Central oxytocin signaling inhibits food reward-motivated behaviors and VTA dopamine responses to food-predictive cues in male rats

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ABSTRACT

Oxytocin potently reduces food intake and is a potential target system for obesity treatment. A better understanding of the behavioral and neurobiological mechanisms mediating oxytocin's anorexigenic effects may guide more effective obesity pharmacotherapy development. The present study examined the effects of central (lateral intracerebroventricular [ICV]) administration of oxytocin in rats on motivated responding for palatable food. Various conditioning procedures were employed to measure distinct appetitive behavioral domains, including food seeking in the absence of consumption (conditioned place preference expression), impulsive responding for food (differential reinforcement of low rates of responding), effort-based appetitive decision making (high-effort palatable vs. low-effort bland food), and postingestive reward value encoding (incentive learning). Results reveal that ICV oxytocin potently reduces food-seeking behavior, impulsivity, and effort-based palatable food choice, yet does not influence encoding of postingestive reward value in the incentive learning task. To investigate a potential neurobiological mechanism mediating these behavioral outcomes, we utilized in vivo fiber photometry in ventral tegmental area (VTA) dopamine neurons to examine oxytocin's effect on phasic dopamine neuron responses to sucrose-predictive Pavlovian cues. Results reveal that ICV oxytocin significantly reduced food cue-evoked dopamine neuron activity. Collectively, these data reveal that central oxytocin signaling inhibits various obesity-relevant conditioned appetitive behaviors, potentially via reductions in food cue-driven phasic dopamine neural responses in the VTA.

57 **Highlights**

58

59 - Central oxytocin inhibits motivated responding for palatable food reinforcement
60
61 - Central oxytocin does not play a role in encoding postingestive reward value
62
63 - Central oxytocin blunts VTA dopamine neuron activity in response to food cues
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Introduction

There is recent interest in targeting the oxytocin system for obesity pharmacotherapy development based on preclinical findings revealing that oxytocin administration reduces caloric intake, increases fat oxidation, and improves insulin sensitivity (Blevins and Ho, 2013; Lawson, 2017; Olszewski et al., 2017; Sabatier et al., 2013). Oxytocin, synthesized centrally in the paraventricular hypothalamic nucleus (PVH) and supraoptic nucleus (SON), reduces food intake through signaling in classic brain feeding centers, including the arcuate nucleus of the hypothalamus (Caquineau et al., 2006; Fenselau et al., 2017; Maejima et al., 2009; Yosten and Samson, 2010), the ventromedial nucleus of the hypothalamus (Noble et al., 2014), and the hindbrain nucleus tractus solitarius, where oxytocin interacts with other feeding-relevant peptides (i.e. neuropeptide Y, alpha-melanocyte-stimulating hormone, glucagon-like peptide-1 and cholecystokinin) (Affleck et al., 2012; Atasoy et al., 2012; Blevins et al., 2003; Katsurada et al., 2014; Larsen et al., 1997; Motojima et al., 2016; Ong et al., 2015; Ong et al., 2017; Rinaman and Rothe, 2002).

These hypothalamic and hindbrain substrates associated with oxytocin's feeding effects are thought to predominantly regulate food intake that is driven by energetic need (Liu and Kanoski, 2018). However, feeding is a complex behavior that requires integration of interoceptive and external environmental cues to coordinate food-directed actions, and therefore must also engage "higher-order" substrates that regulate various cognitive processes. Specifically, learned food-predictive cues provide information about properties of impending food availability, signal affective or incentive properties, and elicit skeletal and autonomic responses to guide complex food-directed behavior (Johnson, 2013). While learning about the food environment is necessary for

89 survival, cues associated with palatable food can also stimulate overeating beyond
90 energetic need and contribute to obesity (Ferrario et al., 2016; Hsu et al., 2018a;
91 Johnson, 2013; Kanoski et al., 2013; Liu and Kanoski, 2018; Petrovich and Gallagher,
92 2007; Rossi and Stuber, 2018). Whether central oxytocin signaling influences higher-
93 order cognitive aspects of feeding that are relevant to excessive caloric intake and
94 obesity is poorly understood. The present study therefore investigated whether central
95 oxytocin signaling influences a multitude of conditioned food-motivated and food cue-
96 associated behavioral tasks that probe [1] palatable food seeking behavior in the absence
97 of consumption, [2] impulsive operant responding for food reinforcement, [3] effort-
98 based decision making (choice between high-effort palatable vs. low-effort bland food),
99 and [4] postingestive reward-value encoding ("incentive learning").

100 Conditioned environmental cues that predict the availability of palatable food
101 activate mesolimbic dopaminergic brain reward pathways to enhance food-directed
102 action and consumption (Berridge, 2007; Hsu et al., 2018a) . Oxytocin receptors are
103 expressed in dopamine neurons in the ventral tegmental area (VTA) and these oxytocin
104 receptor-expressing VTA neurons project to the nucleus accumbens, prefrontal cortex,
105 and extended amygdala (Peris et al., 2017). Furthermore, oxytocin modulates
106 dopaminergic tone in the VTA and can decrease excitatory synaptic transmission
107 through endocannabinoid-dependent mechanisms (Xiao et al., 2018; Xiao et al., 2017).
108 However, whether central oxytocin signaling affects food cue-associated dopamine
109 signaling in awake behaving animals is unknown. Here we explore the effects of central
110 oxytocin signaling on phasic VTA dopamine neuron responses to sucrose-predictive
111 Pavlovian cues as a potential mechanism contributing to oxytocin's anorexigenic effects.

112

113 **Material and Methods**

114 *Subjects*

115 For experiments 1-5, male Sprague-Dawley rats (Envigo, Indianapolis, IN;
116 weighing >250 g) were individually housed in a temperature controlled (22-23°C)
117 vivarium with ad libitum access to water and chow (LabDiet 5001, Lab Diet, St. Louis,
118 MO; except where noted). Given that some experiments involved chronic food
119 restriction, rats were individually housed for precise control of food rationing. Rats were
120 maintained on a 12hr:12hr reverse light/dark cycle (lights off at 1000h). All procedures
121 were approved by the Institute of Animal Care and Use Committee at the University of
122 Southern California.

123 For experiment 6, male Long Evans rats expressing Cre recombinase under the
124 control of the tyrosine hydroxylase promoter [TH:Cre+, Rat Research Resource Center,
125 RRRC#:659] were individually housed in a temperature controlled (22-23°C) vivarium,
126 and were moderately food restricted with 15 g of chow per day throughout the duration
127 of their training and experiments. This modest amount of food restriction permitted
128 gradual weight gain throughout training and testing. Rats were maintained on a
129 12hr:12hr light/dark cycle (lights on at 0700h). Procedures were approved by the
130 Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

131

132 *Surgery*

133 For all surgical procedures, rats were anesthetized and sedated via intramuscular
134 injections of ketamine (90 mg/kg), xylazine (2.8 mg/kg), and acepromazine (0.72
135 mg/kg). Rats were also given analgesic (subcutaneous injection of ketaprofen [5mg/kg]

136 or meloxicam [0.1 mL of 5 mg/mL meloxicam]) after surgery and once daily for 3
137 subsequent days thereafter). All rats recovered for at least one-week post-surgery prior
138 to experiments.

139 For central pharmacological oxytocin delivery, rats were surgically implanted
140 with indwelling guide cannula (26-gauge, Plastics One, Roanoke, VA) targeting the
141 lateral ventricle using the following stereotaxic coordinates, which are relative to the
142 location of bregma and dorsal/ventral (DV) coordinates relative to the skull surface at
143 the cannula implantation site: -0.9 mm anterior/posterior (AP), +1.8 mm medial/lateral
144 (ML), and -2.6 mm DV. Cannula were affixed to the skull as previously described using
145 jeweler's screws and dental cement (Liu et al., 2020). Following one week of recovery,
146 cannula placement was evaluated via measurement of cytoglucopenia-induced
147 sympathoadrenal-mediated glycemic effect that occurs from 210 μg (in 2 μl) of 5-thio-D-
148 glucose (5TG) (Slusser and Ritter, 1980). Food was removed 1 hr prior to the placement
149 test. Rats were injected with 5TG via injectors that extended 2 mm beyond the end of
150 the guide cannula, and blood glucose was measured at 30, 50, 90, and 120 min post-
151 injection. Placement was deemed correct if blood glucose doubled within the
152 measurement period. For rats who did not pass, injector length was adjusted until
153 correct placement was achieved.

