Genetic networks of the oxytocin system in the human brain: A gene expression and large-scale fMRI meta-analysis study

Daniel S. Quintana [a *], Jaroslav Rokicki [a,b], Dennis van der Meer [a], Dag Alnæs [a], Tobias Kaufmann [a], Aldo Córdova Palomera [a], Ingrid Dieset [a], Ole A. Andreassen [a], and Lars T. Westlye [a,b]

[a] NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, University of Oslo, and Oslo University Hospital, Oslo, Norway

[b] Department of Psychology, University of Oslo, Oslo, Norway

* **Corresponding author:** Daniel S. Quintana, NORMENT, KG Jebsen Centre for Psychosis Research, Building 49, Oslo University Hospital, Ullevål, Kirkeveien 166, PO Box 4956 Nydalen, N- 0424 Oslo, Norway, Ph: +47 23 02 73 50, fax: +47 23 02 73 33, email: daniel.quintana@medisin.uio.no

Abstract

Oxytocin is a neuropeptide involved in animal and human reproductive and social behaviour, with potential implications for a range of psychiatric disorders. However, the therapeutic potential of oxytocin in mental health care suggested by animal research has not been successfully translated into clinical practice, partly due to a poor understanding of the expression and distribution of the oxytocin signaling pathway in the human brain, and its complex interactions with other biological systems. Among the genes involved in the oxytocin signaling pathway, three genes have been frequently implicated in human social behavior: *OXT* (structural gene for oxytocin), *OXTR* (oxytocin receptor), and *CD38* (central oxytocin secretion). We characterized the distribution of the *OXT*, *OXTR*, and *CD38* mRNA across the brain, identified putative gene pathway interactions by comparing gene expression patterns across 29131 genes, and assessed associations between gene expression patterns and cognitive states via large-scale fMRI meta-analysis. In line with the animal literature, oxytocin pathway gene expression was enriched in central, temporal, and olfactory regions. Across the brain, there was high co-expression of the oxytocin pathway genes with both dopaminergic (*DRD2*) and muscarinic acetylcholine (*CHRM4*) genes, reflecting an anatomical basis for critical gene pathway interactions. Finally, fMRI meta-analysis revealed that oxytocin pathway maps correspond with motivation and emotion processing, demonstrating the value of probing gene expression maps to identify brain functional targets for future pharmacological trials.

Keywords: Oxytocin, oxytocin receptor, mRNA, brain

Oxytocin is an evolutionarily conserved neuropeptide implicated in an array of social and reproductive behaviors, and its role in the pathophysiology of complex psychological traits and as a putative therapeutic target in mental health conditions has attracted considerable attention (1). Oxytocin is mostly synthesized in neurons in the supraoptic nucleus and the paraventricular nucleus and released both systemically and centrally. Human research has shown beneficial effects of intranasal oxytocin on performance on tests assessing social cognition (2), gaze to the eye region (3), and the retrieval of social cues (4). Moreover, single nucleotide polymorphisms in oxytocin pathway genes have been associated with social behavior and psychiatric disorders (5). Emerging evidence also points to the oxytocin system's role in energy metabolism (6), which may contribute to the increased risk of metabolic dysfunction in mental illness (7).

Despite some encouraging clinical trial results (e.g., 8, 9), unsuccessful trials and failures to replicate early human research have curbed the initial expectations for intranasal oxytocin as a therapeutic target (10). These translation failures have been partly attributed to a poor understanding of human central *OXTR* expression (11). As oxytocin can influence brain circuitry by binding to central oxytocin receptors, the anatomical distribution and density of oxytocin receptors in the brain can denote which neural regions and circuits would be most responsive to oxytocin. Given that *OXTR* mRNA distribution corresponds with *OXTR* locations (12, 13), the distribution of *OXTR* mRNA across the brain can therefore provide a map of central oxytocin binding.

Early animal work using histochemistry and immunohistochemistry revealed high concentrations of *OXTR* mRNA in the hypothalamus, amygdala, olfactory bulb, ventral pallidum, and the dorsal vagal nucleus (14, 15). Experimentally increasing

(16) or decreasing (17) *OXTR* expression in the prairie vole nucleus accumbens also modulated partner preference behavior. While central *OXTR* mRNA localization in the rodent brain has been well-described (18), the anatomical distribution across the human brain is poorly characterized in general, as investigations have tended to sample very few brain regions (19, 20).

In addition to the anatomical distribution of *OXTR* mRNA, characterizing its interactions with other elements of the oxytocin signaling pathway and biological systems beyond this pathway is critical for determining oxytocin's behavioral and functional relevance. Along with *OXTR*, *CD38* and oxytocin-neurophysin I (*OXT*) genes in the oxytocin signaling pathway have been implicated in human social behavior (5). Specifically, *CD38* is involved in central oxytocin secretion (21), and *OXT* encodes the oxytocin prepropeptide containing the nonapeptide oxytocin and the carrier protein neurophysin-I (22). Recent research (23) has reported whole-brain mRNA patterns of two out of three gene constituents of the oxytocin pathway system: *OXTR* and *OXT*. This analysis revealed increased *OXT* gene expression in the paraventricular nucleus of the hypothalamus, the lateral hypothalamic area, and the supraoptic nucleus, along with co-localization of *OXT* and *OXTR* mRNA with μ-opioid and κ-opioid receptor mRNA (23).

