# Effects of chronic oxytocin administration and diet composition on oxytocin and vasopressin 1a receptor binding in the rat brain

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## Abstract

Oxytocin (OT) elicits weight loss in diet-induced obese (DIO) rodents, nonhuman primates, and humans, in part, by reducing food intake. Chronic OT administration produces more sustained weight loss in high fat diet (HFD)-fed DIO rodents relative to chow-fed controls, but the reasons for this effect remain unclear. We hypothesized that HFD-induced obesity is associated with elevated OT receptor (OXTR) binding in brain regions where OT is known to cause decreased food intake and that this sensitized neural system is one mechanism by which OT preferentially elicits weight loss in DIO rodents. We therefore determined the impact of diet (HFD vs chow) and drug treatment (chronic OT infusion vs vehicle) on 1) OXTR binding in hindbrain and forebrain sites where OT suppresses food intake relative to control sites that express OXTR and 2) forebrain vasopressin 1a receptor (AVPR1a) density to evaluate the specificity of any OT effects. Using quantitative receptor autoradiography, we found that 1) diet composition failed to alter OXTR or AVPR1a binding; 2) chronic OT treatment produced largely global reductions in forebrain OXTR and AVPR1a binding without significantly altering hindbrain OXTR binding. These findings suggest that forebrain OXTR and AVPR1a are down-regulated in response to chronic OT treatment. Given that chronic intranasal OT may be used as a therapeutic strategy to treat obesity, future studies should consider the potential downregulatory effect that chronic treatment can have across forebrain and hindbrain nonapeptide receptors and assess the potential contribution of both receptor subtypes to the outcome measures.

## Keywords

Obesity; oxytocin; food intake; receptor autoradiography

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## Introduction

The neurohypophyseal hormone oxytocin (OT) is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus and is well-recognized for its peripheral effects to stimulate uterine contraction during parturition and milk ejection during lactation. Existing studies have extended this knowledge by providing evidence that OT also plays an important role in the central nervous system (CNS) in promoting prosocial behaviors (Striepens et al., 2011, Yamasue et al., 2012) and in the regulation of energy balance (Blevins and Baskin, 2015, Lawson, 2017, Leng and Sabatier, 2017, Leslie et al., 2018, Plessow et al., 2018, Spetter et al., 2018). We and others have demonstrated that OT is effective in reducing weight gain and/or evoking weight loss in both diet-induced obese (DIO) and genetically obese rodent models with impaired (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Zhang and Cai, 2011, Morton et al., 2012, Maejima et al., 2017, Roberts et al., 2017) or defective leptin signaling (Morton et al., 2012, Altirriba et al., 2014, Plante et al., 2015). OT dose-dependently reduces food intake, whether administered systemically, directly into the CNS, or intranasally (Blevins and Baskin, 2015). This effect is observed in rodents fed a standard chow or low fat diet (Rinaman and Rothe, 2002, Maejima et al., 2011, Zhang et al., 2011, Morton et al., 2012, Ho et al., 2014, Noble et al., 2014, Ong et al., 2015, Blevins et al., 2016, Roberts et al., 2017, Klockars et al., 2018), sucrose (Mullis et al., 2013, Herisson et al., 2016), and high fat diet (HFD) (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Zhang and Cai, 2011, Morton et al., 2012, Blevins et al., 2016, Maejima et al., 2017). For these reasons, OT has attracted widespread interest as a therapeutic strategy to treat obesity, given that these effects have been recently translated to DIO nonhuman primates (Blevins et al., 2015) and obese humans (Zhang et al., 2013, Lawson et al., 2015, Thienel et al., 2016, Hsu et al., 2017).

Chronic OT administration (either infusions or repeated administration) elicits sustained weight loss in HFD-fed mice and rats (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Blevins et al., 2016, Maejima et al., 2017, Roberts et al., 2017), but it tends to be ineffective or produce only transient effects on body weight in chow-fed or low fat diet-fed rodents (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Blevins et al., 2016, Maejima et al., 2017, Roberts et al., 2017). These effects on weight loss are mainly attributed to reductions in food intake (Maejima et al., 2011, Blevins et al., 2016, Roberts et al., 2017). The presence of fat (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Blevins et al., 2016, Maejima et al., 2017, Roberts et al., 2017) and carbohydrates (i.e. sugar) (Miedlar et al., 2007, Sclafani et al., 2007, Olszewski et al., 2010, Mullis et al., 2013, Herisson et al., 2014, Blevins et al., 2015, Olszewski et al., 2015, Cherepanov et al., 2017) in the HFD may contribute to the effectiveness of chronic OT to elicit lasting suppression of food intake in HFD subjects, but differences in cholesterol levels between diets have also been hypothesized to play a role. Gimpl (Gimpl and Fahrenholz, 2001) has reported that cholesterol in the diet may enhance the affinity state of the OXTR, which may partly explain the increased effectiveness of chronic OT in HFD-fed rodents relative to chow-fed rodents in reducing food intake and eliciting weight loss. Based on these collective findings, we tested the hypothesis that HFD-induced obesity is associated with elevated OXTR binding in brain regions where OT reduces food intake: the arcuate nucleus (ARC (Maejima et al., 2014)),

the ventromedial hypothalamus (VMH (Noble et al., 2014, Klockars et al., 2017)), and the nucleus of the solitary tract (NTS (Ong et al., 2015)). This sensitized neural OT system could be one mechanism by which OT could have enhanced effectiveness to elicit weight loss in DIO rodents.

