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Postpartum Lactation-Mediated Behavioral Outcomes and Drug Responses in a Spontaneous Mouse Model of Obsessive–Compulsive Disorder

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Abstract

Using a spontaneous mouse model of obsessive–compulsive disorder (OCD), the current study evaluated the influence of postpartum lactation on the expression of compulsive-like behaviors, SSRI effectiveness, and the putative role of oxytocin and dopamine in mediating these lactation specific behavioral outcomes. Compulsive-like lactating mice were less compulsive-like in nest building and marble burying and showed enhanced responsiveness to fluoxetine (50 mg/kg) in comparison to compulsive-like nonlactating and nulliparous females. Lactating mice exhibited more anxiety-like behavior in the open field test compared to the nulliparous females, while chronic fluoxetine reduced anxiety-like behaviors. Blocking the oxytocin receptor with L368-899 (5 mg/kg) in the lactating mice exacerbated the compulsive-like and depression-like behaviors. The dopamine D2 receptor (D2R) agonist bromocriptine (10 mg/kg) suppressed marble burying, nest building, and central entries in the open field, but because it also suppressed overall locomotion in the open field, activation of the D2R receptor may have inhibited overall activity nonspecifically. Lactation- and fluoxetine-mediated behavioral outcomes in compulsive-like mice, therefore, appear to be partly regulated by oxytocinergic mechanisms. Serotonin immunoreactivity and serum levels were higher in lactating compulsive-like mice compared to nonlactating and nulliparous compulsive-like females. Together, these results suggest behavioral modulation, serotonergic alterations, and changes in SSRI effectiveness during lactation in compulsive-like mice. This warrants further investigation of postpartum events in OCD patients.

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Author Contributions

S.M., M.M., and S.O. designed the experiments and collected the data. S.M. and M.M. analyzed the data. A.B.-I. as the senior researcher provided guidance for all experiments and assisted with data analysis, data presentation, and manuscript preparation. S.M. wrote the first draft of the manuscript, and all other authors contributed to subsequent revisions and the final submission.

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Graphical Abstract

Keywords

Lactating; nonlactating; nulliparous; compulsive-like; serotonin; anxiety-like; depression-like; oxytocin; dopamine

INTRODUCTION

Obsessive–compulsive disorder (OCD) is one of the most prevalent neuropsychiatric disorders that affects 1–3% of the population.¹ OCD causes significant interference with quality of life, which leads to functional impairments,² disability, increased use of health care services, and financial difficulties.³ Mood alterations and psychopathology in the postpartum condition can adversely impact both the mother and the child.⁴ In females, the onset and exacerbation of OCD symptoms are associated with the premenstruum, pregnancy, and postpartum periods.⁵ In addition, mood disorders and depression, which are often comorbid conditions in OCD,⁶ precipitate during the postpartum phase in females.⁷ Sex differences also contribute to the phenotypic expression, heterogeneity, and drug response variations in OCD,⁸ which may be partially due to physiological states influencing the expression of OCD and related symptoms in females. Hence, a spontaneous mouse model of OCD⁹ is employed to explore this potential explanation.

The brain undergoes dramatic changes in neuronal mechanisms during pregnancy to accommodate parturition and lactation.¹⁰ Secretion of the hormone prolactin, which stimulates milk production, from the pituitary gland lactotrophs is regulated by tuberoinfundibular neurons of the arcuate nucleus (TIDA) in the hypothalamus that secrete dopamine.¹¹ Dopamine secreted from the TIDA neurons acts on the D2 (dopamine type 2) receptor (D2R) on lactotrophs causing inhibition of prolactin secretion.¹² Oxytocin is produced and released in the posterior pituitary gland in response to suckling. Suckling leads to stimulation of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) regions of the hypothalamus, which in turn signal more oxytocin production and release.¹³ Oxytocin facilitates contraction of the cells surrounding the alveoli in the mammary glands by binding to its receptor (OXTR) causing milk flow through the duct system.¹⁴ Physiological events such as parturition and lactation also trigger extensive morphological plasticity in the oxytocinergic systems.¹⁵ This is characterized by less astrocyte coverage of the oxytocin neurons and increase in juxtaposition of oxytocin neurons with other synapses thereby impacting transmission of neurotransmitters such as glutamatergic systems. This neuroglia remodeling of the oxytocin system can thereby have physiological consequences¹⁵

ultimately driving phenotypic expression. With the already established role of oxytocin in regulating anxiety-like¹⁶ and depression-like behaviors,¹⁷ physiological events such as postpartum could further influence oxytocin driven behavioral outcomes. In addition, a novel serotonergic biosynthetic system in the mammary glands has been shown to be up-regulated during late pregnancy and lactation.¹⁸ This is consistent with higher serum levels of serotonin in lactating female C57BL6/J mice.¹⁹ Lactation also resulted in lower serotonin reactive serotonergic neurons in the dorsal raphe nucleus (DRN) of the brain, while behavioral responsiveness to selective serotonin reuptake inhibitors (SSRIs) for anxiety-like and depression-like behaviors in lactating C57BL6/J females was enhanced when compared to virgin females.¹⁹ Therefore, a possible interaction between the central and peripheral serotonin systems that results in modulation of behavior has been proposed.¹⁹ The serotonergic system has also been shown to influence prolactin secretion.²⁰ These findings together suggest that hormones that regulate pregnancy and postpartum periods also affect critical neurotransmitter systems, which play a role in the expression of compulsive-like behaviors.^{9a,21}

The unique neuroendocrinological events as a result of lactation are known to produce profound effects on healthy lactating mothers, which include reduced stress, positive mood states, and less anxiety.²² However, these behavioral attributes are not universal for all mothers. Studies have shown that a certain subpopulation of women experience emotional lability during postpartum periods²³ explaining the higher prevalence of general anxiety disorders and OCD in postpartum conditions when compared to general population.²⁴ Rodent studies have further corroborated this claim where assessments of anxiety-like measures have shown increased^{16,25} versus decreased²⁶ responses in postpartum conditions. A study on mice selected bidirectionally for high and low thigmotaxis behavior in the open field test revealed higher anxiety-like behavior during lactation in the high thigmotaxis strain when compared to the low thigmotaxis strain.²¹ Environmental conditions also influence maternal care and behavioral responses to novelty.²⁷ Overall, this indicates an influence of genetic variation and environmental conditions on behavioral responses during postpartum conditions. Studies on depression-like and compulsive-like behaviors are limited, where lactation has been shown to abolish drug induced compulsive-like behaviors²¹ and reduce depression-like behaviors.¹⁹ Cessation of breastfeeding or lactation during the postpartum period on the other hand has been associated with anxiety and depression in human studies.²⁸ Animal studies focusing on investigating the effects of the nonlactation phase have been few. Some studies have shown that impeding lactation or major neurohormones associated with the lactation process can impair learning and memory and exacerbate anxiety and depression,^{19,29} while others have found that physical contact between the mothers and their pups is more important than suckling for reducing maternal anxiety.^{22h,j}

The dopaminergic and the serotonergic systems have garnered substantial support in relation to their role in OCD,³⁰ anxiety,³¹ and depression.³² Drugs that target serotonin transporters, such as SSRIs, have been effective in treating both OCD and postpartum depression.³³ Pharmacologically, agonists of autoinhibitory serotonin receptor 5-HT_{1A} and dopamine receptor D₂/D₃ have produced compulsive-like phenotypes.³⁴ Neuropeptides oxytocin and prolactin, which are typically upregulated in lactation, have been implicated in increasing maternal care and aggression, while decreasing anxiety and depression in human^{28b,35} and

animal studies.^{28b,35,36} However, their role in obsessions and compulsions remains inconclusive in human studies. Some studies have linked central³⁷ and peripheral³⁸ oxytocin levels to OCD, while others found no significant correlation among endogenous oxytocin levels³⁹ or exogenous administration of oxytocin on OCD symptomology.⁴⁰ Plasma prolactin levels in response to the serotonin 5-HT_{2C} receptor agonist mCPP among OCD patients has been contradictory with one study showing higher levels^{37–40,41a} and another study finding the opposite.^{41a} Interestingly, 8-OHDPAT-induced compulsive-like perseveration was abolished in the postpartum lactation phase of rats.²¹ Hence, probing the neuropeptide or -transmitter modulation during the postpartum phase in compulsive-like female mice can unravel critical neurobiological mechanisms that drive differential behavioral responses during various physiological states in a compulsive-like phenotype.

Currently there is lack of understanding how predisposition to psychiatric conditions such as OCD results in behavioral repertoire during postpartum breastfeeding and nonbreastfeeding conditions. A comparison of various postpartum (lactating versus nonlactating) and nonpregnant (nulliparous) phases in a compulsive-like mouse model can therefore provide a foundation for understanding the role of physiological status and lactation in influencing compulsive-like, anxiety-like, and depression-like behaviors. Further, due to the convincing role of serotonin in influencing compulsive and affective behaviors and lactation specific events, evaluation of central versus peripheral serotonin levels among various physiological stages in the compulsive-like mice could provide crucial understanding of serotonin specific neuromodulation and concomitant behavioral attributes during postpartum stages. We therefore used a mouse model exhibiting face, predictive, and construct validity for compulsive-like phenotype.⁹ The current mouse model was generated by selective breeding for high and low levels of nest-building behavior. The nest-building phenotypes are influenced by polygenetic^{40,75} and environmental factors⁴² and therefore can serve as an excellent heuristic tool to study a disease like OCD, which has strong polygenic and environmental influences.^{30c} In the current study, within the context provided above, we hypothesized that in spontaneously compulsive-like female mice lactation will enhance responsiveness to SSRIs and protect against excessive behavioral responses, which are mediated by dopamine and oxytocin receptor mediated pathways. We predicted that lactating females will display less compulsive-like, anxiety-like, and depression-like behaviors compared to nonlactating and nulliparous compulsive-like female mice. We also predicted that the compulsive-like lactating mice will exhibit enhanced responsiveness to the SSRI fluoxetine in all these behaviors compared to the other experimental groups. Finally, we predicted that activating D₂Rs and blocking of OXTRs will modulate the compulsive-like, anxiety-like, and depression-like behaviors in lactating compulsive-like females.

RESULTS AND DISCUSSION

Postpartum Lactating Compulsive-like Female Mice Exhibited Less Compulsive-like Behavior and Enhanced SSRI Effectiveness When Compared to the Nonlactating and

Nulliparous Females, and This Reduced Compulsive-like Behavior Was Abolished When Lactating Females Were Treated with OXTR Antagonist L368-899

Lactating females buried significantly fewer marbles in weeks 1, 2, and 3 (Figure 1) when compared to the nonlactating and the nulliparous female mice ($F_{2,66} = 99.80$, $p < 0.001$). The fluoxetine treatment effect was also significant ($F_{1,66} = 204.70$, $p < 0.001$). In the presence of fluoxetine, attenuation in marble burying was observed for lactating females in weeks 1 ($t_{22} = 4.651$, $p < 0.001$), 2 ($t_{22} = 8.001$, $p < 0.001$), and 3 ($t_{22} = 22.84$, $p < 0.001$). The nonlactating and nulliparous females showed no attenuation effect due to fluoxetine in week 1 (Figure 1a). Nonlactating females showed a decrease in marble burying in weeks 2 ($t_{22} = 2.830$, $p < 0.001$) (Figure 1b) and 3 ($t_{22} = 7.210$, $p < 0.001$) (Figure 1c), while nulliparous females showed a response to fluoxetine only in week 3 ($t_{22} = 10.41$, $p < 0.001$) (Figure 1c). This accounted for the physiological status of the female by treatment interaction effect ($F_{2,66} = 34.29$, $p < 0.001$), indicating that lactating females had higher responsiveness to fluoxetine for marble burying when compared to nonlactating and nulliparous females.