Evidence suggests that the oxytocin system also interacts with other neurotransmitter circuits, such as the dopaminergic (24) and muscarinic cholinergic (19) circuits, with possible implication for mental health. For instance, the dopamine D2-receptor subtype (*DRD2*), has been implicated with dysfunctional motivational processing in psychiatric illness (25) and pair bonding in animal models (26, 27). Moreover, the cholinergic muscarinic M4 receptor (*CHRM4*) has been associated with schizophrenia (28) and implicated in cognitive flexibility (29), social impairment

(30), and dopamine release (31). Finally, oxytocin can also bind to *AVPR1A* receptors (32), which has also been linked to social functioning (33, 34). However, the mRNA co-expression of these systems, and potentially others, with the oxytocin system are not well characterized.

While research has identified brain regions associated with high oxytocin pathway gene expression (19, 20, 23), inferring specific mental states to a single brain region can be problematic due to poor specificity. For instance, commonly observed increases in medial and lateral frontal region activity during emotion and pain processing seem to be better explained by more general sustained attention processes (35). The Neurosynth framework provides a tool (35) which can be used to decode which mental states are associated with the whole-brain oxytocin pathway network. Via the reverse inference of data from over 11,000 fMRI studies, this approach can reveal the probability of specific mental state patterns for a given whole-brain oxytocin pathway map with high specificity. Establishing the specific functional role of central oxytocin pathway network will provide a deeper understanding of the central human oxytocin system and aid translational research by more accurately identifying mental state targets for oxytocin treatment.

By leveraging the Allen Human Brain Atlas, which offers a uniquely comprehensive gene expression survey from six neurotypical adult human brains, we first characterize the anatomical distribution of the central oxytocin network by assessing mRNA expression of *OXT*, *OXTR* and *CD38.* Second, we explore putative gene pathway interactions by identifying genes with overlapping anatomical distributions with our target genes. Third, we decode the functional relevance of the oxytocin gene expression network using quantitative reverse inference via large-scale fMRI meta-analysis.

Materials and Methods

Post-mortem brain samples. mRNA distribution data was collected from the Allen Human Brain Atlas (http://human.brain-map.org/). Three donors were Caucasian males, one donor was a Hispanic female and two donors were African-American males. Mean donor age was 42.5 (S.D.=11.2) years. Data was collected on average 22.3 (S.D.=4.5) hours after demise (See Table S1 for details). Each brain was sampled in 363-946 distinct locations, either in the left hemisphere only (4 donor brains), or over both hemispheres (2 donor brains) using a custom Agilent 8 × 60K cDNA array chip. More details on procedures are available at http://help.brain-map.org/display/humanbrain/Documentation.

mRNA expression maps. The Allen Human Brain Atlas provides open-access expression profiles for oxytocin pathway genes, reporting $\log_2$ transformed measures of the amount of hybridized RNA found through microarray analysis. While this is a comprehensive dataset, expression data is missing from some regions or not available from all donor samples. To assess accurate cognitive correlates of gene expression using meta-analysis via the Neurosynth tool, voxel-by-voxel mRNA maps are necessary. To create these voxel-by-voxel volumetric expression maps, we first marked all the sample locations and expression values in native image space. To interpolate missing voxels, we labeled brain borders with the sample expression value that had the closest distance to a given border point (Fig. S1A). Next, we linearly interpolated the space between scattered points by dividing that space into simplices based on Delaunay triangulation (Fig S1B), then linearly interpolated each simplex with values to yield a completed map (Fig. S1C). All maps were computed in

Matlab 2015a (The Mathworks Inc., Natick, MA, USA). Next, we registered all the individual brains to the MNI152 (Montreal Neurological Institute) template using ANTs (Advanced Normalization Tools; 36). To extract region specific statistics, we used regions extracted based on the Automated Anatomical Label (AAL) atlas.

Data extraction. Data for the oxytocin pathway was extracted from the Allen Human Brain Atlas for 3 different mRNA probes that have been associated with social behavior: *OXTR*, *CD38*, and *OXT* (5). Three other mRNA probes, which were hypothesized to co-express with oxytocin pathway mRNA, were also extracted *a priori*: *DRD2* (24-27), *CRHM4 (28-31)*, and *AVPR1A* (33, 34). Since more than one probe for each mRNA was available, we selected the probe with the highest signal-to-noise ratio (mean/standard deviation), which represented the probe with least amount of spatial variability among donors (see Table S2).

