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

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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 suppresses food-seeking behavior, impulsivity, and effort-based palatable food choice, yet does not influence postingestive reward value encoding 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 suppressed food cue-evoked dopamine neuron activity and increased latency to consume the sucrose reinforcement. 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.

Highlights

- Central oxytocin inhibits motivated responding for palatable food reinforcement
- Central oxytocin does not play role in regulating postingestive reward value encoding
- Central oxytocin blunts VTA dopamine neuron activity in response to food cues

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, 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 survival,

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

Conditioned environmental cues that predict the availability of palatable food activate mesolimbic dopaminergic brain reward pathways to enhance food-directed action and consumption (Berridge, 2007; Hsu et al., 2018a) . Oxytocin receptors are expressed in dopamine neurons in the ventral tegmental area (VTA) and these oxytocin-receptor expressing VTA neurons project to the nucleus accumbens, prefrontal cortex, and extended amygdala (Peris et al., 2017). Furthermore, oxytocin has been shown to modulate dopaminergic tone in the VTA and can decrease excitatory synaptic transmission through endocannabinoid-dependent mechanisms (Xiao et al., 2018; Xiao et al., 2017). However, whether central oxytocin signaling affects food cue-associated dopamine signaling in awake behaving animals is unknown. Here we explore the effects of central oxytocin signaling on phasic VTA dopamine neuron responses to sucrose-

predictive Pavlovian cues as a potential mechanism contributing to oxytocin's anorexigenic effects.

Material and Methods

Subjects

For experiments 1-4, male Sprague-Dawley rats (Envigo, Indianapolis, IN; weighing >250 g) were individually housed in a temperature controlled (22-23$_{o}$C) vivarium with ad libitum access to water and chow (LabDiet 5001, Lab Diet, St. Louis, MO; except where noted). Rats were maintained on a 12hr:12hr reverse light/dark cycle (lights off at 1000h). All procedures were approved by the Institute of Animal Care and Use Committee at the University of Southern California.

For experiment 5, male Long Evans rats expressing Cre recombinase under the control of the tyrosine hydroxylase promoter [TH:Cre+, Rat Research Resource Center, RRRC#:659] were individually housed in a temperature controlled (22-23$_{o}$C) vivarium, and were moderately food restricted with 15 g of chow per day throughout the duration of their training and experiments. This modest amount of food restriction permitted gradual weight gain throughout training and testing. Rats were maintained on a 12hr:12hr light/dark cycle (lights on at 0700h). Procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

Surgery

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

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

For central pharmacological oxytocin delivery, rats were surgically implanted with indwelling guide cannula (26-gauge, Plastics One, Roanoke, VA) targeting the lateral ventricle using the following stereotaxic coordinates, which are relative to the location of bregma and dorsal/ventral (DV) coordinates relative to the skull surface at the cannula implantation site: -0.9 mm anterior/posterior (AP), +1.8 mm medial/lateral (ML), and -2.6 mm DV. Cannula were affixed to the skull as previously described using jeweler's screws and dental cement (Liu et al., 2020). Following one week of recovery, cannula placement was evaluated via measurement of cytoglucopenia-induced sympathoadrenal-mediated glycemic effect that occurs from 210 µg (in 2 µl) of 5-thio-D-glucose (5TG) (Ritter et al., 1981). Food was removed 1 hr prior to the placement test. Animals were injected with 5TG via injectors that extended 2 mm beyond the end of the guide cannula, and blood glucose was measured at 30, 50, 90, and 120 min post-injection. Placement was deemed correct if blood glucose doubled within the measurement period. For animals who did not pass, injector length was adjusted until correct placement was achieved.

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

dependent construct and transgenic rat leads to strong and selective construct expression in dopamine neurons (Konanur et al. 2020; Decot et al. 2017). Next, an optic fiber (flat 400um core, 0.48 NA, Doric Lenses Inc.) was implanted directly above the injection site (AP -5.4, ML -0.7, DV -8.00). Lastly, an ICV cannula was implanted as described above. Animals were given 2-week recovery prior to behavioral training procedures.

Behavior

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

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

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

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

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

were on a DRL0 schedule, where each active lever press was reinforced with a 0 sec time delay. Animals were then switched to a DRL5 schedule for five days, where rats had to withhold presses on the active lever for at least a 5 sec interval for each active press to be reinforced. Active lever presses that occurred before the 5 sec had elapsed were not reinforced and the timer was restarted. Animals were then switched to 5 days of DRL10 (10 sec withholding period) and 10 days of DRL20 (20 sec withholding period). Efficiency in DRL was calculated as the number of pellets earned/the number of active lever presses.

The DRL test was conducted using a within-subjects design. Oxytocin (Bachem, Torrance, CA, USA) or aCSF vehicle treatment order was randomized and counterbalanced based on efficiency during training, and treatments were separated by 72 h. On test days, home cage chow was removed 1 h prior and testing began at dark onset. ICV injections of 1 μg/μl oxytocin or vehicle were administered immediately prior to testing on a DRL20 schedule.

Progressive Ratio (PROG) / Chow Feeding Choice Task: Training and testing in the PROG chow choice task (Fig 3A) was adapted from Salamone and colleagues (Randall et al., 2012; SanMiguel et al., 2018; Yohn et al., 2016). Following surgical recovery, rats (n=11) were brought down to and maintained at 85% of their free-feeding body weight. Animals were habituated to twenty 45 mg sucrose pellets (F0023, Bio-Serv, Flemington, NJ, USA) in the home cage prior to training. Behavioral sessions (30 min, 5 days/wk) were conducted in operant conditioning chambers (Med Associates, Fairfax, VT, USA) and began at the onset of the dark cycle. Rats were initially trained to

lever press on a FR1 schedule (one active lever press for 1 sucrose pellet) for 5 days, and then shifted to a PROG schedule for 10 days. During the PROG session, the ratio started at FR1 and sequentially increased by one every time 15 reinforcers were obtained (FR1 x 15, FR2 x 15, FR3 x 15….). Animals were then introduced to free chow (Laboratory Rodent Diet 5001, St. Louis, MO, USA) concurrently available in addition to the PROG sessions for 5 days. Active lever presses, inactive lever presses, pellets earned, highest PROG ratio achieved, and chow intake (including spill) were recorded throughout training.