For the compulsive-like nest building behavior (Figure 1), lactating females had significantly lower nesting scores in comparison to the nonlactating and the nulliparous females ($F_{2,66} = 125.62$, $p < 0.001$). The fluoxetine treatment effect was also significant ($F_{1,66} = 74.53$, $p < 0.001$). Lactating females in the fluoxetine group had lower nesting scores in weeks 1 ($t_{22} = 4.473$, $p < 0.001$), 2 ($t_{22} = 4.247$, $p < 0.001$), and 3 ($t_{22} = 4.479$, $p < 0.001$) when compared to the vehicle group (Figure 1d,e,f). The nonlactating ($t_{22} = 3.113$, $p < 0.01$) and the nulliparous ($t_{22} = 4.280$, $p < 0.001$) females showed a treatment effect compared to the respective vehicle groups only in week 3 (Figure 1f). Though there was a strong trend in enhanced responsiveness to fluoxetine by the lactating females, the physiological status of the female by treatment interaction effect was not significant ($F_{2,66} = 2.53$, $p > 0.05$).

Administration of OXTR antagonist L368-899 increased compulsive-like marble burying ($F_{2,28} = 76.74$, $p < 0.001$) in the lactating females when compared to the vehicle ($t_{18} = 5.305$, $p < 0.001$) and D2 agonist bromocriptine ($t_{19} = 12.33$, $p < 0.001$) treated lactating females (Figure 2a).

For compulsive-like nest-building behavior, there was an overall treatment effect for hours 1 ($F_{2,31} = 117.42$, $p < 0.001$), 2 ($F_{2,31} = 52.54$, $p < 0.001$), 3 ($F_{2,31} = 14.05$, $p < 0.001$), 5 ($F_{2,31} = 9.36$, $p < 0.001$), and 24 ($F_{2,31} = 66.56$, $p < 0.001$) (Figure 2b,c). The drug effect in hours 1 and 2 was due to lactating females treated with L368-899 showing higher nesting scores than the lactating females treated with vehicle ($t_{21} = 6.260$, $p < 0.001$; $t_{21} = 5.102$, $p < 0.001$) or bromocriptine ($t_{21} = 7.307$, $p < 0.001$; $t_{21} = 7.603$, $p < 0.001$). Significantly higher nesting scores for vehicle ($t_{20} = 5.528$, $p < 0.001$) and L368-899 ($t_{20} = 4.509$, $p < 0.001$) treated lactating females when compared to bromocriptine treated females accounted for the drug effect in hour 3. Drug effect in hour 5 was due to an increase in the nesting scores of bromocriptine treated animals in comparison to vehicle ($t_{20} = 4.173$, $p < 0.001$) and L368-899 ($t_{21} = 4.081$, $p < 0.001$) treated females. After 24 h, the overall nesting scores were significantly higher in L368-899 treated lactating females in comparison to the vehicle ($t_{21} = 5.579$, $p < 0.001$) and bromocriptine ($t_{21} = 4.473$, $p < 0.001$) treated ones (Figure 2c).

The reduced compulsive-like marble burying and nest-building in lactating compulsive-like mice aligns with a prior study in rats where 8-OHDPAT-mediated compulsive-like spontaneous alternation in T maze was abolished in the lactation phase.²¹ Chronic fluoxetine treatment resulted in a greater attenuation of compulsive-like marble burying behavior in the lactating females in comparison to the nonlactating and nulliparous females. This greater responsiveness to fluoxetine observed in compulsive-like marble burying behavior also showed a trend in the compulsive-like nest-building behavior, though not statistically significant. This is an interesting finding since an unpublished study from our lab has shown that the compulsive-like mouse strain can exhibit trait specific responses to SSRI treatment. Hence, a trait specific enhanced responsiveness to fluoxetine cannot be ruled out where lactation was anticomulsive for both marble burying and nesting (in absence of fluoxetine), but the fluoxetine response varied between the two compulsive-like behaviors. Whether enhanced responsiveness to SSRI treatment in the compulsive-like mice for one trait over the other represents clinical heterogeneity in postpartum OCD patients remains to be elucidated. The nonlactating females on the other hand did not exhibit an enhanced responsiveness to fluoxetine in compulsive-like behaviors, indicating that compulsive-like behaviors and drug efficacy in the postpartum phases can be influenced by the state of lactation and not pregnancy per se.¹⁹ A study comparing postpartum OCD patients and normal postpartum females revealed that rates of breastfeeding were lower in OCD patients, which was concomitant with precipitation of marital distress, depression, and lack of social interactions.⁴³ Though this study⁴³ did not assess the effect of breastfeeding on expressions of OCD and comorbid symptomology, it is plausible that breastfeeding can improve clinical OCD symptoms as seen with other studies on postnatal anxiety where breastfeeding has been implicated as an antianxiety factor.^{24b}

Various neurobiological possibilities may be contributing to the enhanced responsiveness to fluoxetine. SSRIs are known to increase oxytocin release in humans,⁴⁴ which along with the oxytocin surge due to suckling action, might provide a possible explanation for greater responsiveness to fluoxetine by lactating females.¹⁹ Alternatively, it has been postulated that serotonin type 1A (5-HT1A) autoreceptors are desensitized both by SSRIs⁴⁵ and by lactation specific modulation of oxytocinergic or prolactinergic systems.⁴⁶ Hence, a concerted action of lactation specific events and SSRI-mediated modulation of the serotonergic system might explain the enhanced responsiveness of lactating females when compared to nonlactating and nulliparous females. Enhanced responsiveness to fluoxetine during lactation could also be attributed to the unique sex steroid milieu during lactation that can further enhance the SSRI responsiveness.⁴⁷ Estrogens and progesterone, which are known to desensitize 5-HT1A receptors are altered during parturition and lactation and can account for enhanced serotonergic transmission.²¹

The reduced compulsive-like behavior is contrary to a previous study in which lactating wild-type C57BL/6J females treated with daily vehicle injections exhibited more marble burying behavior than nulliparous females.¹⁹ Considering the low number of marbles buried, that is, about 3 in nulliparous and about 7 on average in lactating females during the 20 min test,¹⁹ C57BL/6J mice can be considered non-compulsive-like mice.^{9a} Possibly the lower rates of marble burying reflect anxiety-like behavior while higher rates of digging better reflect a compulsive-like phenotype as seen in our mouse strains.⁹ Alternatively, the

contradictory effect of lactation on compulsive-like digging may indicate a strain effect due to genetic differences as a result of selection for expression of compulsive-like phenotype or different genetic correlation structures due to founder effects or random genetic drift.⁴⁸ Hence, the current results might provide an explanation as to how genetic variability in healthy subjects and OCD patients could produce differential outcomes in expression of obsessions and compulsions during postpartum.

The current data indicates that blocking OXTR during lactation in compulsive-like mice can significantly exacerbate compulsive-like behaviors. The neuropeptide oxytocin has been investigated in connection to OCD in clinical studies.^{37–40} However, the association of oxytocin and OCD has been contradictory.^{37–40} OXTRs on the other hand are widely distributed in various brain regions,^{14b,49} which is suggestive of its wide range of effects in the CNS.^{36f} In fact, a study comparing human OCD patients with controls linked OXTR epigenetic modulation to OCD severity.⁵⁰

The current finding of the role of oxytocinergic modulation during postpartum lactation in animal models that exhibit compulsive-like phenotype is novel. Emerging evidence from rodent studies suggest that oxytocinergic and serotonergic systems interact to regulate behavioral responses in a context-dependent manner.^{36f,51} For example, oxytocin produced anxiolytic effects in mice appear to be regulated by serotonin transmission through OXTRs expressed on raphe neurons.^{36f} This serotonin release and anxiolytic response was positively regulated by 5-HT_{2A/2C} receptors.^{36f} OXTR deletion on raphe neurons influenced aggression in a sex-dependent fashion with male mice exhibiting enhanced aggression and females exhibiting no changes in aggression.^{51a} This oxytocinergic and serotonergic interaction has also been reported during lactation⁴⁶ in addition to the lactation specific extensive neuroglia remodeling of the oxytocinergic systems.^{10a,19} Moreover, spontaneous alternation due to activation of 5-HT_{1A} receptor by 8OH-DPAT was abolished during lactation in rats.²¹ One of the likely explanations for this response was desensitization of the 5-HT_{1A} receptor due to oxytocinergic or prolactinergic modulation specific to lactation.^{21,46,52} The result from our study is suggestive of a possible interaction of oxytocinergic and serotonergic systems to exhibit behavioral modulation in compulsive-like mice during postpartum lactation. However, to what extent this interaction is involved in regulating spontaneous compulsive-like behavior remains to be determined.

Attenuation of marble burying and nesting behavior observed in bromocriptine treated lactating females was most likely due to the overall behavioral suppression as locomotion was suppressed in the open field (see below). This was unexpected, since we selected a dose that was used in a prior study with mice and did not influence motor behaviors,⁵³ which suggests that compulsive-like mice may be more sensitive to bromocriptine. Further, in another study, a bromocriptine dose of 32 mg/kg evoked climbing behavior in mice.⁵⁴ It should be considered that the acute effect of dopamine agonists on locomotor functions is biphasic with initial depression followed by excitation,⁵⁵ which the lactating compulsive-like mice also displayed for nest-building behavior. The behavioral depression due to bromocriptine in the compulsive-like lactating females could be attributed to the stimulation of the presynaptic dopamine D₂Rs⁵⁶ in brain regions implicated in motor functions such as dorsal striatum and nucleus accumbens.⁵⁷

Lactating Compulsive-like Females Exhibited Higher DRN Serotonin (5-HT) Immunoreactivity and Serum Levels than Nonlactating and Nulliparous Females

The physiological status effect was significant on the total number of 5-HT positive neurons in the dorsal raphe nucleus ($F_{2,12} = 15.30$, $p < 0.001$). Lactating females had higher 5-HT immunoreactivity when compared to the nonlactating ($t_8 = 6.788$, $p < 0.01$) and nulliparous ($t_8 = 6.763$, $p < 0.001$) females (Figure 3). The serum levels of 5-HT in lactating females were also significantly higher ($F_{2,15} = 5.56$, $p < 0.05$) in comparison to the nonlactating ($t_{10} = 4.161$, $p < 0.05$) and nulliparous females ($t_{10} = 4.000$, $p < 0.05$) (Figure 4).

The higher level of serum 5-HT in lactating compared to nonlactating compulsive-like female mice aligns with a similar finding in C57BL6/J mice.¹⁹ Peripheral 5-HT is predominantly produced in the enterochromaffin cells of the gut that enter the bloodstream from where they get transported into platelets.⁵⁸ Two probable sources of 5-HT during lactation could account for the observed high serum 5-HT levels in lactating females. One source of higher 5-HT could be due to enhanced 5-HT synthesis in the mammary glands during lactation,¹⁸ while another possibility could be due to elevated mucosal hyperplasia in the intestine of lactating females.^{19,59}

Serotonergic neurons pertaining to the lateral region in DRN are known to project into a distributed system that is associated with stress and anxiety related behavioral responses.⁶⁰ The DRN serotonergic neurons also innervate brain regions implicated in compulsive behaviors.⁶¹ The increased 5-HT immunoreactivity in the lateral regions of DRN in lactating females versus decreased 5-HT immunoreactivity in non-lactating and nulliparous females is indicative of altered central serotonergic activity during lactation. However, this finding was opposite of what was found in C57BL6/J mice, in which lactating females had decreased DRN 5-HT immunoreactivity compared to virgin females.¹⁹ The discrepancy in DRN immunoreactivity could be due to the collection of brain tissues at different time points during the lactation period, that is, 10 days after parturition¹⁹ and 18 days after parturition in our study. Lactation specific modulation of serotonergic system pertaining to DRN at various stages in postpartum cannot be ruled out. Alternatively, selective breeding for compulsive-like phenotype might have resulted in higher 5-HT immunoreactivity when compared to nulliparous or nonlactating mice. The possibility of higher serotonin immunoreactivity could be related to higher serotonergic firing typically seen during lactation which is further facilitated by desensitization of the auto inhibitory receptor 5-HT_{1A} by ovarian steroid milieu during lactation.^{21,62} A prior study has shown that depleting serotonin in the brain by knocking out tryptophan hydroxylase 2, the initial rate-limiting enzyme in serotonin synthesis, increased compulsive-like behaviors, including nestlet shredding and marble burying,⁶³ compared to wild-type mice, which establishes that activation of the serotonergic system is necessary to reduce compulsive-like behaviors. However, a direct correlation cannot be drawn explaining higher 5-HT immunoreactivity and lower compulsive-like nest-building and marble burying behaviors in the lactating females. It should be noted that compulsive-like, anxiety-like, and depression-like behaviors are probably due to a complex set of interactions involving more than one factor, such as neurotransmitter systems, sex differences, physiological stages, genetic background, and environmental conditions. Moreover, in the current study we investigated a specific region of

DRN and not the entire raphe nucleus. Region specific serotonin alterations during postpartum can therefore be one of the many factors influencing the behavioral phenotypes in the compulsive-like mice. We also believe that alterations in central versus peripheral serotonin levels during lactation are independent of each other.

