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Hippocampus Oxytocin Signaling Promotes Prosocial Eating in Rats

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ABSTRACT

BACKGROUND: Oxytocin (OT) is a hypothalamic neuropeptide involved in diverse physiological and behavioral functions, including social-based behavior and food intake control. The extent to which OT's role in regulating these 2 fundamental behaviors is interconnected is unknown, which is a critical gap in knowledge given that social factors have a strong influence on eating behavior in mammals. Here, we focus on OT signaling in the dorsal hippocampus (HPCd), a brain region recently linked to eating and social memory, as a candidate system where these functions overlap.

METHODS: HPCd OT signaling gain- and loss-of-function strategies were used in male Sprague Dawley rats that were trained in a novel social eating procedure to consume their first nocturnal meal under conditions that varied with regard to conspecific presence and familiarity. The endogenous role of HPCd OT signaling was also evaluated for olfactory-based social transmission of food preference learning, sociality, and social recognition memory.

RESULTS: HPCd OT administration had no effect on food intake under isolated conditions but significantly increased consumption in the presence of a familiar but not an unfamiliar conspecific. Supporting these results, chronic knockdown of HPCd OT receptor expression eliminated the food intake–promoting effects of a familiar conspecific. HPCd OT receptor knockdown also blocked social transmission of food preference learning and impaired social recognition memory without affecting sociality.

CONCLUSIONS: Collectively, the results of the current study identify endogenous HPCd OT signaling as a novel substrate in which OT synergistically influences eating and social behaviors, including the social facilitation of eating and the social transmission of food preference.

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Oxytocin (OT) is an evolutionarily conserved neuropeptide produced in the paraventricular and supraoptic nuclei of the hypothalamus. Its release either into the periphery or within the brain plays a role in a diverse set of behavioral functions, including regulation of food intake (1,2). Acting as an anorexigenic signal, administration of OT reduces food intake in experimental rodent models, as well as in nonhuman primates and humans (1,3). In rodents, either central or peripheral administration of OT reduces food intake, especially for highly palatable foods (4,5) and in diet-induced obese models (6). These findings have identified OT as a potential therapeutic for the treatment of obesity (7–10).

OT has also been extensively studied for its effects on modulating social behaviors, including maternal bonding, pair bonding, sociability, and social-based memory (11,12). While OT's influence on social behaviors is generally considered to be prosocial (e.g., cooperation, caregiving), recent findings suggest that its effects are complex and highly dependent on the social context, with OT administration increasing outgroup discrimination and reducing affiliative behaviors under some conditions (12–14). Given that OT plays a role in mediating both eating and social behaviors, it is highly plausible but unknown whether critical overlap exists in these functions.

Eating behavior is strongly influenced by social factors in humans. For example, the social facilitation of eating (SFE) effect is a phenomenon in which both the number of people at a meal, as well as the familiarity with and the sex of those present during the meal, can powerfully modulate caloric consumption (15–20). Rats, like humans, are highly social eaters because food choice and preference, the amount consumed, and foraging strategies in rats are influenced by the behavior of conspecifics (21,22). The SFE effect has also been documented in rodent models (23,24), thus supporting that results from mechanistic rodent models on interactions between eating and social factors have translational relevance. Despite evidence that social factors influence eating behavior in both humans and rodents, the overwhelming majority of rodent model research on food intake control has evaluated consumption with subjects in isolated conditions. Because social-based eating is a more ecologically valid model of food intake in both humans and rats than isolated eating conditions, it is critical to understand the extent to which OT's effects on food intake are modulated by the social environment.

We hypothesized that the dorsal subregion of the hippocampus (HPCd) is a substrate where OT synergistically

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influences eating and social behaviors. The HPCd has recently been linked to food intake control in rodents (25,26), and OT receptors (OXTRs) are expressed in the HPCd and have been established as mediators of social behaviors (26–35). To investigate the mechanisms through which HPCd OT influences food intake, social factors, memory, and potential interactions between these behaviors, we developed a novel eating paradigm in rats that allows for evaluations of consumption under conditions that vary with regards to social presence, conspecific familiarity, and context familiarity. By combining this paradigm with established rodent social behavioral procedures (e.g., social transmission of food preference, social recognition memory [SRM]) and neuropharmacological and virogenetic manipulations, our results identify neurobiological mechanisms that connect the OT system with food intake and socially relevant behaviors.

METHODS AND MATERIALS

Animals

Male Sprague Dawley rats (Envigo; postnatal day 60–70; 250–275 g on arrival) were individually housed in a temperature-controlled vivarium with ad libitum access (except where noted) to food and water (LabDiet 5001; LabDiet) on a 12-hour reverse light/dark cycle. All procedures were approved by the Institute of Animal Care and Use Committee at the University of Southern California.

Experiment 1: The Effects of Hippocampal OT and Social Presence on Eating

To determine the effects of hippocampal OT administration on eating, rats implanted with bilateral HPCd cannulae underwent the social eating procedure depicted in Figure 1 (surgical and behavioral procedures are described in Supplemental Methods, and an experimental design summary is provided in Table S1). Animals in the isolated home cage (n = 8), isolated neutral cage (n = 8), and familiar conspecific (n = 8) groups were injected with either artificial cerebral spinal fluid (aCSF) or 0.05 μg OT (within-subjects drug design, counterbalanced treatments) in the HPCd approximately 15 minutes prior to the start of the test (OT dose selection is described in Supplemental Methods). Food was returned to the animals at the start of the testing period, which coincided with the onset of the dark cycle, and food intake and meal pattern parameters were recorded for 1 hour.

Experiment 2: The Effects of Social Presence and Conspecific Familiarity on Eating

Animals with bilateral HPCd cannulae (n = 12) underwent social eating procedure training. Animals were then divided into 2 groups that differed by conspecific familiarity. Those in the familiar group (n = 6) received counterbalanced aCSF and 0.05 μg OT drug treatments in the presence of the familiar conspecific from training. The unfamiliar group (n = 6) received the treatments in the presence of a novel, unfamiliar conspecific that had also undergone social eating procedure training.

Experiment 3: The Effects of Hippocampal OXTR Reduction on Social Eating

To examine the physiological role of hippocampal OT signaling on social eating, we utilized a viral-mediated approach (described in Supplemental Methods) to reduce OXTR expression within the HPCd (dentate gyrus [DG] subregion). Following surgery, body weight changes were measured for 36 days and home cage food intake (including meal patterns) was tracked for 5 days (~3 weeks after surgery) (Supplemental Methods) before animals completed the social eating procedure. During training, all animals were paired with the same conspecific for 12 training days. On test days, control (CON) (n = 6) and OXTR knockdown (KD) (n = 8) animals were placed into the social eating cage with either the same familiar conspecific from training or a novel unfamiliar conspecific

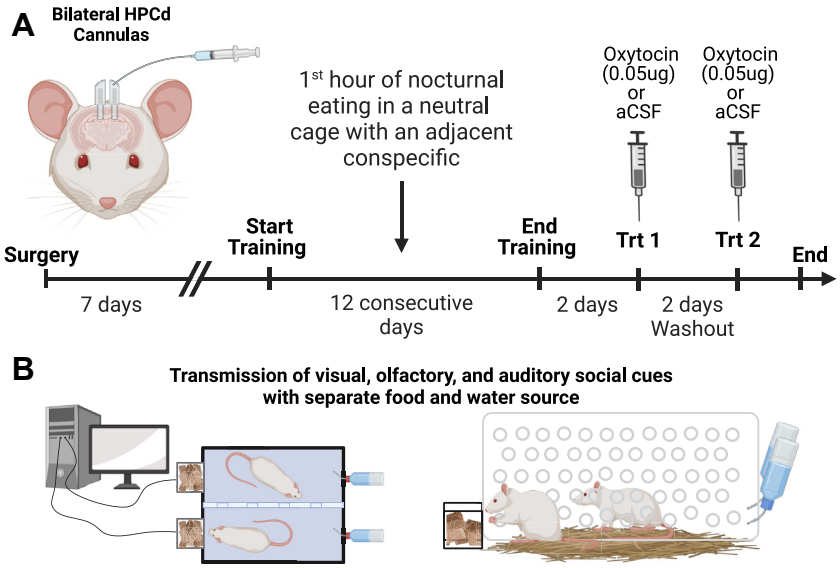

Figure 1. Overview of social eating procedures. **(A)** An experimental timeline for surgeries and training in social eating procedures. **(B)** In a 2-chamber food intake monitoring system, spontaneous meal patterns are evaluated during the first hour of the nocturnal feeding period under isolated conditions that vary with regards to context familiarity or under social conditions that vary with regards to conspecific familiarity. A divider separates the chambers and the animals physically to allow for precise consumption measures for individual animals, absent competition for the food source. The divider is transparent, with various holes to allow for transmission of visual, olfactory, and auditory social cues between chambers. aCSF, artificial cerebral spinal fluid; HPCd, dorsal hippocampus.

(within-subjects design, counterbalanced tests), and 1-hour food intake was measured (experimental conditions summarized in Table S1).

Experiment 4: The Effects of Hippocampal OXTR Reduction on Social Transmission of Flavor Preference

To examine the effects of HPCd OXTR KD on food preference memory based on olfactory and social cues, we utilized the social transmission of flavor preference (STFP) task (described in Supplemental Methods) (36–39). Nonexperimental animals with intact OT signaling were used as demonstrator animals. Observer animals had either control AAV (adeno-associated virus) (n = 6) or OXTR KD AAV (n = 9) injected into the HPCd at least 3 weeks prior to completing the STFP protocol.

Experiment 5: The Effects of Hippocampal OXTR Reduction on Sociability, SRM, and Object Recognition Memory

In this study, we utilized a social discrimination (SD) task (described in Supplemental Methods) to assess both sociability and SRM (40–43). SD tests were completed in CON AAV (n = 7) and OXTR KD (n = 7) animals that had undergone viral-mediated HPCd OXTR KD surgery at least 3 weeks prior to testing.

After SD testing, the rats underwent novel object recognition (NOR) memory testing (described in Supplemental Methods) to evaluate novelty-based learning, absent social factors (44). CON AAV (n = 7) and OXTR KD (n = 7) animals both underwent the NOR procedure at least 3 weeks after surgery.

RESULTS

HPCd Oxytocin Effects on Food Intake Are Dependent on the Social Context

Under isolated home cage conditions, OT administration to the HPCd had no effect on cumulative 1-hour food intake (Figure 2A), first meal size (Figure 2B), or 1-hour meal frequency (Figure 2C). HPCd OT administration also did not influence 1-hour cumulative food intake (Figure 2D), first meal size (Figure 2E), or meal frequency (Figure 2F) for animals in the isolated neutral cage condition.

However, for animals in the familiar conspecific–neutral cage group, HPCd OT administration in the presence of a familiar conspecific significantly increased 1-hour cumulative food intake (Figure 2G), an effect driven by a significant increase in first meal size (Figure 2H) because there was no significant difference in meal frequency (Figure 2I), meal duration, number of eating bouts, average eating bout size, or average eating bout duration (Figure S2).