154 For fiber photometry, a Cre-dependent virus containing the construct for a
155 genetically encoded Ca^{2+} indicator (AAV1.Syn.Flex.GCaMP6f.WPRE.SV40) was
156 unilaterally administered to the VTA of TH:Cre+ rats (VTA; 1 μl of 0.5e13 GC/mL: AP -
157 5.4, ML -0.7, DV -815, mm) using a rate of 0.1 μl/min and a 5 min post infusion period
158 to allow for diffusion before the injector was removed. The combination of Cre-

159 dependent construct and transgenic rat leads to strong and selective construct
160 expression in dopamine neurons (Konanur et al. 2020; Decot et al. 2017). Next, an optic
161 fiber (flat 400um core, 0.48 NA, Doric Lenses Inc.) was implanted directly above the
162 injection site (AP -5.4, ML -0.7, DV -8.00). Lastly, an ICV cannula was implanted as
163 described above. Rats were given 2-week recovery prior to behavioral training
164 procedures. Rats with missed fiber optic placement were excluded from the experiment
165 (n=3 removed).

166

167 *Behavior*

168 *Conditioned Place Preference (CPP):* CPP training and testing procedures (Fig
169 1A) were conducted as described previously (Hsu et al., 2015; Kanoski et al., 2014b;
170 Kanoski et al., 2011b). Briefly, the CPP apparatus consisted of two conjoined plexiglass
171 compartments with a guillotine door separating the two sides (Med Associates, Fairfax,
172 VT, USA). The two sides (contexts) were distinguished by wall color and floor texture.
173 Rats (n=18, n=8/aCSF, n=9/oxytocin) were given one 15 min habituation session with
174 the guillotine door open and video recording to measure time spent in each context. For
175 each rat, the least preferred context during habituation was designated as the food-
176 paired context for subsequent training.

177 Training occurred in the early phase of the dark cycle and home-cage chow was
178 pulled 2.5 hr prior to each training session. CPP training consisted of 16 daily, 20-min
179 sessions: eight sessions isolated in the food-paired context and eight sessions isolated in
180 the non-food-paired context. Context training order was randomized. During the food-
181 paired sessions, 5 g of 45% kcal high fat/sucrose diet (D12451, Research Diets, New
182 Brunswick, NJ, USA) was placed on the chamber floor, and no food was presented

183 during non-food-paired sessions. All rats consumed the entire 5 g of food during each
184 food-paired session.

185 CPP testing occurred 2 days after the last training session using a between-
186 subjects design, and groups were matched for baseline context preference. Immediately
187 before the testing session, rats received either lateral ICV 1 μg/1μl oxytocin (Bachem,
188 Torrance, CA, USA) or artificial cerebrospinal fluid (aCSF) vehicle injections. During
189 testing, the guillotine door remained open and rats were allowed to freely explore both
190 contexts for 15 min. No food was present during testing. Time spent in each context
191 during the test was later calculated from video recordings by an experimenter blind to
192 the group and context-food assignments. The dependent variable was the percentage
193 shift in preference for the food-associated context during testing compared with the
194 baseline session.

195

196 *Differential reinforcement of low rates of responding (DRL):* DRL training and
197 testing procedures (Fig 2A) were conducted as described previously (Hsu et al., 2018b;
198 Noble et al., 2019). Rats (n=12, within-subjects design) were first habituated in their
199 home cage to consume twenty 45 mg sucrose pellets (F0023, Bio-Serv, Flemington, NJ,
200 USA)). Throughout DRL training (5 days/week), home cage chow was removed 1 h prior
201 to training, which began at the onset of the dark cycle, and was returned to the rats
202 following training. During training, rats were placed in an operant chamber (Med
203 Associates, Fairfax, VT, USA) containing an active lever (reinforced with one 45 mg
204 sucrose pellet) and an inactive lever (non-reinforced). Training sessions were 45 min
205 long, during which both levers were extended at the start of the session and retracted at

206 the end of the session. Neither lever retracted at all during the session, and no visual or
207 audio cues were used during training or testing. For the first five days of training, rats
208 were on a DRL0 schedule, where each active lever press was reinforced with a 0 sec time
209 delay. They were then switched to a DRL5 schedule for five days, where rats had to
210 withhold presses on the active lever for at least a 5 sec interval for each active lever press
211 to be reinforced. Active lever presses that occurred before the 5 sec had elapsed were not
212 reinforced and the timer was restarted. They were then switched to 5 days of DRL10 (10
213 sec withholding period) and 10 days of DRL20 (20 sec withholding period). Efficiency in
214 DRL was calculated as the number of pellets earned divided by the number of active
215 lever presses.

216 The DRL test was conducted using a within-subjects design and no training
217 sessions occurred on days between tests. Oxytocin (Bachem, Torrance, CA, USA) or
218 vehicle treatment order was counterbalanced based on efficiency during training, and
219 treatments were separated by 72 h. On test days, home cage chow was removed 1 h prior
220 and testing began at dark onset. ICV injections of 1 μg/μl oxytocin or vehicle were
221 administered immediately prior to testing on a DRL20 schedule.

222

223 *Progressive Ratio (PROG) / Chow Feeding Choice Task:* Training and testing in
224 the PROG/Chow choice task (Fig 3A) was adapted from Salamone and colleagues
225 (Randall et al., 2012; SanMiguel et al., 2018; Yohn et al., 2016). Following surgical
226 recovery, rats (n=11, within-subjects design) were brought down to and maintained at
227 85% of their free-feeding body weight. Rats were habituated to twenty 45 mg sucrose
228 pellets (F0023, Bio-Serv, Flemington, NJ, USA) in the home cage prior to training.

229 Behavioral sessions (30 min, 5 days/week) were conducted in operant conditioning
230 chambers (Med Associates, Fairfax, VT, USA) and began at the onset of the dark cycle.
231 Rats were initially trained to lever press on a FR1 schedule (one active lever press for
232 one sucrose pellet) for 5 days, and then shifted to a PROG schedule for 10 days. During
233 the PROG session, the ratio started at FR1 and sequentially increased by one every time
234 15 reinforcers were obtained (FR1 x 15, FR2 x 15, FR3 x 15….). The rats were then
235 introduced to free chow (Laboratory Rodent Diet 5001, St. Louis, MO, USA)
236 concurrently available in addition to the PROG sessions for 5 days. Active lever presses,
237 inactive lever presses, pellets earned, highest PROG ratio achieved, and chow intake
238 (including spill) were recorded throughout training.

239 Testing in the PROG/Chow feeding choice task was conducted using a within-
240 subjects design, and treatments were separated by 72 h. Drug treatment order was
241 counterbalanced and matched by performance during the last day of training (ratio of
242 sucrose to chow intake, kCal). The rats received lateral ICV 1 μg/μl oxytocin (Bachem,
243 Torrance, CA, USA) or vehicle immediately prior to 30 min test session, where PROG
244 performance and chow intake were measured (just as in training sessions).

245 In addition to the PROG/Chow feeding choice task, the effect of ICV oxytocin on
246 preference between consumption of free chow and free sucrose concurrently available in
247 the apparatus was also examined (n=10, within-subjects design). Rats were maintained
248 at 85% of their free-feeding body weight and tested at the onset of the dark cycle.
249 Treatments were separated by 48 h and drug treatment order was counterbalanced.
250 Using the same apparatus (but without extension of the levers) and drug injection
251 parameters as the PROG/Chow feeding choice task, intake of freely available chow and

252 freely available sucrose was measured in a 30 min test session and ratio of intake was
253 calculated.

254

255 *Incentive Learning:* Rats were trained in an instrumental incentive learning task,
256 modified from (Wassum et al., 2011a; Wassum et al., 2011b; Wassum et al., 2009). Rats
257 (n=21, n=6/dep aCSF, n=8/dep OT, n=8/sated aCSF) were habituated to twenty 45 mg
258 sucrose pellets (F0023, Bio-Serv, Flemington, NJ, USA) in the home cage prior to
259 training. Training sessions occurred daily at the onset of the dark cycle, and food was
260 pulled 1 hr prior to the start of the session and returned 1 hr after completion of the
261 session. Each session started with insertion of the levers where appropriate and ended
262 with retraction of the levers.

263 For magazine and single-action instrumental training, rats received 3 d of
264 magazine training where they received 20 noncontingent sucrose deliveries in the
265 operant chamber with the levers retracted. Following magazine training, rats received 3
266 d of single-action instrumental training, where right lever presses were continuously
267 reinforced up to 20 pellets or 30 min elapsed.

268 Rats were next trained in the reward-delivery chain phase (Fig 4A). Initially, only
269 the left (seeking) lever was retracted. One left lever press was rewarded by presentation
270 of the right (taking) lever, and one right lever press was rewarded with delivery of one
271 45 mg sucrose pellet. This reward-delivery chain lasted until 20 pellets were earned or
272 30 min elapsed. The seeking lever was continuously rewarded with the taking lever for
273 four sessions, then the reinforcement schedule was increased to random ratio (RR)-2 for
274 four sessions, and then to RR-4 until stable lever pressing was obtained (approximately

275 three to five sessions). The taking lever was always continuously rewarded (FR-1) and
276 retracted after sucrose delivery. Rats that did not earn 20 sucrose pellets on a RR-4
277 schedule were pulled out from the experiment.