Postpartum Lactating Compulsive-like Female Mice Exhibited More Anxiety-like Behavior in the Open Field than Nulliparous Females, Which Was Not Influenced by L368-899, while Bromocriptine Reduced Overall Locomotor Functions

For the anxiety-like behavior in the open field, significant physiological status and fluoxetine treatment effects on central zone entries were observed ($F_{2,64} = 8.52$, $p < 0.01$, and $F_{2,64} = 22.03$, $p < 0.001$, respectively) (Figure 5a). Compulsive-like lactating females had significantly lower central zone entries when compared to nulliparous females in the vehicle group ($t_{22} = 2.591$, $p < 0.05$). In the fluoxetine treated groups, the lactating females showed fewer number of central entries ($t_{21} = 3.213$, $p < 0.001$) when compared to the nulliparous females. The significant treatment effect was due to the improvement in the central square entries for fluoxetine treated lactating ($t_{21} = 2.532$, $p < 0.05$) and nulliparous ($t_{22} = 3.307$, $p < 0.05$) females when compared to their vehicle treated counterparts. This was not seen for the nonlactating females. No significant effect of physiological status ($F_{2,64} = 1.72$, $p = 0.18$), treatment ($F_{1,64} = 2.59$, $p = 0.11$), and physiological status by treatment interaction ($F_{2,64} = 1.12$, $p = 0.33$) was observed for locomotor activity, measured as total distance traveled (Figure 5b).

A significant treatment effect was observed for the number of central entries ($F_{2,29} = 7.60$, $p < 0.01$) (Figure 5c) and total locomotion ($F_{2,29} = 12.43$, $p < 0.001$) (Figure 5d) in the anxiety-like open field measure. This was mainly due to the suppression of total locomotion in the bromocriptine treated lactating females that also contributed to a lower number of central entries when compared to the vehicle ($t_{19} = 3.243$, $p < 0.01$) and L368-899 ($t_{20} = 4.924$, $p < 0.001$) treated lactating females. No significant differences were observed for number of central entries ($t_{20} = 0.039$, $p > 0.05$) and locomotion ($t_{20} = 1.722$, $p > 0.05$) between vehicle and L368-899 treated females.

Contrary to our hypothesis, the data indicates that lactating compulsive-like mice exhibited more anxiety-like behaviors in comparison to nulliparous ones. Lower levels of anxiety during postpartum is considered to be a maternal adaptation due to neuroendocrine modulation,⁶⁴ which facilitates social bonding and offspring acceptance.⁶⁵ However, the component of emotionality can be perceived very differently by lactating females as evaluated through behavioral tests. For example, evaluation of anxiety-like behavior between postpartum and virgin mice and rats in the anxiety-like light–dark test revealed no differences,⁶⁶ while locomotion was reduced in lactating rats when compared to virgin ones.⁶⁷ Further, when isolated from pups, lactating wild-type C57BL6/J mice were found to be more anxious in marble burying behavior.¹⁹ It is interesting to note that though lactating females were more anxiety-like, locomotor activity among vehicle treated lactating, non-lactating, and nulliparous compulsive-like females were not different, which supports that the anxiety-like differences were not due to activity level differences. Hence, motivation to explore a novel environment was not affected in the lactation phase. The timing of anxiety-

like evaluation in our study, which was the 18th day of lactation, also needs to be considered, since it has been reported that the anxiolytic effect of lactating rodents such as rats last only through the first postpartum week.^{22j} Alternatively, selective breeding for compulsive-like phenotype could have resulted in greater anxiety in our mouse strains during the postpartum lactating phase. It could be due to a genetic correlation between genes that affect compulsivity and postpartum anxiety or different genetic correlation structures due to founder effects or random genetic drift.⁴⁸ Further, strain specific anxiety-like behavioral differences have been observed in rodents.⁶⁸ A study with high and low anxiety-like lines of rats showed that innate anxiety produced a differential response in anxiety-like behaviors during the postpartum phase.²⁵ Hence, genotype by physiological effect interactions cannot be ruled out for responses to anxiety-like behaviors in the compulsive-like mice.

Though improvement in the number of central entries was observed in lactating and nulliparous females following fluoxetine treatment, no significant effect was seen for the nonlactating females (although it trended in the same direction). It points toward a lesser response to flin the compulsive-like nonlactating mice. This is interesting because the state of lactation or the presence of pups has been proposed to be an important factor in influencing SSRI responsiveness in a study with C57BL6/J mice.¹⁹ The fact that both lactating and nulliparous compulsive-like mice responded to fluoxetine indicates that the state of lactation is not the only factor that drives SSRI responsiveness for anxiety-like behavior. However, we believe that removal of pups and therefore cessation of lactation can result in reduced responsiveness to SSRIs for anxiety-like behaviors. The current data could provide enhanced understanding as to how OCD patients might vary in their emotional responses during postpartum with potentially greater vulnerability for lactating females and poor response to SSRI for nonlactating ones.

Lack of effect on anxiety-like behavior due to blocking of OXTRs in lactating compulsive-like mice is in agreement with other rodent studies where OXTR antagonists did not cause differences in various anxiety-like assessments.⁶⁹ Further, a study with OXTR knockout mice did not reveal significant differences in the anxiety-like measures from wild-type controls.^{65b} However, it has to be considered that these studies were not conducted in the postpartum phase. In fact, a study in rats showed that blocking OXTR can produce anxiogenic behaviors in pregnant and lactating females when compared to virgin females.⁷⁰ A likely explanation for this differential response could be due to low oxytocin system activity during nonreproductive and stress-free events.¹⁶ This indicates that the oxytocinergic system might not maintain basal levels of anxiety-like responses but can regulate such behaviors during specific physiological conditions, such as pregnancy and lactation.¹⁶ Studies on wild-type mice have shown that chronic intra-cerebroventricular infusions or nasal application of oxytocin resulted in downregulation of oxytocin receptors in brain regions regulating anxiety⁷¹ probably due to OXTR desensitization.⁷² Interestingly, chronic oxytocin treatment upregulates vasopressin receptor binding in the lateral septum where vasopressin is known to exert anxiogenic effects.^{71b,73} An oxytocin surge during lactation that desensitizes OXTRs in specific brain regions could therefore have increased anxiogenic behavior in the lactating compulsive-like mice. In that scenario, blocking OXTRs would not be expected to alter anxiety-like behavior as observed in the compulsive-like

lactating females indicating no role of OXTRs in driving anxiety-like responses during lactation. Though oxytocin is known to reduce anxiety-like responses for maternal care,^{23b} it could be one of the many factors contributing toward these responses.⁷⁴ The anxiogenic behavior observed in bromocriptine treated lactating females was predominantly due to the overall suppression of locomotory activity as discussed in the context of compulsive-like behavior. The association between OCD and general anxiety is controversial.⁷⁵ The ego-dystonic and intrusive nature of obsessions differ largely from general anxiety.⁷⁶ Further, the implicated neurocircuitries for anxiety and OCD differ.⁷⁷ This makes it an ambiguous indicator for OCD. Recently, OCD has been declassified as an anxiety disorder.⁷⁸ Our results in the compulsive-like female mice indicate that compulsive-like and anxiety-like phenotypes share a complex correlation that might be influenced by more than one factor, such as genetic background, physiological status, environmental influence, or a combination of all these elements.

Physiological Status Did Not Influence Overall Depression-like Behavior in the Compulsive-like Mice, while OXTR Antagonist L368-899 Increased Depression-like Behavior in the Lactating Females

For depression-like tail suspension behavior, a significant effect of the physiological status ($F_{2,64} = 8.38$, $p < 0.001$) was found in the compulsive-like mice (Figure 6a). There were no significant treatment ($F_{1,64} = 0.14$, $p = 0.71$) and physiological status by treatment interaction ($F_{2,64} = 1.60$, $p = 0.21$) effects. The physiological effect was primarily due to lactating ($t_{20} = 3.840$, $p < 0.001$) and nonlactating ($t_{22} = 2.692$, $p < 0.001$) females having lower immobility in fluoxetine treated groups when compared to fluoxetine treated nulliparous females. A similar trend was observed in the vehicle treated groups, but no significant pairwise comparisons were found.

Overall, the data indicates that physiological status of the compulsive-like mice did not influence depression-like behavior under normal conditions. However, in the presence of fluoxetine, depression-like behavior increased in the nulliparous mice when compared to the fluoxetine treated nonlactating and lactating compulsive-like females. The data also shows that fluoxetine did not attenuate depression-like behavior in the nonlactating, lactating, and nulliparous compulsive-like mice. In an inbred mouse strain, lactating females exhibited less depression-like behavior.¹⁹ In another study, chronic treatment of fluoxetine (SSRI) in wild-type mice exposed to chronic stress conditions exacerbated the depression-like behavior when compared to mice exposed to enriching conditions, which improved performance in depression-like behavior.⁷⁹ We believe that the responses of the lactating, nonlactating, and nulliparous compulsive-like mice in the fluoxetine group is probably due to the selective breeding that resulted in a higher stress response.^{9c} This might be compounded by physiological events, such as postpartum, accounting for lack of anti-depressant effect in the presence of fluoxetine.

An overall treatment effect ($F_{2,64} = 8.38$, $p < 0.001$) was observed for lactating females undergoing tail suspension behavior (Figure 6b). Lactating females administered with L368-899 had higher immobility in the tail suspension when compared to vehicle ($t_{16} = 5.110$, $p < 0.001$) and bromocriptine ($t_{16} = 4.500$, $p < 0.001$) administered females.

This data indicates that blocking OXTR increased depression-like behavior in compulsive-like lactating females. The antidepressant activity of oxytocin has been established in rodent models⁸⁰ and is facilitated through OXTR.⁸¹ Oxytocin is known to control stress adaptation by projecting from the paraventricular region of the hypothalamus to areas like the amygdala, hippocampus, and lateral septum.⁸² This is also in good agreement with the OXTR expression profile in these regions.^{14b} Hence, blocking OXTR in our study increased immobility time in the tail suspension test.

Interestingly, bromocriptine did not depress mobility in the tail suspension test compared to vehicle, although it was measured at the same time after injection as the open field test. Bromocriptine reduces immobility of mice in the tail suspension test⁸³ and has been shown to have antidepressant effects in preclinical and clinical studies.⁸⁴ Therefore, this antidepressant effect of bromocriptine may have compensated for the lower levels of activity observed for the bromocriptine treated lactating compulsive-like mice in the marble burying, nest building, and open field tests.

CONCLUSION

The current study provides a novel understanding of the role of physiological status on treatment and behavioral outcomes in the female compulsive-like mouse model, in which lactation protected compulsive-like female mice from expressing compulsive-like behaviors and increased their response to the SSRI fluoxetine in reducing compulsive-like behaviors. Our findings indicate that postpartum events in lactating female mice can alter central and peripheral serotonin signaling independent of each other and might play a role in influencing behavioral and drug responses. This study also revealed for the first time that the oxytocinergic system during postpartum lactation was at least partially responsible for mediating these effects of lactation on mice with a compulsive-like phenotype. Considering a pressing need for better understanding of OCD in females during physiologically challenging conditions, our data holds clinical importance by providing critical insights into SSRI effectiveness, role of breast feeding and comorbid affective disorders in postpartum OCD patients.

MATERIALS AND METHODS

The University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) approved the animal care and experimental procedures (IACUC assurance number 862663).