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

Incentive Learning: Animals were trained in an instrumental incentive learning task (Fig 4A), modified from (Wassum et al., 2011a; Wassum et al., 2011b; Wassum et al., 2009). Animals (n=21, 6-8/group) were habituated to twenty 45 mg sucrose pellets (F0023, Bio-Serv, Flemington, NJ, USA) in the home cage prior to training. Training sessions occurred daily at the onset of the dark cycle, and food was pulled 1 hr prior to the start of the session and returned 1 hr after completion of the session. Each session started with insertion of the levers where appropriate and ended with retraction of the levers.

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

Rats were next trained in the reward-delivery chain phase. Initially, only the left (seeking) lever was retracted. One left lever press was rewarded by presentation of the right (taking) lever, and one right lever press was rewarded with delivery of one 45 mg sucrose pellet. This reward-delivery chain lasted until 20 pellets were earned or 30 min elapsed. The seeking lever was continuously rewarded with the taking lever for four sessions, then the reinforcement schedule was increased to random ratio (RR)-2 for four sessions, and then to RR-4 until stable lever pressing was obtained (approximately three to five sessions). The taking lever was always continuously rewarded (FR-1) and retracted after sucrose delivery. Animals that did not earn 20 sucrose pellets on a RR-4 schedule were pulled out from the experiment.

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

For the incentive learning test session, all animals were 24 h food-deprived prior to the test session that occurred in the operant chambers. Animals were tested under

extinction in the reward-delivery train on an RR-4 reinforcement schedule for 4 min. Right lever presses, left lever presses, and seconds between right (taking) lever presses were measured.

Cue-predictive reward task for measurement of VTA dopamine neuron activity: After in vivo fiber photometry preparation, food restricted animals (n=7 for behavior; n=4 for fiber photometry) were trained to associate a cue with brief access to sucrose. Training and experimental sessions were conducted during the light phase in standard operant chambers (ENV-009A-CT, Med Associates, Fairfax, VT, USA). Animals underwent daily training for 8 consecutive days. Each training session was comprised of 40 trials where a trial consisted of a 1 sec audio cue (4.5 kHz tone or white noise) followed by either 20 sec availability of a retractable sipper containing a 0.3 M sucrose solution (CS+ trials; n=20) or a dry sipper (CS- trials; n=20). Licks were timestamped using a contact lickometer and controller (ENV-252 M; ENV-250, Med Associates Inc.). The audio cue paired with each sipper was counterbalanced across rats. Trials were separated by a randomly selected, variable inter-trial interval (32-48 sec). Following eight training days, test sessions were conducted identical to training sessions but lateral ventricle injections of vehicle, 0.3 μg/ μl oxytocin, or 1 μg/μl oxytocin (within-subjects) were made immediately prior.

Fiber Photometry and Signal Normalization

For in vivo fiber photometry, LEDs delivered 465 nm (Ca^{2+} dependent) and 405 nm (Ca^{2+} independent) excitation. Intensity of the 465 nm and 405 nm light were

sinusoidally modulated at 211 Hz and 531 Hz, respectively, for all recording sessions. Intensity was then coupled to a filter cube (FMC4 contains excitation filters at 405 nm, 460–490 nm and emission filters at 500–550 nm, Doric Lenses), and converged into an optical fiber patch cord mated to the fiber optic implant. GCaMP6f fluorescence was collected and focused onto a photoreceiver (Visible Femtowatt Photo- receiver Model 2151, Newport). A lock-in amplifier and data acquisition system (RZ5P; Tucker Davis Technologies) was used to demodulate 465 nm and 405 nm excited fluorescence. Behavioral events (e.g. cue, licks) were timestamped and sent as digital inputs to the same data acquisition system and recorded in software (Synapse Suite, Tucker Davis Technologies). The signal was then processed with Fourier transformed subtraction to calculate fluorescence due to fluctuations in Ca^{2+} and bleaching and movement artifacts ($\Delta F/F$). The subtracted signal was smoothed using a custom fifth order bandpass butterworth filter (cutoff frequencies: 0.05 Hz, 2.25 Hz).

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

Statistical Analyses

Experiments 1-3 were analyzed using student's t-test. 15 min time blocks of active presses, pellets earned, and efficiency in experiment 2 (DRL) were analyzed using two-

way analysis of variance (ANOVA) with time and drug as within-subjects variables, and Newman-Keuls post-hoc for multiple comparisons. Experiment 4 was analyzed using a one-way ANOVA and Newman-Keuls post-hoc for multiple comparisons. Statistical analyses were performed using Graphpad Prism 8 with an α level of significance of $p < .05$.