Existing studies indicate that OT may interact with the vasopressin 1a receptors (AVPR1a) as OT is reported to have only a 10-fold selectivity for OXTR relative to vasopressin 1a receptor (AVPR1a) (Kimura et al., 1994, Postina et al., 1996, Gimpl and Fahrenholz, 2001). This is likely due to the structural similarity between OT and AVP, which differ by only two amino acids (Gimpl and Fahrenholz, 2001), and between the mammalian OXTR and AVPR1a, which share approximately 50% sequence homology (Gimpl and Fahrenholz, 2001). Previous studies from rodent models (including mice, rats, hamsters and prairie voles) suggest that acute or chronic OT administration can act through AVPR1a to elicit measured effects on neural activation, behavior and physiology (Yamamoto et al., 2004, Bales et al., 2007, Schorscher-Petcu et al., 2010, Sala et al., 2011, Song et al., 2014, Flicks et al., 2016, Everett et al., 2018, Guoynes et al., 2018). Thus, an important question is whether chronic infusions of OT (at doses that elicit weight loss) may also impact AVPR1a density in brain areas that contribute to the effects of OT to reduce food intake and whether these effects occur in a diet-specific manner. We therefore determined the impact of HFD and chronic OT treatment on OXTR binding in areas where OT reduces food intake relative to control areas that express OXTR, as well as AVPR1a binding in several forebrain areas to evaluate the specificity of action of OT.

## Experimental Procedures

### Materials and Methods

**Animals—**Adult male outbred Sprague-Dawley (CD® IGS) (≈ 6–7 months/490–887 g) were obtained from Charles River Laboratories International (Wilmington, MA). All rats in this study were maintained on a HFD or chow diet and received a 21-day chronic third ventricular (3V) infusions of OT (16 nmol/day) or saline vehicle. These rats are a subset of those used in a previously published metabolic study (Blevins et al., 2016). All rats were housed individually in Plexiglas cages in a temperature controlled room (22±2°C) under a 12:12-h light-dark cycle (lights off at 1 p.m.). Rats had *ad libitum* access to water and either a HFD containing 60% kcal from fat (Research Diets, D12492, New Brunswick, NJ) or a low fat chow diet containing 13% kcal from fat (LabDiet, St. Louis, MO). The current research protocols were approved both by the Institutional Animal Care and Use Committee of the Veterans Affairs Puget Sound Health Care System (VAPSHCS) and the University of Washington in accordance with NIH Guidelines for the Care and Use of Animals.

**Brain removal and slicing—**HFD-fed or chow-fed control rats were euthanized with an overdose of ketamine hydrochloride (214.3 mg/kg), xylazine (10.71 mg/kg) and acepromazine (2.2 mg/kg) (IP) following a 21-day infusion of OT or saline vehicle as previously described (Blevins et al., 2016). Brains were immediately removed, flash frozen in isopentane and stored frozen at −80°C. They were then sliced on a cryostat at 20 μm and

mounted onto Superfrost slides (Fisherbrand, Fisher Scientific, PA). All slides were stored at −80°C in sealed slide boxes with desiccants until the time of assay.

**Receptor autoradiography—**Receptor autoradiography for OXTR and AVPR1a receptor was performed as described previously (Perkeybile et al., 2015). Slides were removed from −80°C and allowed to thaw at room temperature. Next, they were lightly fixed in 0.1% paraformaldehyde (pH 7.4), washed twice in 50 mM Tris base (pH 7.4; to remove endogenous hormones), and incubated for 1 hr in tracer buffer (pH 7.4) containing 50 pM $^{125}$l- ornithine vasotocin analog ($^{125}$l-OVTA) or $^{125}$I- linear vasopressin antagonist ($^{125}$I-LVA) to target OXTR and AVPR1a, respectively (radioligands from PerkinElmer, Waltham, MA). Finally, unbound radioligand was removed after slides were rinsed in several washes of 50 mM Tris base with 10 mM $MgCl_2$ (pH 7.4) and dipped in deionized water before being allowed to air dry. Once dried, the slides were exposed to Carestream BioMax MR film (Kodak, Rochester, NY) for 3 days and then developed and analyzed.

**Quantification—**A rat brain atlas (Paxinos and Watson, 2007) was used to determine the anatomical boundaries of the following regions of interest (ROI): ARC, VMH, central nucleus of the amygdala (CeA), lateral septum (LS), subiculum (Sub), area postrema (AP), dorsal motor nucleus of the vagus (DMV), and NTS. Our analysis focused on ROIs where OT is known to act to affect food intake: ARC, VMH, and NTS. In order to evaluate the specificity of any measurable effects, we also included in our analysis a few forebrain ROIs which have dense receptor binding in rats but have not been linked to OT's effects on food intake: the CeA and Sub (for OXTR) and the CeA and LS (for AVPR1a). In the hindbrain, these control regions included the AP and DMV.

The $^{125}$l-OVTA and $^{125}$I-LVA binding was quantified in the following manner, which has been described previously (Freeman et al., 2017). For the forebrain study, the optical binding density (OBD) was quantified directly from the film using a light box, a top mounted camera, and the MCID Core Digital Densitometry system (Cambridge, UK). For the hindbrain study, which was performed prior to our acquisition of the MCID system, we used ImageJ (NIH; Bethesda, MD, USA) to measure OBD from digitized images of the films. These films were scanned and digitized using an Epson Perfection V500 Photo scanner, and the resulting autoradiogram image files were imported into ImageJ 64 for analysis. In both studies, quantification was carried out in the same way: OBD values from a set of $^{125}$l autoradiography standards (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) were loaded into the software and used to generate a standard curve, from which OBD values for each ROI were extrapolated. For each specimen, average OBD values were calculated for each ROI, as well as for one background area where no binding was detected, which served as a measure of non-specific binding. This average background/non-specific binding was subtracted from the ROI measurements to yield normalized OBDs across specimens and correct for individual variation in non-specific binding across individuals of this outbred strain of rats.

**Data Analysis—**In order to control for the multiple brain regions examined, we divided our analyses into three groups: OXTR forebrain binding, OXTR hindbrain binding, and AVPR1a forebrain binding. We then performed multivariate analysis of variance

(MANOVA) on each group. Analysis was performed in SAS 9.4 (SAS Institute, Cary, NC), using proc glm. Assumptions of analysis of variance were tested. A significant result in the multivariate analysis was followed by separate ANOVAs for each brain area in that analysis. Alpha was set at $p < 0.05$ and all tests were two-tailed.