Animals

For this study, we used the compulsive-like BIG male and female mice.⁹ This compulsive-like mouse model was developed from house mouse strains (*Mus musculus*) through bidirectional selection for nest-building behavior.^{48,85} The HS/Ibg outbred strain,⁸⁶ which was developed through crossing of eight inbred strains (A, AKR, BLB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII) served as the stock population for the selective breeding.⁸⁵ Bidirectional selection resulted in two compulsive-like BIG strains, which consistently exhibit a fortyfold higher level of compulsive-like nest-building behavior^{48,85} and a 3-fold higher level of compulsive-like marble burying behavior^{9a} when compared to the two non-

compulsive-like SMALL strains. The two randomly bred control strains express intermediate levels of these behaviors.^{9a,c,48,85} These excessive, repetitive, and perseverant otherwise normal nest-building and marble burying behaviors by the BIG strains make them a good model to study compulsive-like phenotypes.⁹ All experimental BIG males and females, taken from the one compulsive-like strain that had the highest breeding success of about 85% during colony breeding, were housed in polypropylene cages (27 cm × 17 cm × 12 cm) with same sex littermates and provided with wood shavings under a 12:12 light–dark cycle at 22 ± 1 °C with *ad libitum* food (Purina Mills, Lab Diet Mouse Diet #5015, St. Louis, MO) and water. When the animals reached 60 days of age, each BIG female was paired with a BIG male in a cage with *ad libitum* access to high protein rodent chow (Masuri Rodent Diet #5663, Purina Mills, LLC, St. Louis, MO, USA) and water.

Experimental Design

The entire study was divided into two main phases.

Phase I: Determining the Role of Physiological Status in Behavioral Expression and SSRI Effectiveness—In the first phase of the experiments, compulsive-like lactating, nonlactating, and nulliparous female mice were tested for compulsive-like, anxiety-like and depression-like behaviors. For lactating and nonlactating females, the males were separated 2 weeks after pairing. All the females showed signs of pregnancy within 2 weeks of pairing, which was confirmed through vaginal plug. All lactating females were housed with their pups until the end of all the behavioral assessments. For the lactating group, the litter sizes ranged from 7 to 10 pups. For the nonlactating females, pups were removed immediately after birth and the females were housed individually in cages until the end of all experimentation. Nulliparous females were housed individually in cages after they failed to conceive following pairing with male counterparts for 2 weeks (males were removed after 2 weeks of pairing). All experimental groups of females (lactating, nonlactating, and nulliparous) were treated with either fluoxetine ($n = 12$) or vehicle ($n = 12$). Animals treated with either fluoxetine or vehicle were subjected to compulsive-like marble burying and nest-building behaviors once every week for 3 weeks on days 1, 2, 8, 9, 15, and 16 of lactation, respectively (day 0 was the day of parturition). Anxiety-like open field and depression-like tail suspension tests were conducted only once on days 17 and 18 of lactation, respectively.

Separate groups ($n = 5$ per group) of lactating, nonlactating, and nulliparous females that did not undergo any behavioral assessments and treatments were subjected to transcardial whole body perfusion on day 18 of lactation. Brains were collected for immunohistochemical analysis of 5-HT neurons in the dorsal raphe nucleus (DRN). Other separate groups of lactating, nonlactating, and nulliparous females ($n = 6$ per group) that also were not subjected to any behavioral assessments and treatments were used to collect blood samples on day 18 of lactation from the trunk of cervically dislocated animals for serum analysis of 5-HT through ELISA. Because most of the behavioral assessments (except compulsive-like behaviors, which were performed once every week) were performed in the third week, we wanted to evaluate the 5-HT serum levels and DRN immunoreactivity during the final week

of lactation. Day 18 of lactation was selected for 5-HT measurements since it is considered to be the peak lactation period.⁸⁷

Phase II: Determining the Role of a Dopaminergic Agonist and an Oxytocinergic Antagonist in Lactating Compulsive-like Mice—

For phase two of the experiments, only lactating females (litter size from 7 to 10 pups) were used. All lactating females were divided into 12 groups. Animals were tested for compulsive-like nest-building and marble burying, anxiety-like open field, and depression-like tail suspension. For each of four behaviors, three main treatment groups were used for a total of 12 experimental groups. For each behavior, mice were treated with bromocriptine mesylate, a dopamine D2 receptor agonist,⁸⁸ L368-899, an OXTR antagonist,⁸⁹ or vehicle. All drug or vehicle treated lactating females were subjected to a single behavioral assessment, that is, tested just once for one behavior, on day 18 of lactation. Day 18 being the peak lactation period was selected for behavioral assessments.⁸⁷ Exposing animals to multiple behaviors was avoided to minimize stress to the pups and the females due to multiple injections. All behavioral assessments were carried out in the light phase of the 12:12 L/D cycle, and data were collected by an experimenter blinded to the treatment groups.

Drug Treatment—For phase one of the experiments, all groups (lactating, nonlactating, and nulliparous females) were treated with either fluoxetine or vehicle. The vehicle animals received sucrose in drinking water (2.9 g/L), while the drug groups received 50 mg/kg of fluoxetine in the sucrose vehicle as described previously.^{9a} The dosage of 50 mg/kg was used because it shows the most consistent suppression of compulsive-like behaviors in our compulsive-like mice.^{9a} The route of administration was orally in the drinking water. The average water consumption was measured for 3–5 days before and after the pups were born in lactating and nonlactating females. Treatment for lactating and nonlactating females started on the day the pups were born. The amount of drug given was calculated based on the average body weight and daily average water consumption of the mice. The water levels were measured daily and new water bottles were given every 2 days. To avoid excess consumption of treatment only minimum volumes, determined through measurement of average daily water consumption, were provided and animals were checked every 12 h. For the nulliparous females, the same time frame was used for evaluation of drug and vehicle treatments, and treatment was started when it was confirmed that they were not pregnant, that is, 3 weeks after the males were removed. The total duration of treatment was 18 days because after 3 weeks of SSRI treatment maximum fluoxetine effects are obtained in our mouse model of OCD.^{9a}

In phase two of the experiments, lactating females were divided into three drug treatment groups for a total of four behaviors to a total of 12 experimental groups. Each behavioral group received 10 mg/kg of D2R agonist bromocriptine mesylate, 5 mg/kg of OXTR antagonist L368-899 or vehicle (0.9% sterile saline and 1% Tween) through intraperitoneal (ip) injection 1 h before behavioral assessment. The dosage for L368-899 and bromocriptine was determined from previous studies in mice and rats.^{88–90} The injection volume was adjusted proportionally according to the body weight of each animal with a 0.3 mL injection volume for a 40 g mouse.

Compulsive-like Behaviors. Marble Burying Behavior—The marble-burying test was used to assess compulsive-like behavior.^{9a,91} All mice were individually introduced into a polypropylene cage (37 cm × 21 cm × 14 cm) containing 20 glass marbles (10 mm in diameter) evenly spaced on 5 cm deep wood shavings firmly pressed into a uniform bedding without access to food or water for 10 min. The total number of marbles buried at least 2/3 in the 10 min period was quantified as compulsive-like digging behavior.⁹ Following the 10 min test, animals were returned to their home cages.

Nest-Building Behavior—Nest-building behavior was used to assess the compulsive-like phenotype of the female mice.⁹ All mice were housed individually and were allowed to access a preweighed cotton roll placed in the cage top food hopper. The amount of cotton used by the mice after 1 h was determined by weighing the cotton roll. For phase one of the experiments, nest-building was performed for 1 h only. This was mainly to ensure that the lactating females were not separated from the pups for too long. The same time frame was followed for nonlactating and nulliparous females to have uniform data collection. Nest-building was performed on weeks 1, 2, and 3 of postpartum for lactating and nonlactating females. A similar time frame was also maintained for nulliparous females and was tested three times on weeks 1, 2, and 3.

For phase two of the experiments, all lactating females treated for vehicle, bromocriptine, or L368-899 were subjected to nest-building for 24 h only once starting on day 18 of lactation. Amounts of cotton used for nesting were measured 1, 2, 3, 4, 5, and 24 h after ip injection.

Anxiety-like Open Field Behavior—Anxiety-like behaviors were determined through the open field test.⁹² Mice were individually introduced into an open field (40 cm × 40 cm × 35 cm) with a central zone (20 cm × 20 cm). The apparatus was placed underneath an overhead light illuminating the entire apparatus.⁹ The animals were placed in the central zone of the apparatus and their behavior was videotaped for 3 min and analyzed with the aid of ANYMaze video tracking software (Stoelting Co., Wood Dale, IL, USA). The total number of central zone entries (anxiety-like measure) and total distance traveled (locomotion) in the entire open field were measured. The open field was cleaned before each test with a dilute chlorhexidine solution. Prior experiments⁹ with the BIG mice indicate that 3 min duration provides consistent outcomes for assessment of locomotor activity and anxiety-like behaviors and therefore this duration was considered for the current experiment.

Depression-like Tail Suspension Behavior—Depression-like behavior in the compulsive-like mice was assessed through the tail suspension test.⁹³ Each mouse was individually suspended from a hook of the tail suspension apparatus (Stoelting Co., Wood Dale, IL) above the surface of the table by a 15 cm long adhesive tape. The tape was placed 5 mm from the tip of the tail, and the animal was suspended 60 cm above the base of the apparatus. The immobility duration was recorded for 6 min using a camera. Mice were considered immobile only when they were completely motionless without any movement of body parts or front and hind limbs.⁹⁴ The videos were analyzed by an experimenter blinded to the experimental conditions of the mice.

Serum 5-HT Levels—Trunk blood was collected from females (lactating, nonlactating, and nulliparous) on the 18th day of lactation after cervical dislocation. Blood samples were kept undisturbed at 4 °C overnight. This allowed the 5-HT to be released from the platelets.¹⁹ Serum was then extracted from blood samples by centrifugation at 12 000 rpm for 20 min (4 °C). The extracted serum was stored at –80 °C until analysis. All serum samples were assayed in duplicate for 5-HT levels using an ELISA kit (Kit no. ADI 900-175; Enzo life Sciences) according to the manufacturer instructions. The sensitivity for the kit was 0.293 ng/mL.

5-HT Immunohistochemistry in DRN—Brains collected through whole body transcardial perfusion (8 mL of 0.01 M phosphate buffer saline (PBS) followed by 25 mL of 4% paraformaldehyde in PBS) were fixed overnight in 4% paraformaldehyde. Fixed brains were transferred to 30% sucrose solution and stored until they submerged completely. Brains were then frozen in Tissue-Tek OCT compound in beaker cups (VWR International). Frozen brains were sectioned on a freezing microtome at 20 μ m coronal slices –4.84 Bregma and –5.02 Bregma as per the mouse brain atlas, which followed the procedures of a previous study.¹⁹ Brain slices were then immunostained for 5-HT on slides (VWR Cat. no. 48311-703). Slides containing brain sections were first washed in PBS (five 5 min washes). The slides were then incubated in 30% hydrogen peroxide in PBS for 30 min at room temperature followed by PBS washes (six 5 min washes). Blocking buffer (PBS containing 5% normal goat serum, 2% BSA, and 0.4% Triton X-100) was then added to the slides for 1 h. After 1 h, slides were washed in PBS (three 5 min washes) and incubated with 5-HT primary (Immunostar Cat. no. 20080; 1:20 000 in PBS and 0.4% Triton X-100) overnight for 20 h. On day two, slides were washed in PBS (four 5 min washes) and were incubated with secondary antibody (1:600 biotinylated goat anti-rabbit, Vector laboratories Cat. no. BA1000; in PBS and 0.04% Triton X-100) for 1 h. Sections were finally processed using Vectastain Elite ABC immunoperoxidase system (Vector Laboratories) as per the manufacturer instructions and visualized with Ni²⁺-DAB enzyme substrate. For analysis of 5-HT reactive cell bodies in the DRN, ImageJ software (NIH) was used. In the threshold mode, which was the same for all brain sections analyzed, the number of cell bodies stained positively for 5-HT in the DRN were counted.