For experiment 5, acquisition of CS+/CS- discrimination was measured by 1st lick latency and % of trials containing at least one lick. Analyses here utilized two-way repeated measures ANOVA with cue and day as within-subjects variables. The magnitude of cue-evoked dopamine neuron transients (mean of signal 0 s to 1 s after cue onset) during CS+ compared to CS- were analyzed using student's t-test. In oxytocin treatments involving fiber photometry, mean signal 1 s after cue onset and behavioral measures in each drug treatment were compared using one-way repeated measures ANOVAs. When drug main effects were found, Newman-Keuls post-hoc tests were used to compare individual drug treatments. Behavior was analyzed with two-way repeated measures ANOVA with trial bin (Trial 1-10; Trial 11-20) and drug as within-subjects variables. Sidak's post-hoc tests were used to compare drug treatments. Statistical analyses were computed using Graphpad Prism 6 with an α level of significance of $p < .05$.

Results

Experiment 1: Central oxytocin inhibits food seeking behavior in the absence of consumption (conditioned place preference expression)

ICV oxytocin administration prior to testing significantly decreased conditioned place preference expression for the palatable food-associated context relative to vehicle (expressed as % shift from baseline preference, Fig 1B) ($F_{[8,7]} = 5.087$, $p = 0.03$),

suggesting that central oxytocin signaling reduces palatable food seeking behavior in the absence of consumption.

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

ICV administration of oxytocin significantly suppressed impulsive behavior in the DRL20 schedule (Fig 2). Oxytocin significantly increased the average number of seconds between active lever presses ($t_{[11]} = 6.17$, $p < 0.0001$, fig 2C). Vehicle-treated animals waited an average of 26.05 sec between active lever presses, whereas ICV oxytocin-treated animals waited an average of 92.95 sec between active lever presses. Furthermore, ICV oxytocin significantly decreased the number of sucrose pellets earned ($t_{[11]} = 9.17$, $p < 0.0001$, fig 2D) and active lever presses ($t_{[11]} = 9.04$, $p < 0.0001$, fig 2E) in the DRL test. There were also no significant differences in number of inactive lever presses (fig 2F). Overall efficiency during the test (fig 2G), a measure of impulsive behavior, was calculated by dividing pellets earned by number of active lever presses. ICV oxytocin treatment significantly increased efficiency in the task ($t_{[11]} = 5.11$, $p = 0.0003$, fig 2B), indicating reduced impulsivity. When efficiency is broken down into three 15 min time blocks, there is a significant time ($p < 0.0001$) and drug ($p < 0.01$) main effect, but no significant time by drug interaction. However, post-hoc analyses reveal significant differences in efficiency between oxytocin and vehicle treatments in the first 15 min, but not the mid 15 min or last 15 min (fig 2J).

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

Motivated behaviors are often characterized by increased work, so we tested effort-based decision making using the PROG versus choice task. Oxytocin significantly decreased pellets earned ($t_{[10]} = 7.76$, $p < 0.0001$, Fig 3B), highest PROG ratio achieved ($t_{[10]} = 5.63$, $p = 0.0002$, fig 3C), and free chow consumption ($t_{[10]} = 2.51$, $p = 0.03$, fig 3E). Moreover, oxytocin significantly decreased the ratio of sucrose over total intake ($t_{[10]} = 4.29$, $p = 0.0016$), suggesting a preferential decrease in motivation for high-effort sucrose in favor of low-effort chow.

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

Incentive learning is the process modifying the incentive value of a specific food through outcome-nutritive state learning (Balleine, 2001; Balleine and Dickinson, 1998a; Balleine and Dickinson, 1998b). Results reveal that ICV oxytocin did not influence the capacity of food restriction to update/enhance the incentive value of consuming sucrose. Analysis of reward seeking behavior during testing (as a percent of baseline) revealed a significant group effect ($F_{[2,16]} = 4.52$, $p = 0.005$). Posthoc comparisons revealed that the dep aCSF and dep oxytocin groups were significantly different from the sated aCSF group, but not from each other.

Experiment 5: Central oxytocin administration suppresses VTA phasic dopamine neuron activity in response to cues associated with palatable food.

Animals were trained to discriminate between a CS+ that predicted the presentation of sucrose reward and a CS- that predicted no reinforcement. As training progressed, rats successfully discriminated between CS+ and CS- cues as measured by

significant differences in latency to first lick and percent of trials with a lick (Fig 5A (left): $F_{[7, 42]} = 7.866$, $p<0.05$ main effect of day; $F_{[1, 6]} = 278.7$ main effect of cue; $F_{[7, 42]}= 24.10$ $p<0.05$ day x cue interaction. Fig 5A (right): $F_{[7,42]} = 2.012$ $p = 0.08$ main effect of day; $F_{[1, 6]} = 257.7$ $p<0.05$ main effect of cue; $F_{[7,42]} = 16.44$ $p<0.05$ day x cue interaction). Fiber photometry (Fig 5B) revealed a robust, time-locked dopamine response to the CS+, but not the CS- (Fig 5C; $t(3) = 8.49$ $p<0.05$). Interestingly, we found no effect of central oxytocin on cue-evoked VTA phasic dopamine activity across the entirety of the behavioral session (data not shown). However, upon closer examination of cue-evoked signal on a trial-by-trial basis, we observed that effects of central oxytocin appeared to affect CS+ responses early in the test session (Fig 5D). Indeed, central oxytocin at both the 0.3 μg and 1 μg dose significantly reduced cue-evoked VTA phasic dopamine responses when comparing across the first 10 trials of the test session (Fig 5E; $F_{[2,6]} = 8.880$ $p<0.05$ main effect of drug; Post-hoc: $p<0.05$ vehicle vs. 0.3 μg oxytocin, vehicle vs. 1 μg OT). Importantly, this oxytocin mediated reduction in cue-evoked VTA phasic dopamine activity occurred concomitantly with behavior, where there is a significant increase in latency to first lick (Fig 5F: $F_{[2,12]} = 8.117$, $p<0.05$ main effect of drug; $F_{[1,6]} = 6.520$, $p<0.05$ main effect of trial; Sidak post hoc: $p<0.05$ veh vs. 1ug OT). However, there was no drug x trial interaction ($F_{[2,12]} = 0.742$, $p>0.05$).