278 For the US exposure phase, rats were then split into groups of 1 h restriction
279 (sated) + aCSF, 24 h restriction (dep) + aCSF, or 24 h restriction (dep) + oxytocin (Fig
280 4B). Rats were appropriately food restricted and received 1 μg/μl oxytocin or vehicle ICV
281 injections immediately prior to an exposure session. Exposure session was held in a
282 novel context, where all rats received 30 noncontingent sucrose pellets to consume in 40
283 min. The exposure session was followed by 2 d of ad libitum re-feeding in the home
284 cage.

285 For the incentive learning test session, all rats were 24 h food-deprived prior to
286 the test session that occurred in the operant chambers. Rats were tested under
287 extinction in the reward-delivery train on an RR-4 reinforcement schedule for 4 min.
288 Right lever presses, left lever presses, and seconds between right (taking) lever presses
289 were measured.

290 *Open field:* General activity level and anxiety were measured in open field, which
291 consists of a large gray bin, 60 cm (L) X 56 cm (W), that is placed under diffuse even
292 lighting (30 lux). A center zone is marked in the bin [19 cm (L) X 17.5 cm (W)]. AnyMaze
293 Software (Stoelting Co., Wood Dale, IL) was used to track rat movement. Rats (n=9,
294 within-subjects) were tested under ad lib feeding conditions 1 hr after the onset of the
295 dark cycle. Rats received 1 μg/μl oxytocin or vehicle ICV injections immediately prior to
296 the test, where they were allowed to freely explore the arena for 10 min. The apparatus
297 was cleaned with 10% ethanol after each rat was tested.

298 *Cue-predictive reward task for measurement of VTA dopamine neuron activity:*
299 After in vivo fiber photometry preparation, food restricted rats (n=4) were trained to
300 associate a cue with brief access to sucrose. Training and experimental sessions were
301 conducted during the light phase in standard operant chambers (ENV-009A-CT, Med
302 Associates, Fairfax, VT, USA). While the procedures described above were conducted
303 during the dark cycle, photometry protocols were established and optimized in the light
304 cycle (Konanur et al., 2020) and thus rats underwent daily training for 8 consecutive
305 days during the light cycle. Each training session was comprised of 40 trials where a
306 trial consisted of a 1 sec audio cue (4.5 kHz tone or white noise) followed by either 20
307 sec availability of a retractable sipper containing a 0.3 M sucrose solution (CS+ trials;
308 n=20) or a dry sipper (CS- trials; n=20). Licks were timestamped using a contact
309 lickometer and controller (ENV-252 M; ENV-250, Med Associates Inc.). The audio cue
310 paired with each sipper was counterbalanced across rats. Trials were separated by a
311 randomly selected, variable inter-trial interval (32-48 sec). Following eight training
312 days, test sessions were conducted identical to training sessions but lateral ventricle
313 injections of vehicle, 0.3 μg/ μl oxytocin, or 1 μg/μl oxytocin (within-subjects) were
314 made immediately prior.

315

316 *Fiber Photometry and Signal Normalization*

317 For in vivo fiber photometry, LEDs delivered 465 nm (Ca^{2+} dependent) and 405
318 nm (Ca^{2+} independent) excitation. Intensity of the 465 nm and 405 nm light were
319 sinusoidally modulated at 211 Hz and 531 Hz, respectively, for all recording sessions.
320 Intensity was then coupled to a filter cube (FMC4 contains excitation filters at 405 nm,
321 460–490 nm and emission filters at 500–550 nm, Doric Lenses), and converged into an

322 optical fiber patch cord mated to the fiber optic implant. GCaMP6f fluorescence was
323 collected and focused onto a photoreceiver (Visible Femtowatt Photo- receiver Model
324 2151, Newport). A lock-in amplifier and data acquisition system (RZ5P; Tucker Davis
325 Technologies) was used to demodulate 465 nm and 405 nm excited fluorescence.
326 Behavioral events (e.g. cue, licks) were timestamped and sent as digital inputs to the
327 same data acquisition system and recorded in software (Synapse Suite, Tucker Davis
328 Technologies). The signal was then processed with Fourier transformed subtraction to
329 calculate fluorescence due to fluctuations in Ca^{2+} and bleaching and movement artifacts
330 ($\Delta F/F$). The subtracted signal was smoothed using a custom fifth order bandpass
331 butterworth filter (cutoff frequencies: 0.05 Hz, 2.25 Hz).

332 To compare behavior-related responses across recording sessions, the smoothed
333 Fourier subtracted Ca^{2+} specific signal of each session was normalized by the mean
334 transient Ca^{2+} amplitude of that session (Konanur et al., 2020). A transient was defined
335 as a point 3 standard deviations above the previous point. The normalized signal was
336 then aligned to a 15 sec cue window around cue onset. All data processing was
337 performed using custom MATLAB scripts (available upon request).

338

339 *Statistical Analyses*

340 All experiments met assumptions of normality (Shaprio Wilk), and Experiments
341 2-6 met assumptions for equality of variance (Levene's tests). Experiment 1 did not pass
342 assumption of equality variance test, and was thus analyzed using between-subjects
343 Welch's t-test. Experiments 2, 3, and 5 were analyzed using paired t-tests. 15-min time
344 blocks of active presses, pellets earned, and efficiency in experiment 2 (DRL) were

345 analyzed using two-way analysis of variance (ANOVA) with time and drug as within-
346 subjects variables, with Bonferroni post-hoc tests for multiple comparisons. Experiment
347 4 was analyzed using a one-way ANOVA and Bonferroni post-hoc tests for multiple
348 comparisons.

349 All photometry data were analyzed using parametric repeated measures one or
350 two-way ANOVAs. In all photometry experiments, signal windows were averaged across
351 trials for individual rats, then between rats. For experiment 5, acquisition of CS+/CS-
352 discrimination was measured by 1st lick latency and % of trials containing at least one
353 lick. Analyses here utilized two-way repeated measures ANOVA with cue and day as
354 within-subjects variables. The magnitude of cue-evoked dopamine neuron transients
355 (mean of signal 0 s to 1 s after cue onset) during CS+ compared to CS- were analyzed
356 using student's t-test. In oxytocin treatments involving fiber photometry, mean signal 1
357 s after cue onset and behavioral measures in each drug treatment were compared using
358 one-way repeated measures ANOVAs. When drug main effects were found, Bonferroni
359 post-hoc tests were used to compare individual drug treatments.

360 Effect size estimates were calculated for all significant group differences.
361 Statistical analyses were performed using Graphpad Prism 8 with an α level of
362 significance of $p < .05$.

363

364 **Results**

365 *Experiment 1: Central oxytocin reduces food seeking behavior in the absence of*
366 *consumption (conditioned place preference expression)*

367 ICV oxytocin administration prior to testing significantly decreased conditioned
368 place preference expression for the palatable food-associated context relative to vehicle
369 (expressed as % shift from baseline preference, Fig 1B) ($t_{[15]} = 2.47$, $p = 0.03$; effect size
370 $d = -1.151$), suggesting that central oxytocin signaling reduces palatable food seeking
371 behavior in the absence of consumption.

372

373 *Experiment 2: Central oxytocin decreases food impulsivity (differential reinforcement*
374 *of low rates of responding task)*

375 ICV administration of oxytocin significantly reduced impulsive behavior in the
376 DRL20 schedule (Fig 2). Oxytocin significantly increased the average number of seconds
377 between active lever presses ($t_{[11]} = 6.17$, $p = 7E-05$, fig 2G). Vehicle-treated rats waited
378 an average of 26.05 sec between active lever presses, whereas ICV oxytocin-treated rats
379 waited an average of 92.95 sec between active lever presses. Furthermore, ICV oxytocin
380 significantly decreased the number of sucrose pellets earned ($t_{[11]} = 9.17$, $p = 1.7E-06$, fig
381 2F) and active lever presses ($t_{[11]} = 9.04$, $p = 2E-06$, fig 2E) in the DRL test. There were
382 also no significant differences in number of inactive lever presses (fig 2D). Overall
383 efficiency during the test (fig 2C), a measure of impulsive behavior, was calculated by
384 dividing pellets earned by number of active lever presses. ICV oxytocin treatment
385 significantly increased efficiency in the task ($t_{[11]} = 5.11$, $p = 0.0003$, fig 2C; effect size d
386 $= 1.759$), indicating reduced impulsivity. When efficiency is broken down into three 15
387 min time blocks, there is a significant time ($p < 0.0001$) and drug ($p = 0.0075$) main
388 effect, but no significant time by drug interaction. However, post-hoc analyses reveal
389 significant differences in efficiency between oxytocin and vehicle treatments in the first
390 15 min, but not the mid 15 min or last 15 min (fig 2J).