Statistical Analysis—Statistical system software (SAS; Version 9.4 NC Carey) was used for analysis of all the results. For the first phase of experiments, a generalized linear model (GLM) repeated measures analysis of variance (ANOVA) for physiological status (lactating, nonlactating, and nulliparous), treatment (vehicle, fluoxetine), and physiological status by treatment interaction effects was used to statistically evaluate the nest building and marble burying behaviors. A GLM two-way ANOVA for physiological status, treatment, and physiological status by treatment interaction effects was used to statistically evaluate the open field and tail suspension behaviors. For serum and DRN 5-HT levels, GLM one-way ANOVA was conducted for the effect of physiological status. For the second phase of the experiments, a GLM one-way ANOVA was performed to test for the effect of treatment (vehicle, bromocriptine, and L368-899) on each of four behaviors. When significance was found in any of the ANOVA measures, appropriate pairwise comparisons were performed using the Studentized Range test. The nesting scores were square root transformed to obtain

a more normal distribution.^{9,48,95} All data are represented as mean $\pm$ standard error of the mean (SEM). A probability level of $p < 0.05$ was used to determine statistical significance in all cases.

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References

1. Pallanti S, Grassi G, Sarrecchia ED, Cantisani A, Pellegrini M. Obsessive-compulsive disorder comorbidity: clinical assessment and therapeutic implications. *Frontiers in psychiatry*. 2011; 2:70. [PubMed: 22203806]
2. Huppert JD, Simpson HB, Nissenson KJ, Liebowitz MR, Foa EB. Quality of life and functional impairment in obsessive-compulsive disorder: a comparison of patients with and without comorbidity, patients in remission, and healthy controls. *Depression Anxiety*. 2009; 26(1):39–45. [PubMed: 18800368]
3. (a) Koran LM. Quality of life in obsessive-compulsive disorder. *Psychiatric clinics of North America*. 2000; 23(3):509–17. [PubMed: 10986724] (b) Bobes J, Gonzalez MP, Bascaran MT, Arango C, Saiz PA, Bousono M. Quality of life and disability in patients with obsessive-compulsive disorder. *European psychiatry: the journal of the Association of European Psychiatrists*. 2001; 16(4):239–45. [PubMed: 11418275]
4. Doornbos B, Fekkes D, Tanke MA, de Jonge P, Korf J. Sequential serotonin and noradrenalin associated processes involved in postpartum blues. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2008; 32(5):1320–5.
5. (a) Abramowitz JS, Schwartz SA, Moore KM. Obsessional Thoughts in Postpartum Females and Their Partners: Content, Severity, and Relationship with Depression. *Journal of Clinical Psychology in Medical Settings*. 2003; 10(3):157–164. (b) Brandes M, Soares CN, Cohen LS. Postpartum onset obsessive-compulsive disorder: diagnosis and management. *Arch Women's Ment Health*. 2004; 7(2):99–110. [PubMed: 15083345] (c) House SJ, Tripathi SP, Knight BT, Morris N, Newport DJ, Stowe ZN. Obsessive-compulsive disorder in pregnancy and the postpartum period: course of illness and obstetrical outcome. *Arch Women's Ment Health*. 2016; 19(1):3–10. [PubMed: 26173597] (d) Labad J, Menchon JM, Alonso P, Segalas C, Jimenez S, Vallejo J. Female reproductive cycle and obsessive-compulsive disorder. *J Clin Psychiatry*. 2005; 66(4):428–35. quiz 546. [PubMed: 15816784] (e) Maina G, Albert U, Bogetto F, Vaschetto P, Ravizza L. Recent life events and obsessive-compulsive disorder (OCD): the role of pregnancy/delivery. *Psychiatry Res*. 1999; 89(1): 49–58. [PubMed: 10643877] (f) Vulink NC, Denys D, Bus L, Westenberg HG. Female hormones affect symptom severity in obsessive-compulsive disorder. *International clinical psychopharmacology*. 2006; 21(3):171–5. [PubMed: 16528139] (g) Miller ES, Chu C, Gollan J, Gossett DR. Obsessive-compulsive symptoms during the postpartum period. A prospective cohort. *Journal of reproductive medicine*. 2013; 58(3–4):115–22. [PubMed: 23539879]
6. Tükel R, Meteris H, Koyuncu A, Tecer A, Yazici O. The clinical impact of mood disorder comorbidity on obsessive-compulsive disorder. *European archives of psychiatry and clinical neuroscience*. 2006; 256(4):240–5. [PubMed: 16683062]

7. (a) Smith MV, Shao L, Howell H, Lin H, Yonkers KA. Perinatal depression and birth outcomes in a Healthy Start project. *Maternal and child health journal*. 2011; 15(3):401–9. [PubMed: 20300813] (b) Alipour Z, Lamyian M, Hajizadeh E. Anxiety and fear of childbirth as predictors of postnatal depression in nulliparous women. *Women and birth: journal of the Australian College of Midwives*. 2012; 25(3):e37–43. [PubMed: 21959041]
8. (a) Noshirvani HF, Kasvikis Y, Marks IM, Tsakiris F, Monteiro WO. Gender-divergent aetiological factors in obsessive-compulsive disorder. *Br J Psychiatry*. 1991; 158:260–3. [PubMed: 2012918] (b) Bogetto F, Venturello S, Albert U, Maina G, Ravizza L. Gender-related clinical differences in obsessive-compulsive disorder. *European psychiatry: the journal of the Association of European Psychiatrists*. 1999; 14(8):434–41. [PubMed: 10683629] (c) Mundo E, Bareggi SR, Pirola R, Bellodi L. Effect of acute intravenous clomipramine and antiobsessional response to proserotonergic drugs: is gender a predictive variable? *Biol Psychiatry*. 1999; 45(3):290–4. [PubMed: 10023504]
9. (a) Greene-Schloesser DM, Van der Zee EA, Sheppard DK, Castillo MR, Gregg KA, Burrow T, Foltz H, Slater M, Bult-Ito A. Predictive validity of a non-induced mouse model of compulsive-like behavior. *Behav Brain Res*. 2011; 221(1):55–62. [PubMed: 21316394] (b) Mitra S, Bastos CP, Bates K, Pereira GS, Bult-Ito A. Ovarian Sex Hormones Modulate Compulsive, Affective and Cognitive Functions in A Non-Induced Mouse Model of Obsessive-Compulsive Disorder. *Front Behav Neurosci*. 2016; 10:215. [PubMed: 27881956] (c) Mitra S, Bastos CP, Chesworth S, Frye C, Bult-Ito A. Strain and sex based characterization of behavioral expressions in non-induced compulsive-like mice. *Physiol Behav*. 2017; 168:103–111. [PubMed: 27838311] (d) Mitra S, Mucha M, Khatri SN, Glenon R, Schulte MK, Bult-Ito A. Attenuation of Compulsive-Like Behavior Through Positive Allosteric Modulation of $\alpha 4\beta 2$ Nicotinic Acetylcholine Receptors in Non-Induced Compulsive-Like Mice. *Front Behav Neurosci*. 2017; 10:244. [PubMed: 28105008]
10. (a) Theodosis DT. Oxytocin-secreting neurons: A physiological model of morphological neuronal and glial plasticity in the adult hypothalamus. *Front Neuroendocrinol*. 2002; 23(1):101–35. [PubMed: 11906204] (b) Merchenthaler I. Induction of enkephalin in tuberoinfundibular dopaminergic neurons during lactation. *Endocrinology*. 1993; 133(6):2645–51. [PubMed: 7694844]
11. Romano N, Guillou A, Hodson DJ, Martin AO, Mollard P. Multiple-scale neuroendocrine signals connect brain and pituitary hormone rhythms. *Proc Natl Acad Sci U S A*. 2017; 114(9):2379–2382. [PubMed: 28193889]
12. Fitzgerald P, Dinan TG. Prolactin and dopamine: what is the connection? A review article. *J Psychopharmacol*. 2008; 22:12–9. [PubMed: 18477617]
13. (a) Zingg HH, Lefebvre DL. Oxytocin and vasopressin gene expression during gestation and lactation. *Mol Brain Res*. 1988; 4:1–6. (b) Mezey E, Kiss JZ. Coexpression of vasopressin and oxytocin in hypothalamic supraoptic neurons of lactating rats. *Endocrinology*. 1991; 129(4):1814–20. [PubMed: 1915070] (c) Caldwell JD, Greer ER, Johnson MF, Prange AJ Jr, Pedersen CA. Oxytocin and vasopressin immunoreactivity in hypothalamic and extrahypothalamic sites in late pregnant and postpartum rats. *Neuroendocrinology*. 2004; 46(1):39–47.
14. (a) Zingg HH, Laporte SA. The oxytocin receptor. *Trends Endocrinol Metab*. 2003; 14(5):222–7. [PubMed: 12826328] (b) Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev*. 2001; 81(2):629–83. [PubMed: 11274341]
15. Catheline G, Touquet B, Lombard MC, Poulain DA, Theodosis DT. A study of the role of neuroglial remodeling in the oxytocin system at lactation. *Neuroscience*. 2006; 137(1):309–16. [PubMed: 16216421]
16. Neumann ID, Slattery DA. Oxytocin in General Anxiety and Social Fear: A Translational Approach. *Biol Psychiatry*. 2016; 79(3):213–21. [PubMed: 26208744]
17. Cochran DM, Fallon D, Hill M, Frazier JA. The role of oxytocin in psychiatric disorders: a review of biological and therapeutic research findings. *Harvard review of psychiatry*. 2013; 21(5):219–47. [PubMed: 24651556]
18. Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, Bailey JP, Nieport KM, Walther DJ, Bader M, Horseman ND. Serotonin regulates mammary gland development via an autocrine-paracrine loop. *Dev Cell*. 2004; 6(2):193–203. [PubMed: 14960274]

19. Jury NJ, McCormick BA, Horseman ND, Benoit SC, Gregerson KA. Enhanced responsiveness to selective serotonin reuptake inhibitors during lactation. *PLoS One*. 2015; 10(2):e0117339. [PubMed: 25689282]
20. (a) Balsa JA, Sanchez-Franco F, Pazos F, Lara JJ, Lorenzo MJ, Maldonado G, Cacicedo L. Direct action of serotonin on prolactin, growth hormone, corticotropin and luteinizing hormone release in cocultures of anterior and posterior pituitary lobes: autocrine and/or paracrine action of vasoactive intestinal peptide. *Neuroendocrinology*. 1998; 68(5):326–33. [PubMed: 9822800] (b) Chaiseha Y, Kang SW, Leclerc B, Kosonsiriluk S, Sartsoongnoen N, El Halawani ME. Serotonin receptor subtypes influence prolactin secretion in the turkey. *Gen Comp Endocrinol*. 2010; 165(1):170–5. [PubMed: 19540238]
21. Agrati D, Fernandez-Guasti A, Zuluaga MJ, Uriarte N, Pereira M, Ferreira A. Compulsive-like behaviour according to the sex and the reproductive stage of female rats. *Behav Brain Res*. 2005; 161(2):313–9. [PubMed: 15922059]
22. (a) Altemus M, Deuster PA, Galliven E, Carter CS, Gold PW. Suppression of hypothalamic-pituitary-adrenal axis responses to stress in lactating women. *J Clin Endocrinol Metab*. 1995; 80(10):2954–9. [PubMed: 7559880] (b) Altemus M, Redwine LS, Leong YM, Frye CA, Porges SW, Carter CS. Responses to laboratory psychosocial stress in postpartum women. *Psychosom Med*. 2001; 63(5):814–21. [PubMed: 11573030] (c) Carter CS, Altemus M. Integrative functions of lactational hormones in social behavior and stress management. *Ann N Y Acad Sci*. 1997; 807:164–74. [PubMed: 9071349] (d) Altshuler LL, Hendrick V, Cohen LS. An Update on Mood and Anxiety Disorders During Pregnancy and the Postpartum Period. Primary care companion to the *Journal of clinical psychiatry* 2. 2000; 6:217–222. (e) Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *Jama*. 2002; 287(3):356–9. [PubMed: 11790215] (f) Heinrichs M, Meinschmidt G, Neumann I, Wagner S, Kirschbaum C, Ehler U, Hellhammer DH. Effects of suckling on hypothalamic-pituitary-adrenal axis responses to psychosocial stress in postpartum lactating women. *J Clin Endocrinol Metab*. 2001; 86(10):4798–804. [PubMed: 11600543] (g) Fleming AS, Ruble DN, Flett GL, Shaul DL. Postpartum adjustment in first-time mothers: Relations between mood, maternal attitudes, and mother-infant interactions. *Developmental psychology*. 1988; 24(1):71. (h) Stern JM, Mackinnon DA. Postpartum, hormonal, and non-hormonal induction of maternal behavior in rats: effects on T-maze retrieval of pups. *Horm Behav*. 1976; 7(3):305–16. [PubMed: 992586] (i) Pereira M, Uriarte N, Agrati D, Zuluaga MJ, Ferreira A. Motivational aspects of maternal anxiety in lactating rats. *Psychopharmacology*. 2005; 180(2): 241–8. [PubMed: 15778891] (j) Lonstein JS. Reduced anxiety in postpartum rats requires recent physical interactions with pups, but is independent of suckling and peripheral sources of hormones. *Horm Behav*. 2005; 47(3):241–55. [PubMed: 15708752]
23. (a) Barrett J, Fleming AS. Annual research review: All mothers are not created equal: Neural and psychobiological perspectives on mothering and the importance of individual differences. *Journal of Child Psychology and Psychiatry*. 2011; 52(4):368–397. [PubMed: 20925656] (b) Lonstein JS. Regulation of anxiety during the postpartum period. *Front Neuroendocrinol*. 2007; 28(2–3):115–41. [PubMed: 17604088]
24. (a) Agrati D, Lonstein JS. Affective changes during the postpartum period: influences of genetic and experiential factors. *Horm Behav*. 2016; 77:141–152. [PubMed: 26210061] (b) Ross LE, McLean LM. Anxiety disorders during pregnancy and the postpartum period: A systematic review. *J Clin Psychiatry*. 2006; 67(8):1285, 98. [PubMed: 16965210]
25. Ragan C, Lonstein J. Differential postpartum sensitivity to the anxiety-modulating effects of offspring contact is associated with innate anxiety and brainstem levels of dopamine beta-hydroxylase in female laboratory rats. *Neuroscience*. 2014; 256:433–444. [PubMed: 24161285]
26. (a) Maestripieri D, D'Amato FR. Anxiety and maternal aggression in house mice (*Mus musculus*): a look at interindividual variability. *J Comp Psychol*. 1991; 105(3):295–301. [PubMed: 1935008] (b) Miller SM, Piasecki CC, Lonstein JS. Use of the light-dark box to compare the anxiety-related behavior of virgin and postpartum female rats. *Pharmacol, Biochem Behav*. 2011; 100(1):130–7. [PubMed: 21851834]
27. (a) Champagne FA, Meaney MJ. Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. *Behav Neurosci*. 2007; 121(6):1353–63. [PubMed: 18085888] (b) Wang Z, Storm DR. Maternal behavior is impaired in female mice