Hippocampus OT Signaling Augments the SFE by a Familiar but Not an Unfamiliar Conspecific

Here, we sought to assess whether conspecific familiarity influences food intake under social eating conditions and whether this effect is influenced by HPCd OT signaling. All animals were trained to eat their first nocturnal meal with a familiar conspecific in the social eating procedure. When we compared intake at the start of training with home cage nonsocial conditions, there was no significant difference in 1-hour cumulative intake. However, at the end of social eating training, cumulative intake was significantly increased in comparison to both nonsocial and start-of-training conditions

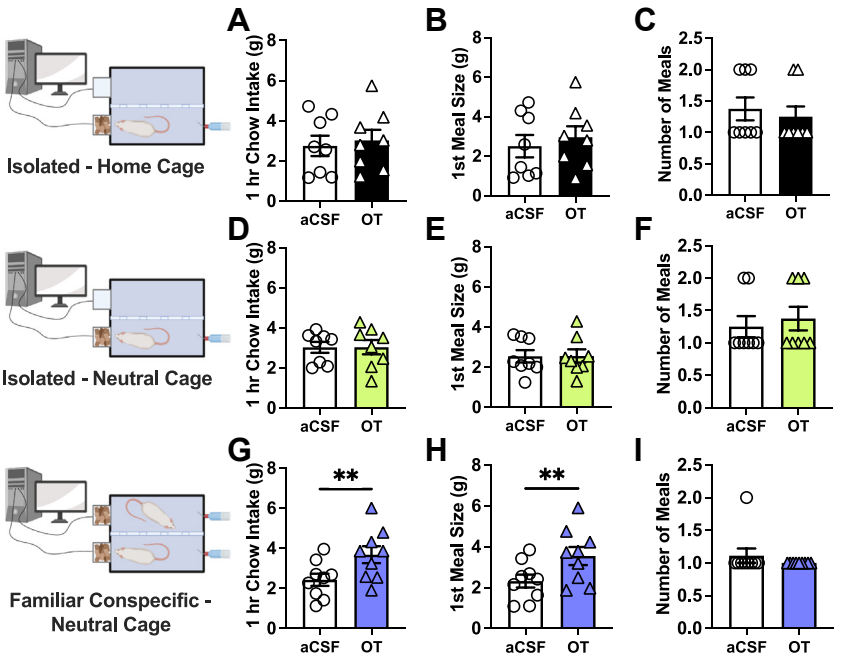

Figure 2. Dorsal hippocampus OT effects on food intake differ by social context. Under isolated home cage **(A–C)** and isolated neutral cage **(D–F)** conditions, hippocampal administration of 0.05 μg OT did not affect 1-hour cumulative caloric intake, first meal size, or meal frequency. **(G)** OT administration to the dorsal hippocampus increased 1-hour cumulative chow intake when consumed in the presence of a familiar conspecific (t_8 = 3.937, p = .004). **(H, I)** This effect was mediated by a significant increase in the first nocturnal meal size (t_8 = 4.319, p = .003), without affecting meal frequency (home cage n = 9, isolated neutral n = 8, familiar conspecific n = 9; all between-subjects design for eating condition and within-subjects design for drug treatments). Data are means ± SEM. **p < .01. aCSF, artificial cerebral spinal fluid; OT, oxytocin.

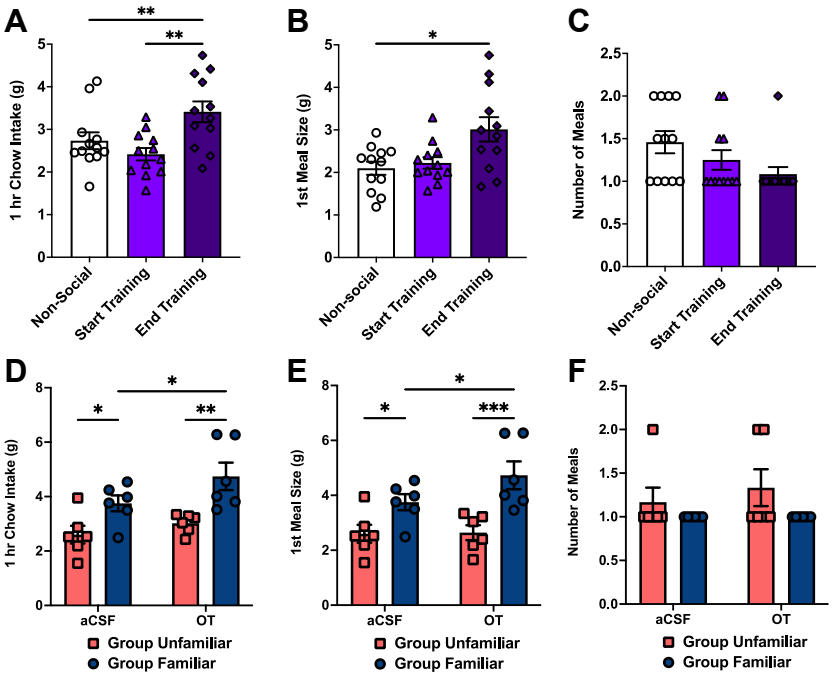

Figure 3. Social facilitation of eating in the presence of a familiar conspecific is augmented by hippocampal OT. **(A)** Compared with average isolated nonsocial home cage intake, there was no significant difference in 1-hour cumulative chow intake at the start of the social eating training (start training); however, by the end of training (end training), rats consumed significantly more chow than under isolated conditions as well as compared with the start of the social eating training (n = 12, 1-way repeated-measures ANOVA, days: $F_{1.590,17.49}$ = 9.587, p = .0026, Sidak's adjusted p = .0048 nonsocial vs. end training and p = .0092 start training vs. end training). **(B, C)** These outcomes were based on an increase in first meal size by the end of training in comparison to the nonsocial and start of training (n = 12, 1-way repeated-measures ANOVA, days: $F_{1.512,16.63}$ = 5881, p = .0171, Sidak's adjusted p = .0281 nonsocial vs. end training) because there were no significant differences in meal frequency. **(D)** During the pharmacological testing phase, rats assigned to the familiar group that received both drug treatments in the presence of a familiar conspecific consumed significantly more food than rats assigned to the unfamiliar group with an unfamiliar conspecific's presence under both the vehicle (aCSF) and OT conditions. The familiar group also showed a significant increase in food intake with the administration of OT in comparison to vehicle (aCSF) conditions while the unfamiliar group showed no effect of OT administration (2-way repeated-measures ANOVA, conspecific familiarity: $F_{1,10}$ = 12.63, p = .0052; treatment: $F_{1,10}$ = 7.083, p = .0238; Fisher's least significant difference planned comparisons aCSF: t_{20} = 2.381, p = .0273 unfamiliar vs. familiar; OT: t_{20} = 3.569, p = .0019 unfamiliar vs. familiar; and familiar: t_{10} = 2.649, p = .0244 aCSF vs. OT). **(E–F)** These outcomes were based on the presence of a familiar but not an unfamiliar conspecific increasing first nocturnal meal size (2-way repeated-measures ANOVA, conspecific familiarity: $F_{1,10}$ = 15.17, p = .003; Fisher's least significant difference planned comparisons aCSF: t_{20} = 2.247 p = .0361 unfamiliar vs. familiar; OT: t_{20} = 4.086, p = .0006 unfamiliar vs. familiar; and familiar: t_5 = 2.325, p = .0424 aCSF vs. OT) without influencing meal frequency and hippocampal OT treatment enhancing these effects (within-subjects n = 12 design for training comparing intake in contexts differing in social presence; mixed design for testing with conspecific familiarity as between-subjects and drug treatment as within-subjects; unfamiliar group n = 6, familiar group n = 6). Data are means ± SEM. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$. aCSF, artificial cerebral spinal fluid; ANOVA, analysis of variance; OT, oxytocin.

(Figure 3A). This familiarity-dependent SFE effect was mediated by an increase in first meal size because end-of-training first meal size was significantly larger than in the nonsocial condition and trended higher than in the start-of-training condition (Figure 3B). There were also no significant differences in meal frequency between the 3 conditions (Figure 3C). There was a significant increase in first meal duration between nonsocial and both training conditions and an increase in average bout size between nonsocial and end-of-training conditions, with no differences in bout frequency or duration (Figure S3A–D). Collectively, these results establish a critical component of the SFE effect in rats: eating more in the presence of familiar (end of training) than unfamiliar (start of training) individuals or eating alone.

To determine whether elevated SFE induced by HPCd OT administration (Figure 2G) was driven simply by social presence or whether familiarity is an important component of this effect, animals were divided into 2 groups after training, differing with regard to conspecific familiarity for drug tests (unfamiliar vs. familiar group). Results revealed that animals in the familiar group consumed more food than animals in the unfamiliar group following both aCSF and OT treatments (Figure 3D). Animals in the unfamiliar group showed no difference in intake between drug treatments, whereas OT significantly increased 1-hour cumulative intake compared with aCSF treatment in the familiar group. Similar results were seen for first meal size, where the familiar group consumed a larger first meal than the unfamiliar group following aCSF, and OT increased intake in the familiar group but not the unfamiliar group (Figure 3E). There was a significant main effect of group but not drug treatment for first meal size. The familiar group had a significantly larger first meal size under aCSF and OT conditions, with OT treatment significantly increasing first meal size in comparison to aCSF in the familiar but not the unfamiliar group. There were no significant differences in meal frequency between groups or treatments (Figure 3F). OT administration significantly increased bout frequency in the familiar but not the unfamiliar group, with no effect on average bout size or duration (Figure S3F–H). Collective results reveal that rats exhibited hyperphagia when eating in the presence of a familiar conspecific compared with either eating alone or in the presence of an unfamiliar conspecific and that this familiarity-dependent SFE effect is augmented by HPCd OT administration.

SFE by a Familiar Conspecific Requires Endogenous Hippocampal OXTR Signaling

The viral-mediated approach to KD HPCd OXTR (Figure 4A) involved OXTR and control (scrambled) short hairpin RNA