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, however, 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 consumption in a nonreinforced conditioned place preference test. ICV oxytocin also suppressed effort-based palatable food-directed operant responding under ad libitum-fed (nonrestricted) conditions in the DRL task, resulting in a higher task efficiency which is indicative of reduced impulsivity. Consistent with these results, ICV oxytocin reduced effort-based operant responding for palatable food under food-restricted conditions in the PROG vs. chow choice task, where oxytocin shifted the ratio of food consumed from effort-based responding (lever pressing) on a PROG schedule for a preferred food (sucrose) to the less preferred lab chow that is freely available in the apparatus. Additional results show that central oxytocin signaling did not influence incentive learning, or the process in which animals learn about the value of rewards based on postingestive feedback. Overall these findings show that central oxytocin signaling reduces palatable food-directed responses under various conditions but does not significantly influence the postingestive reinforcing properties of sucrose consumption.

Modulation of phasic dopamine neural responses is a feasible mechanism through which oxytocin suppresses palatable food-directed responses, as the conditioned place preference test, DRL impulsivity test, and PROG vs. chow choice test are all influenced by pharmacological manipulations targeting dopamine receptors (Randall et al., 2012; Simon et al., 2013; Spyraki et al., 1982). Moreover, oxytocin neurons in the paraventricular hypothalamic nucleus (PVH) directly project to VTA dopamine neurons (Beier et al., 2015; Peris et al., 2017; Xiao et al., 2017) and oxytocin administration to the VTA suppresses sucrose intake (Mullis et al., 2013). Here we recorded phasic dopamine neuron calcium activity in awake and behaving male rats in an appetitive Pavlovian conditioning task and results revealed that central oxytocin administration suppressed food (sucrose) cue-evoked dopamine neuron responses and associated measures of appetitive behaviors (1st lick latency). These effects were observed during the first ten trials only (within ~20 min into the session), which suggests that oxytocin-mediated inhibition of food reward seeking behavior, consistent with effects on reducing chow intake (Ho et al., 2014; Liu et al., 2020; Ong et al., 2015), is immediate and short-lasting. By providing an immediate and brief anorexigenic signal, oxytocin may be involved in attention-redirection for need-based prioritization of behavior (e.g., contextual and energetic state-driven shifting between appetitive and social behavior) (Burnett et al., 2019), which is an area for future investigation.

Electrophysiological studies demonstrate that oxytocin administration, as well as optogenetic stimulation of oxytocinergic terminals, enhances activity of VTA dopamine neurons (Xiao et al., 2017). In contrast, systemic oxytocin administration reduces dopamine release (as measured by in vivo fixed potential amperometry) in the nucleus

accumbens under baseline conditions and following nomifensine, a dopamine reuptake inhibitor (Estes et al., 2019). Consistent with this latter report, present results reveal reduced dopamine neuron activity in response to presentation of a sucrose-conditioned cue. It is possible these conflicting results occur because, while oxytocin reduces motivated behavior for palatable food, oxytocin enhances social reward and modulates attention-orienting responses to external contextual social cues (Shamay-Tsoory and Abu-Akel, 2016). Indeed, VTA oxytocin administration increases dopamine release in the nucleus accumbens in response to sociality and activation of this circuit enhances prosocial behaviors (Hung et al., 2017; Shahrokh et al., 2010), an effect that occurs through coordinated activity with serotonin (Dolen et al., 2013). Thus, the influence of oxytocin signaling on dopamine neural responses may be dependent on the conditions of behavioral testing, with oxytocin enhancing dopamine signaling in the context of sociality, and reducing dopamine signaling in the context of food-associated stimuli. In addition to a complex reinforcement-dependent relationship between oxytocin and dopamine signaling, oxytocin has also been proposed to be conditionally anorexigenic depending on social context (Olszewski et al., 2016). For example, intranasal oxytocin increases food sharing in the common vampire bat (Carter and Wilkinson, 2015) and peripheral oxytocin has been shown to enhance the social transmission of food preference in rats (Popik and Van Ree, 1993). Moreover, hierarchy social factors influence the effectiveness of oxytocin receptor ligands on food intake. Specifically, administration of an oxytocin receptor antagonist in dominant mice increases the amount of sugar consumed regardless of social or nonsocial contexts, whereas in subordinate animals, it only increases the amount of sugar consumed in a setting devoid of social cues (Olszewski et al., 2015). Future studies are needed to directly examine

whether oxytocin's influence on dopamine signaling and motivated behavior are dependent on the nature of the reinforcement and context.

Our findings demonstrate that while dopamine-associated conditioned food reward-directed behaviors were suppressed under various conditions following ICV oxytocin administration, central oxytocin did not influence incentive learning. Specifically, ICV oxytocin did not alter food reward seeking behavior following learned postingestive-mediated changes in sucrose value that occur following an energetic motivational shift (from satiety to hunger). These results are consistent with previous literature demonstrating that instrumental incentive learning, using procedures similar to those in the present study, is not affected by treatment of flupenthixol, a D1 and D2 receptor antagonist (Wassum et al., 2011b). Furthermore, our incentive learning results suggest that oxytocin may act downstream via a ventral and not dorsal striatal pathway to influence food-motivated behavior. For example, Tellez and colleagues identified dissociable basal ganglia sensorimotor circuits that encode hedonic versus metabolic values of reward (Tellez et al., 2016), where dorsal striatal descending pathways are recruited to encode the nutritional (postingestive) aspects of food, and ventral striatum descending pathways transmit hedonic (flavor) value of food. These findings combined with present results support a putative model in which oxytocin signals downstream to ventral striatal dopaminergic pathways to reduce conditioned food cue-directed motivated behaviors, without influencing dorsal striatum-mediated postingestive incentive learning.