391

392 *Experiment 3: Central oxytocin shifts food choice from high-effort palatable towards*
393 *low-effort bland food (PROG versus chow choice task)*

394 Motivated behaviors are often characterized by increased work to obtain
395 reinforcement, so we tested effort-based decision making using the PROG versus choice
396 task. A 2-treament within-subjects analysis demonstrates that oxytocin significantly
397 decreased highest PROG ratio achieved ($t_{[10]} = 5.63, p = 0.0002$, fig 3C), pellets earned
398 ($t_{[10]} = 7.76$, $p < 0.0001$, Fig 3D), and free chow consumption ($t_{[10]} = 2.51$, $p = 0.03$, fig
399 3E). Moreover, oxytocin significantly decreased the ratio of sucrose over total intake
400 ($t_{[10]} = 4.29$, $p = 0.0016$; effect size $d = -0.889$, Fig 3F), suggesting a preferential
401 decrease in motivation for high-effort sucrose in favor of low-effort chow. In addition to
402 examining effort-based choice, the effect of oxytocin on preference between freely
403 available chow and freely available sucrose in the conditioning chamber was also
404 measured. ICV oxytocin significantly reduced sucrose intake ($t_{[9]} = 3.77$, $p = 0.0045$, Fig
405 3G). Interestingly, ICV oxytocin had no effect on chow intake (Fig 3H). Thus, oxytocin
406 significantly reduced the ratio of sucrose intake relative to chow intake ($t_{[9]} = 2.37$, $p = 0$
407 0.042; effect size $d = -1.412$, Fig 3I).

408

409 *Experiment 4: Central oxytocin does not affect postingestive reward value encoding*
410 *(incentive learning task)*

411 Incentive learning is the process of modifying the incentive value of a specific
412 food through outcome-nutritive state learning (Balleine, 2001; Balleine and Dickinson,
413 1998a; Balleine and Dickinson, 1998b). Results reveal that ICV oxytocin did not
414 influence the capacity of food restriction to update/enhance the incentive value of
415 consuming sucrose. Analysis of reward seeking behavior during testing (as a percent of

416 baseline) revealed a significant group effect ($F_{[2,16]} = 4.52$, $p = 0.005$; effect size d =
417 1.24). Posthoc comparisons revealed that the dep aCSF and dep oxytocin groups were
418 significantly different from the sated aCSF group, but not from each other.

419

420 *Experiment 5: Central oxytocin administration does not affect general activity levels*
421 *or anxiety-like behavior in an open field test*

422 ICV oxytocin or vehicle was administered immediately prior to an open field test,
423 where rats are allowed to freely move in a novel chamber devoid of food or food-related
424 cues. Results reveal that ICV oxytocin did not significantly affect the distance traveled
425 (Fig 5A), nor did it affect time spent in the center zone (Fig 5B).

426

427 *Experiment 6: Central oxytocin administration suppresses VTA phasic dopamine*
428 *neuron activity in response to cues associated with palatable food.*

429 Rats were trained to discriminate between a conditioned stimulus plus (CS+) that
430 predicted the presentation of sucrose reward and a conditioned stimulus minus (CS-)
431 that predicted no reinforcement. As training progressed, rats successfully discriminated
432 between CS+ and CS- cues as measured by significant differences in latency to first lick
433 and percent of trials with a lick (Fig 6A (top): $F_{[7, 21]} = 5.30$, $p = 0.0013$ main effect of
434 day; $F_{[1, 3]} = 212.77$, $p = 0.0007$ main effect of cue; $F_{[7, 21]} = 19.95$ $p < 0.0001$ day x cue
435 interaction. Fig 6A (bottom): $F_{[7,21]} = 1.405$ $p = 0.26$ no main effect of day; $F_{[1, 3]} =$
436 253.50 $p = 0.0005$ main effect of cue; $F_{[7,42]} = 17.06$ $p<0.0001$ day x cue interaction).
437 Fiber photometry (Fig 6B, histology and fiber optic placement in Fig 6C-D) revealed a
438 robust, time-locked dopamine response to the CS+, but not the CS- (Fig 6E; $t(3) = 8.49$
439 $p = 0.0034$). Further, differences between the CS- and CS+ dopamine response 1 sec

post-cue had an (effect size d = 1.025). We found no effect of central oxytocin on cue-evoked VTA phasic dopamine activity across the entirety of the behavioral session (veh: 0.22±0.05; 0.3μg OT: 0.18±0.03; 1μg OT: 0.18±0.05; data are mean±SEM. $F_{[2,6]}$ = 2.089, p = 0.21). However, a priori analyses were also conducted across the 1st 10 trials (minutes 0-20) based on the short half-life of central oxytocin (Mens et al., 1983) and based on our impulsivity data revealing maximum drug effect in the 1st 15-min epoch (Fig 2H-J). Results from this analysis revealed that both the 0.3 μg and 1 μg dose significantly reduced cue-evoked VTA phasic dopamine responses relative to vehicle treatment (Fig 6F, 6G; $F_{[2,6]}$ = 8.880 p= 0.016 main effect of drug; Bonferroni post-hoc: p = 0.036 vehicle vs. 0.3 μg oxytocin, p = 0.015 vehicle vs. 1 μg OT). Additionally, we demonstrate that central oxytocin has no impact on 1st lick-evoked VTA phasic dopamine activity (Fig 6H, Ca2+ signal aligned to first lick, F[2,6]=0.17, p=0.85, no main effect of treatment), suggesting that central oxytocin signaling impacts VTA phasic dopamine responses to cues associated with food, but not consummatory behaviors.

Discussion and Conclusions

Central oxytocin signaling potently reduces food intake and plays a key role in regulating overall energy balance (Blevins and Ho, 2013; Leng et al., 2008). The behavioral and neuronal mechanisms mediating these effects are poorly understood. In order to probe the behavioral intricacies through which oxytocin inhibits food intake, we looked at the effect of central (lateral ICV) oxytocin administration in a number of distinct conditioned palatable food reinforcement-associated behavioral tasks. Results reveal that central oxytocin reduced palatable food seeking behavior in the absence of

463 consumption in a nonreinforced conditioned place preference test. ICV oxytocin also
464 reduced effort-based palatable food-directed operant responding under ad libitum-fed
465 (nonrestricted) conditions in the DRL task, resulting in a higher task efficiency which is
466 indicative of reduced impulsivity. These findings may also result from decreased
467 motivation for sucrose, and thus we also examined the effect of oxytocin in an effort-
468 based decision-making task. We found that ICV oxytocin reduced effort-based operant
469 responding for palatable food under food-restricted conditions in the PROG vs. chow
470 choice task, where oxytocin shifted the ratio of food consumed from effort-based
471 responding (lever pressing) on a PROG schedule for a preferred food (sucrose) to the
472 less preferred lab chow that is freely available in the apparatus. Consistent with a
473 preference shift in favor of chow over sucrose, ICV oxytocin also significantly reduced
474 consumption of sucrose but not chow in a choice test in which both foods were freely
475 available in the training apparatus. Additional results show that central oxytocin
476 signaling did not influence incentive learning, or the process in which rats learn about
477 the value of rewards based on postingestive feedback. Furthermore, oxytocin did not
478 affect general activity levels in an open field test, suggesting that the reductions in
479 sucrose-motivated behavior were not secondary to lethargy or impaired motor
480 responses. Overall these findings show that central oxytocin signaling reduces palatable
481 food-directed responses under various conditions but does not significantly influence
482 learning about the postingestive reinforcing properties of sucrose consumption.

483 Modulation of phasic dopamine neural responses is a feasible mechanism
484 through which oxytocin reduces palatable food-directed responses, as the conditioned
485 place preference test, DRL impulsivity test, and PROG vs. chow choice test are all

486 influenced by pharmacological manipulations targeting dopamine receptors (Randall et
487 al., 2012; Simon et al., 2013; Spyraki et al., 1982). Moreover, oxytocin neurons in the
488 paraventricular hypothalamic nucleus (PVH) directly project to VTA dopamine neurons
489 (Beier et al., 2015; Peris et al., 2017; Xiao et al., 2017) and oxytocin administration to the
490 VTA suppresses sucrose intake (Mullis et al., 2013). Here we recorded phasic dopamine
491 neuron calcium activity in awake and behaving male rats in an appetitive Pavlovian
492 conditioning task and results revealed that central oxytocin administration suppressed
493 food (sucrose) cue-evoked dopamine neuron responses. These effects were observed
494 during the first ten trials only (within ~20 min into the session), which suggests that
495 oxytocin-mediated inhibition of food reward seeking behavior, consistent with effects on
496 reducing chow intake (Ho et al., 2014; Liu et al., 2020; Ong et al., 2015), is immediate
497 and short-lasting. By providing an immediate and brief anorexigenic signal, oxytocin
498 may be involved in attention-redirection for need-based prioritization of behavior (e.g.,
499 contextual and energetic state-driven shifting between appetitive and social behavior)
500 (Burnett et al., 2019), which is an area for future investigation.