- lacking type 3 adenylyl cyclase. *Neuropsychopharmacology*. 2011; 36(4):772–81. [PubMed: 21150908] (c) Bosch OJ. Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. *Philos Trans R Soc, B*. 2013; 368(1631):20130085. (d) Bosch OJ, Neumann ID. Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: from central release to sites of action. *Horm Behav*. 2012; 61(3):293–303. [PubMed: 22100184]
28. (a) Ystrom E. Breastfeeding cessation and symptoms of anxiety and depression: a longitudinal cohort study. *BMC Pregnancy Childbirth*. 2012; 12:36. [PubMed: 22621668] (b) Skrundz M, Bolten M, Nast I, Hellhammer DH, Meinschmidt G. Plasma oxytocin concentration during pregnancy is associated with development of postpartum depression. *Neuropsychopharmacology*. 2011; 36(9):1886–93. [PubMed: 21562482] (c) Nissen E, Uvnäs-Moberg K, Svensson K, Stock S, Widström AM, Winberg J. Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by caesarean section or by the vaginal route. *Early Hum Dev*. 1996; 45(1–2):103–118. [PubMed: 8842644] (d) Nissen E, Gustavsson P, Widström A, Uvnäs-Moberg K. Oxytocin, prolactin, milk production and their relationship with personality traits in women after vaginal delivery or Cesarean section. *Journal of Psychosomatic Obstetrics & Gynecology*. 1998; 19(1):49–58. [PubMed: 9575469] (e) Stuebe AM, Grewen K, Pedersen CA, Proper C, Meltzer-Brody S. Failed lactation and perinatal depression: common problems with shared neuroendocrine mechanisms? *Journal of women's health*. 2012; 21(3):264–72.
29. Fraga MC, Moura EG, Silva JO, Bonomo IT, Filgueiras CC, Abreu-Villaca Y, Passos MC, Lisboa PC, Manhaes AC. Maternal prolactin inhibition at the end of lactation affects learning/memory and anxiety-like behaviors but not novelty-seeking in adult rat progeny. *Pharmacol, Biochem Behav*. 2011; 100(1):165–73. [PubMed: 21777608]
30. (a) Denys D, Zohar J, Westenberg HG. The role of dopamine in obsessive-compulsive disorder: preclinical and clinical evidence. *Journal of clinical psychiatry*. 2004; 65(Suppl 14):11–7. (b) Koo MS, Kim EJ, Roh D, Kim CH. Role of dopamine in the pathophysiology and treatment of obsessive-compulsive disorder. *Expert Rev Neurother*. 2010; 10(2):275–90. [PubMed: 20136383] (c) Pauls DL, Abramovitch A, Rauch SL, Geller DA. Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective. *Nat Rev Neurosci*. 2014; 15(6):410–24. [PubMed: 24840803] (d) van Dijk A, Klomp makers A, Denys D. Role of serotonin in obsessive-compulsive disorder. *Future Neurol*. 2008; 3(5):589–603.
31. (a) Gordon JA, Hen R. The serotonergic system and anxiety. *NeuroMol Med*. 2004; 5(1):27–40. (b) Zarrindast MR, Khakpai F. The Modulatory Role of Dopamine in Anxiety-like Behavior. *Archives of Iranian medicine*. 2015; 18(9):591–603. [PubMed: 26317601]
32. (a) Mann JJ. Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology*. 1999; 21(2 Suppl):99S–105S. [PubMed: 10432495] (b) Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry*. 2007; 64(3):327–37. [PubMed: 17339521]
33. (a) Delgado PL, Price LH, Miller HL, Salomon RM, Licinio J, Krystal JH, Heninger GR, Charney DS. Rapid serotonin depletion as a provocative challenge test for patients with major depression: relevance to antidepressant action and the neurobiology of depression. *Psychopharmacology bulletin*. 1991; 27(3):321–30. [PubMed: 1775606] (b) Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clinical chemistry*. 1994; 40(2):288–95. [PubMed: 7508830] (c) Newport DJ, Owens MJ, Knight DL, Ragan K, Morgan N, Nemeroff CB, Stowe ZN. Alterations in platelet serotonin transporter binding in women with postpartum onset major depression. *J Psychiatr Res*. 2004; 38(5):467–73. [PubMed: 15380396] (d) Albert U, Aguglia A, Bramante S, Bogetto F, Maina G. Treatment-resistant obsessive-compulsive disorder (OCD): Current knowledge and open questions. *Clin Neuropsychiatry*. 2013; 10(1):19–30. (e) Marazziti D, Picchetti M, Baroni S, Ceresoli D, Consoli G, Catena Dell'Osso M. Current pharmacological and non pharmacological treatments for obsessive-compulsive disorder. *Journal of Psychopathology*. 2012; 18:5–18. (f) Fernandez CE, López-Ibor AJ. Use of monochlorimipramine in psychiatric patients who are resistant to other therapy. *Actas luso-espanolas de neurologia y psiquiatria*. 1967; 26(2):119–147. (g) Goodman WK, Price LH, Rasmussen SA, Delgado PL, Heninger GR, Charney DS. Efficacy of fluvoxamine in obsessive-compulsive disorder. A double-blind comparison with placebo. *Arch Gen Psychiatry*. 1989; 46(1):36–44. [PubMed: 2491940]
34. (a) Yadin E, Friedman E, Bridger WH. Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? *Pharmacol, Biochem Behav*. 1991; 40(2):311–5. [PubMed:

- 1839567] (b) Szechtman H, Sulis W, Eilam D. Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behav Neurosci*. 1998; 112(6):1475–85. [PubMed: 9926830]
35. Groer MW. Differences between exclusive breastfeeders, formula-feeders, and controls: a study of stress, mood, and endocrine variables. *Biol Res Nurs*. 2005; 7(2):106–17. [PubMed: 16267372]
36. (a) Giovenardi M, Padoin MJ, Cadore LP, Lucion AB. Hypothalamic paraventricular nucleus modulates maternal aggression in rats: effects of ibotenic acid lesion and oxytocin antisense. *Physiol Behav*. 1998; 63(3):351–9. [PubMed: 9469726] (b) Neumann ID, Torner L, Wigger A. Brain oxytocin: differential inhibition of neuroendocrine stress responses and anxiety-related behaviour in virgin, pregnant and lactating rats. *Neuroscience*. 2000; 95(2):567–75. [PubMed: 10658637] (c) Torner L, Toschi N, Nava G, Clapp C, Neumann ID. Increased hypothalamic expression of prolactin in lactation: involvement in behavioural and neuroendocrine stress responses. *European journal of neuroscience*. 2002; 15(8):1381–9. [PubMed: 11994132] (d) Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc Natl Acad Sci U S A*. 2001; 98(22):12736–41. [PubMed: 11606726] (e) Bosch OJ, Meddle SL, Beiderbeck DI, Douglas AJ, Neumann ID. Brain oxytocin correlates with maternal aggression: link to anxiety. *J Neurosci*. 2005; 25(29):6807–15. [PubMed: 16033890] (f) Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, Nishimori K. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci*. 2009; 29(7):2259–71. [PubMed: 19228979] (g) Finkenwirth C, Martins E, Deschner T, Burkart JM. Oxytocin is associated with infant-care behavior and motivation in cooperatively breeding marmoset monkeys. *Horm Behav*. 2016; 80:10–8. [PubMed: 26836769] (h) Salais-Lopez H, Lanuza E, Agustin-Pavon C, Martinez-Garcia F. Tuning the brain for motherhood: prolactin-like central signalling in virgin, pregnant, and lactating female mice. *Brain Struct Funct*. 2017; 222(2): 895–921. [PubMed: 27344140]
37. Leckman JF, Goodman WK, North WG, Chappell PB, Price LH, Pauls DL, Anderson GM, Riddle MA, McSwiggan-Hardin M, McDougle CJ, et al. Elevated cerebrospinal fluid levels of oxytocin in obsessive-compulsive disorder. Comparison with Tourette's syndrome and healthy controls. *Arch Gen Psychiatry*. 1994; 51(10):782–92. [PubMed: 7524462]
38. Humble MB, Uvnas-Moberg K, Engstrom I, Bejerot S. Plasma oxytocin changes and anti-obsessive response during serotonin reuptake inhibitor treatment: a placebo controlled study. *BMC Psychiatry*. 2013; 13:344. [PubMed: 24359174]
39. Altemus M, Jacobson KR, Debellis M, Kling M, Pigott T, Murphy DL, Gold PW. Normal CSF oxytocin and NPY levels in OCD. *Biol Psychiatry*. 1999; 45(7):931–3. [PubMed: 10202583]
40. (a) den Boer JA, Westenberg HG. Oxytocin in obsessive compulsive disorder. *Peptides*. 1992; 13(6):1083–5. [PubMed: 1494489] (b) Epperson CN, McDougle CJ, Price LH. Intranasal oxytocin in obsessive-compulsive disorder. *Biol Psychiatry*. 1996; 40(6):547–9. [PubMed: 8879477]
41. (a) Khanna S, John JP, Reddy LP. Neuroendocrine and behavioral responses to mCPP in Obsessive-Compulsive Disorder. *Psychoneuroendocrinology*. 2001; 26(2):209–23. [PubMed: 11087965] (b) de Leeuw AS, Westenberg HG. Hypersensitivity of 5-HT₂ receptors in OCD patients. An increased prolactin response after a challenge with meta-chlorophenylpiperazine and pre-treatment with ritanserin and placebo. *J Psychiatr Res*. 2008; 42(11):894–901. [PubMed: 18533183]
42. Lynch CB. Clinal variation in cold adaptation in *Mus domesticus*: verification of predictions from laboratory populations. *Am Nat*. 1992; 139(6):1219–1236.
43. Challacombe FL, Salkovskis PM, Woolgar M, Wilkinson EL, Read J, Acheson R. Parenting and mother-infant interactions in the context of maternal postpartum obsessive-compulsive disorder: Effects of obsessional symptoms and mood. *Infant behavior & development*. 2016; 44:11–20. [PubMed: 27259042]
44. Marazziti D, Baroni S, Giannaccini G, Betti L, Massimetti G, Carmassi C, Catena-Dell'Osso M. A link between oxytocin and serotonin in humans: supporting evidence from peripheral markers. *Eur Neuropsychopharmacol*. 2012; 22(8):578–83. [PubMed: 22297159]
45. (a) Hervas I, Vilario MT, Romero L, Scorza MC, Mengod G, Artigas F. Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of