While present results identify a role for central oxytocin signaling in modulating palatable food cue-directed behavior and mesolimbic dopamine signaling, the mediating

sites of action require further investigation. Oxytocin receptors are expressed widely in various feeding-associated regions throughout the brain, including in the amygdaloid complex, VTA, hippocampus, basal ganglia, prefrontal cortex, caudal brainstem, and hypothalamus (Freund-Mercier et al., 1987; Otero-Garcia et al., 2016). In addition to the VTA, one region that may be relevant to present results is the ventral subregion of the hippocampus, which has robust oxytocin receptor expression (Freund-Mercier et al., 1987) and has been shown to be involved in food impulsivity (Noble et al., 2019), conditioned place preference for palatable food (Kanoski et al., 2011b; Zhou et al., 2019), and striatal dopamine (Kanoski et al., 2013). Additionally, the prefrontal cortex plays a critical role in effort-related food choice behavior (Walton et al., 2002) as well as food impulsivity (Hsu et al., 2018b). These and other potential sites of action should be explored in future studies, as they may act in conjunction and/or in parallel with dopaminergic mesolimbic ventral striatal pathways to mediate oxytocin's suppression of palatable food cue-directed responses.

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

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Competing Interests

The authors have nothing to disclose.

Figure Captions

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

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

Figure 3: ICV oxytocin suppresses effort-based consumption of sucrose over freely available chow in a PROG chow choice task. (A) Schematic of the PROG chow choice task. (B) Ratio of sucrose intake over total intake (kCal) over the course of five days training in decision-making task. (C-E) Highest PROG ratio achieved, number of sucrose pellets earned, and free chow intake following ICV oxytocin administration. (F)

Ratio of sucrose intake over total intake (kCal) following ICV oxytocin administration. Data are mean ± SEM; *p<0.05.

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

Figure 5: ICV oxytocin suppresses food cue-evoked dopamine neuron response in the ventral tegmental area. (A) First lick latency and % trials with licks following CS+ and CS- during training, where CS+ precedes sucrose presentation and CS- precedes no food reward. (B) Experimental schematic of fiber photometry and oxytocin pharmacology in TH:Cre+ rats. (C) Representative trace (left) and quantitative ΔF/F (right) of phasic dopamine activity aligned to the CS+ and CS- cues following training. (D) Color plot of dopamine fluctuations during 15 sec window surrounding presentation of the CS+ and following ICV administration of 0.3 μg or 1 μg oxytocin. Red boxes highlight first ten trials, where oxytocin had greatest effect on phasic dopamine signal. (E) Representative traces (left) and quantitative ΔF/F (right) of cue-evoked phasic dopamine during 15 sec window surrounding presentation of the CS+ and following ICV administration of 0.3 μg or 1 μg oxytocin. (F) Average latency to the first lick following ICV administration of 0.3

μg or 1 μg oxytocin in the first ten trials and last ten trials. Data are mean ± SEM;

*$p<0.05$.

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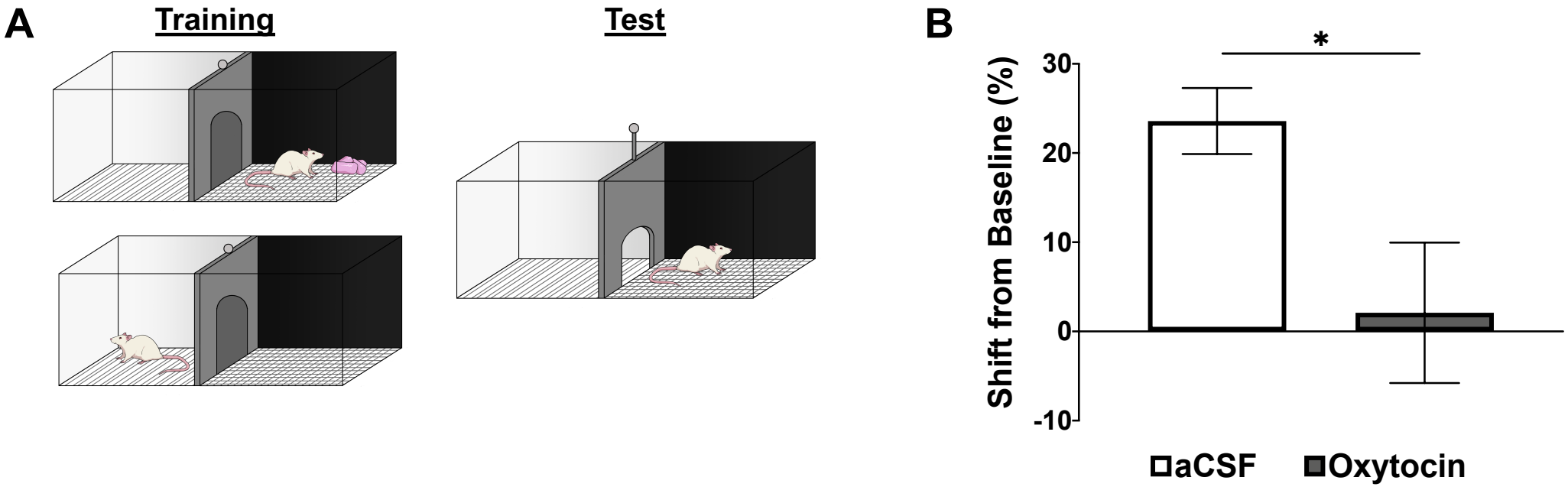
Figure 1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Training
Test
B
*
Shift from Baseline (%)
30
20
10
0
-10
aCSF
Oxytocin

