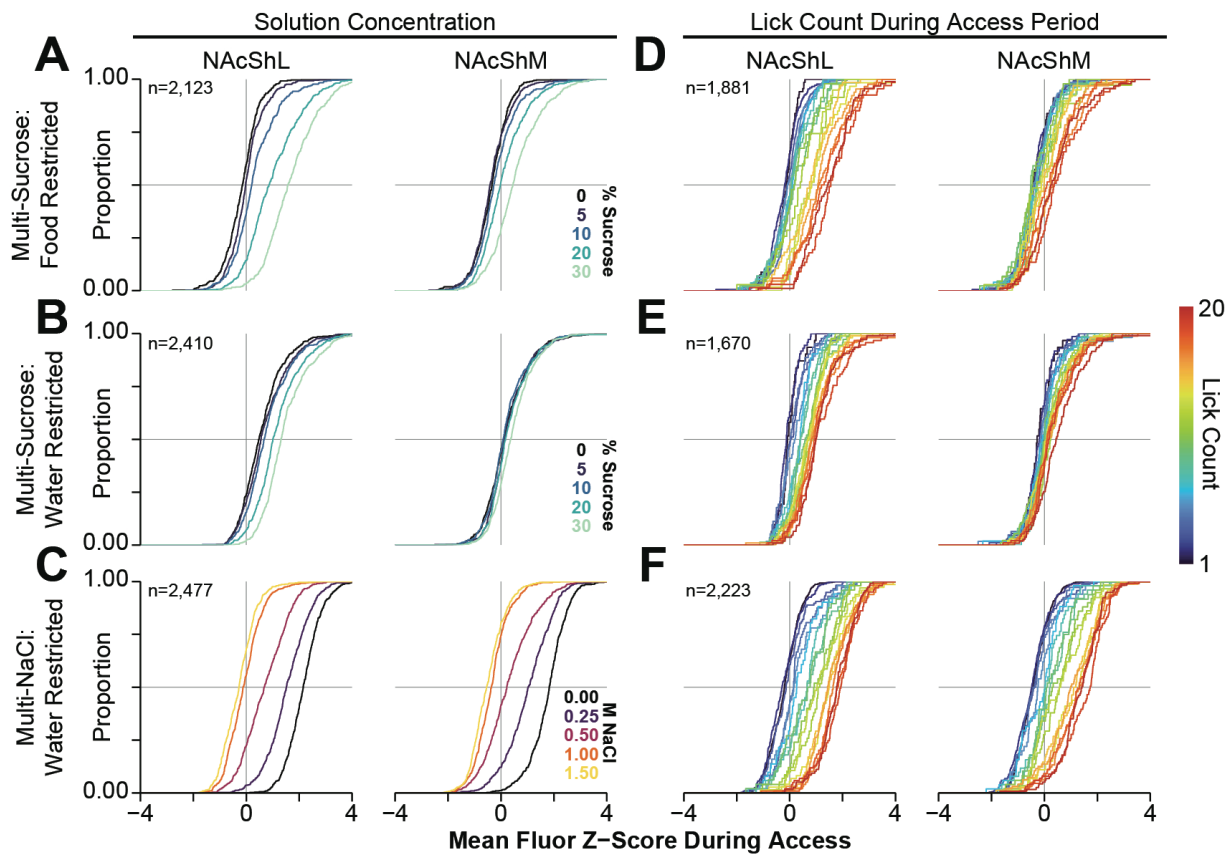
1320

1321 **Multi-Spout Licking Behavior:** Multi-spout licking behavior corresponding to Figure 7. A) Mean binned
1322 lick rate for all mice for each concentration during multi-spout consumption of sucrose under food-
1323 restriction (color indicates concentration of sucrose). B) The mean number of licks per trial for each
1324 concentration of sucrose under food-restriction. C) Mean binned lick rate for all mice for each
1325 concentration during multi-spout consumption of sucrose under water-restriction (color indicates
1326 concentration of sucrose). D) The mean number of licks per trial for each concentration of sucrose under
1327 water-restriction. E) Mean binned lick rate for all mice for each concentration during multi-spout
1328 consumption of NaCl under water-restriction (color indicates concentration of NaCl). F) The mean number

1329 of licks per trial for each concentration of NaCl under water-restriction (*Asterisks listed on plots are the*
1330 *results from One-Way RM ANOVA; see stats table for details*).