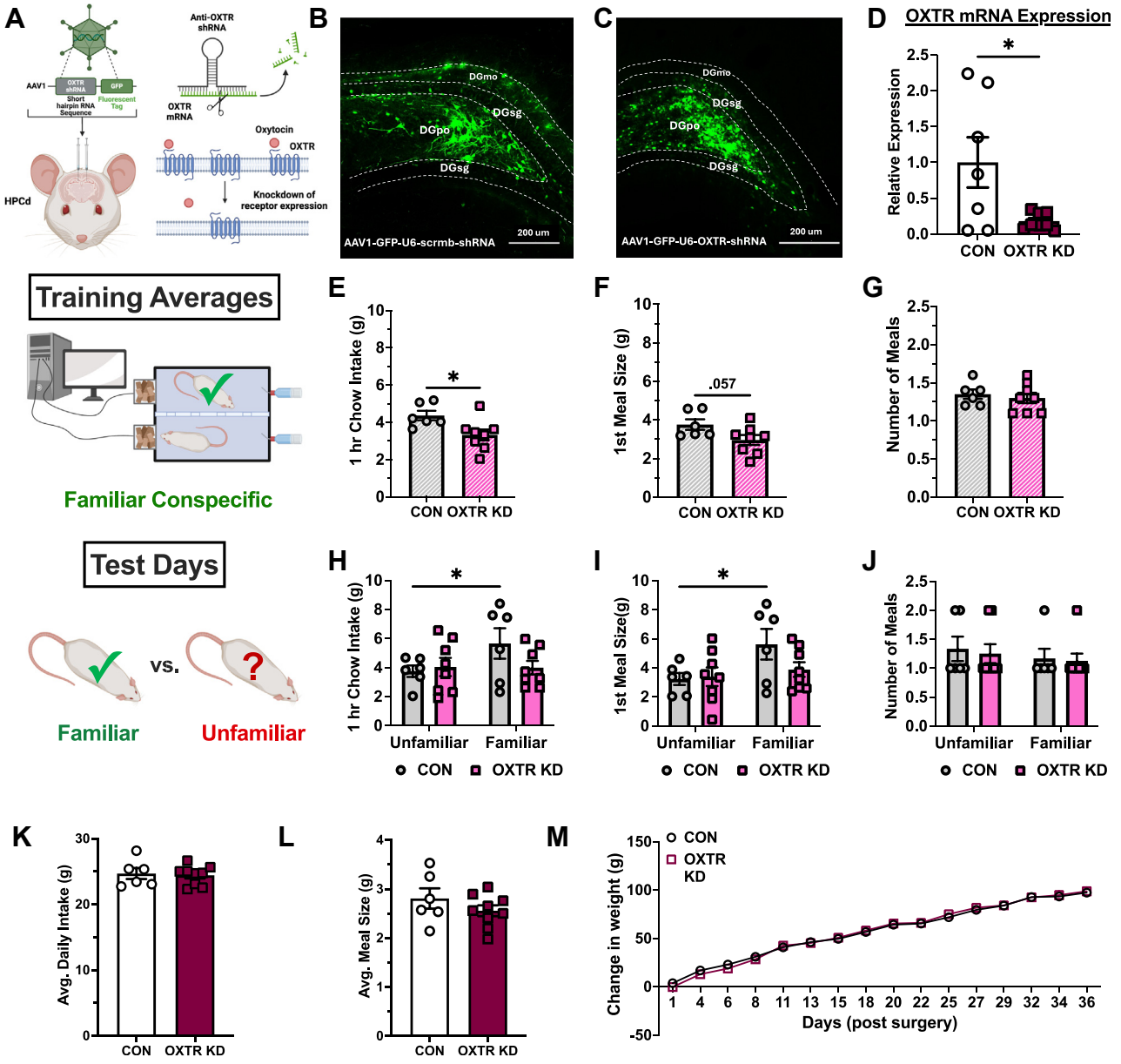

Figure 4. Conspecific familiarity–based social facilitation of eating requires endogenous hippocampal OXTR signaling. **(A)** Diagram depicting viral vector–mediated OXTR shRNA for chronic KD of OXTR expression in the HPCd. **(B, C)** Following the infusion of scrambled sequence CON (scrmb) or OXTR shRNA AAVs, DG target sites and viral-induced GFP expression were confirmed using immunohistochemistry (representative photomicrographs from each group depicted). **(D)** Quantification of relative OXTR mRNA expression showed a significant reduction in KD animals of approximately 80% compared with CON (t_{13} = 2.496, p = .027; CON n = 7, KD n = 7). **(E–G)** During the training phase of the social eating procedure, OXTR KD animals consumed significantly less food within the hour in the social arena than CON (t_{12} = 2.553, p = .025), with a trend toward a reduction in first meal size (t_{12} = 2.10, p = .057) but no effect on meal frequency. **(H–J)** CON but not OXTR KD animals consumed more food within the hour-long test in the presence of a familiar than an unfamiliar conspecific (Fisher's least significant difference planned comparisons CON: t_{12} = 2.217, p = .0467 unfamiliar vs. familiar), an effect driven by an increased first meal size (Fisher's least significant difference planned comparisons CON: t_{12} = 2.655, p = .021 unfamiliar vs. familiar) with no change in meal frequency. **(K–M)** KD of dorsal hippocampal OXTRs did not yield long-term changes in daily caloric intake, meal frequency, or body weight under isolated conditions in the home cage (between-subjects design for group; CON n = 6, OXTR KD n = 9). Data are means ± SEM. *p < .05. AAV, adeno-associated virus; CON, control; DGmo, dentate gyrus molecular layer; DGpo, dentate gyrus polymorph layer; DGsg, dentate gyrus granule layer; GFP, green fluorescent protein; HPCd, dorsal hippocampus; KD, knockdown; mRNA, messenger RNA; OXTR, oxytocin receptor; shRNA, short hairpin RNA.

conjugated to GFP (green fluorescent protein) to confirm viral transfection in the HPCd DG (Figure 4B, C). The OXTR short hairpin RNA (KD) significantly reduced OXTR messenger RNA expression in the HPCd compared with CON by approximately 80% (Figure 4D). This targeted viral KD approach had no effect on OXTR expression in adjacent cortex (CON mean = 1.00,

SEM = 0.3794; OXTR KD mean = 0.878, SEM = 0.2209; t_5 = 0.2966, p = .779) or in the ventral hippocampus subregion (CON mean = 1.00, SEM = 0.2741; OXTR KD mean = 4.119, SEM = 1.323; t_{11} = 2.136, p = .056). Thus, this vector-mediated approach was effective in significantly reducing OXTR expression in the HPCd without affecting OXTR expression in adjacent brain regions.

The OXTR KD group consumed significantly less food during the social eating training sessions than the CON group (Figure 4E). There was a trend toward a reduction in average first meal size during training (Figure 4F), with no group effect on meal frequency (Figure 4G). These results suggest that endogenous HPCd OXTR signaling mediates SFE by a familiar conspecific, and consistent with our pharmacological data, this effect is driven by meal size and not by eating frequency.

On test days, CON animals consumed significantly more food within the 1-hour social eating test in the presence of a familiar conspecific than an unfamiliar conspecific, an effect driven by increased first meal size, whereas OXTR KD animals showed no effect of conspecific familiarity on 1-hour food intake or meal size (Figure 4H, I). There were no significant between- or within-group differences based on conspecific familiarity in meal frequency (Figure 4J). Overall results show that CON animals consumed more with a familiar conspecific whereas OXTR KD animals did not, thus further supporting that endogenous hippocampal OXTR signaling mediates familiarity-based SFE.

Reduced expression of HPCd OXTR did not significantly influence long-term daily caloric intake, average meal size, or change in body weight under isolated home cage conditions (Figure 4K–M). There were also no significant differences in other meal pattern parameters (first meal size, average first meal duration) (Figure S4). These results, consistent with the pharmacological data, highlight the selectivity of HPCd OT signaling in influencing food intake under conditions that involve social interactions.

Hippocampal OXTR KD Impairs STFP Learning

In addition to influencing the amount of food consumed, social factors can influence food preference and choice (20,45,46). An STFP protocol (depicted in Figure 5A) was used to assess the role of HPCd OXTR signaling in social-based learning about food-associated olfactory cues. Data from the social interactions following demonstrator consumption revealed no difference between the CON and OXTR KD groups in the amount of time spent investigating the demonstrators (Figure 5B), which is indicative of comparable sociability between groups. For food preference testing, consistent with previous STFP studies (36,37,47), CON animals showed a strong preference for the demonstrator-paired flavor during the 30-minute consumption test. In contrast, OXTR KD animals showed no significant preference for either of the 2 food choices, as indicated by no significant difference from chance (Figure 5C). The CON group had a significantly higher paired flavor preference than the OXTR KD group. There was no significant group difference in total consumption during the 2-choice preference test (Figure 5D). Taken together, these data indicate that hippocampal OXTR KD impaired social-based learning about food preference.

Hippocampal OT Administration Has No Effect on Risk-Associated Appetitive and Consummatory Behavior in a Nonsocial Context

To determine whether the effects of HPCd OT administration on SFE and STFP were due to reduced anxiety in a food-related environment (i.e., functioning as a safety signal to eat), animals completed an approach-avoidance consumption task (detailed in Supplemental Methods) in which animals enter a brightly lit environment, leaving the safety of a darkened familiar space, to consume a highly palatable peanut butter food reward. Results showed that there was no effect of HPCd

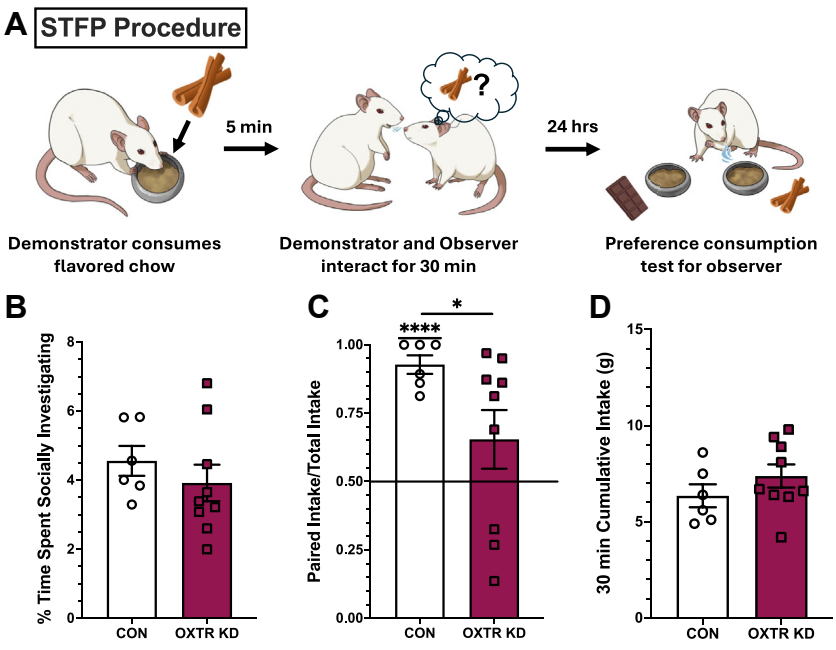

Figure 5. Endogenous dorsal hippocampus OXTR signaling is required for STFP learning. **(A)** Diagram depicting STFP procedure in which an experimental or observer rat is exposed to a novel food flavor from the breath of a demonstrator that has recently consumed the flavored chow in a separate room. Twenty-four hours after exposure to demonstrator rats, observer rats are tested in the 2-choice preference consumption test. **(B)** There was no difference between CON and OXTR KD rats in the time spent investigating the demonstrator during social interaction. **(C)** CON animals successfully demonstrated a significant preference for the flavor that their demonstrator had consumed, whereas OXTR KD rats showed no flavor preference (Welch's t test CON vs. KD $t_{9.532}$ = 2.429, p = .037; 1-sample t test comparing 0.50 to CON: t_5 = 12.58, $p < .0001$). **(D)** The total amount of food consumed during the preference test did not differ by group (between-subjects design for group; CON n = 6, OXTR KD n = 9). Data are means ± SEM. *$p < .05$, ****$p < .0001$. CON, control; KD, knockdown; OXTR, oxytocin receptor; STFP, social transmission of flavor preference.