## Results

### Effects on OXTR density in the hindbrain

We conducted this study to examine the extent to which diet (HFD vs chow) and chronic drug treatment (OT vs vehicle) may impact OXTR binding in a hindbrain area (NTS) known to express OTR (Loup et al., 1989, Verbalis et al., 1995, Gould and Zingg, 2003, Yoshida et al., 2009) and that contributes to the effects of OT on food intake relative to control sites (AP, DMV) that express OXTR (Vaccari et al., 1998, Yoshida et al., 2009) but have not been directly linked to the central effects of OT to reduce food intake (AP, DMV). OT treatment did not significantly predict OXTR binding in the hindbrain (Wilks' lambda = 0.547, $F_{3,9}$ = 2.49, $p = 0.127$; **Figure 1**). There was no effect of diet (Wilks' lambda = 0.604, $F_{3,9}$ =1.97, $p$ = 0.190), and no diet by drug interaction (Wilks' lambda = 0.833, $F_{3,9}$ = 0.60, $p = 0.631$). Due to the overall lack of drug effect, we do not claim significance for individual hindbrain areas. However, it is worth noting that had they been examined alone, OT would have reduced OXTR binding in both the DMV ($F_1$ =8.11, $p = 0.016$) and the NTS ($F_1$ = 5.52, $p = 0.039$).

### Effects on OXTR density in the forebrain

We conducted this study to examine the extent to which diet (HFD vs chow) and drug treatment (OT vs vehicle) may impact OXTR binding in forebrain areas that express OXTR (Vaccari et al., 1998, Yoshida et al., 2009, Hidema et al., 2016), are linked to the control of food intake (ARC, VMH, CeA) and potentially contribute to the metabolic effects of OT in HFD-fed rats as has been reported previously in chow-fed rats and mice (Maejima et al., 2014, Noble et al., 2014, Klockars et al., 2017, Klockars et al., 2018). To determine whether the neural effects were specific only to brain areas that are known to contribute to the effects of OT on food intake, we also included the Sub, given that this site expresses OXTR but has not been linked to the central effects of OT to reduce food intake. We found that OT treatment significantly down-regulated OXTR binding in the forebrain (Wilks' lambda = 0.340, $F_{4,20}$ = 9.69, $p < 0.001$; **Figures 2–3).** There was no effect of diet (Wilks' lambda = 0.839, $F_{4,20}$ = 0.96, $p = 0.453$), and no diet by drug interaction (Wilks' lambda = 0.792, $F_{4,20}$ = 1.31, $p = 0.299$). OXTR binding was significantly affected by treatment in the ARC ($r_2 = 0.566$, $F_1 = 29.00$, $p < 0.0001$), the CeA ($r_2 = 0.501$, $F_1 = 18.74$, $p < 0.001$), the VMH ($r_2 = 0.593$, $F_1 = 32.34$, $p < 0.0001$), and Sub ($r_2 = 0.587$, $F_1 = 26.93$, $p < 0.0001$).

### Effects on AVPR1a density in the forebrain

In addition, we examined the structurally similar AVPR1a in forebrain sites with previously reported AVP binding (Dorsa et al., 1984, Szot et al., 1990) and AVP1a mRNA expression (Ostrowski et al., 1992, Ostrowski et al., 1994), in order to evaluate the specificity of any OT effects, given the precedent for cross-talk between these two systems (Yamamoto et al., 2004, Bales et al., 2007, Gupta et al., 2008, Schorscher-Petcu et al., 2010, Sala et al., 2011,

Song et al., 2014, Guoynes et al., 2018). To determine whether the neural effects were specific to forebrain areas that contribute to the central effects of OT on food intake (ARC, VMH, CeA) (Maejima et al., 2014, Noble et al., 2014, Klockars et al., 2017, Klockars et al., 2018), we also included the lateral septum (LS) because it expresses AVPR binding sites (Baskin et al., 1983) [including AVPR1a (Grundwald et al., 2016)] in rats but has not been linked to the central effects of OT to reduce food intake. OT treatment also significantly down-regulated AVPR1a receptor binding in the forebrain (Wilks' lambda = 0.516, $F_{4,25}$ = 5.87, p = 0.002; **Figures 4–5).** There was no effect of diet (Wilks' lambda = 0.889, $F_{4,25}$ = 0.78, p = 0.548), and no diet by drug interaction (Wilks' lambda = 0.943, $F_{4,25}$ = 0.38, p = 0.0823). AVPR1a was significantly affected by treatment in the ARC ($r_2$ = 0.293, $F_1$ = 10.44, p = 0.003), the VMFI ($r_2$ = 0.279, $F_1$ = 10.50, p = 0.003), and the LS ($r_2$ = 0.326, $F_1$ = 11.24, p = 0.002). However, OT treatment did not affect AVPR1a binding in the CeA ($r_2$ = 0.072, $F_1$ = 0.75, p = 0.393).

## Discussion

Chronic OT administration produces more sustained effects to evoke weight loss in HFD-fed DIO rodents relative to chow-fed controls (Deblon et al., 2011, Maejima et al., 2011, Zhang et al., 2011, Blevins et al., 2016, Maejima et al., 2017, Roberts et al., 2017), but the reasons for this effect remain unclear. We hypothesized that HFD-induced obesity is associated with elevated OXTR binding in brain regions where OT has been shown to be effective in reducing food intake (ARC, VMH, CeA, NTS) and that this sensitized neural OT system is one mechanism by which OT could have enhanced effectiveness to elicit weight loss in DIO rodents. To test this hypothesis, we determined the impact of HFD diet and chronic OT treatment on OXTR binding in hindbrain (NTS) and forebrain (ARC, CeA, VMH) sites where OT suppresses food intake, relative to control sites that express OXTR (AP, DMV, Sub) but have not been linked to the central effects of OT on food intake. Additionally, we also measured forebrain vasopressin 1a receptor (AVPR1a) density in the ARC, CeA and VMH relative to the LS to evaluate the specificity of any OT effects.