501 Electrophysiological studies demonstrate that oxytocin administration, as well as
502 optogenetic stimulation of oxytocinergic terminals, enhances activity of VTA dopamine
503 neurons (Xiao et al., 2017). In contrast, systemic oxytocin administration reduces
504 dopamine release (as measured by in vivo fixed potential amperometry) in the nucleus
505 accumbens under baseline conditions and following nomifensine, a dopamine reuptake
506 inhibitor (Estes et al., 2019). Consistent with this latter report, present results reveal
507 reduced dopamine neuron activity in response to presentation of a sucrose-conditioned
508 cue. It is possible these conflicting results occur because, while oxytocin reduces

509 motivated behavior for palatable food, oxytocin enhances social reward and modulates
510 attention-orienting responses to external contextual social cues (Shamay-Tsoory and
511 Abu-Akel, 2016). Indeed, VTA oxytocin administration increases dopamine release in
512 the nucleus accumbens in response to sociality and activation of this circuit enhances
513 prosocial behaviors (Hung et al., 2017; Shahrokh et al., 2010), an effect that occurs
514 through coordinated activity with serotonin (Dolen et al., 2013). Thus, the influence of
515 oxytocin signaling on dopamine neural responses may be dependent on the conditions
516 of behavioral testing, with oxytocin enhancing dopamine signaling in the context of
517 sociality, and reducing dopamine signaling in the context of food-associated stimuli.

518 In addition to a complex reinforcement-dependent relationship between
519 oxytocin and dopamine signaling, oxytocin has also been proposed to be conditionally
520 anorexigenic depending on social context (Olszewski et al., 2016). For example,
521 intranasal oxytocin increases food sharing in the common vampire bat (Carter and
522 Wilkinson, 2015) and peripheral oxytocin enhances the social transmission of food
523 preference in rats (Popik and Van Ree, 1993). Moreover, hierarchy social factors
524 influence the effectiveness of oxytocin receptor ligands on food intake. Specifically,
525 administration of an oxytocin receptor antagonist in dominant mice increases the
526 amount of sugar consumed regardless of social or nonsocial contexts, whereas in
527 subordinate rats, it only increases the amount of sugar consumed in a setting devoid of
528 social cues (Olszewski et al., 2015).

529 In contrast to the enhancement of social reinforcement and similar to its effects
530 on reducing food intake and food-motivated responses, oxytocin also potently inhibits
531 ethanol, methamphetamine, and cocaine seeking and consumption (Cox et al., 2017;

532 Kohtz et al., 2018; Peters et al., 2017). Oxytocin has been proposed to promote social
533 affiliation, in part, by reducing behavioral effects of drugs of abuse via alterations in
534 dopamine neurotransmission (Insel, 2003; Mattson et al., 2001), a model that can
535 potentially apply to reduction of food-motivated behaviors. Consistent with this
536 framework, suckling stimulation in lactating dams and cocaine exposure in virgin
537 females activate the dopamine system. However, lactating dams exposed to cocaine
538 show reduced activity in dopaminergic-associated pathways, which the authors suggest
539 may be in part due to oxytocin (Ferris et al., 2005). These studies collectively suggest
540 that oxytocin signaling may prioritize behavior towards socially-relevant cues in favor or
541 other reinforcers. Future studies are needed to further examine whether oxytocin's
542 influence on dopamine signaling and food-motivated behavior are dependent on the
543 nature of the reinforcement and the context.

544 Our findings demonstrate that while dopamine-associated conditioned food
545 reward-directed behaviors were reduced under various conditions following ICV
546 oxytocin administration, central oxytocin did not influence incentive learning.
547 Specifically, ICV oxytocin administered prior to consuming sucrose after an energetic
548 motivational shift (from satiety to hunger/fasted) did not affect sucrose-motivated
549 behavior when subsequently tested after a fast. When considered together with our
550 results showing that central oxytocin reduced dopamine neuron responses to a sucrose-
551 associated cue, these results are consistent with previous literature demonstrating that
552 instrumental incentive learning, using procedures similar to those in the present study,
553 is not affected by treatment of flupenthixol, a D1 and D2 receptor antagonist (Wassum
554 et al., 2011b). Furthermore, our incentive learning results suggest that oxytocin may act

555 downstream via a ventral and not dorsal striatal pathway to influence food-motivated
556 behavior. For example, Tellez and colleagues identified dissociable basal ganglia
557 sensorimotor circuits that encode hedonic versus metabolic values of reward (Tellez et
558 al., 2016), where dorsal striatal descending pathways are recruited to encode the
559 nutritional (postingestive) aspects of food, and ventral striatum descending pathways
560 transmit hedonic (flavor) value of food. These findings combined with present results
561 support a putative model in which oxytocin signals downstream to ventral striatal
562 dopaminergic pathways to reduce conditioned food cue-directed motivated behaviors,
563 without influencing dorsal striatum-mediated postingestive incentive learning.

564 While present results identify a role for central oxytocin signaling in modulating
565 palatable food cue-directed behavior and mesolimbic dopamine signaling, one
566 limitation of the present study is that the mediating sites of CNS oxytocin action were
567 not investigated. Oxytocin receptors are expressed widely in various feeding-associated
568 regions throughout the brain, including in the amygdaloid complex, VTA, hippocampus,
569 basal ganglia, prefrontal cortex, caudal brainstem, and hypothalamus (Freund-Mercier
570 et al., 1987; Otero-Garcia et al., 2016). In addition to the VTA, one region that may be
571 relevant to present results is the ventral subregion of the hippocampus, which has
572 robust oxytocin receptor expression (Freund-Mercier et al., 1987) and is involved in
573 food impulsivity (Noble et al., 2019), conditioned place preference for palatable food
574 (Kanoski et al., 2011a; Zhou et al., 2019), and striatal dopamine signaling (Kanoski et al.,
575 2013). Additionally, the prefrontal cortex plays a critical role in effort-related food
576 choice behavior (Walton et al., 2002) as well as food impulsivity (Hsu et al., 2018b), and
577 may therefore be a likely candidate site of action for oxytocin's effects on food reward-

578 directed behavior. In addition to these forebrain regions, oxytocin signaling potently
579 reduces feeding in the hindbrain nucleus tractus solitarius (mNTS). While this region is
580 more classically associated with meal size and satiation control, recent results link the
581 mNTS in the control of conditioned food reward-directed behaviors (Kanoski et al.,
582 2014a; Liu and Kanoski, 2018). These and other potential sites of action should be
583 explored in future studies, as they may act in conjunction and/or in parallel with
584 dopaminergic mesolimbic ventral striatal pathways to mediate oxytocin's suppression of
585 palatable food cue-directed responses. A second limitation of the present study is that,
586 with the exception of the photometry experiment, we utilized a single dose (1 μg) of
587 oxytocin for behavioral analyses. While this dose was selected based on our previous
588 work where it reliably reduced 30 min chow intake in rats following ICV administration
589 (Liu et al., 2020), oxytocin has been shown to have dose-related U-shaped relationship
590 with social trust behavior in humans (Zhong et al., 2012). Thus, a future direction is to
591 examine whether such U-shaped relationships occur when various doses of central
592 oxytocin are tested on conditioned food reward-directed behaviors. Lastly, with regards
593 to the photometry, the present study recorded dopamine neuron activity in the VTA,
594 however, it is unclear if this activity resulted in dopamine release and, if so, in which
595 terminal regions (e.g. nucleus accumbens, medial prefrontal cortex, etc.). A number of
596 recent investigations have demonstrated dopamine release can occur independent of
597 cell body activity (Mateo et al., 2017; Mohebi et al., 2019; Threlfell et al., 2012). Future
598 work will need to utilize fluorescent dopamine sensors (dlight, GRAB-DA) to determine
599 downstream dopamine release dynamics in response to central oxytocin signaling.

600 Collectively, our results reveal that central oxytocin administration reduces
601 motivated behaviors in response to conditioned food cues under various conditions.
602 Furthermore, oxytocin suppresses food cue-evoked phasic VTA dopamine neuron
603 responses, thus providing a potential neurobiological mechanism for the capacity of
604 central oxytocin signaling to rapidly inhibit palatable food-directed behavior. These
605 results illuminate novel behavioral and neurochemical mechanisms through which
606 oxytocin inhibits food intake, thus further supporting oxytocin's potential as a target
607 system for obesity pharmacotherapy development.

608

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614

615 **Competing Interests**

616 The authors have nothing to disclose.

617 **Figure Captions**

618

619 *Figure 1: ICV oxytocin suppresses food seeking behavior in the conditioned place*
620 *preference (CPP) test*. (A) Schematic of CPP training and test procedures. (B) Effect of
621 ICV oxytocin on time spent on the food-paired side during the test (represented as a
622 percent shift from baseline). Data are mean ± SEM; *p<0.05.