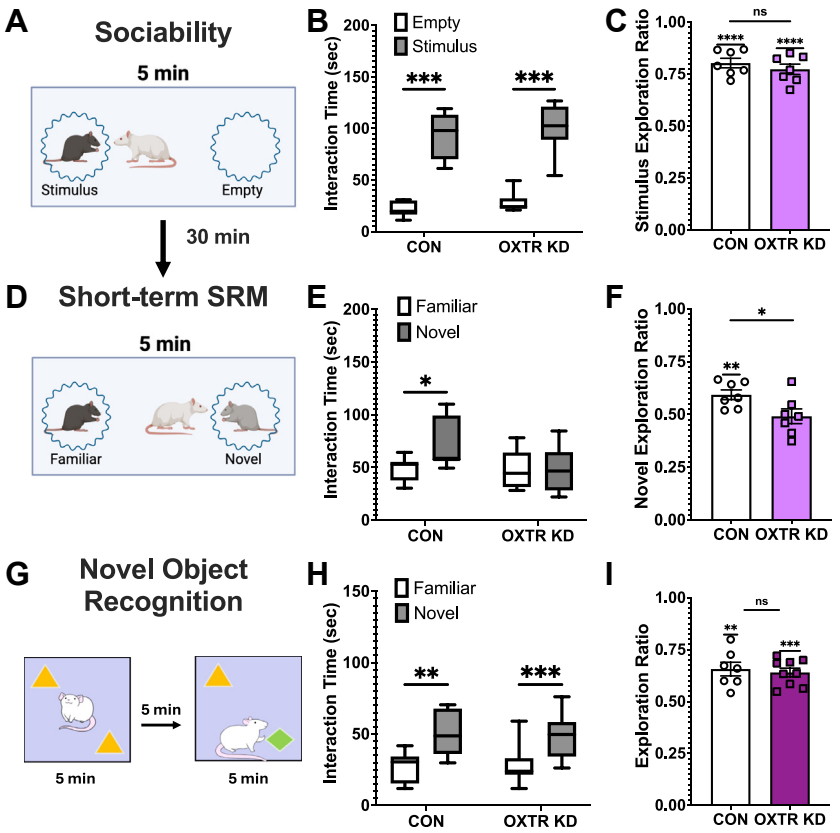

Figure 6. Reduction in dorsal hippocampus OXTR expression impairs social recognition memory but not sociability. **(A)** Sociability is assessed by placing experimental animals into an arena for 5 minutes with an empty enclosure and another containing a stimulus animal. The time spent investigating each enclosure is measured. **(B, C)** Both CON and HPCd OXTR KD animals spent significantly more time investigating the stimulus animal than the empty enclosure, indicating normal sociability in both groups (CON: t_6 = 8.352, p = .00032 empty vs. stimulus; OXTR KD: t_6 = 7.317, p = .00033 empty vs. stimulus). **(D)** Following an interval of 30 minutes, animals are placed back into the arena to assess social recognition memory, now with the previously experienced familiar stimulus animal or a new novel animal. **(E–F)** CON animals spent more time investigating the novel stimulus animal while the HPCd OXTR KD animals did not [**(E)** CON: t_6 = 3.358, p = .03 familiar vs. novel; **(F)** t_{12} = 2.432, p = .032 CON vs. OXTR KD, 1-sample t test comparing 0.50 to CON: t_6 = 4.046, p = .0068]. **(G)** Animals were also tested in a novel object recognition task to assess nonsocial recognition memory. **(H, I)** The CON and OXTR KD groups performed similarly in the novel object recognition test (CON: t_6 = 4.719, p = .00326 familiar vs. novel, OXTR KD: t_6 = 6.457, p = .00039 familiar vs. novel) (between-subjects for group; CON n = 7, OXTR KD n = 7). Data are means ± SEM. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. CON, control; HPCd, dorsal hippocampus; KD, knockdown; ns, nonsignificant; OXTR, oxytocin receptor; SRM, social recognition memory.

OT administration on latency to enter the light side (Figure S5C), time spent in the light side (Figure S5D), or peanut butter consumption (Figure S5E). Overall, these results show that HPCd OT did not appear to promote SFE and STFP by reducing anxiety in a food-related context.

Hippocampal OXTR Signaling Mediates SRM but Not Sociability or Object Recognition

In the sociability portion of the SD task (Figure 6A), both CON and OXTR KD animals spent significantly more time investigating the novel stimulus animal than the empty enclosure (Figure 6B). Both groups were significantly above chance (0.5) in their investigation of the stimulus animal, and there were no significant group differences in the exploration ratio (Figure 6C). Thus, HPCd OXTR KD did not impact sociability.

Results of the subsequent SRM test (Figure 6D) revealed that while the CON group spent significantly more time investigating the novel than the familiar animal, the OXTR KD group did not (Figure 6E). Likewise, the CON group had a novel stimulus exploration ratio that was significantly above chance, while the OXTR KD group did not, consistent with the significant group difference in exploration ratio (Figure 6F). These results indicate that HPCd OXTR KD impaired SRM without affecting measures of sociability.

The NOR procedure was used to evaluate novelty-based memory outside of a social context (Figure 6G). Results showed that both CON and OXTR KD animals spent significantly more time investigating the novel than the familiar object (Figure 6H), that both CON and OXTR KD were significantly above chance (0.5) for exploration ratio, and that there were no group differences in the exploration ratio (Figure 6I). Thus, reduction of HPCd OXTR expression had no effect on object recognition memory, suggesting that OT's action in the hippocampus preferentially encodes social cue-related memory processes.

DISCUSSION

OT administration reduces food intake in rodents and in humans (3). However, the overwhelming majority of previous studies evaluating OT's effects on food intake involved eating under isolated conditions. Given that social factors potently influence eating behavior in both rodents and humans and that both species regularly consume food in the presence of conspecifics (16,20–24), social-based eating is a more ecologically valid model to assess the impact of OT on food intake control, particularly given that OT has been extensively studied for its role in mediating social behaviors (2,12,48), including those related to food intake (14,49,50). Here, we examined how OT signaling in the DG subregion of the HPCd, an OXTR-expressing brain area linked to social-based memory and more recently to control of food intake (33–35,51–54), influences consumption in rats in a novel behavioral paradigm

that varies eating testing conditions with regard to social presence and familiarity. Results from neuropharmacological studies have revealed that while HPCd OT administration had no effect on food intake under isolated conditions in either the home cage or in a familiar neutral cage, HPCd OT significantly increased consumption in the presence of a familiar conspecific in a social eating arena, an effect based on increasing the size of the first nocturnal meal. These findings differ from previous studies conducted under isolated eating conditions that revealed a potent anorexigenic role for central OT driven by a reduction in meal size (3,55–58). However, previous studies did not examine OT's effects on food intake specifically within the hippocampus but rather its action in traditional feeding centers in the brain, including the caudal brainstem and the hypothalamus (1,59,60). Our findings taken together with previous work reveal that central OT signaling bidirectionally influences meal size, depending on the targeted brain region and the social context.

The SFE effect is a robust phenomenon studied in humans that involves 2 primary components: eating more in the presence of a group than in isolation and eating more in the presence of familiar than unfamiliar individuals (15,17,18). While the former component has been demonstrated in rats (23,24), to our knowledge, the latter has not. Here, we demonstrated that rats consumed more in the presence of a familiar conspecific than in either isolated conditions or in the presence of an unfamiliar conspecific. Furthermore, using complementary gain- and loss-of-function approaches, we revealed that familiarity-based SFE was mediated by OT signaling in the HPCd. These findings are consistent with previous work showing that OT, which has typically been thought to elicit predominantly prosocial effects, has a more complicated role in social interactions based on social familiarity and perceived in- versus outgroup dynamics (12,13). Relatedly, previous findings with mice revealed increased sucrose consumption following peripheral OXTR antagonist injections in both nonsocial and social contexts in dominant mice but only in a nonsocial context in subordinate mice (50). Results from the current study expand these findings by revealing that HPCd OXTR signaling promotes prosocial effects on eating that are dependent on conspecific familiarity. Additional research is needed to determine whether either the number of conspecifics present or the dominant versus submissive status of the animals influences HPCd OXTR-mediated effects on eating.

The hippocampus is important in mediating social behaviors, including SRM (61,62), and knockout of OXTR in the CA2/CA3 region in mice impairs SRM (30). Here, we focused on the DG subregion in the HPCd, a region that has been established as an important center for adult neurogenesis and memory function, particularly for context recognition and memory tasks that involve pattern separation (63–66). The ability to distinguish individuals based on auditory, visual, and olfactory cues is essential for SRM and can be considered an analogous process to DG-mediated pattern separation. Adult-born DG neurons are associated with social memory maintenance in mice (53,67). Here, we extended these previous studies by identifying a novel role for DG OXTR signaling in mediating SRM. Using a viral vector–mediated approach to KD HPCd DG OXTRs by ∼80%, our results revealed normal sociability but an absence of SRM in the KD group. Given these findings, taken together with our additional results showing that DG OXTR KD eliminated the familiarity-based SFE effect, HPCd OT signaling may promote prosocial eating by enhancing the mnemonic familiarity of a conspecific. Additional work is required to determine whether these effects are mediated by OXTR expressed on adult-born DG neurons.

Social-based learning about eating behavior is extremely beneficial from an evolutionary perspective because it allows an animal to mitigate food-related risks by learning from another individual's experience. This is especially true for rodents such as rats that lack the ability to dispel harmful chemicals after consumption through emesis. Therefore, rats exhibit robust food neophobia (68–71). In the STFP procedure, animals learn to prefer food with a flavor that was previously experienced through an interaction with a demonstrator animal that had recently consumed food with that flavor (36,38,39,72). OT facilitates STFP learning in rats (47); however, this previous work did not provide insight into the neural site(s) of action. Given our results indicating a role for HPCd OT signaling in SFE and previous findings that the HPCd is critical for STFP (51), we hypothesized here that HPCd OT signaling mediates STFP. Our results confirmed this because HPCd OXTR KD prevented STFP learning without influencing sociability during the social interaction or the total amount consumed during the preference test.

It is possible that HPCd OT signaling functions as a general safety signal to eat (SFE) or to eat specific foods (STFP). However, this is unlikely because HPCd OT administration had no effect on either appetitive or consummatory behaviors in a nonsocial risk-associated approach-avoidance task. Moreover, effects of HPCd OT signaling on familiarity-dependent SFE or STFP are unlikely to be based on a general role in novelty recognition memory because HPCd OT KD did not impact NOR memory. Rather, given that both SFE and STFP involve learning about social experiences relevant to eating, we hypothesize that endogenous HPCd OT signaling facilitates the acquisition and/or retention of social-based food-relevant memories to guide future eating behavior.

A limitation of the current study is the exclusion of females. We recently identified sex differences in the effect of intracerebroventricular OT administration on food intake, which was mediated by gonadal hormones and the estrus cycle (73). Moreover, there are known sex differences in social behavior, feeding behavior, OT production, and central OXTR expression (74,75). Investigating whether HPCd OT signaling mediates SFE in females is an important direction for future research. Given that intracerebroventricular OT preferentially reduces consumption of highly palatable foods under isolated conditions (76–78), it would also be interesting for future work to examine how macronutrient composition alters the effect of OT administration on food intake in social contexts.

Our results demonstrate a novel SFE effect in a rat model that is dependent on conspecific familiarity, similar to the phenomenon that has been observed in humans (15,17,20). Both gain- and loss-of-function approaches identify OT signaling in the HPCd DG as a neural substrate mediating SFE, as well as STFP, potentially via SRM-mediated processes. Thus, the collective findings reveal novel mechanisms through which OT intersects the control of food intake and social

behaviors. Given that OT is currently being investigated as a potential therapy for the treatment of obesity (8–10), the current results should be taken into consideration regarding clinical obesity relevance because OT's role as an anorexigenic system may be more complex than it was previously considered to be.

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ARTICLE INFORMATION

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SUPPLEMENTARY INFORMATION

Hippocampus Oxytocin Signaling Promotes Prosocial Eating in Rats

Rea *et al.*

Supplemental Methods & Materials

Surgical Procedures

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). Rats were also given analgesic (subcutaneous injection of ketoprofen [5mg/kg]) after surgery and once daily for 3 subsequent days thereafter. All rats recovered for at least one-week post-surgery prior to experiments.

I.C.V. cannula implantation

For delivery of oxytocin into the lateral ventricle (LV) for intracerebroventricular (ICV) drug administration , rats were surgically implanted with a unilateral indwelling guide cannula (26-gauge, Plastics One, Roanoke, VA) using the stereotaxic coordinates, relative to the location of bregma: -0.90 mm anterior/posterior (AP), +1.80 mm medial/lateral (ML), and -2.60 mm dorsal/ventral (DV) with the DV coordinate zeroed at the surface of the skull before being lowered into the brain. Cannula were affixed to the skull as previously described using jeweler's screws and dental cement (1). Following a week of recovery, the optimal injector tip length for infusion into the LV was determined by injecting 5-Thio-D-Glucose (5-TG) (Sigma-Aldrich, St. Louis MO) into the LV and measuring change in blood glucose. Animals had their food pulled 1hr prior to the onset of the dark cycle so that they were mildly food restricted before testing. An initial blood glucose reading was taken an hour after the dark cycle onset using a OneTouch monitor with blood taken from the tip of the tail cut by a razor blade. Starting with an injector that extends 2.0 mm beyond the end of the guide cannula, 2uL

of a 105mg/mL 5-TG solution was then infused into the LV using a Hamilton microinjector. Blood glucose readings were taken 30 min and 1 hr following infusion of 5-TG. An animal was considered to pass with that tip length if their blood glucose approximately doubled in that time. Procedure was repeated with injector tip lengths of 2.50 mm and 3.00 mm until animal passed. This tip length was then used for all subsequent ICV drug deliveries.