We found that diet composition failed to alter OXTR or AVPR1a density and that chronic OT treatment down-regulated OXTR density in forebrain. In addition, chronic OT treatment down-regulated forebrain AVPR1a density with the exception of the CeA. Collectively, these findings suggest that both forebrain OXTR and AVPR1a are down-regulated in response to chronic OT treatment. Given that chronic intranasal OT may be used as a therapeutic strategy, future studies should assess the extent to which both receptor subtypes may contribute to outcome measures. Future plans for the use of chronic OT treatment in humans or animal studies also be aware of the the potential downregulatory effect that this course of treatment can have across forebrain nonapeptide receptors.

Our findings indicate that chronic OT treatment was not associated with a significant downregulation of hindbrain OXTR in both HFD-fed or chow-fed rats, although it is worth noting that OT would have reduced OXTR binding in DMV and the NTS had the ROIs been examined alone. Moreover, there was also no significant impact of diet on OXTR binding within the hindbrain. We acknowledge the possibility that there may have been a floor effect that prevented the detection of OXTR downregulation. It will be important in future studies

to address this possibility with a larger sample size and to assess the temporal effects of chronic OT on hindbrain OXTR binding and whether OXTR downregulation coincides with OT's waning effectiveness to reduce food intake in HFD-fed rats.

OXTRs in NTS are anatomically positioned to control energy balance by reducing food intake and stimulating energy expenditure by increasing brown adipose tissue (BAT) thermogenesis. The NTS is a CNS site with sympathetic outflow to BAT and is linked to the control of BAT thermogenesis (Bamshad et al., 1999, Li et al., 2007, Morrison and Nakamura, 2011). Recent studies have identified an anatomical circuit comprised of projections from the parvocellular PVN (pPVN) to the NTS (Kong et al., 2012) that then project to the raphe pallidus (RPa), a brain area known to regulate sympathetic control of BAT thermogenesis via projections to spinal cord sympathetic pre-ganglionic neurons (Morrison and Nakamura, 2011, Morrison, 2016). Existing studies have extended these anatomical findings to provide more direct support for a role of endogenous OT action at hindbrain NTS OXTRs in the control of food intake (Blouet et al., 2009, Baskin et al., 2010, Matarazzo et al., 2012, Ong et al., 2015) and thermogenesis (Ong et al., 2017) in rats and mice. Furthermore, acute 4V (Ho et al., 2014, Ong et al., 2015) or NTS (Ong et al., 2015) administration of OT reduces food intake and stimulates BAT temperature (Roberts et al., 2017) as well as core temperature (Ong et al., 2017). In addition, our recent studies show chronic fourth ventricular (4V) OT is associated with prolonged weight loss that is mediated, in part, by reductions in food intake (Roberts et al., 2017). OXTRs in both the NTS and DMV may contribute to the effects of OT to reduce food intake, in part, by inhibiting gastric motility or gastric emptying (Rogers and Hermann, 1987, McCann and Rogers, 1990, Flanagan et al., 1992, Cruz et al., 2007, Holmes et al., 2013). Future studies that assess the effects of OT to reduce food intake in rats with gastric cannulas that remain open (sham feeding; no gastric distension) or closed (real feeding) would help identify whether decreases in gastric emptying are required for OT to reduce food intake.

Two key questions remain to better understand the role of specific populations of OXTR within the brain in contributing to the prolonged effects of chronic CNS administration of OT on weight loss:1) at what point does the OXTR downregulation occur following continuous OT infusion and 2) does this coincide with the inability of chronic CNS OT to produce sustained reductions in food intake that extend beyond 2–3 weeks? The extent to which downregulation of OXTRs in forebrain, midbrain and hindbrain areas contribute to the inability of chronic CNS OT to elicit long-term reductions of food intake await further investigation (Leslie et al., 2018).

Another mechanism that may contribute to the more prolonged effectiveness of OT to evoke weight loss in DIO animals maintained on a HFD includes an enhanced affinity state of OXTR, due to cholesterol in the HFD. OXTRs are found in either a high or low affinity state, and cholesterol is required for high-affinity binding of OT (Gimpl and Fahrenholz, 2001). The presence of cholesterol in media that contains solubilized OXTRs can potentially enhance high-affinity OT binding and stabilize the OXTR in a high affinity state (Gimpl et al., 1995). Gimpl indicated that "cholesterol acts as an allosteric modulator and stabilizes the receptor in a high-affinity state for agonists and antagonists". Given that the HFDs used across many studies (60% kcal from fat, D12492) may contain up to 279.6 to 300.8 mg/kg

cholesterol (D12492 from 2006 or 2011 formulations) relative to 200 mg/kg in chow diet (13% kcal from fat; 5001) the potential role of cholesterol levels is a possible complicating factor in interpreting the results of studies on the impact of OT on HFD-fed animals. However, with the exception of the CeA, we did not find evidence of enhanced binding in the CNS in vehicle-treated DIO rats relative to chow-fed controls. Future studies that examine the role of cholesterol on both OXTR binding and OXTR mRNA and protein expression in DIO rodents maintained on a HFD relative to chow-fed controls will be helpful in identifying whether enhanced OXTR binding may contribute to the more prolonged effectiveness of OT in DIO animals.