623 *Figure 2: ICV oxytocin reduces impulsive responding in the differential reinforcement*
624 *of low rates of responding (DRL) task*. (A) Schematic of DRL training and testing
625 procedures. (B) Efficiency (pellets earned divided by number of active lever presses)
626 over the last 10 days of DRL20 training. (C) Efficiency for entire test session (45 min)
627 following ICV administration of oxytocin. (D-F) Total inactive lever presses, active lever
628 presses, and sucrose pellets earned for entire test session following ICV administration
629 of oxytocin. (G) Average seconds between active lever presses following ICV
630 administration of oxytocin for entire test session. (H-J) Active lever presses, pellets
631 earned, and efficiency broken into 15 min time blocks following ICV administration of
632 oxytocin. Data are mean ± SEM; *p<0.05.

633 *Figure 3: ICV oxytocin reduces effort-based consumption of sucrose over freely*
634 *available chow in a PROG/Chow choice task*. (A) Schematic of the PROG/Chow choice
635 task. (B) Ratio of sucrose intake over total intake (kCal) over the course of five days
636 training in the choice task. (C-F) Highest PROG ratio achieved, PROG sucrose pellets
637 earned, free chow intake, and ratio of PROG sucrose intake over total intake in the
638 PROG/Chow choice task following ICV oxytocin administration. (G-I) Free sucrose
639 pellets consumed, free chow intake, and ratio of free sucrose intake over total intake in

640 free sucrose/free chow choice task following ICV oxytocin administration. Data are
641 mean ± SEM; *p<0.05.

642 *Figure 4: Oxytocin does not play a role in postingestive reward value encoding in an*
643 *instrumental incentive learning task.* (A) Schematic of reward-delivery chain in an
644 instrumental incentive learning task. (B) Table outlining drug treatment and
645 deprivation state for three experimental groups: sated aCSF, 24 hr food deprived (dep)
646 aCSF, 24 hr food deprived (dep) oxytocin. (C) Reward seeking behavior (% baseline) on
647 the reward delivery chain represented by rate of presses on the right (taking) lever
648 during 4 min extinction session. All rats tested under 24 hr food deprivation. Data are
649 mean ± SEM; *p<0.05.

650 *Figure 5: ICV oxytocin does not affect locomotion or anxiety-like behavior in the open*
651 *field test.* (A) Distance traveled in the open field following ICV oxytocin. (B) Total time
652 spent in the center zone in the open field following ICV oxytocin.

653 *Figure 6: ICV oxytocin reduces food cue-evoked dopamine neuron response in the*
654 *ventral tegmental area.* (A) First lick latency and % trials with licks following the CS+
655 and the CS- during training, where CS+ precedes sucrose presentation and CS- precedes
656 no food reward. (B) Experimental schematic of fiber photometry and oxytocin
657 pharmacology in TH:Cre+ rats (C) Representative AAV1-FLEX-gCaMP (green) and
658 tyrosine hydroxylase (red) expression in the VTA. (D) VTA fiber optic placement. (E)
659 Representative trace (top) and quantitative ΔF/F (bottom) of phasic dopamine activity
660 aligned to the CS+ and CS- cues following training. (F) Color plot of dopamine
661 fluctuations during 15 sec window surrounding presentation of the CS+ and following
662 ICV administration of 0.3 μg or 1 μg oxytocin. Red boxes highlight first ten trials, where

663 oxytocin had greatest effect on phasic dopamine signal. (G) Representative traces (left)
664 and quantitative ΔF/F (right) of cue-evoked phasic dopamine during 15 sec window
665 surrounding presentation of the CS+ and following ICV administration of 0.3 μg or 1 μg
666 oxytocin. (H) Quantitative ΔF/F of first lick-evoked phasic dopamine during 15 sec
667 window surrounding first lick after sipper presentation and following ICV
668 administration of 0.3 μg or 1 μg oxytocin.