1331

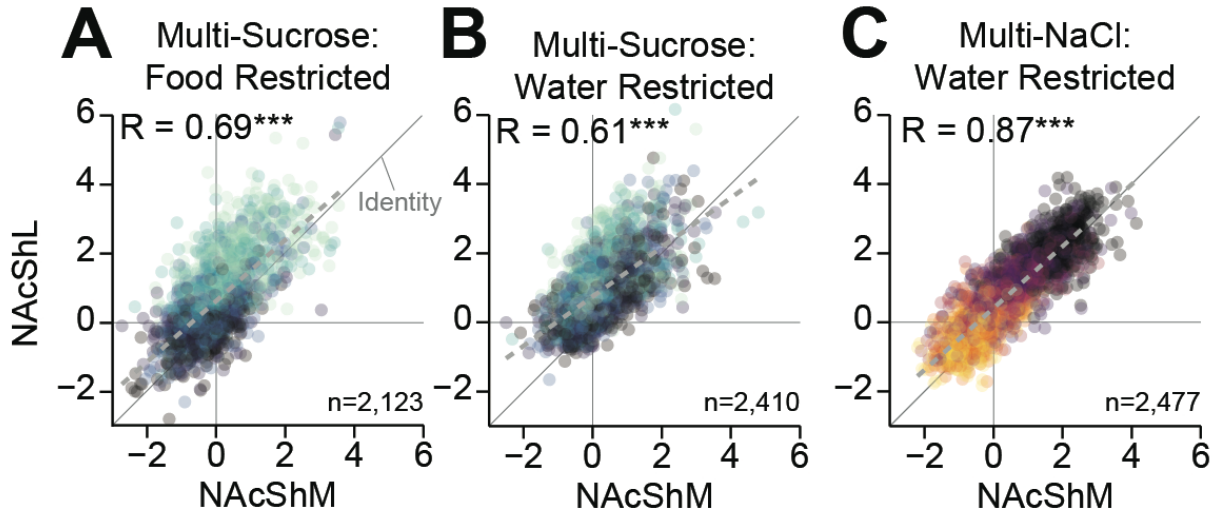
1332 **Figure 7- Figure Supplement 2**



1333

1334 **Cumulative Distribution Functions of GRAB-DA Responses in the NAcSh During Multi-Spout**
1335 **Consumption Behavior:** (A-C) Cumulative distribution functions of mean GRAB-DA fluorescence during
1336 the access period for all trials with at least 1 lick during multi-spout sucrose under food-restriction (A),
1337 multi-spout sucrose under water-restriction (B), and multi-spout NaCl under water-restriction (C) (color
1338 indicates the solution ID as indicated in right inset). (D-F) Cumulative distribution functions of mean
1339 GRAB-DA fluorescence during the access period for all trials with 1-20 licks during multi-spout sucrose
1340 under food-restriction (D), multi-spout sucrose under water-restriction (E), and multi-spout NaCl under
1341 water-restriction (F) (color indicates the number of licks during the trial as indicated in right inset).