Although the majority of existing studies provide data to support that cholesterol may certainly contribute to the enhanced effectiveness of OT in DIO rodent models it does not appear to be required as others have demonstrated a more robust effect of OT to elicit weight loss following chronic subcutaneous administration in mice on a cholesterol-free HFD relative to a low fat diet (Maejima et al., 2011, Maejima et al., 2017). Maejima and colleagues further demonstrated that OT-elicited weight loss was negatively correlated with initial body weight in mice maintained on the HFD such that OT was more effective in those mice on the HFD that were more obese. In contrast, there was no correlation between OT-elicited weight loss and initial body weight in mice maintained on the low fat diet. While we did not directly measure changes in CNS OXTR mRNA or OXTR protein expression in this study, others have found that 7-week exposure to a HFD in a genetic model of obesity is associated with increased OXTR mRNA in peripheral tissues, including adipose tissue (Gajdosechova et al., 2014, Yi et al., 2015) and skeletal muscle (Gajdosechova et al., 2014). This may be a response to the reduction of circulating OT that is observed in certain genetic models of obesity (Gajdosechova et al., 2014, Plante et al., 2015). Given that others have demonstrated a direct action of OT on adipocytes (Deblon et al., 2011, Yi et al., 2015), this may also explain, in part, the more robust effectiveness of chronic systemic OT to evoke weight loss in DIO mice. These findings are also consistent with those that have found reductions in serum OT in DIO mice (Zhang et al., 2011, Zhang and Cai, 2011) although this finding is not always evident in DIO mice (Maejima et al., 2017) and rats (Morton et al., 2012) for reasons that may be related to length of exposure to HFD or time when measurements occurred. It will be important to examine the extent to which OXTRs on adipocytes are necessary for systemic OT to elicit weight loss in DIO rodents in future studies.

Our findings that show a largely global pattern of downregulation of CNS OXTRs in response to chronic OT infusion are largely consistent with what others have shown in the CNS following chronic intracerebroventricular (ICV) infusions in rats (Insel et al., 1992) and mice (Peters et al., 2014) fed a standard low fat or chow diet. Insel and colleagues reported that lateral ventricular administration of OT over 10 days, at a dose that enhanced social behavior (≈ 2.38 nmol/day) (Witt et al., 1992), produced a global reduction of OXTR binding in the VMH, anterior olfactory nucleus (AOP), bed nucleus of the stria terminalis (BNST), CeA, and the ventral septum (VS) in rats (Insel et al., 1992). Similarly, Peters and colleagues demonstrated that lateral ventricular infusion of OT over 15 days, at a dose that produced anxiogenic behavior ≈ 0.0238 or 0.238 nmol/day), produced a global downregulation of OXTR in the dorsolateral septum (DLS), ventrolateral septum (VLS),

basolateral amygdala, medial amygdala, CeA, and median raphe nucleus in mice (Peters et al., 2014). In contrast, Insel and colleagues demonstrated that repeated administration (2x daily over 4 days) of OT (1000 ng) did not produce any obvious alterations in OXTR binding in the AOP, BNST, amygdala, VMH, and VS at 24 h following the last injection although there appeared to be a slight reduction in OXTR binding in the AOP at 72 h post-treatment (Insel et al., 1992).

We and others have found that 2–4x daily administration of OT is also effective in reducing body weight in obese nonhuman primates and humans (Zhang et al., 2013, Blevins et al., 2015) suggesting that repeated intermittent administration of OT might be an effective strategy to promote weight loss. Moreover, others have shown that long-term intermittent delivery of anorexigenic peptides (2–4x daily 3-h infusions) [e.g. PYY(3–36), exendin-4] into the periphery is an effective strategy to produce sustained reductions in food intake and weight loss in DIO rodents (Reidelberger et al., 2011, Henry et al., 2015). Now that OT is currently being tested as a weight-loss therapy in pre-clinical trials in humans, it will be important to consider delivery approach (long-term intermittent delivery vs continuous delivery) and the potential impact on OXTR downregulation when optimizing a treatment regimen to elicit weight loss in obese humans.

The finding that chronic OT infusion downregulates AVPR1a binding is also consistent with what others have suggested in regards to specificity of OT action. The effects of *in vitro* bath application of OT to increase contractions in rabbit ejaculatory tissues was blocked by the V1a antagonist, SR49059 (Gupta et al., 2008). In addition, ICV administration of OT appears to control certain forms of social behavior in hamsters and mice through the AVPR1a (Sala et al., 2011, Song et al., 2014). Recent reports also indicate that chronic intranasal OT reduces vasopressin-immunoreactive cells in the paraventricular nucleus of male prairie voles (Guoynes et al., 2018). This does not appear to be limited to only chronic administration of OT as a single acute injection of OT on postnatal day 1 produces changes in both OT (Yamamoto et al., 2004) and AVP (Yamamoto et al., 2004, Bales et al., 2007) immunoreactive cells in the hypothalamus of prairie voles. Furthermore, acute intraperitoneal administration of OT is reported to elicit 1) c-Fos in various CNS sites (Hicks et al., 2016), locomotor activity (Hicks et al., 2016), analgesia and scratching (Schorscher-Petcu et al., 2010), as well as 2) inhibit methamphetamine-elicited behaviors (Everett et al., 2018), in part, through AVPR1a in rodent models. However, in contrast to the adult-onset obesity phenotype observed with OT or OXTR null mice (Takayanagi et al., 2008, Camerino, 2009), AVPR1a null mice are hypermetabolic (Hiroyama et al., 2007) and existing data from acute studies in our lab and others suggest that the effects of CNS OT to reduce food intake (Rinaman and Rothe, 2002, Ho et al., 2014, Ong et al., 2015, Klockars et al., 2017), sucrose consumption (Mullis et al., 2013), and increase BAT temperature (Roberts et al., 2017) are mediated by OXTRs. However, further tests will need to rule out the possibility that effects of chronic 4V OT may be mediated, in part, by AVPR1a receptors in the NTS. It will also be important to examine when the OXTR and AVPR1a downregulation occurs and whether this coincides with the waning of OT-elicited changes in food intake. If so, this may explain, in part, why the effects of chronic OT to reduce food intake do not typically extend beyond a 2–3 week period.