669

Figure 1

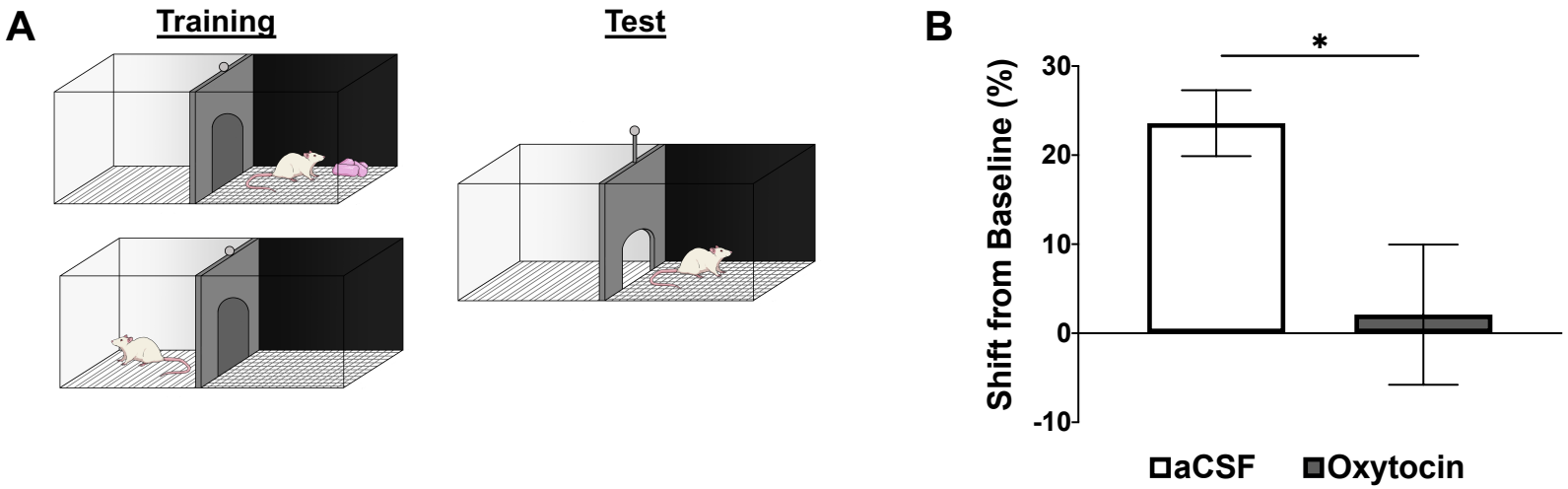

A
Training
Test
B
*
Shift from Baseline (%)
30
20
10
0
-10
aCSF
Oxytocin

Figure 2

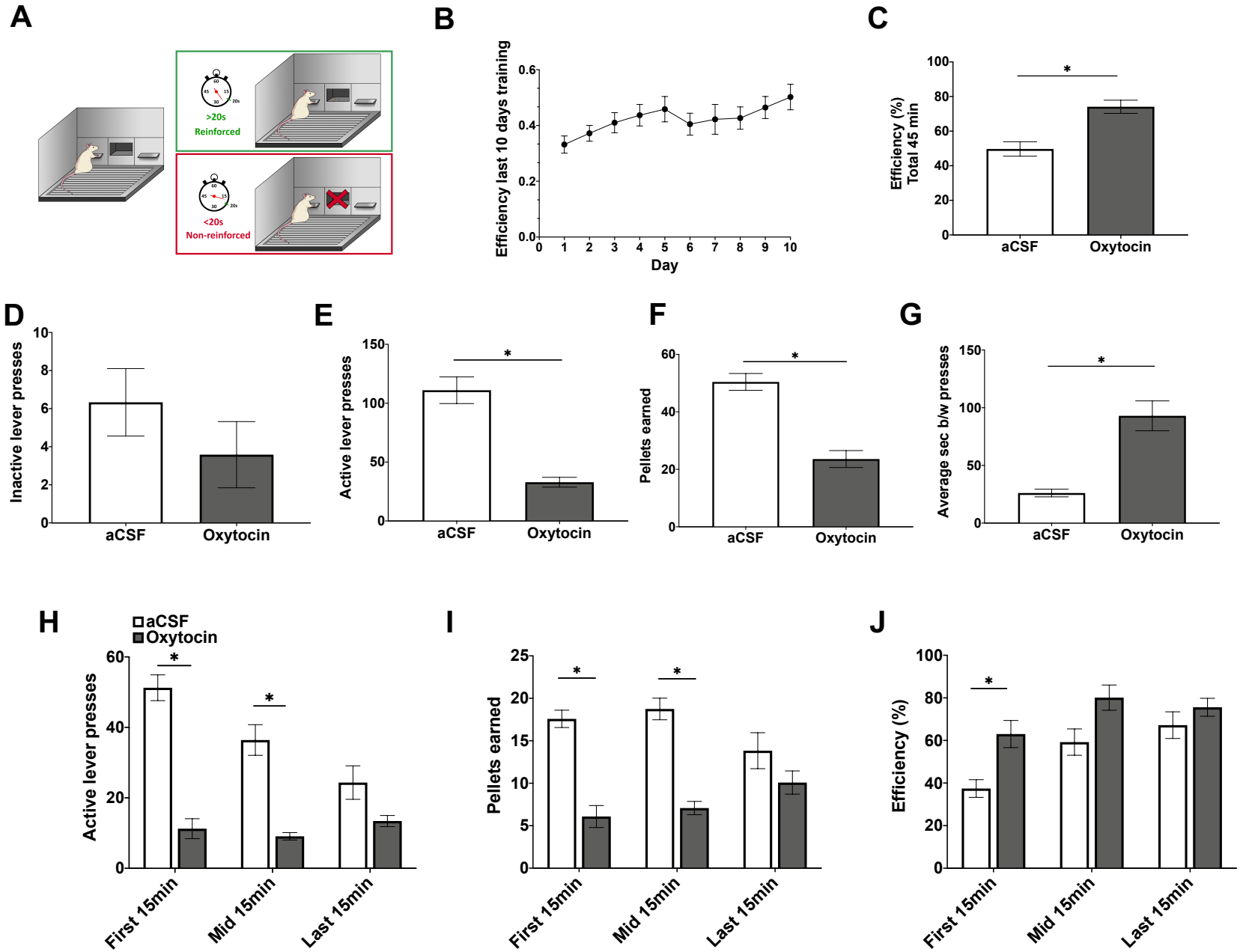

A
>20s
Reinforced
<20s
Non-reinforced
B
Efficiency last 10 days training
Day
C
Efficiency (%)
Total 45 min
aCSF
Oxytocin
D
Inactive lever presses
E
Active lever presses
F
Pellets earned
G
Average sec b/w presses
H
aCSF
Oxytocin
Active lever presses
First 15min
Mid 15min
Last 15min
I
Pellets earned
J
Efficiency (%)

Figure 3

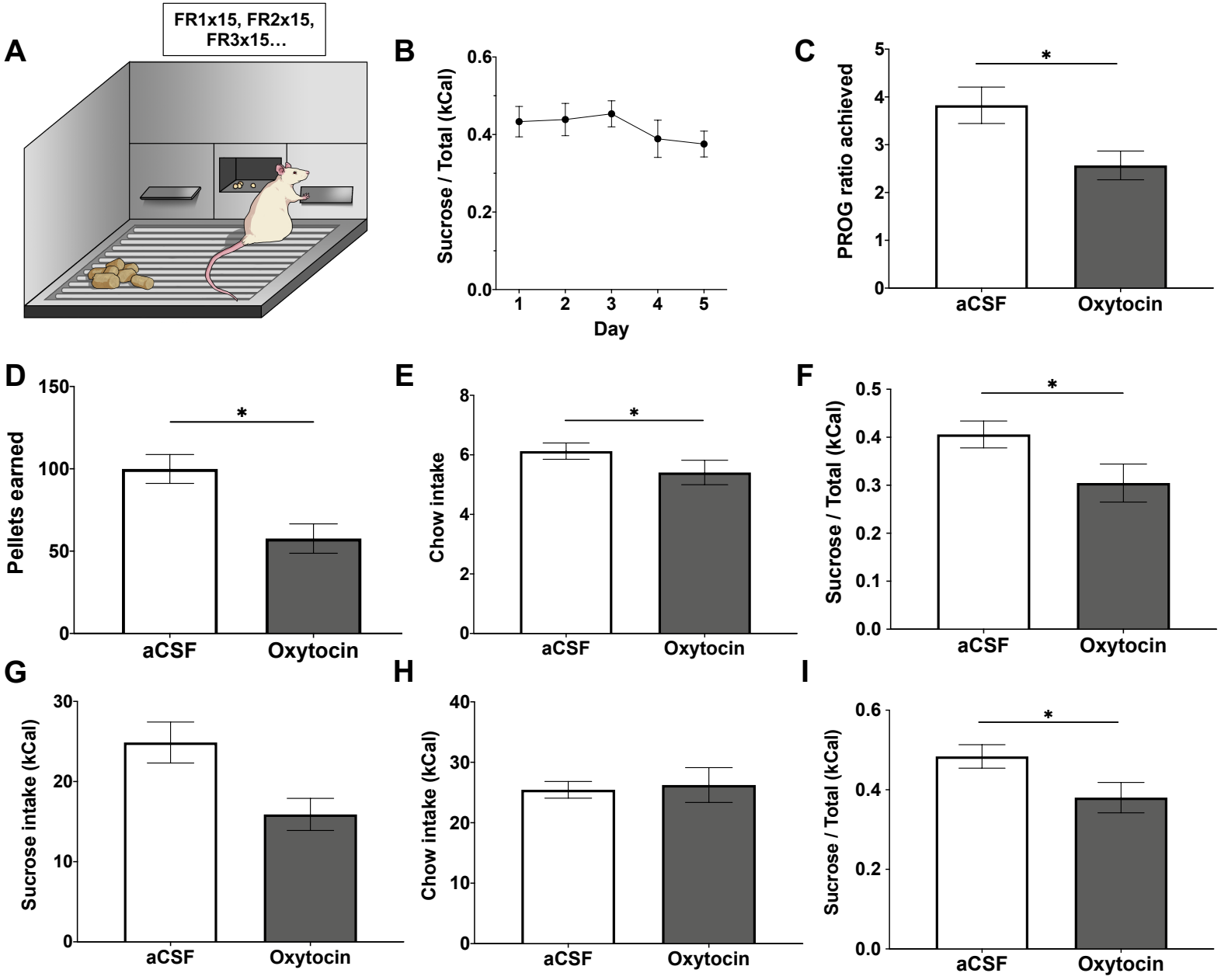
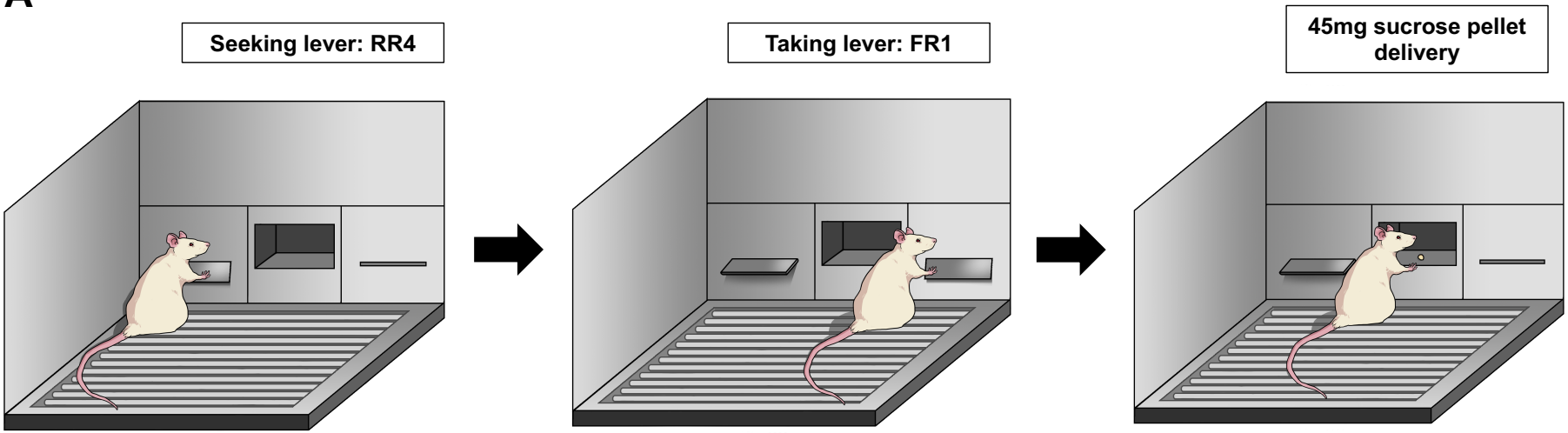

A
FR1x15, FR2x15, FR3x15…
B
Sucrose / Total (kCal)
0.6
0.4
0.2
0.0
1
2
3
4
5
Day
C
PROG ratio achieved
5
4
3
2
1
0
*
aCSF
Oxytocin
D
Pellets earned
150
100
50
0
*
aCSF
Oxytocin
E
Chow intake
8
6
4
2
0
*
aCSF
Oxytocin
F
Sucrose / Total (kCal)
0.5
0.4
0.3
0.2
0.1
0.0
*
aCSF
Oxytocin
G
Sucrose intake (kCal)
30
20
10
0
aCSF
Oxytocin
H
Chow intake (kCal)
40
30
20
10
0
aCSF
Oxytocin
I
Sucrose / Total (kCal)
0.6
0.4
0.2
0.0
*
aCSF
Oxytocin

Figure 4

A



B

Group	Train	Exposure state	Exposure drug	Test state
Sated aCSF	Sated	Sated	aCSF	24 hr food dep
Dep aCSF	Sated	24 hr food dep	aCSF	24 hr food dep
Dep Oxytocin	Sated	24 hr food dep	Oxytocin	24 hr food dep