1342 **Figure 7- Figure Supplement 3**

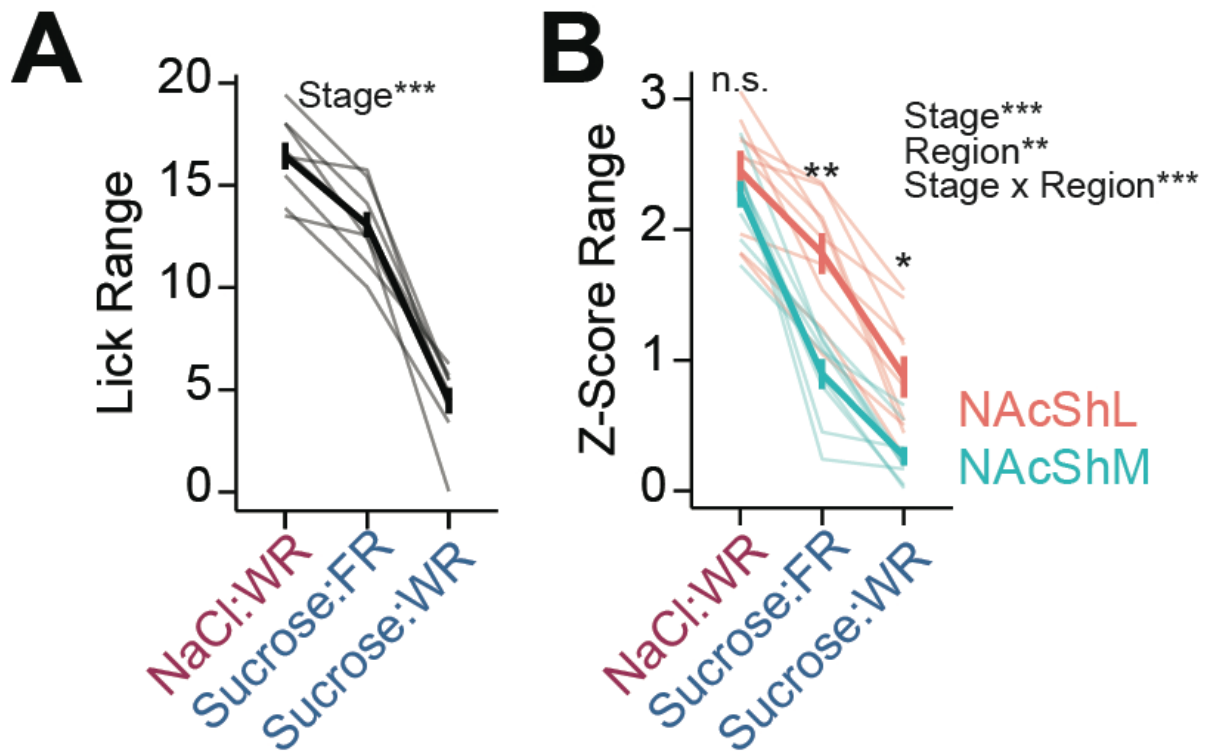


1343

1344 **Linear Correlation of Dopamine Dynamics during Multi-Spout Consumption:** (A-C) Correlations
1345 between NAcShM and NAcShL mean GRAB-DA fluorescence during the access period for all trials with
1346 at least 1 lick during multi-sucrose under food-restriction (A), multi-sucrose under water-restriction (B),
1347 and multi-NaCl under water-restriction (C) (*** Correlation $P < 0.001$; see stats table for details).