## Conclusions

Collectively, these findings indicate that chronic OT, at a dose that elicits weight loss and reductions in fat mass (Blevins et al., 2015), produces global reductions in OXTR binding in forebrain sites where OT has been shown to be effective in reducing food intake (ARC, CeA, VMH) in addition to control sites that are also linked to the control of food intake and express OXTRs (Sub). Furthermore, OT also produced largely similar reductions in AVPR1a binding in the ARC and VMH as well as a control site that is linked to the control of food intake that expresses AVPR1a (LS), but it failed to produce significant reductions in AVPR1a binding in the CeA. Our findings indicate that sustained OT treatment was not associated with a significant downregulation of hindbrain OXTR in both HFD-fed or chow-fed rats nor was there a significant impact of diet on OXTR binding within the hindbrain. It will be important to assess whether OXTR and AVPR1a downregulation may be causally related to OT's waning effectiveness to reduce food intake in HFD-fed animals or whether these effects occur afterwards. Given the excitement over the use of chronic intranasal OT as potential therapeutic to treat obesity (Ott et al., 2013, Zhang et al., 2013, Blevins et al., 2015, Lawson et al., 2015, Kuppens et al., 2016, Striepens et al., 2016, Thienel et al., 2016, Hsu et al., 2017, Lawson, 2017, Plessow et al., 2018, Spetter et al., 2018), autism spectrum disorder (Striepens et al., 2011, Yamasue et al., 2012, Lacivita et al., 2017), and schizophrenia (Striepens et al., 2011, Montag et al., 2013, Ota et al., 2018) and the precedent for cross-talk between these OT and AVP, future studies should also assess the extent to which both receptor subtypes may contribute to the effects of OT when it is administered chronically.

## ACKNOWLEDGMENTS

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### Highlights

- Diet composition failed to alter oxytocin (OXTR) or vasopressin 1a receptor (AVPR1a) binding in forebrain and hindbrain.

- Chronic oxytocin (OT) treatment was associated with largely global reductions in forebrain OXTR and AVPR1a binding.

- It will be important to consider the effect that chronic OT treatment can have across forebrain nonapeptide receptors.
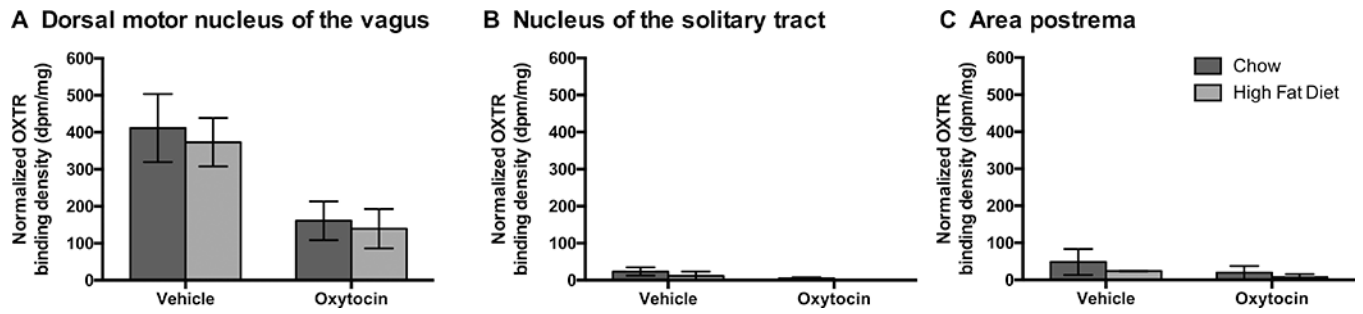
**Figure 1. Optical binding density of OXTR from the hindbrain following chronic 3V OT and vehicle infusions in rats that were fed a high fat diet (HFD) or chow.**

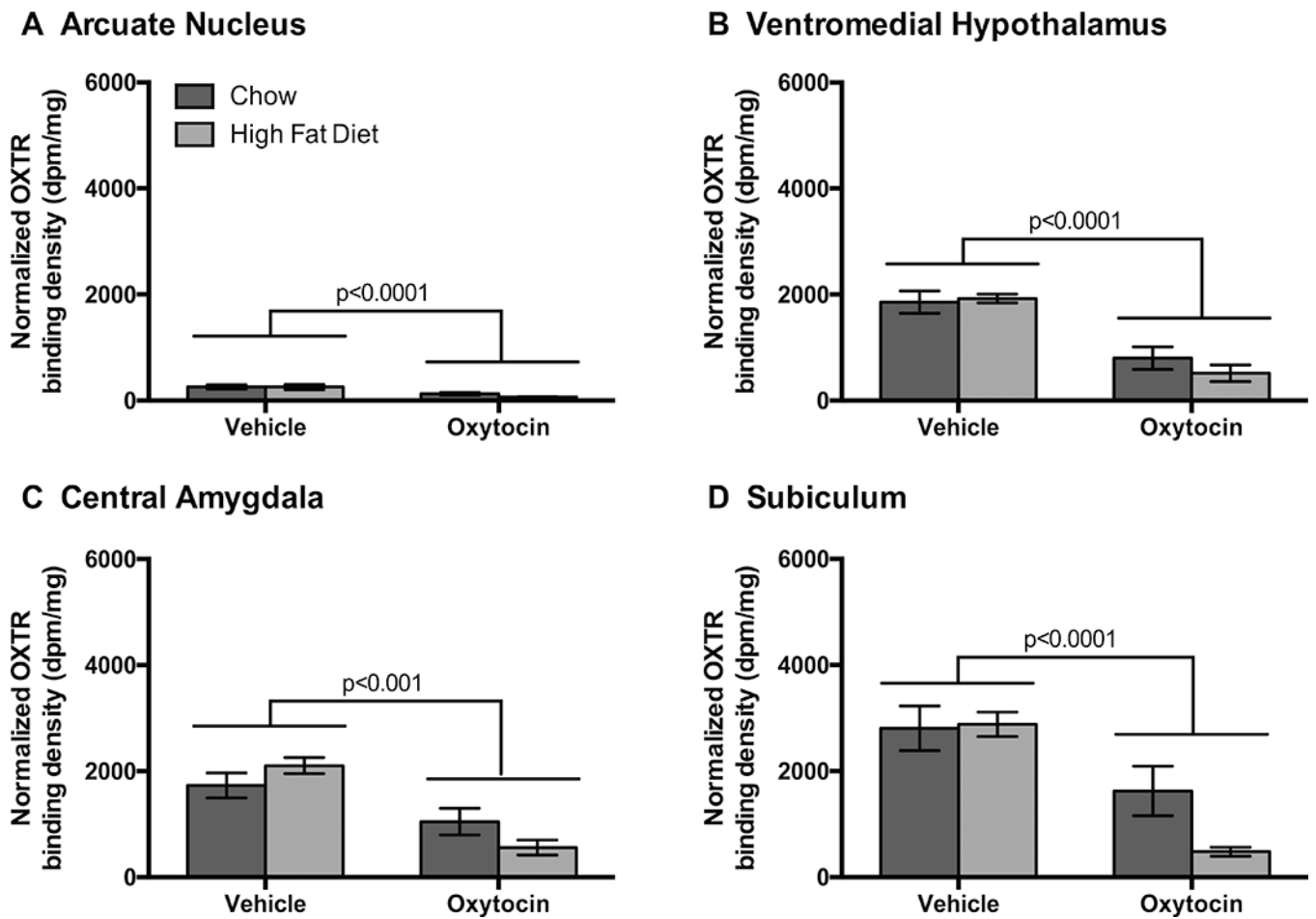Data are expressed as mean ± SEM (n=2–6 rats per group). *$P$<0.05 HFD vs. chow.