Figure 2

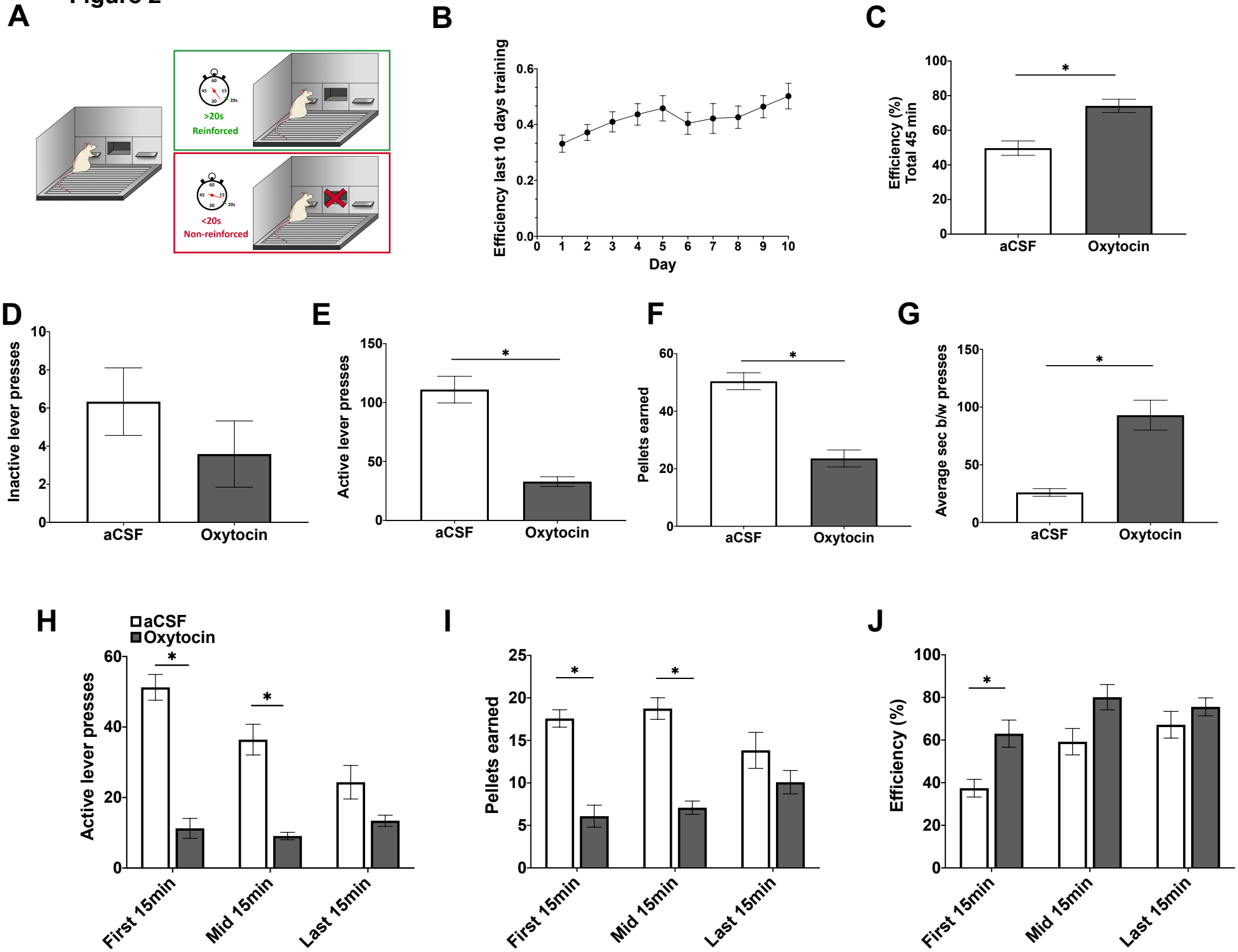

A
>20s
Reinforced
<20s
Non-reinforced
B
Efficiency last 10 days training
Day
C
Efficiency (%)
Total 45 min
aCSF
Oxytocin
D
Inactive lever presses
E
Active lever presses
F
Pellets earned
G
Average sec b/w presses
H
aCSF
Oxytocin
Active lever presses
First 15min
Mid 15min
Last 15min
I
Pellets earned
J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