Hippocampal cannula implantation

For parenchymal pharmacological OT delivery, rats were surgically implanted with bilateral indwelling guide cannula (26-gauge, Plastics One, Roanoke, VA) using the following stereotaxic coordinates, which are relative to the location of bregma: -4.08 mm AP, +/-2.50 mm ML, and -2.60 mm DV. Cannula were affixed to the skull as previously described using jeweler's screws and dental cement (1). Drug injections were made with injectors that projected 1.0 mm beyond the end of the guide cannula. Experiments involving pharmacological administration included bilateral HPCd (dentate gyrus [DG] region) injections of oxytocin (Bachem, Torrance, CA) which was dissolved in artificial cerebral spinal fluid (aCSF) and diluted to a 0.05 µg dose (concentration 0.25 µg/µL). Injections were administered using a microinfusion pump (Harvard Apparatus, Holliston, MA) connected to a 26-gauge microsyringe injector through the indwelling guide cannulae. Flow rate was calibrated and set to 5 ml/min and 100nl injection volume per hemisphere. Injectors were left in place for 30-sec to allow for complete infusion of the drug.

Viral preparation and delivery

For in vivo knockdown of OXTR expression, short hairpin RNAs targeting rat OXTR mRNA were cloned and packaged into an adeno-associated virus (AAV1; Vector Biolabs) under the control of a U6 promoter and co-expressing green fluorescent protein (GFP) downstream of the U6 promoter (titer 1.4 x 10^{13} GC/mL). OXTR shRNA sequence is: CACC-GCTTCTGCCTTCATCATTGCCCTCGAGGGCAATGATGAAGGCAGAAGC-TTTTT. A scrambled shRNA, GFP expressing AAV1 downstream of a U6 promoter (titer 1.0 x 10^{13} GC/mL) was used as a control (Vector Biolabs). AAVs were then delivered bilaterally to the HPCd DG subregion (AP: -4.08 ML: ±2.50 DV: -3.60) at an injection volume of 300nL per hemisphere via pressure injection with the micro infusion pump setup described above. Animals recovered for 3 weeks prior to behavioral experiments to allow ample time for viral expression and protein knockdown.

Histology

Placements for HPCd cannulae were verified post-mortem by injection of 100nl blue dye (100nl, 2% Chicago sky blue ink) through the guide cannulae followed by removal of brain tissue that was postfixed in 10% formalin solution. Animals were included if dye was confined to the HPCd DG or within 0.20mm of the region bilaterally. A representative image of ink placement can be found in Supplemental Figure 5B. Animals were also included if there were clear bilateral cannula tracts directly above the correct HPCd region, in the absence of ink. In total, 10% of rats with HPCd cannulae were removed from the data because of misplaced cannulae.

In animals that received AAV injections following all experimental procedures, animals were anesthetized and transcardially perfused with ice-cold 0.9% saline, followed by 4% paraformaldehyde (PFA) in 0.1M borate buffer (pH 9.5). Brains were removed and immersed in 12% sucrose in PFA fixative for 20-24h at 4°C. The brains were then flash-frozen in dry-ice cooled isopentane before sectioning on a sliding microtome at 30μm. For histological verification, transverse sections of the HPCd were slide mounted and viewed under a fluorescent microscope (Nikon 80i) until GFP-expressing cells were visualized in the DG. Overall, 13% of rats were removed from analyses for lack of GFP expression or missed AAV placement.

Fluorescence in situ hybridization

Tissue sections were sectioned and mounted on subbed glass slides (Fisher brand Superfrost Plus, Fisher Scientific, Hampton, NH, USA) and desiccated overnight (16h) in a vacuum desiccant chamber. Following 1h and 45min postfix in 4% PFA, sections were washed 5 x 5 min in KPBS and incubated for 30 min at 37C in a solution of 100nM Tris (pH 8), 50nM EDTA (pH8) and 0.1% Proteinase K (10mg/ml, sigma P2308), then rinsed for 3 min in the same Tris and EDTA solution without Proteinase K. Sections were washed 3 min in a solution of 100nM triethanolamine (pH 8) in water and then incubated for 10 min at room temperature with 0.25% acetic anhydride in 100mM triethanolamine, then washed 2 x 2 min in 10% 20Xsaline-sodium citrate buffer. Prior to hybridization, sections were dehydrated in increasing concentrations of ethanol (50%, 70%, 95%, 100%, 100%). Sections were incubated with probes for 3 h (OXTR Cat # 483671), Reagents from the RNAscope Fluorescent Multiplex Detection Reagent Kit v2

(Advanced Cell Diagnostics, Newark, CA, USA; Cat #:323100) were used to amplify the probe as per the kit's instructions. Slides were coverslipped using Prolong Gold Antifade Reagent (Cell Signaling, Danvers, MA, USA; Cat #: 9071s). Photomicrographs were acquired using a Nikon 80i (Nikon DS- QI1,1280X1024 resolution, 1.45 megapixel) under epifluorescent illumination using the Nikon Elements BR Software (Supp Fig 5A).

Verification of AAV-mediated knockdown

A separate cohort of animals was used to validate the viral-mediated knockdown technique through quantitative polymerase chain reaction (PCR) analyses. Either control AAV (n=7) or OXTR KD (n=8) shRNAs were injected into the HPCd (as described above), and following 3 weeks to allow viral expression, bilateral micro-punches (2 x 2 x 2mm) were taken from the HPCd, adjacent cortex and ventral hippocampus (HPCv) regions of the flash frozen brains. OXTR mRNA levels were quantified using Taqman gene expression kits (OXTR: Rn00563503_m1; GapDH: Rn01775763_g1; Life Technologies) and PCR reagents (Applied Biosystems). qPCR was conducted using an Eppendorf Mastercycler ep realplex2 and the comparative threshold cycle method was used to quantify relative mRNA expression.

Subthreshold ventricular dose establishment

Isolated home cage food intake

To establish a dose of HPCd oxytocin in which effects on food intake (standard chow) under standard testing conditions (isolated in the home cage) are unlikely to be based on leakage into the ventricular system, a dose-response within-subjects

preliminary experiment was performed with ventricular injections delivered immediately prior to dark onset. To look at the effect of ICV administration of OT on food intake, animals were implanted with a guide cannula in the right lateral ventricle as described above. Immediately prior to the start of the intake measurements, animals received an ICV infusion of either aCSF, 0.50μg or 0.05μg oxytocin, counterbalanced by order into the LV using the injector tip length previously established for each rat as described above. Doses were based off of previous work in the lab, along with others to find a dose of OT with moderate effects on food intake (1-3).

Treatment days were separated by 2 days to allow for a washout of drug effects. At the start of the experiment food hoppers were weighed and then placed inside cages at the start of the dark cycle. Tech-board sheets were placed underneath hanging wire-bottom cages to be able to collect spill measurements at 30 min, 1hr and 2hr time points. At these time points the food hoppers were weighed before being replaced in the cage and spill was measured, and the spill sheets replaced. Results revealed that 0.50μg, but not 0.05μg LV oxytocin significantly reduced chow intake 30 min after delivery, (Supplemental Figure 1A). Based on results from this preliminary experiment (data not shown) and those of the Conditioned flavor avoidance experiment described immediately below, all HPCd-targeted pharmacological experiments utilized a dose of 0.05μg OT.

Conditioned flavor avoidance

Next, given that central oxytocin signaling has been shown to produce a conditioned flavor avoidance (CFA) (4, 5), we evaluated whether 0.05μg oxytocin, which

did not influence food intake following LV administration, produced a CFA following HPCd administration. Rats were first habituated to a water deprivation schedule for 7 days during which water access was given once daily for 90 min in two water bottles. Water intake was monitored on day 6 to ensure that rats were drinking from each bottle. For training, drug or vehicle treatments were counterbalanced (within-subjects design) across two training days separated by an intervening day. On each training day during the normal 90-min water access period, rats were given two bottles containing approximately 100 ml of a Kool-Aid mix (0.16 oz unsweetened Kool-Aid, 10 ml saccharin, 3.5 l water). Rats received one flavor of Kool-Aid, cherry or grape, on each training day. These flavors have previously been shown to be equally preferred in rats (e.g., (6)). Immediately after the 90-min flavor exposure period, rats were given injections of vehicle or drug (flavor/drug pairings counterbalanced with respect to groups). For the HPCd OT group animals received infusions of either aCSF or 0.05μg OT through their guide cannula. For the lithium chloride (LiCl; a positive control for CFA paradigm) control group animals received either saline or 0.15M LiCl intraperitoneally (IP). The volume of IP injections was 1.33mL/100g bodyweight with a LiCl concentration of 6.36mg/ml. Two days after the second training day, rats were given a 2-bottle preference test during the normal 90-min water access period; one bottle was presented containing the cherry Kool-Aid mix, the other containing the grape Kool-Aid mix. The side (left vs. right) of the initial flavor presentation was counterbalanced with respect to groups and treatment orders. At 45 min fluid intake was recorded and the side of flavor presentation was switched. Fluid intake was recorded again at 90 min.

Results revealed that while 0.15M lithium chloride yielded a robust CFA relative to vehicle injections, 0.05μg oxytocin delivered to the HPCd did not (Supplemental Figure 1B-C). Thus, 0.05μg was selected as the dose for all HPCd oxytocin administration experiments as this dose (1) did not influence food intake under standard testing conditions following ventricular delivery, and (2) did not produce a CFA following HPCd delivery. For all subsequent pharmacological test treatments (Experiments 1-5), rats received either 0.05μg oxytocin or aCSF vehicle immediately before food intake testing session, with meal parameters were recorded by the BioDAQ system.

Behavioral Procedures

Food intake and body weight measures

Animals were housed in cages in the BioDAQ food monitoring system, where their daily intake patterns could be recorded and were weighed three days a week (MWF) to monitor body weight changes. Following a 3-day period of habituation to the BioDAQ system, data was analyzed from 5 consecutive days to examine home cage isolated meal patterns. Analysis of meal patterns included daily (24 h) cumulative intake, meal frequency, average meal size, average meal duration, 1st meal size, 1st meal duration, bout frequency, average bout size, and average bout duration. Meals were defined using a 900 sec inter-meal interval (IMI) to separate the last bout of one meal and the start of a new meal. First meal was defined as the first meal after the onset of the dark cycle, as rats are nocturnal animals.

Social eating procedure

Rats were individually housed in BioDAQ Food and Water intake monitoring system (Research Diets Inc. New Brunswick, NJ) which recorded episodic ad libitum feeding activity of rats in their home cages and social eating cage. Rats were habituated to the BioDAQ for at least 3 days and henceforth housed in BioDAQ chambers for the remainder of experiments. In social feeding experiments rats were paired off and socialized with the same conspecific in a neutral cage for 12 consecutive daily training sessions followed by 2 test sessions, each separated by two washout days between tests, as depicted in Figure 1A. For each training day and subsequent test days, access to the food hopper was restricted 1 hour prior to the start of the social eating session which began at the onset of the dark cycle, as preliminary data (not shown) demonstrated that all rats consume the first meal of the dark cycle within the first hour. During the 1-hour social eating sessions in the neutral cage, rats were separated from their conspecific using a clear plexiglass divider with evenly interspersed 13mm diameter holes that allowed for transmission of visual, auditory, and olfactory cues between the two sub-chambers (Figure 1B). For isolated groups (Experiment 1) the clear plexiglass divider was placed either directly into the animals' home cage for both training and test days to confine them to one half of the cage simulating conditions in the social cages (Isolated Home Cage) or animals were placed on one side of the plexiglass divider within a neutral cage for each day of training and testing with no conspecific present in the adjacent compartment (Isolated Neutral Cage). Subjects were given ad libitum access to food and water and food intake was measured at the end of each one-hour training session. Experimental conditions varied

on test days, as summarized in Supplemental Table 1. Each of two test days consisted of recording the first hour of food intake from the onset of the dark cycle, with tests separated by two days to allow for drug washout. Pharmacological administration of drug treatments on test days were done approximately 15 min prior to the start of the test. In experiments where the test conspecific differed from training (Experiments 2 and 3) social cages were thoroughly cleaned and new bedding replaced. To control for potential olfactory variables, home cage bedding of each experimental animal was mixed into the clean bedding in the testing arena on their perspective side of the cage the day before each test day.