- WAY-100635. *Neuropsychopharmacology*. 2001; 24(1):11–20. [PubMed: 11106871] (b) Descarries L, Riad M. Effects of the antidepressant fluoxetine on the subcellular localization of 5-HT1A receptors and SERT. *Philos Trans R Soc, B*. 2012; 367(1601):2416–25.
46. (a) Higuchi T, Honda K, Fukuoka T, Negoro H, Hosono Y, Nishida E. Pulsatile secretion of prolactin and oxytocin during nursing in the lactating rat. *Endocrinol Jpn*. 1983; 30(3):353–9. [PubMed: 6662071] (b) Picazo O, Rosenblatt JS, Fernandez-Guasti A. The differential effect of the anxiolytic agent 8-OH-DPAT during lactation is independent of pup withdrawal and maternal behavior. *Psychoneuroendocrinology*. 2000; 25(7):693–706. [PubMed: 10938449]
47. Fernandez-Guasti A, Agrati D, Reyes R, Ferreira A. Ovarian steroids counteract serotonergic drugs actions in an animal model of obsessive-compulsive disorder. *Psychoneuroendocrinology*. 2006; 31(8):924–34. [PubMed: 16839691]
48. Bult A, Lynch CB. Breaking through artificial selection limits of an adaptive behavior in mice and the consequences for correlated responses. *Behav Genet*. 2000; 30(3):193–206. [PubMed: 11105393]
49. (a) Yoshimura R, Kiyama H, Kimura T, Araki T, Maeno H, Tanizawa O, Tohyama M. Localization of oxytocin receptor messenger ribonucleic acid in the rat brain. *Endocrinology*. 1993; 133(3):1239–46. [PubMed: 8396014] (b) Vaccari C, Lolait SJ, Ostrowski NL. Comparative distribution of vasopressin V1b and oxytocin receptor messenger ribonucleic acids in brain. *Endocrinology*. 1998; 139(12):5015–33. [PubMed: 9832441]
50. Cappi C, Diniz JB, Requena GL, Lourenco T, Lisboa BC, Batistuzzo MC, Marques AH, Hoexter MQ, Pereira CA, Miguel EC, Brentani H. Epigenetic evidence for involvement of the oxytocin receptor gene in obsessive-compulsive disorder. *BMC Neurosci*. 2016; 17(1):79. [PubMed: 27903255]
51. (a) Pagani JH, Williams Avram SK, Cui Z, Song J, Mezey E, Senerth JM, Baumann MH, Young WS. Raphe serotonin neuron-specific oxytocin receptor knockout reduces aggression without affecting anxiety-like behavior in male mice only. *Genes, brain, and behavior*. 2015; 14(2):167–76. (b) Ferrari PF, van Erp AM, Tornatzky W, Miczek KA. Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *European journal of neuroscience*. 2003; 17(2):371–8. [PubMed: 12542674] (c) Nakazato T. Dual modes of extracellular serotonin changes in the rat ventral striatum modulate adaptation to a social stress environment, studied with wireless voltammetry. *Exp Brain Res*. 2013; 230(4):583–96. [PubMed: 22791197]
52. (a) Fernandez-Guasti A, Picazo O, Ferreira A. Blockade of the anxiolytic action of 8-OH-DPAT in lactating rats. *Pharmacol, Biochem Behav*. 1998; 59(1):45–50. [PubMed: 9443535] (b) Ferreira A, Picazo O, Uriarte N, Pereira M, Fernandez-Guasti A. Inhibitory effect of buspirone and diazepam, but not of 8-OH-DPAT, on maternal behavior and aggression. *Pharmacol, Biochem Behav*. 2000; 66(2):389–96. [PubMed: 10880695]
53. Rogers DC, Costall B, Domeney AM, Gerrard PA, Greener M, Kelly ME, Hagan JJ, Hunter AJ. Anxiolytic profile of ropinirole in the rat, mouse and common marmoset. *Psychopharmacology*. 2000; 151(1):91–7. [PubMed: 10958122]
54. Zarrindast MR, Shahed-Dirin K. Bromocriptine induces climbing behaviour: possible D-1 or D-2 dopamine receptor involvement. *Psychopharmacology*. 1990; 100(2):275–80. [PubMed: 2305016]
55. (a) Harkin A, Kelly JP, Frawley J, O'Donnell JM, Leonard BE. Test conditions influence the response to a drug challenge in rodents. *Pharmacol, Biochem Behav*. 2000; 65(3):389–98. [PubMed: 10683478] (b) Kelsey JE, Carlezon WA Jr. Prior experience with bromocriptine in the home cage attenuates locomotor sensitization in rats. *Behav Brain Res*. 2002; 134(1–2):1–8. [PubMed: 12191786]
56. (a) Clark D, Hjorth S, Carlsson A. Dopamine receptor agonists: mechanisms underlying autoreceptor selectivity. II. Theoretical considerations. *Journal of neural transmission*. 1985; 62(3–4):171–207. [PubMed: 2863323] (b) Richtand NM, Woods SC, Berger SP, Strakowski SM. D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev*. 2001; 25(5):427–43. [PubMed: 11566480]
57. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. *Trends Neurosci*. 2007; 30(5):194–202. [PubMed: 17408759]
58. (a) Barter R, Everson Pearse A. Mammalian enterochromaffin cells as the source of serotonin (5-hydroxytryptamine). *J Pathol Bacteriol*. 1955; 69(1):25–31. [PubMed: 13243168] (b) Lesch KP,

- Wolozin BL, Murphy DL, Riederer P. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. *J Neurochem*. 1993; 60(6):2319–22. [PubMed: 7684072]
59. Hammond KA. Adaptation of the maternal intestine during lactation. *Journal of mammary gland biology and neoplasia*. 1997; 2(3):243–52. [PubMed: 10882308]
 60. Lowry CA, Hale MW, Evans AK, Heerkens J, Staub DR, Gasser PJ, Shekhar A. Serotonergic systems, anxiety, and affective disorder: focus on the dorsomedial part of the dorsal raphe nucleus. *Ann N Y Acad Sci*. 2008; 1148:86–94. [PubMed: 19120094]
 61. Aouizerate B, Guehl D, Cuny E, Rougier A, Burbaud P, Tignol J, Bioulac B. Updated overview of the putative role of the serotonergic system in obsessive-compulsive disorder. *Neuropsychiatric disease and treatment*. 2005; 1(3):231–43. [PubMed: 18568072]
 62. (a) Ford JJ, Takahashi M, Yoshinaga K, Greep RO. Progesterone levels after inhibition of postpartum ovulation in rats. *Biol Reprod*. 1975; 12(5):584–9. [PubMed: 766839] (b) Hansen S, Södersten P, Eneroth P. Mechanisms regulating hormone release and the duration of dioestrus in the lactating rat. *J Endocrinol*. 1983; 99(2):173–180. [PubMed: 6655401]
 63. Angoa-Pérez M, Kane MJ, Sykes CE, Perrine SA, Church MW, Kuhn DM. Brain serotonin determines maternal behavior and offspring survival. *Genes, Brain and Behavior*. 2014; 13(7):579–591.
 64. (a) Bale TL, Davis AM, Auger AP, Dorsa DM, McCarthy MM. CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. *Journal of neuroscience: the official journal of the Society for Neuroscience*. 2001; 21(7):2546–52. (b) Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, Luo B, Schechter LE, Rizzo S, Rahman Z, Rosenzweig-Lipson S. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology*. 2006; 185(2):218–25. [PubMed: 16418825]
 65. (a) Caldwell HK. Neurobiology of sociability. *Advances in experimental medicine and biology*. 2012; 739:187–205. [PubMed: 22399403] (b) Rich ME, deCardenas EJ, Lee HJ, Caldwell HK. Impairments in the initiation of maternal behavior in oxytocin receptor knockout mice. *PLoS One*. 2014; 9(6):e98839. [PubMed: 24892749]
 66. (a) Gammie SC, Seasholtz AF, Stevenson SA. Deletion of corticotropin-releasing factor binding protein selectively impairs maternal, but not intermale aggression. *Neuroscience*. 2008; 157(3):502–12. [PubMed: 18929624] (b) Zuluaga MJ, Agrati D, Pereira M, Uriarte N, Fernandez-Guasti A, Ferreira A. Experimental anxiety in the black and white model in cycling, pregnant and lactating rats. *Physiol Behav*. 2005; 84(2):279–86. [PubMed: 15708779]
 67. Habr SF, Bernardi MM, Conceição IM, Freitas TA, Felício LF. Open field behavior and intra-nucleus accumbens dopamine release in vivo in virgin and lactating rats. *Psychol Neurosci*. 2011; 4:115–121.
 68. (a) Savignac HM, Dinan TG, Cryan JF. Resistance to early-life stress in mice: effects of genetic background and stress duration. *Front Behav Neurosci*. 2011; 5:13. [PubMed: 21519375] (b) Tarantino LM, Sullivan PF, Meltzer-Brody S. Using animal models to disentangle the role of genetic, epigenetic, and environmental influences on behavioral outcomes associated with maternal anxiety and depression. *Frontiers in psychiatry*. 2011; 2:44. [PubMed: 21811473]
 69. (a) Lukas M, Toth I, Reber SO, Slattery DA, Veenema AH, Neumann ID. The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology*. 2011; 36(11):2159–68. [PubMed: 21677650] (b) Slattery DA, Neumann ID. Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. *Neuropharmacology*. 2010; 58(1):56–61. [PubMed: 19589349]
 70. Neumann ID, Wigger A, Torner L, Holsboer F, Landgraf R. Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus. *J Neuroendocrinol*. 2000; 12(3):235–43. [PubMed: 10718919]
 71. (a) Peters S, Slattery DA, Uschold-Schmidt N, Reber SO, Neumann ID. Dose-dependent effects of chronic central infusion of oxytocin on anxiety, oxytocin receptor binding and stress-related parameters in mice. *Psychoneuroendocrinology*. 2014; 42:225–36. [PubMed: 24636519] (b) Huang H, Michetti C, Busnelli M, Manago F, Sannino S, Scheggia D, Giancardo L, Sona D, Murino V, Chini B, Scattoni ML, Papaleo F. Chronic and acute intranasal oxytocin produce