Figure 3

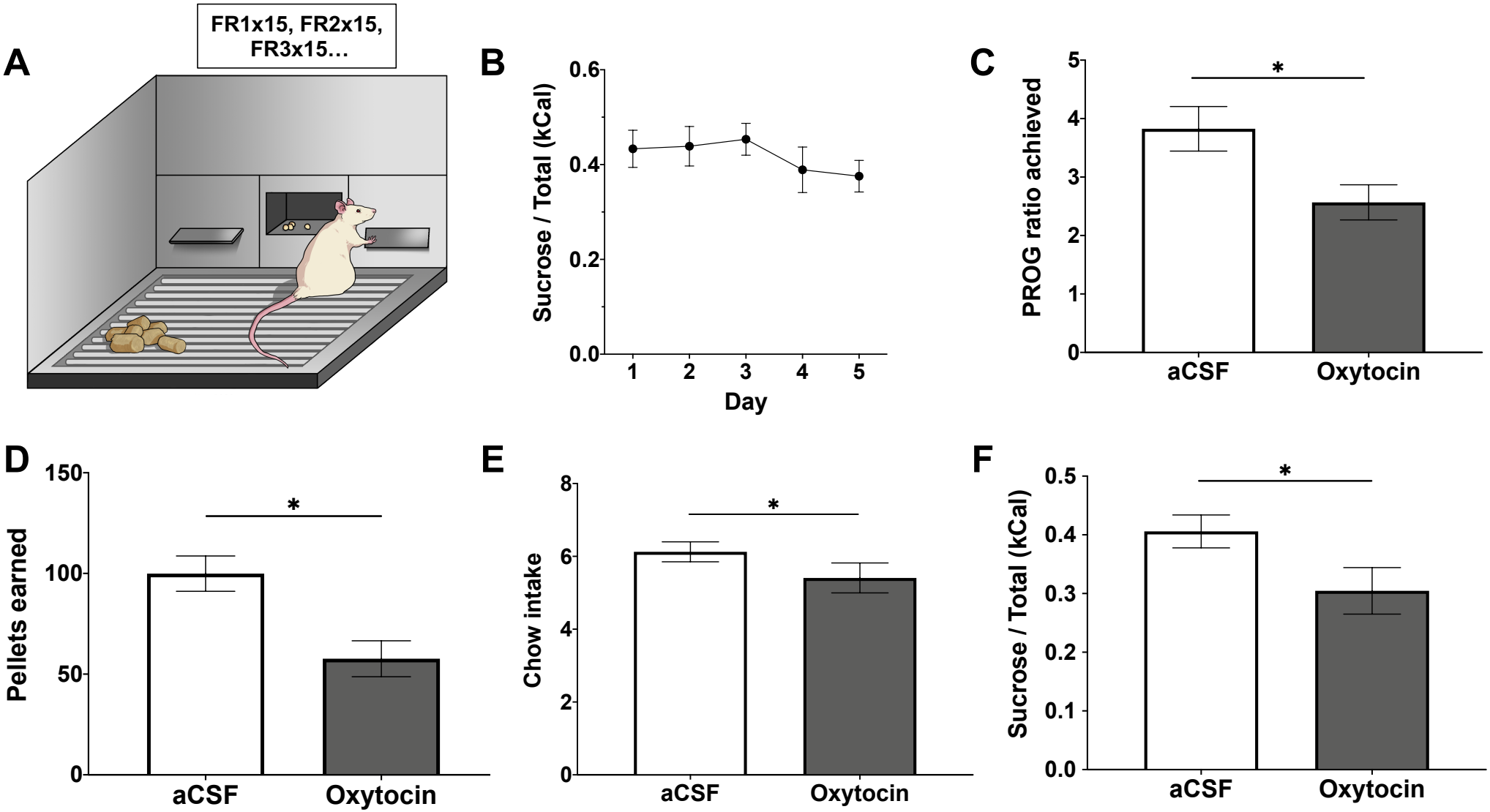
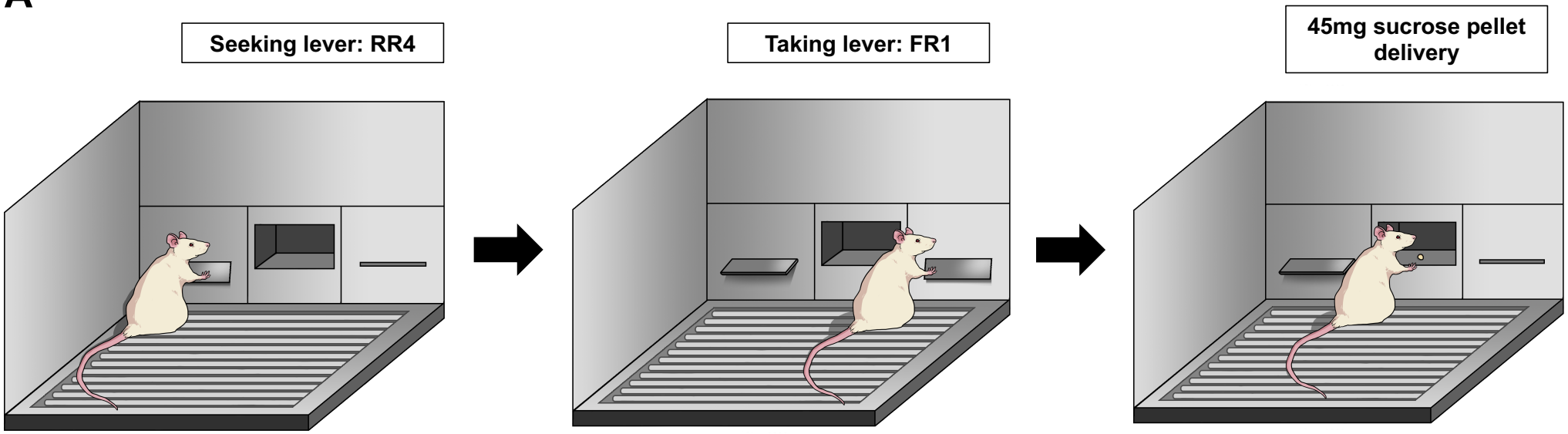

A
FR1x15, FR2x15,
FR3x15…
B
Sucrose / Total (kCal)
0.6
0.4
0.2
0.0
1
2
3
4
5
Day
C
PROG ratio achieved
5
4
3
2
1
0
*
aCSF
Oxytocin
D
Pellets earned
150
100
50
0
*
aCSF
Oxytocin
E
Chow intake
8
6
4
2
0
*
aCSF
Oxytocin
F
Sucrose / Total (kCal)
0.5
0.4
0.3
0.2
0.1
0.0
*
aCSF
Oxytocin