To evaluate potential changes in intake across training with increasing levels of conspecific familiarity (Experiment 2), meal intake parameters were averaged from the first two days of training ("Start Training") and compared to the average of the last two days, days 11 & 12, of training ("End Training). Eating parameters from each of these periods were also compared to intake in the absence of a conspecific averaged across the 2-day interval between training and testing ("Non-Social").

Social transmission of food preference (STFP)

We utilized the STFP task adapted from (7-10). First, untreated normal adult male rats, separate from our experimental group, are designated as "Demonstrators", while experimental groups are designated as "Observers". Demonstrators and Observers are first habituated to a powdered rodent chow [Lab Diet 5001 (ground pellets), Lab Diet, St. Louis, MO] overnight. 24hr later, Observers are then individually assigned to Demonstrators and are allowed to freely interact in an arena (23.5cm W x

44.45cm L x 27cm H clear plastic bin with Sani-chip bedding) and allowed to interact for 30min as a form of social interaction habituation. Both Observers and Demonstrators are returned to their home cages and food is withheld for both groups. 23hrs later, in a room separate from Observers, the food-restricted Demonstrators are given the opportunity to consume one of two flavors of powdered chow (flavored with 1% cinnamon or 2% cocoa, 2% marjoram or 0.5% thyme; counterbalanced according to group assignments). The Demonstrator rat is then placed in the social interaction arena with the Observer rat and allowed to socially interact for 30 min. Observers are then returned to their home cage and allowed to eat standard maintenance chow ad libitum for 1hr. 23hr later, the food-restricted Observer animals are given a home cage food choice test for either powdered chow that contains the flavor paired with the Demonstrator animal, vs. a novel, unpaired flavor of chow that was not consumed by the Demonstrator animal (1% cinnamon vs. 2% cocoa or 2% marjoram vs. 0.5% thyme; counterbalanced according to group assignments). 30 min food intake is recorded with spillage accounted for by weighing crumbs collected from Tech-board paper that is placed under the cages of each animal prior to feeding. The % preference for the paired flavor is calculated as: 100*Demonstrator paired flavored chow intake/Demonstrator + Novel flavored chow intake. In this procedure, normal untreated animals learn to prefer the Demonstrator paired flavor based on social interaction and smelling the breath of the Demonstrator rat (9-11).

Videos were recorded during the 30 min social interaction between Observers and their Demonstrators after they had consumed their designated flavored chow. These videos were hand scored by experimenters blinded to experimental group for

time spent in social interaction. Social interaction measures are adapted from (12) and include time spent in the following activities: time spent grooming and sniffing the partner rat, especially in orofacial region.

Social discrimination (SD) and social recognition memory (SRM)

In this study we utilized an SD task to assess both sociability and SRM adapted from (13-16). A semi-transparent box (78.7 cm L × 39.4 cm W × 31.1 cm H), placed in a room with dim ambient lighting, was used as the SRM apparatus. Rats were habituated to the empty apparatus for 10 min 1-2 days prior to testing. On the day of testing, the SRM apparatus contained two plastic enclosures 6” in diameter that were used to confine the stimulus animals. The enclosures were slotted to allow for visual and olfactory investigation. During the first phase of testing, the test animal is placed in the apparatus with one enclosure remaining empty while the other contained a novel, non-experimental, adult male rat (previously habituated over several days to being in confined in the enclosure). The test animal is allowed to freely explore the apparatus for 5 min during which time video is taken and hand scored for time spent investigating the empty enclosure vs. stimulus animal. This was used to assess general sociability or tendency to approach other conspecifics as normal adult rats typically show a proclivity towards investigating the stimulus animal over the empty enclosure. The test animal is then removed and placed in a neutral cage for 30 min during which the bin and enclosures are cleaned with 10% ethanol. The animal is then placed back in the arena with the two enclosures, one of which contains the previously encountered conspecific from the previous social interaction now referred to as the “familiar” stimulus. The

previously empty enclosure now contains a new novel stimulus animal (male rat of similar age and size as “familiar” rat) not previously encountered by the experimental animal, termed the “novel” stimulus. The test animal is allowed to explore for another 5 min interval, which is video recorded and scored by an individual blinded to experimental group for time spent investigating each enclosure. Investigations were defined as the rat sniffing or touching the enclosure with the nose or forepaws. A normal adult rat should spend more time investigating the novel stimulus animal, indicating social recognition of the previously encountered animal and an intact social memory.

Novel object recognition (NOR)

The apparatus utilized for the NOR procedure was a semi-transparent box (78.7 cm L × 39.4 cm W × 31.1 cm H), placed in a room with dim ambient lighting. Procedures followed as described in (17), modified from (18). Rats were habituated to the empty apparatus and conditions for 10 min 1-2 days prior to testing. The test consisted of a 5-min familiarization phase during which rats were placed in the center of the apparatus (facing a neutral wall to avoid biasing them toward either object) with two identical objects and allowed to explore. The objects used were either two identical soda cans or two identical stemless wine glasses. Rats were then removed from the apparatus and placed in a neutral cage for 5 min. During this period, the apparatus and objects were cleaned with 10% ethanol solution and one of the objects was replaced with a “novel object” (either the can or glass) that the animal had not previously been exposed to. Rats were then placed in the center of the apparatus again and allowed to explore both objects for 5 min. The novel object and side on which the novel object was placed were

counterbalanced per treatment group. The time each rat spent exploring the objects was quantified by hand-scoring of video recordings by an experimenter blinded to the animal group assignments. Object exploration was defined as the rat sniffing or touching the object with the nose or forepaws.

Approach-avoidance Task

Rats were surgically implanted with bilateral HPCd guide cannulae as previously described. Procedures were adapted from an established protocol (19). Following surgical recovery, a place preference box was used to measure risk-food reward behavior in animals. One side of the box (dark side) was covered with a black nontransparent sheet, and the other side (light side) was surrounded by white walls with a transparent cover. The box was divided by a wall with a door with a handle on top of the box for the experimenters to control the opening and closing of the door. Standing lights illuminated the light side. Light was adjusted to a level of approximately 40 lumens on the light side during testing session. Rats were habituated to peanut butter (Jif Creamy Peanut Butter) in the home cages for 15 minutes a day during the dark cycle for seven days prior to testing. Following the peanut butter habituation, rats were habituated to the dark side of the place preference box for five minutes for two days prior to testing. On the test day, food was removed from rats' cages two hours before the dark cycle and testing. Animals received infusions of either aCSF or 0.05ug OT as previously described, approximately 5 min prior to start of testing with the order of drug treatments counterbalanced across the day. A shallow metal dish containing peanut butter was placed on the light side of the box. Rats were placed and remained in the

dark side of the box until the experimenters opened the door for rats to move freely between both sides of the box for five minutes. Peanut butter intake, latency to enter the light side, and the time spent on the light side was measured. Experimenters cleaned the box with 10% ethanol after each trial. Testing was recorded and analyzed using ANYmaze software (Stoelting, CO, USA).

BioDAQ food monitoring system and analysis parameters

Rats were individually housed in BioDAQ Food and Water intake monitoring system (Research Diets Inc. New Brunswick, NJ) which recorded episodic ad libitum feeding activity of rats in their home cages. Social eating procedures were also conducted in BioDAQ cages as described below to measure meal pattern parameters during the 1hr feeding sessions. All peripheral sensor controllers (PSCs) were validated for accuracy using 10.00g standard weights with an allowable margin of error +/- 0.05g prior to the start of the experiment as well as before test days. All PSCs were required to have QUIET readings (i.e., no movement at food hopper) prior to the start of any session. BioDAQ data was analyzed using an inter meal interval (IMI) of 900 seconds to separate meals, and bouts were filtered to be between -9.00 and 9.00g in size. Data was also set to have a period that started at the onset of the dark cycle when the behavioral task would begin, in this case 11:00AM to coincide with the light schedule in the room in which they were housed. The number of periods in the day was set to 24 resulting in 1hr long period bins from which cumulative intake as well as average meal size and duration could be calculated. Following exportation to Excel values were obtained from the “PSC by period” tab to record individual animals’ intake. First meal

size was taken from the “Meals” tab as it was an important measure given OT’s proposed role in regulating meal size by affecting satiation signals.

Experiment 6: The effects of ICV OT administration on social eating

To assess the effect of ICV oxytocin administration on social feeding, animals were implanted with ICV cannula as previously described above. Following surgical recovery, animals underwent social eating procedure (described above) where they were trained for 12 consecutive days to spend the first hour of the dark cycle consuming food in the presence of a familiar conspecific in a neutral cage. Following training there were 3 separate test days, each separated by 2 washout days between treatments, on which animals received either aCSF, 0.05ug OT or 0.50ug OT approximately 15 min prior to the start of the test period and with the order of drug treatments counter-balanced across test days. Results revealed that while ICV administration of 0.05ug OT had no effect on food intake in social feeding setting, a dose of 0.50ug OT significantly reduced first meal size (Supplemental Figure 1E) while having no effect on 1hr intake (Supplemental Figure 1D), likely due to a significant increase in meal frequency (Supplemental Figure 1F).

Statistical Analyses

Data are presented as means ± standard errors of the mean (SEM; error bars in all figures). Statistical analyses were performed using Prism software (GraphPad, Inc., version 10.0.2, San Diego, CA, USA). For all statistical tests the α level for significance was 0.05.