## A Arcuate Nucleus

## B Ventromedial Hypothalamus

## C Central Amygdala

## D Subiculum

**Figure 2. Optical binding density of OXTR binding from the forebrain following chronic 3V OT and vehicle infusions in rats that were fed a high fat diet (HFD) or chow.**

Data are expressed as mean ± SEM (n=6–9 rats per group). *$P$<0.05 HFD vs. chow.
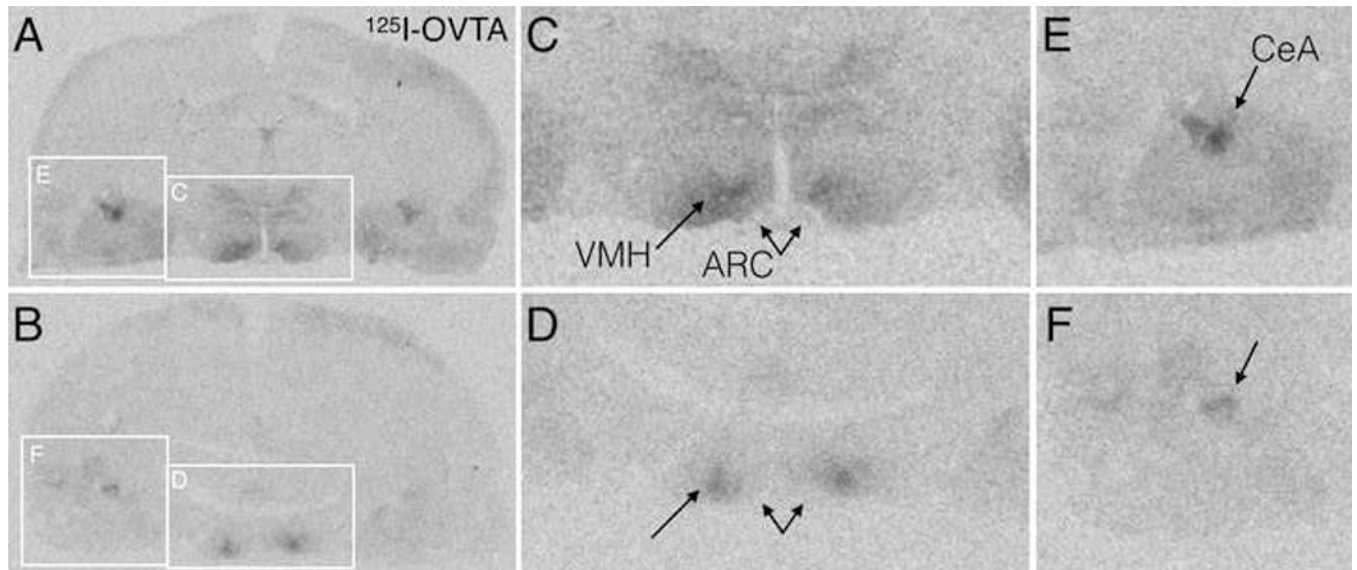
**Figure 3. Representative autoradiograms of forebrain OXTR binding in saline (A,C,E) and OT (B,D,F) treated rats.**