C

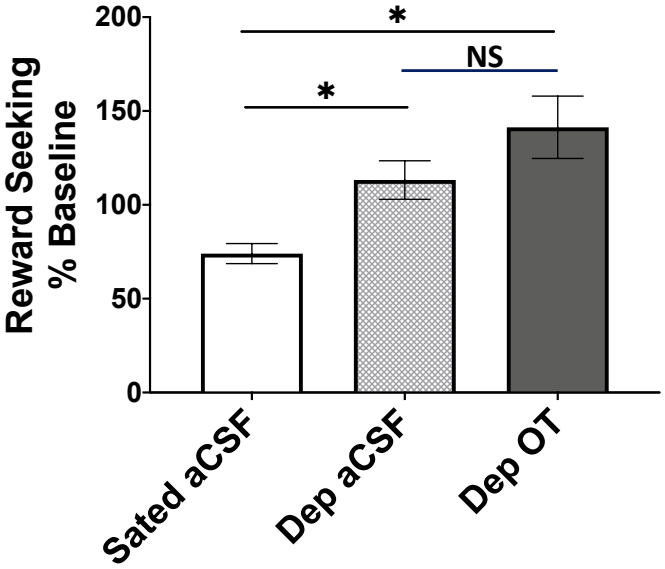


Figure 5

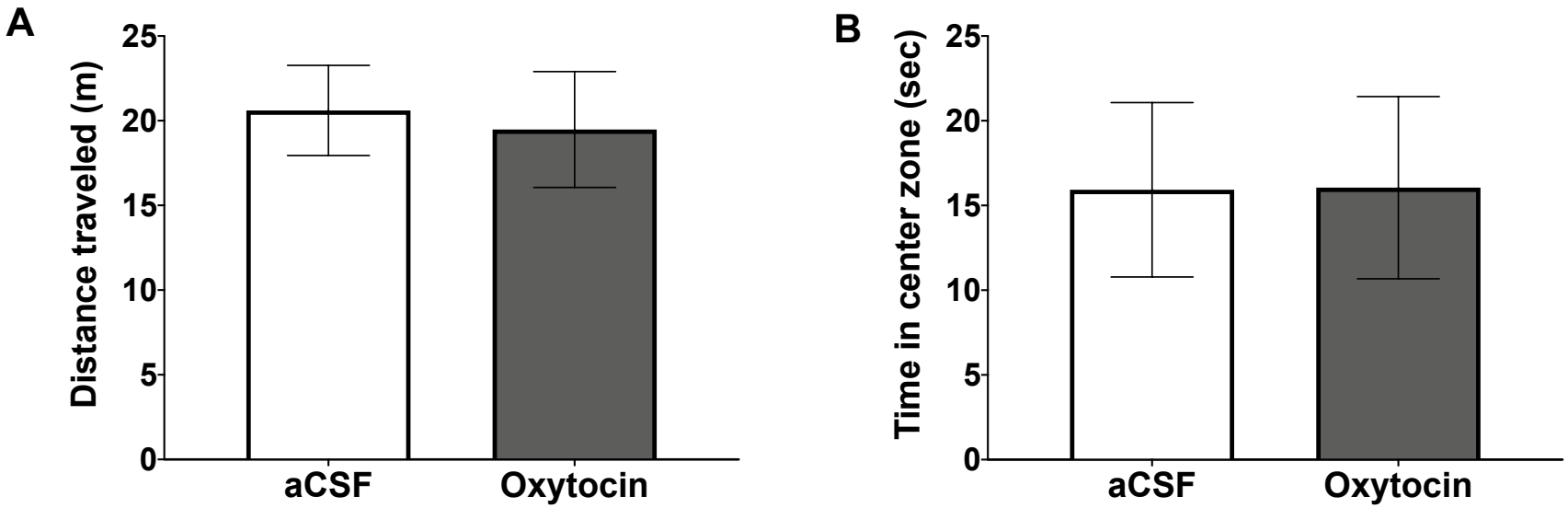
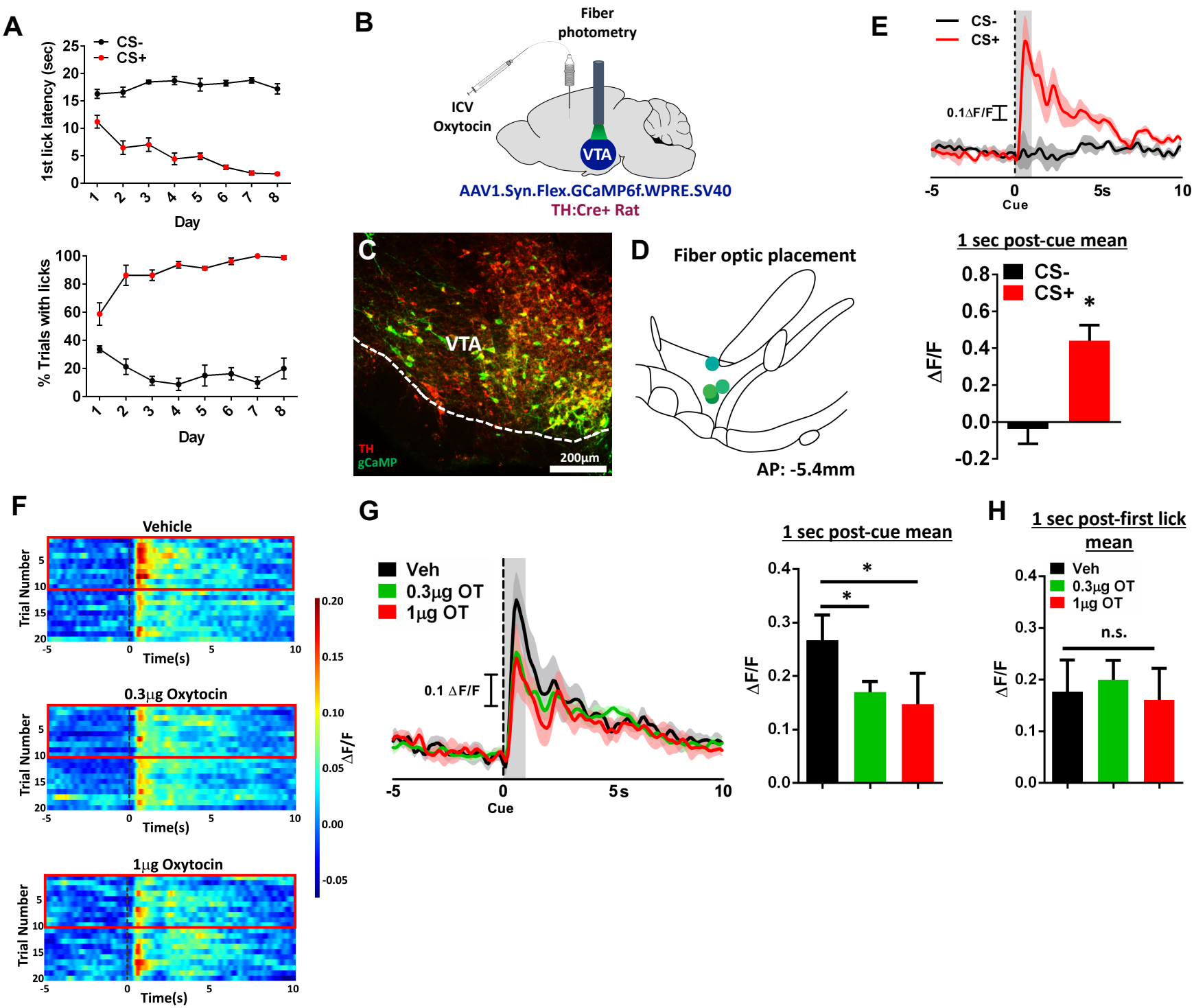

A
Distance traveled (m)
25
20
15
10
5
0
aCSF
Oxytocin
B
Time in center zone (sec)
25
20
15
10
5
0
aCSF
Oxytocin

Figure 6


A
CS-
CS+
1st lick latency (sec)
Day
% Trials with licks
Day
B
Fiber photometry
ICV Oxytocin
VTA
AAV1.Syn.Flex.GCaMP6f.WPRE.SV40
TH:Cre+ Rat
C
VTA
TH
gCaMP
200µm
D
Fiber optic placement
AP: -5.4mm
E
CS-
CS+
0.1ΔF/F
Cue
1 sec post-cue mean
ΔF/F
F
Vehicle
0.3µg Oxytocin
1µg Oxytocin
Trial Number
Time(s)
ΔF/F
G
Veh
0.3µg OT
1µg OT
0.1 ΔF/F
Cue
1 sec post-cue mean
ΔF/F
H
1 sec post-first lick mean
Veh
0.3µg OT
1µg OT
n.s.
ΔF/F

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