- divergent social effects in mice. *Neuropsychopharmacology*. 2014; 39(5):1102–14. [PubMed: 24190025]
72. Conti F, Sertic S, Reversi A, Chini B. Intracellular trafficking of the human oxytocin receptor: evidence of receptor recycling via a Rab4/Rab5 “short cycle. *American Journal of Physiology - Endocrinology And Metabolism*. 2009; 296(3):E532. [PubMed: 19126785]
 73. Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F, Engelmann M. V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. *Journal of neuroscience: the official journal of the Society for Neuroscience*. 1995; 15(6):4250–8.
 74. Sabihi S, Dong SM, Durosko NE, Leuner B. Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Front Behav Neurosci*. 2014; 8:258. [PubMed: 25147513]
 75. Stein DJ, Fineberg NA, Bienvenu OJ, Denys D, Lochner C, Nestadt G, Leckman JF, Rauch SL, Phillips KA. Should OCD be classified as an anxiety disorder in DSM-V? *Depression Anxiety*. 2010; 27(6):495–506. [PubMed: 20533366]
 76. Langlois F, Freeston MH, Ladouceur R. Differences and similarities between obsessive intrusive thoughts and worry in a non-clinical population: study 1. *Behaviour research and therapy*. 2000; 38(2):157–73. [PubMed: 10661001]
 77. Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*. 2010; 35(1):169–91. [PubMed: 19625997]
 78. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5. American Psychiatric Association; Washington, DC: 2015.
 79. Alboni S, van Dijk RM, Poggini S, Milior G, Perrotta M, Drenth T, Brunello N, Wolfer DP, Limatola C, Amrein I, Cirulli F, Maggi L, Branchi I. Fluoxetine effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. *Mol Psychiatry*. 2017; 22(4):552–561. [PubMed: 26645631]
 80. (a) Arletti R, Bertolini A. Oxytocin acts as an antidepressant in two animal models of depression. *Life Sci*. 1987; 41(14):1725–30. [PubMed: 3657379] (b) Amini-Khoei H, Mohammadi-Asl A, Amiri S, Hosseini MJ, Momeny M, Hassanipour M, Rastegar M, Haj-Mirzaian A, Mirzaian AH, Sanjarimoghaddam H, Mehr SE, Dehpour AR. Oxytocin mitigated the depressive-like behaviors of maternal separation stress through modulating mitochondrial function and neuroinflammation. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2017; 76:169–178. (c) Kim TK, Lee JE, Kim JE, Park JY, Choi J, Kim H, Lee EH, Han PL. G9a-Mediated Regulation of OXT and AVP Expression in the Basolateral Amygdala Mediates Stress-Induced Lasting Behavioral Depression and Its Reversal by Exercise. *Mol Neurobiol*. 2016; 53(5):2843–2856. [PubMed: 25863961]
 81. Lesse A, Rether K, Groger N, Braun K, Bock J. Chronic Postnatal Stress Induces Depressive-like Behavior in Male Mice and Programs second-Hit Stress-Induced Gene Expression Patterns of OxtR and Avpr1a in Adulthood. *Mol Neurobiol*. 2017; 54:4813. [PubMed: 27525673]
 82. Burbach, JPH., Young, LJ., Russell, JA. Oxytocin: synthesis, secretion, and reproductive functions. In: Knobil, E., Neill, JD., editors. *Physiology of Reproduction*. Vol. 2. Elsevier; St. Louis: 2006. p. 3055-3128.
 83. (a) Mori A, Ohashi S, Nakai M, Moriizumi T, Mitsumoto Y. Neural mechanisms underlying motor dysfunction as detected by the tail suspension test in MPTP-treated C57BL/6 mice. *Neurosci Res*. 2005; 51(3):265–74. [PubMed: 15710490] (b) Rana DG, Galani VJ. Dopamine mediated antidepressant effect of *Mucuna pruriens* seeds in various experimental models of depression. *Ayu*. 2014; 35(1):90–97. [PubMed: 25364207]
 84. (a) Sarkisova KY, Kulikov MA, Midzyanovskaya IS, Folomkina AA. Dopamine-dependent nature of depression-like behavior in WAG/Rij rats with genetic absence epilepsy. *Neurosci Behav Physiol*. 2008; 38(2):119–28. [PubMed: 18197376] (b) Porsolt RD. Animal model of depression. *Biomedicine*. 1979; 30(3):139–40. [PubMed: 573643] (c) Bouras N, Bridges PK. Bromocriptine in depression. *Curr Med Res Opin*. 1982; 8(3):150–3. [PubMed: 7128188] (d) Muscat R, Papp M, Willner P. Antidepressant-like effects of dopamine agonists in an animal model of depression. *Biol Psychiatry*. 1992; 31(9):937–46. [PubMed: 1353377]
 85. Lynch CB. Response to divergent selection for nesting behavior in *Mus musculus*. *Genetics*. 1980; 96(3):757–65. [PubMed: 7196362]

86. McClearn, GE., Wilson, JR., Meredith, JE. The Use of Isogenic and Heterogenic Mouse Stocks in Behavioral Research. Appleton-Century-Crofts; New York, NY: 1970.
87. (a) Speakman, JR., Johnson, MS. Relationships between Resting Metabolic Rate and Morphology in Lactating Mice: What Tissues are the Major Contributors to Resting Metabolism?. In: Heldmaier, G., Klingenspor, M., editors. Life in the Cold: Eleventh International Hibernation Symposium. Springer; Berlin, Heidelberg: 2000. p. 479-486. (b) Johnson M, Thomson S, Speakman J. Limits to sustained energy intake. *Journal of Experimental Biology*. 2001; 204(11): 1925–1935. [PubMed: 11441034]
88. Johnson AM, Loew DM, Vigouret JM. Stimulant properties of bromocriptine on central dopamine receptors in comparison to apomorphine, (+)-amphetamine and L-DOPA. *British journal of pharmacology*. 1976; 56(1):59–68. [PubMed: 943193]
89. Choe HK, Reed MD, Benavidez N, Montgomery D, Soares N, Yim YS, Choi GB. Oxytocin Mediates Entrainment of Sensory Stimuli to Social Cues of Opposing Valence. *Neuron*. 2015; 87(1):152–63. [PubMed: 26139372]
90. (a) Boccia ML, Goursaud AP, Bachevalier J, Anderson KD, Pedersen CA. Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. *Horm Behav*. 2007; 52(3):344–51. [PubMed: 17583705] (b) Durant S, Coulaud J, Homo-Delarche F. Bromocriptine-induced hyperglycemia in nonobese diabetic mice: kinetics and mechanisms of action. review of diabetic studies: RDS. 2007; 4(3):185–94. [PubMed: 18084676]
91. (a) Takeuchi H, Yatsugi S, Yamaguchi T. Effect of YM992, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT 2A receptor antagonistic activity, on a marble-burying behavior test as an obsessive-compulsive disorder model. *Jpn J Pharmacol*. 2002; 90(2):197–200. [PubMed: 12419892] (b) Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*. 2009; 204(2):361–73. [PubMed: 19189082] (c) Angoa-Perez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM. Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. *J Visualized Exp*. 2013; (82):50978.
92. (a) Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav Brain Res*. 1994; 61(1):59–64. [PubMed: 7913324] (b) Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol*. 2003; 463(1–3):3–33. [PubMed: 12600700]
93. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, Gould TD. The tail suspension test. *J Visualized Exp*. 2012; (59):e3769.
94. Chatterjee M, Jaiswal M, Palit G. Comparative evaluation of forced swim test and tail suspension test as models of negative symptom of schizophrenia in rodents. *ISRN Psychiatry*. 2012; 2012:595141. [PubMed: 23738205]
95. (a) Bult A, Lynch CB. Multiple selection responses in house mice bidirectionally selected for thermoregulatory nest-building behavior: crosses of replicate lines. *Behav Genet*. 1996; 26(4):439–46. [PubMed: 8771904] (b) Bult A, Lynch CB. Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. *Behav Genet*. 1997; 27(3):231–40. [PubMed: 9210794]

Figure 1.

Lactating compulsive-like female mice exhibited less compulsive-like marble burying and nest-building behaviors. Compulsive-like marble burying behavior represented as the total number of marbles 2/3rd buried among lactating, nonlactating, and nulliparous compulsive-like females administered with vehicle or fluoxetine in (a) week 1, (b) week 2, and (c) week 3. *** ($p < 0.001$) indicates significant differences between fluoxetine and vehicle groups; # ($p < 0.05$), ## ($p < 0.01$), and ### ($p < 0.001$) indicate significant differences between lactating and nonlactating females within each treatment group. + ($p < 0.05$) and +++ ($p < 0.001$) indicate significant differences between lactating and nulliparous females within each treatment group. Compulsive-like nest-building behavior represented as the total nesting score in grams among lactating, nonlactating, and nulliparous compulsive-like mice in (d) week 1, (e) week 2, and (f) week 3. ** ($p < 0.01$) and *** ($p < 0.001$) indicate significant differences between fluoxetine and vehicle groups. ### ($p < 0.001$) indicates significant differences between lactating and nonlactating females within each treatment group. +++ ($p < 0.001$) indicates significant differences between lactating and nulliparous females within each treatment group. All data is expressed as the mean $\pm$ SEM.

Figure 2.

The OXTR antagonist increased compulsive-like marble burying and nest-building in lactating compulsive-like female mice. (a) Compulsive-like marble burying behavior represented as the total number of marbles 2/3rd buried in lactating females 1 h after bromocriptine ($n = 11$), vehicle ($n = 10$), or L368-899 ($n = 10$) administration. *** ($p < 0.001$) and ### ($p < 0.001$) indicate significant differences between L368-899 and vehicle or bromocriptine groups, respectively. +++ ($p < 0.001$) indicates a significant difference between vehicle and bromocriptine groups. Compulsive-like nest-building behavior expressed as total nesting score in grams in lactating compulsive-like female mice (b) 1–5 h and (c) 0–24 h after administration with bromocriptine ($n = 11$), vehicle ($n = 11$), or L368-899 ($n = 12$). ** ($p < 0.01$) and *** ($p < 0.001$) represent significant differences between L368-899 and vehicle groups. ## ($p < 0.01$) and ### ($p < 0.001$) represent significant differences between L368-899 and bromocriptine groups. +++ ($p < 0.001$) represents significant differences between vehicle and bromocriptine groups. All data is expressed as the mean $\pm$ SEM.

Figure 3.

Lactating compulsive-like female mice had higher 5-HT immunoreactivity in the dorsal raphe nucleus. 5-HT immunoreactivity represented as 5-HT positive cell count in the DRN of naive lactating ($n = 5$), nonlactating ($n = 5$), and nulliparous ($n = 5$) compulsive-like female mice. ** ($p < 0.01$) represents the significant differences of the 5-HT positive cells in lactating females in comparison to both nonlactating and nulliparous females. All data is expressed as the mean $\pm$ SEM.

Figure 4.

Lactating compulsive-like female mice had higher 5-HT levels in serum. 5-HT levels in serum represented as ng/mL in naive lactating ($n = 6$), nonlactating ($n = 6$), and nulliparous ($n = 6$) compulsive-like female mice. * ($p < 0.05$) represents the significant differences of the 5-HT serum levels in lactating females in comparison to both nonlactating and nulliparous females. All data is expressed as the mean $\pm$ SEM.

Figure 5.

Lactating compulsive-like female mice exhibited more anxiety-like behavior and were not influenced by OXTR antagonist, while D2R agonist suppressed overall locomotion. Open field behavior represented as (a) anxiety-like total number of central entries among the lactating, nonlactating, and nulliparous females administered with vehicle or fluoxetine. (b) Locomotor activity as the total distance traveled in cm among lactating, nonlactating, and nulliparous female mice treated with vehicle or fluoxetine. * ($p < 0.05$) and ** ($p < 0.01$) indicate significant differences between vehicle and fluoxetine groups. # ($p < 0.01$) and ## ($p < 0.001$) indicate significant differences between lactating and nulliparous females within each treatment group. (c) Total number of central entries and (d) locomotor activity in lactating females 1 h after bromocriptine ($n = 10$), vehicle ($n = 11$), or L368-899 ($n = 11$) administration. ** ($p < 0.01$) and *** ($p < 0.001$) indicate significant differences between L368-899 and bromocriptine groups. ## ($p < 0.01$) indicates significant differences between vehicle and bromocriptine groups. All data is expressed as the mean $\pm$ SEM.

Figure 6.

Depression-like tail suspension behavior in lactating compulsive-like female mice was increased by the OXTR antagonist. The depression-like tail suspension test is represented as total immobility time. (a) Lactating, nonlactating, and nulliparous females administered with vehicle or fluoxetine. * ($p < 0.05$) represents a significant difference between nulliparous and nonlactating females in the fluoxetine group. ### ($p < 0.001$) represents a significant difference between nulliparous and lactating females in the fluoxetine group. (b) Lactating females administered with bromocriptine ($n = 10$), vehicle ($n = 10$), or L368-899 ($n = 10$). *** ($p < 0.001$) represents a significant difference between L368-899 and vehicle groups. ### ($p < 0.001$) represents a significant difference between L368-899 and bromocriptine groups. All data is expressed as the mean $\pm$ SEM.