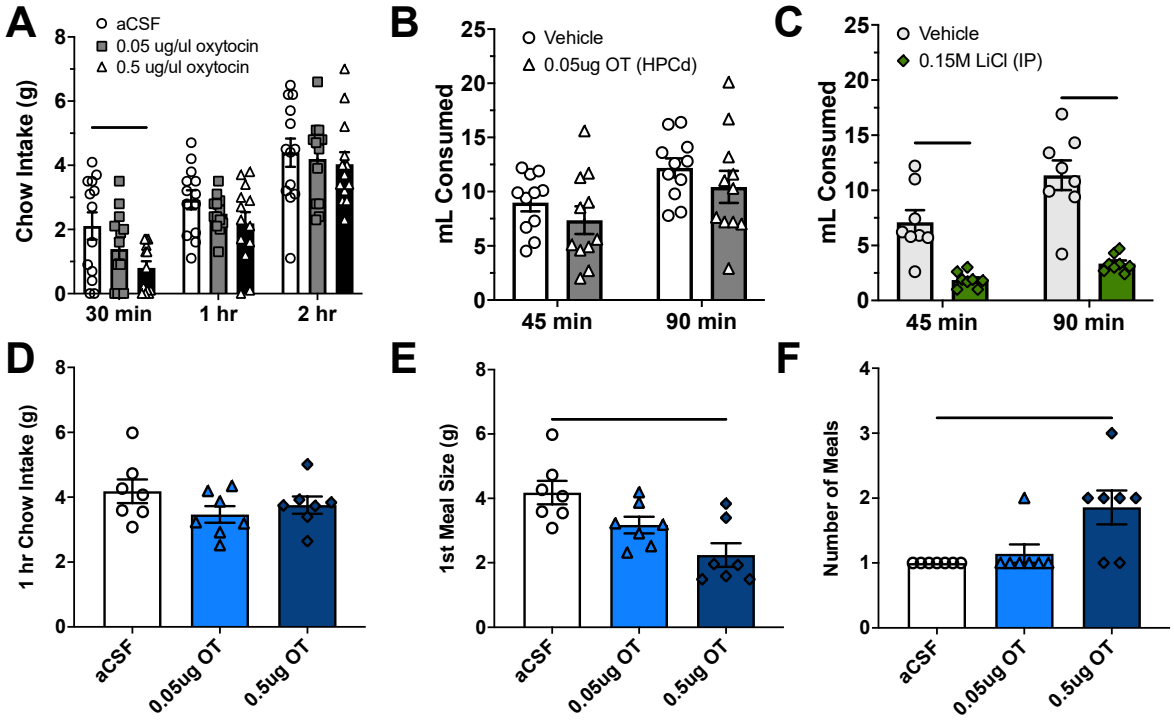
For Experiment 1, intake data and meal analyses were analyzed using a paired two-sample Student's t-test for each within-subject comparison of treatments. In Experiment 2 training intake data were analyzed with a repeated measures one-way analysis of variance (ANOVA) and then Sidak's correction for multiple comparisons when significant main effects or interactions were found. For social facilitation of eating (SFE) test data (Experiments 2 and 3), cumulative intake and meal patterns were analyzed using two-way ANOVA and Fisher's LSD tests to perform planned comparisons between groups, based on a priori hypotheses prior to conducting experiments for effects on cumulative food intake and meal patterns. Experiment 3 utilized an unpaired Student's t-test to analyze qPCR, social eating training, and home cage intake data. Analyzing changes in bodyweight over time used a repeated measures two-way ANOVA with Sidak's multiple comparisons test. Analyses in Experiment 4 were conducted using an unpaired Welch's t-test due to the unequal variance in 30min paired intake ratio. The 30min paired ratio was also analyzed using one sample t- and Wilcoxon tests to determine whether the preference was above chance (50%). For Experiments 5 and 6, multiple paired t-tests were used to analyze interaction time in the sociability, SRM, and NOR tasks. Exploration ratios were analyzed using unpaired Student's t-tests and one-sample t Wilcoxon test comparing to chance (50%).

Supplemental Table 1.

Experiment	Group Name	Context	Training Conspecific	Test Conspecific	Test Conditions
1	Isolated - Home Cage (n=8)	Home Cage	None	None	aCSF vs. 0.05ug OT
	Isolated - Neutral Cage (n=8)	Neutral Cage	None	None	aCSF vs. 0.05ug OT
	Familiar Conspecific - Neutral Cage (n=9)	Neutral Cage	Present/consistent	Familiar	aCSF vs. 0.05ug OT
2	Group Unfamiliar (n=6)	Neutral Cage	Present/consistent	Unfamiliar	aCSF vs. 0.05ug OT
	Group Familiar (n=6)	Neutral Cage	Present/consistent	Familiar	aCSF vs. 0.05ug OT
3	CON (n=6)	Neutral Cage	Present/consistent	Present	Unfamiliar vs. Familiar Conspecific
	OXTR KD (n=8)	Neutral Cage	Present/consistent	Present	Unfamiliar vs. Familiar Conspecific

Supplemental Table 1. Summary overview of experimental designs. A summary overview of training and testing conditions in experiments 1-3 including the context in which it was performed, whether there was a conspecific present during training and what test conditions were being compared.

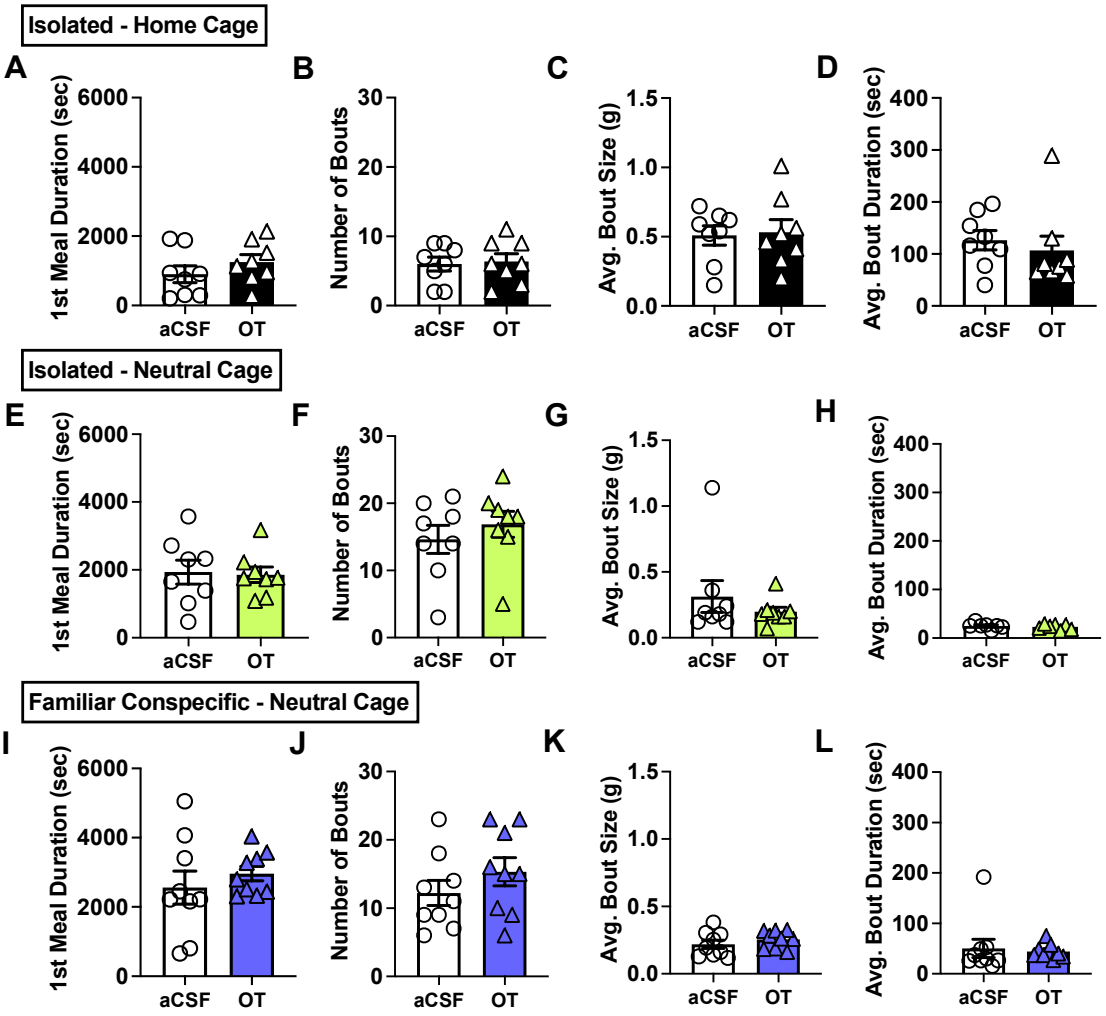
Supplemental Figure 1.



Supplemental Figure 1. HPCd oxytocin dose selection. (A) Administration of o.50ug but not 0.05ug OT into the lateral ventricle (LV) significantly reduces 30-min cumulative chow intake, and thus 0.05ug OT is without effect on food intake following ventricular administration under standard testing conditions (Within-subjects design for drug treatment, n=9; Data are means ± SEM; *p<0.05). **(B-C)** Administration of 0.05ug OT into the HPCd does not result in a conditioned flavor avoidance (CFA) while the IP administration of 0.15M lithium chloride results in a strong CFA (RM two-way ANOVA, treatment: $F_{(1,14)}$=29.36, p<0.0001, Sidak's adjusted p=0.0005 at 45 min and p<0.0001 at 90 min). (Between-subjects for drug treatment; HPCd vehicle n=11; HPCd 0.05ug OT n=11; IP vehicle n=8; IP 0.15M LiCl n=8; Data are means ± SEM; ***p<0.001, ****p<0.0001). **(D-F)** Administration of higher dose of 0.50ug OT ICV in the social eating paradigm had no effect on 1 h cumulative intake while significantly reducing 1st meal

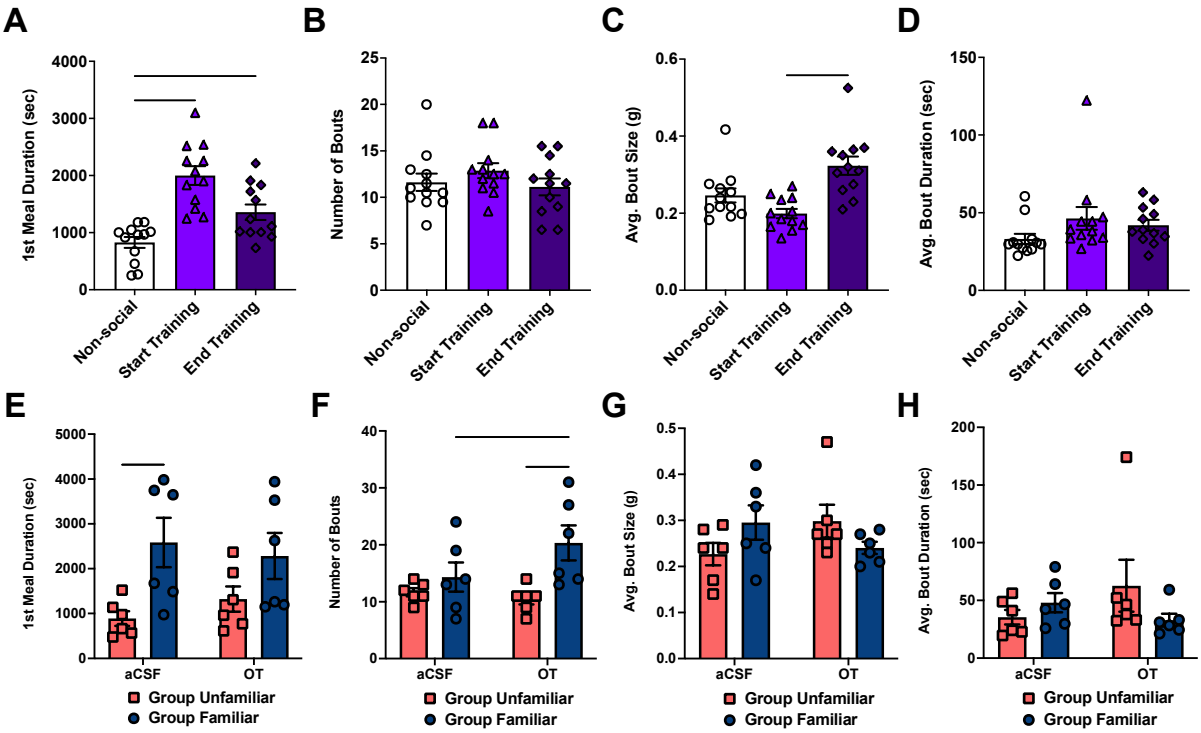
size (RM one-way ANOVA Treatment: $F_{(1.950,11.70)}$=8.386, p=0.0057, Sidak's adjusted p=0.0133 aCSF vs. 0.50ug OT) and significantly increasing meal frequency (RM one-way ANOVA Treatment: $F_{(1.140,6.842)}$=5.813, p=0.0448, Sidak's adjusted p=0.0492 aCSF vs. 0.50ug OT) while a lower dose of 0.05ug OT had no effect on intake under social eating when administered ICV. (Within-subjects design for drug treatment, n=7; Data are means ± SEM; *p<0.05).