A,B. Full coronal sections at the level of the ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and central amygdala (CeA), with white boxes indicating the zoomed in regions in panels C-F. C,D. Digitally zoomed images of the hypothalamus showing reduced OXTR binding after OT treatment (D) compared to saline (C). E,F. Digitally zoomed images of the amygdala showing reduced OXTR binding after OT treatment (F) compared to saline (D).
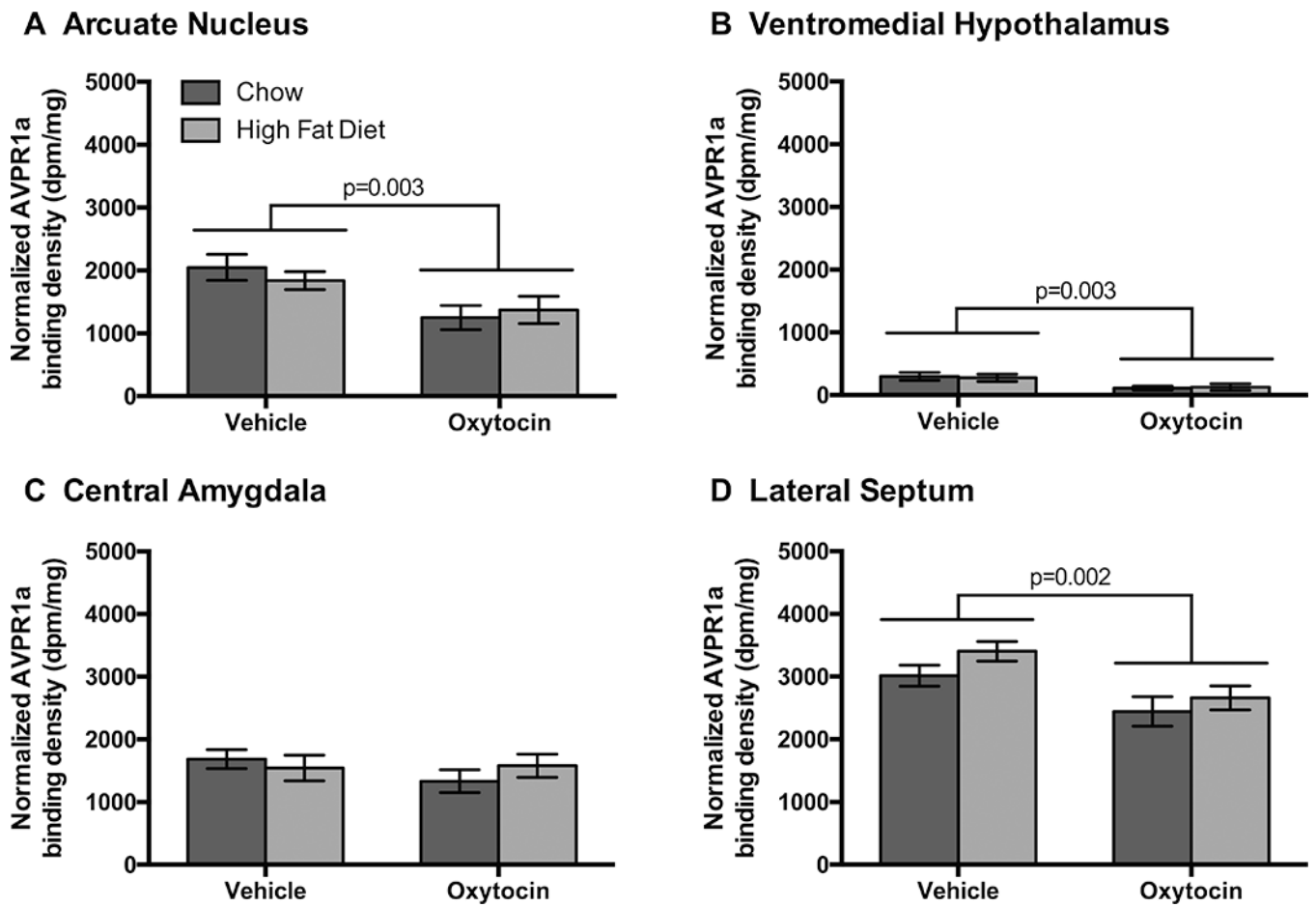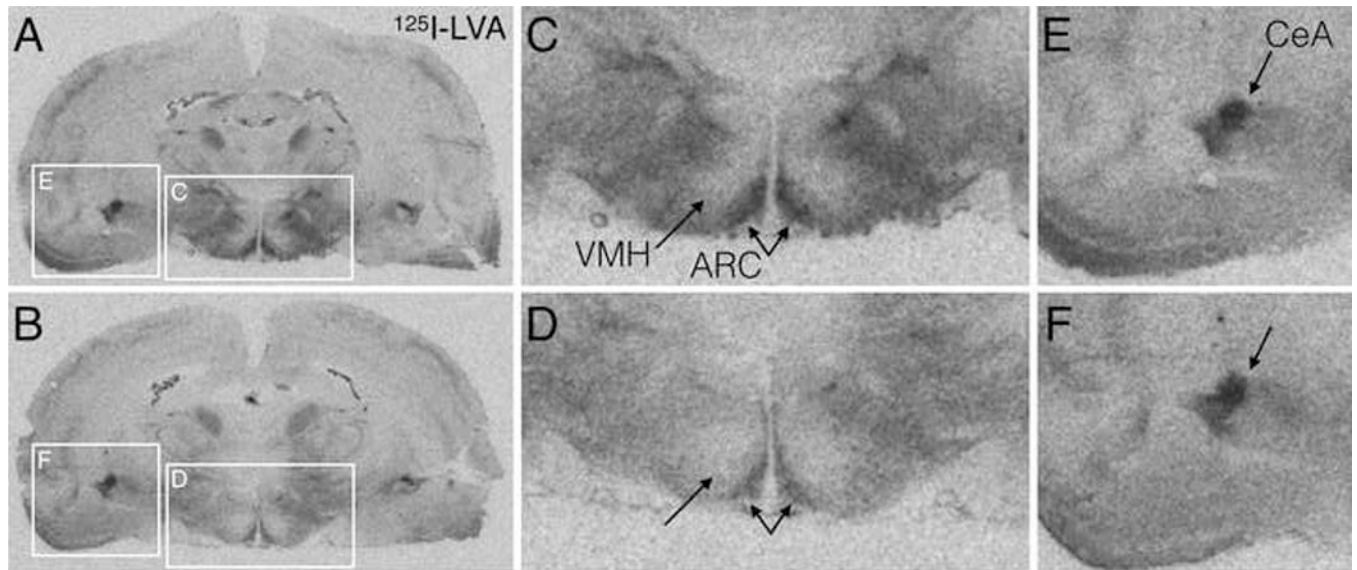
**Figure 4. Optical binding density of AVPR1a binding from the forebrain following chronic 3V OT infusions in rats that were fed a high fat diet (HFD) or chow.**
Data are expressed as mean ± SEM (n=7–9 rats per group). *$P<0.05$ HFD vs. chow.

**Figure 5. Representative autoradiograms of forebrain AVPR1a binding in saline (A,C,E) and OT (B,D,F) treated rats.**

A,B. Full coronal sections at the level of the ventromedial hypothalamus (VMH), arcuate nucleus (ARC), and central amygdala (CeA), with white boxes indicating the zoomed in regions in panels C-F. C,D. Digitally zoomed images of the hypothalamus showing reduced AVPR1a binding after OT treatment (D) compared to saline (C). E,F. Digitally zoomed images of the amygdala showing no change in AVPR1a binding between saline treated (E) and OT treated (F) rats.