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

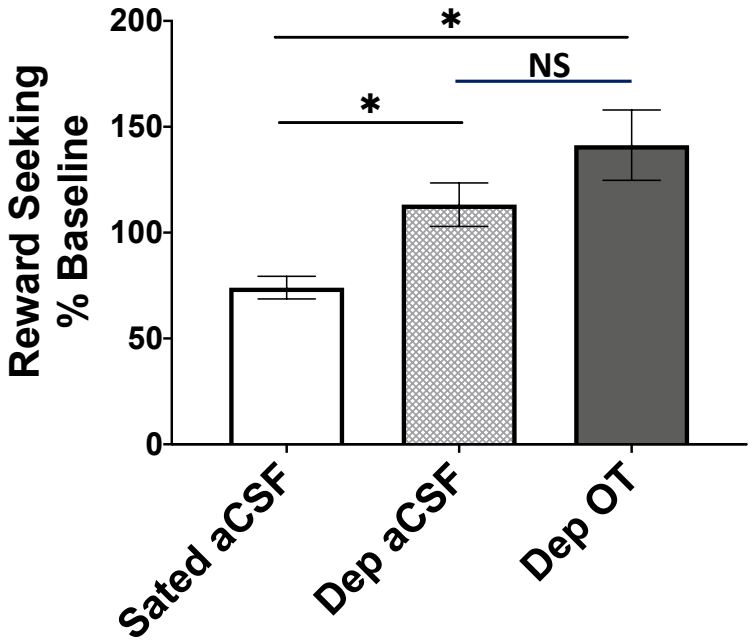
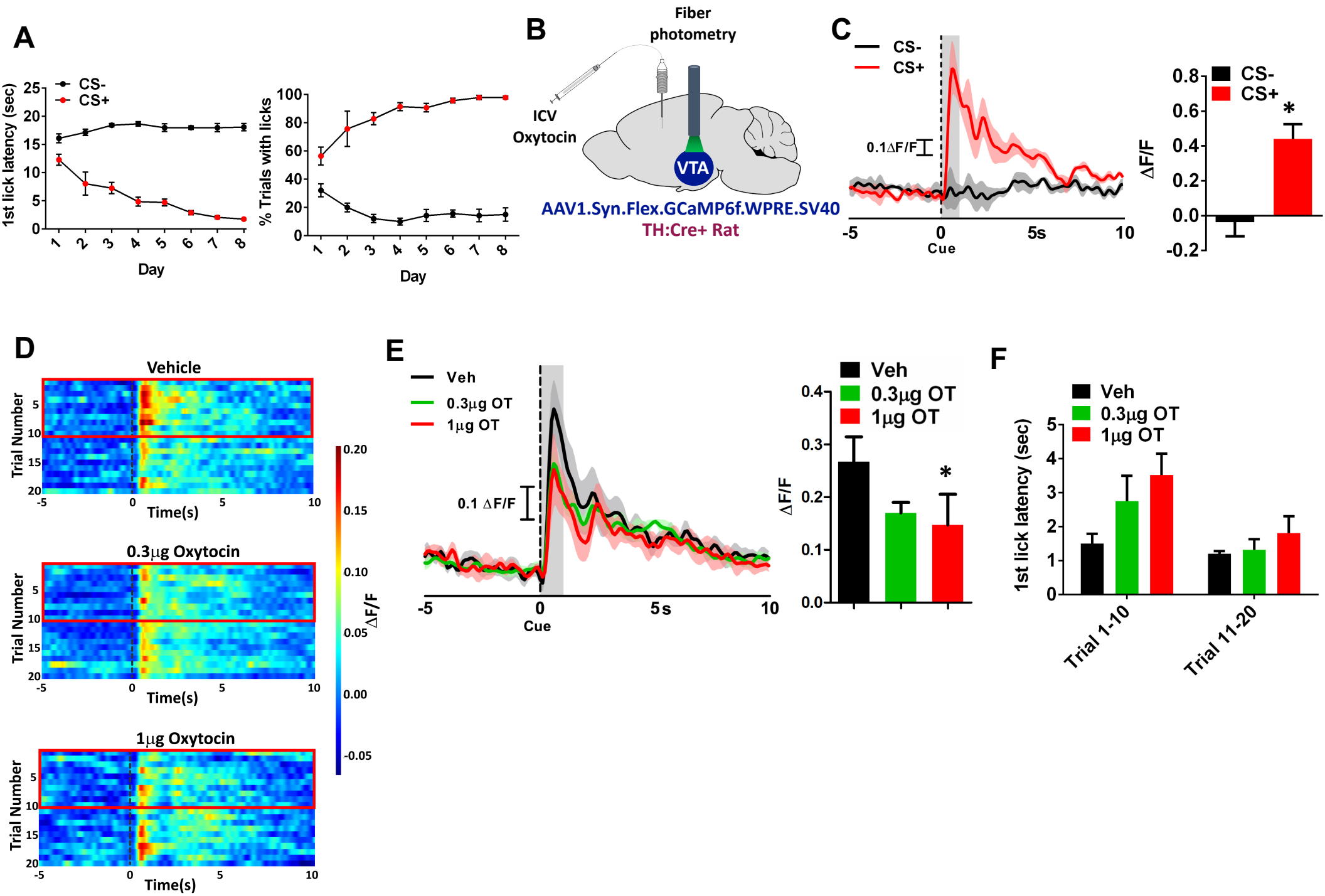


Figure 5


A
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
CS-
CS+
0.1ΔF/F
Cue
ΔF/F
D
Vehicle
0.3μg Oxytocin
1μg Oxytocin
Trial Number
Time(s)
ΔF/F
E
Veh
0.3μg OT
1μg OT
0.1 ΔF/F
Cue
ΔF/F
F
Veh
0.3μg OT
1μg OT
1st lick latency (sec)
Trial 1-10
Trial 11-20