Supplemental Figure 2.



Supplemental Figure 2. HPCd oxytocin administration did not influence meal duration of eating bout parameters. Under all three conditions: isolated home cage **(A-D,)** isolated neutral cage **(E-H),** and in the presence of a familiar conspecific **(I-L)**, hippocampal administration of 0.05 µg oxytocin did not affect 1st meal duration, bout frequency, average bout size or average bout duration during the 1hr social eating period. (home cage n=9; isolated neutral n=8; familiar conspecific n=9; all within-subject design for drug treatments; Data are means ± SEM; *p<0.05, **p<0.01).

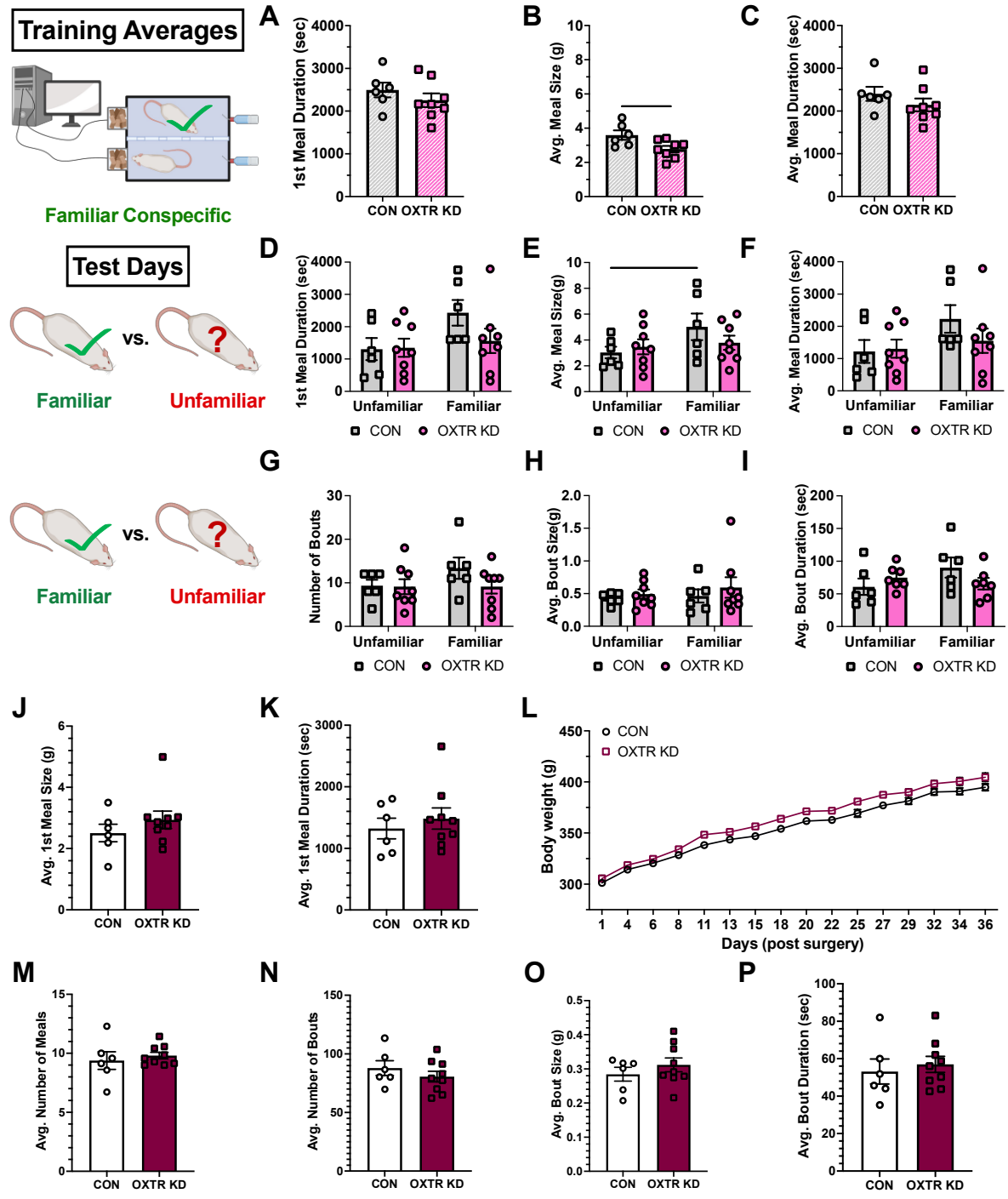
Supplemental Figure 3.



Supplemental Figure 3. Endogenous HPCd oxytocin receptor signaling increases eating bout number in the presence of a familiar conspecific. (A) When compared to average isolated "Non-social" home cage intake, there is a significant increase in 1st meal duration and both the start of the social eating training ("Start Training") and the end of training ("End Training"). There is no significant difference in 1st meal duration between the start and end of training (n=12, RM one-way ANOVA, Days: $F_{(1.485,16.04)}$=16.43, p=0.0003, Sidak's adjusted p=0.0002 non-social vs. start training and p=0.0191 non-social vs. end training). **(B,D)** There is no significant differences in bout frequency or average bout duration between Non-social, start training and end training conditions. **(C)** There is a significant increase in average bout size between the start of training and the end of training with no differences between non-social and start or non-social and end of training (n=12, RM one-way ANOVA, Days: $F_{(1.363,14.99)}$=10.25,

p=0.0034, Sidak's adjusted p=0.0007 start training vs. end training). (within-subject design for level of conspecific familiarity, Data are means ± SEM; n=12; *p<0.05, **p<0.01, ***p<0.001).). **(E)** During pharmacological testing, rats assigned to Group Familiar had a longer 1st meal duration with the aCSF treatment compared to rats assigned to the Group Unfamiliar with aCSF treatment, but not with OT in both groups (RM two-way ANOVA, conspecific familiarity: $F_{(1,10)}$=5.925, p=0.0352; Fisher's LSD planned comparisons aCSF: t(20)=2.926 p=0.0083 unfamiliar vs. familiar). **(F)** Rats in the Group Familiar had a significantly increased bout frequency with OT was administered compared to Group Unfamiliar OT treatment. Administration of OT significantly increased bout frequency compared to aCSF treatment in Group Familiar but not Group Unfamiliar (RM two-way ANOVA, conspecific familiarity: $F_{(1,10)}$=5.039, p=0.0486, treatment: $F_{(1,10)}$=5.80, p=0.0368, treatment x conspecific familiarity: $F_{(1,10)}$=12.75, p=0.0051; Fisher's LSD planned comparisons OT: t(20)=3.323, p=0.0034 unfamiliar vs. familiar, and Familiar: t(10)=4.228, p=0.0017 aCSF vs. OT). **(G,H)** There was no significant effect of either group or drug treatment on either average bout size or bout duration. (between-subject design for conspecific familiarity, within-subject for drug treatment; Group Unfamiliar n=6; Group Familiar n=6; Data are means ± SEM; *p<0.05, **p<0.01, ***p<0.001).

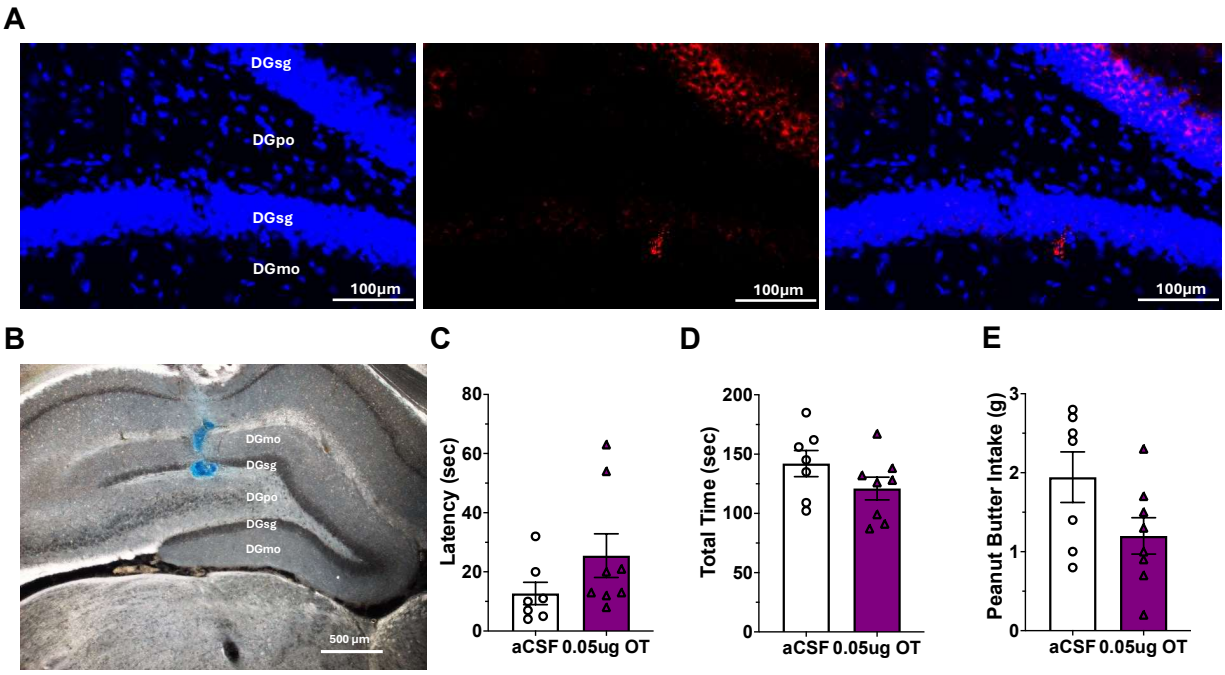
Supplemental Figure 4.



Supplemental Figure 4. Chronic hippocampal oxytocin receptor knockdown reduces meal size under in the presence of a familiar conspecific. (A-C) During the training phase of the social eating procedure, OXTR KD animals saw a reduction in

average meal size (t(12)=2.545, p=0.026) during the hour in the social arena compared to controls while there was no significant difference in either 1st meal duration or average meal duration. **(D-I**) Control, but not OXTR KD animals had a larger average meal size (RM two-way ANOVA, group: $F_{(1,12)}$=4.213, p=0.0626; Fisher's LSD planned comparisons CON: t(12)=2.344 p=0.0371 unfamiliar vs. familiar) during the hour-long test in the presence of a familiar vs. an unfamiliar conspecific, while showing no effect of 1st meal duration, average meal duration, bout frequency, average bout size or average bout duration. **(J-P)** Knockdown of dorsal hippocampal oxytocin receptors did not yield long-term changes in daily meal parameters or body weight under isolated conditions in the home cage. (Between subjects control n=6; OXTR KD n=9; Data are means ± SEM; *p<0.05).

Supplemental Figure 5.



Supplemental Figure 5. Pharmacological activation of OXTR in the HPCd DG region has no effect on general anxiety in an approach-avoidance task. (A) Confirmation of OXTR expression within the DG region of the HPCd using FISH to visualize OXTR (red) and Dapi (blue) (Abbreviations, DGmo = dentate gyrus molecular layer, DGsg = dentate gyrus granule layer, DGpo = dentate gyrus polymorph layer). **(B)** Representative image of inclusion criteria for pharmacological administration experiments into the HPCd including the dentate gyrus. **(C-D)** Administration of 0.05ug OT into the HPCd prior to an approach-avoidance task has no significant effect on latency to enter the light side of the apparatus or time spent in the light side compared to aCSF controls. **(E)** There was a non-significant trend towards decreasing peanut butter intake with the administration of 0.05ug OT compared to aCSF (Between Subjects aCSF = 7; 0.05ug OT = 8; Data are means ± SEM; *p<0.05)

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