1348

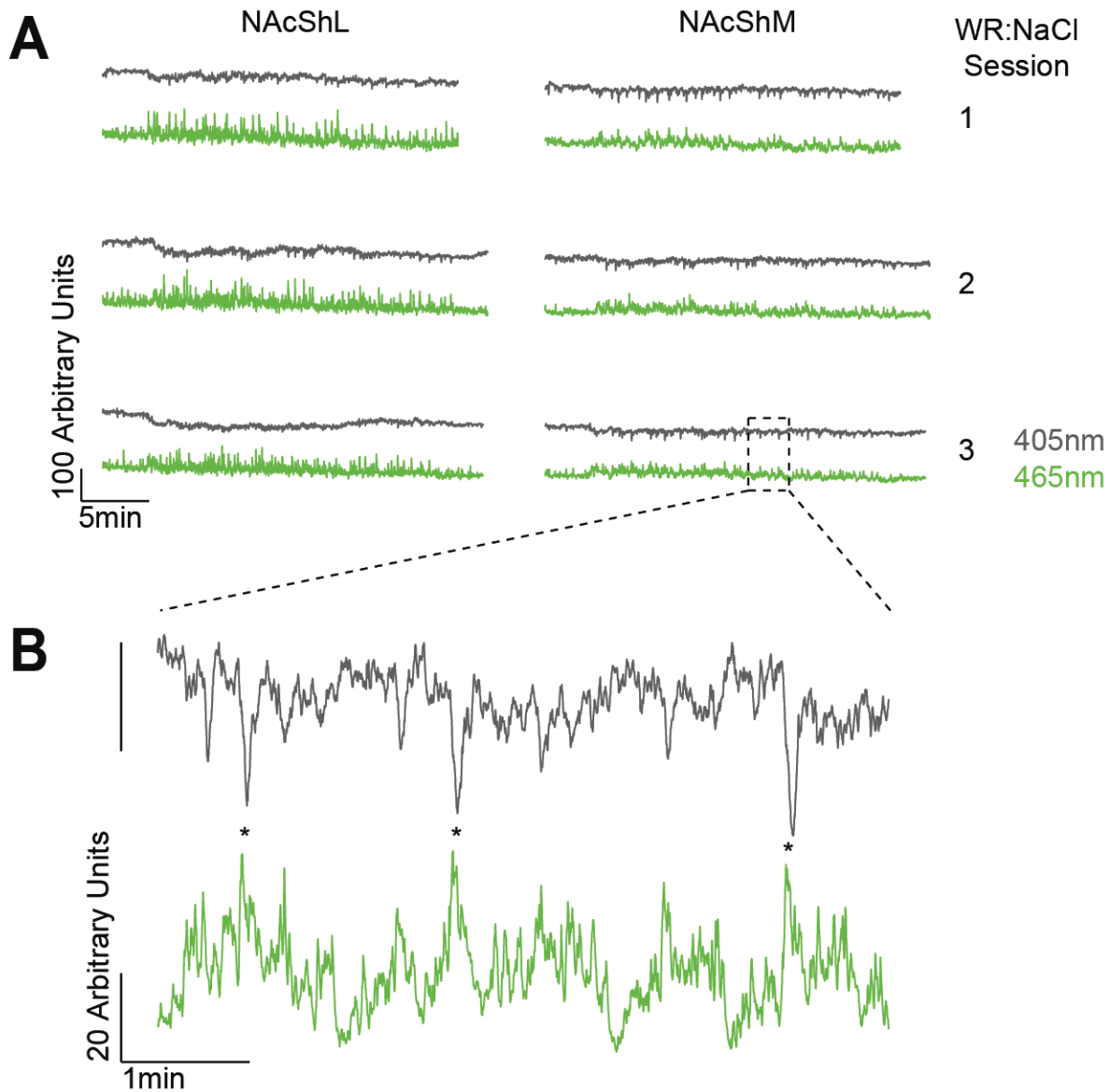
1349 **Figure 7- Figure Supplement 4**



1350

1351 **Range of Licking and NAcSh Dopamine Signals during Multi-Spout Consumption Behavior:** (A)
1352 Range of licking (absolute difference in licking during access to highest and lowest concentrations) across
1353 each stage of the task (WR: water-restricted, FR: food-restricted) (*One-Way RM ANOVA: set****). (B)
1354 Range of GRAB-DA fluorescence signals (absolute difference in mean z-score during access to highest
1355 and lowest concentrations) across each stage of the task (*Stats listed on plots are the results from One-*
1356 *Way RM ANOVA; asterisks indicate differences between NAcShM and NAcShL at a corresponding set;*
1357 *all comparisons across stages within a brain region are significant; see stats table for details*).

1358 **Figure 7- Figure Supplement 6**

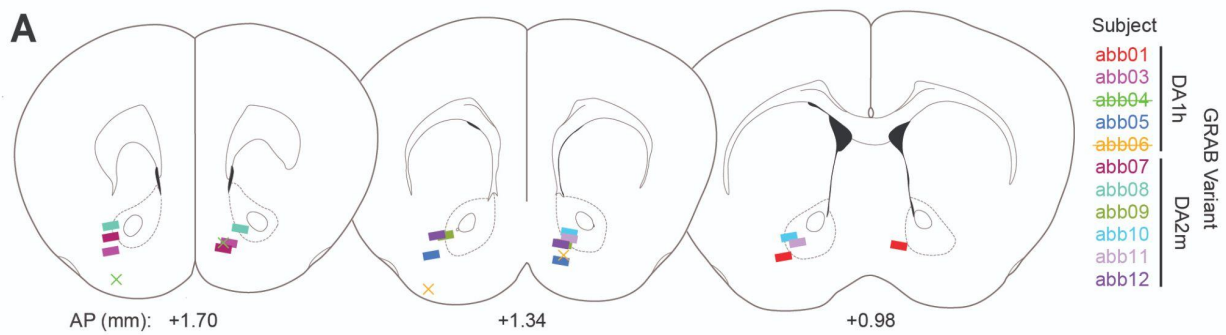


1359

1360 **Representative Full-Session Traces:** (A) Full-session raw traces from mouse abb11 across three
1361 sessions of multi-NaCl under water-restricted conditions (WR:NaCl). Both the 465 nm channel (used for
1362 GRAB-DA2m imaging) and the 405 nm channel (imprecise isosbestic) show a high degree of stability
1363 over the course of the session. B) Zoomed in portion of trace shown in (A). Note that the 405 nm channel
1364 shows negative deflections during positive deflections in the 465 nm channel, which is likely due to the
1365 fact that 405 nm differs from the isosbestic wavelength for GRAB-DA2m of 440 nm (Sun et al., 2020).

1366

1367 **Figure 7- Figure Supplement 6**



1368

1369 **Fiber Placements for Fiber-Photometry:** A) Position of fibers for fiber-photometry experiments shown
1370 in **Figure 7** (AP relative to bregma). Rectangles depict the fiber position determined by histology for mice
1371 included in the analysis, while the “X” symbols depict the fiber position of two mice that were removed
1372 from the experiment for having missed NAcShL placements. In the experiment, the lateralization of
1373 placements for the NAcShL and NAcShM were randomized across mice. For this figure, fiber positions
1374 on the left side of the diagram depict fibers targeting in the NAcShL, and fibers on the right side of the
1375 diagram depict fibers targeting in the NAcShM.

1376

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