Central gene expression. Statistical analysis was performed with R statistical package (version 3.3.2). Across the brain, gene expression for the right hemisphere (n = 2) was highly correlated with the corresponding left hemisphere (n = 6) gene expression values for all oxytocin pathway genes (*OXTR*, *OXT*, *CD38*; all p values < .00001, all r values ≥ 0.9; Fig S2). Thus, the left hemisphere was used for analysis given its higher sample size. One-sample t-tests (two-tailed) were conducted to assess which of the 54 left hemisphere regions from six donor samples expressed mRNA to a significantly greater or lesser degree compared to average expression across the brain. To correct for multiple tests (54 in total), reported p-values were adjusted using a false discovery rate (FDR) threshold. Cohen's d values for one-sample t-tests were calculated to yield a measure of effect size. Pearson correlation

coefficients were also calculated to assess the relationship of mRNA for 54 brain regions for *OXTR, OXT, CD38, DRD2, CHRM4*, and *AVPR1A*. P-values for correlational analyses were adjusted using a FDR threshold to correct for multiple tests (15 in total). Finally, we performed exploratory correlational analysis computing relationship between brain sample set of each subject and averaging over six individuals of all available mRNA probes (n = 58691) against three oxytocin pathway mRNA probes to assess putative gene-gene interactions based on spatial similarities in gene expression.

Cognitive term correlates. To identify the cognitive correlates of mRNA whole-brain expression maps, we performed quantitative reverse inference via large-scale meta-analysis of functional neuroimaging data using the NeuroSynth framework (35). As NeuroSynth favors the input of whole-brain voxel maps, we created a composite brain map representing an average of 6 individuals on the left hemisphere and average of 2 individuals on right hemisphere. Using naïve Bayes classification, NeuroSynth estimated the likelihood that mRNA maps were associated with mental state terms based on text-mining a corpus of 11,406 neuroimaging studies. The top 5 strongest non-anatomical correlations (Pearson's *r*) were identified for *OXTR*, *CD38*, and *OXT* mRNA.

Data and analysis script availability. mRNA expression data is available from Allen Human Brain Atlas (http://human.brain-map.org/). The Matlab script for producing the brain region-specific data, the resulting dataset, and the R script used for statistical analysis is available at https://osf.io/jp6zs/.

Results

Central gene expression. Compared to average expression across the brain, high expression of *OXTR* mRNA levels were observed in the olfactory bulbs (p = .0007, d = 6.9), caudate (p = .0009, d = 5.7), putamen (p = .002, d = 3.8), pallidum (p = .006, d = 3), amygdala (p = .009, d = 2.6), rectus (p = .02, d = 2.2), parahippocampal region (p = .02, d = 2.1), hippocampus (p =.02, d = 2), and anterior cingulate (p = .03, d = 1.9; Fig 1A; Fig 2A; Supplemental Dataset S1 contains all test statistics). There was lower *OXTR* expression in the cerebral crus I (p = .009, d = 2.5), cerebral crus II (p = .001, d = 4.9), Cerebellum 7b (p = .002, d = 3.7), and Cerebellum 8 (p = .001, d = 4.5). High expression of *CD38* mRNA was observed in the caudate (p = .003, d = 5.7), pallidum (p = .003, d = 3) olfactory bulbs (p = .01, d = 6.9), putamen (p = .01, d = 3.8), thalamus (p = .02, d = 1.5), and cingulate anterior (p = .02, d = 1.9; Fig 1B; Fig 2A). There was lower *CD38* expression in the middle temporal pole (p = .02, d = .7) and Cerebellum 7b (p = .03, d = 3.7).

High expression of *CHRM4* mRNA was observed in the thalamus (p = .0002, d = 9.6), pallidum (p = .02, d = 2.8), and olfactory bulbs (p = .02, d = 2.7; Fig S3C). There was lower *CHRM4* expression in the cerebral crus I (p = .04, d = 2.2), cerebral crus II (p = .001, d = 6.8) and Cerebellum 7b (p = .002, d = 4.8) High expression of *DRD2* mRNA was observed in the putamen (p < .0001, d = 13.1), caudate (p < .0001, d = 10.3), thalamus (p = .0002, d = 7.2), pallidum (p = .004, d = 3.3), olfactory bulbs (p = .009, d = 2.4), insula (p = .01, d = 2.4), amygdala (p = .01, d = 2.1), and hippocampus (p = .01, d = 2.1; Fig S3B). There was lower *DRD2* expression in several areas in the cerebellum (cerebellum 7b, cerebellum 8, cerebellum 9, cerebral crus I, cerebral crus II), frontal region (orbital superior frontal gyrus, precentral area, dorsolateral superior frontal gyrus, orbital superior frontal gyrus, triangular inferior

frontal gyrus, supplementary motor area, medial superior frontal gyrus, medial orbital superior frontal gyrus), Occipital lobe (calcarine, cuneus), and Parietal lobe (postcentral gyrus, superior parietal gyrus, precuneus, paracentral lobule; all FDR corrected *p*-values < .05; Fig S3B; Dataset S1). There were no brain areas with significantly increased *OXT* (Fig. 1C; Fig 3A) or *AVPR1A* (Fig. S3A) expression which survived FDR correction (Table S1), however there was a considerable increase of *OXT* mRNA expression in the olfactory region (p = .13, d = 2.5).

Analysis revealed statistically significant relationships in gene expression over 54 brain regions (FDR corrected *p*-values) between all six genes (Fig. 3). Gene expressions were positively correlated for all gene pairs, except for the relationship in gene expression between AVPR1A and CHRM4, which was negatively related. Exploratory correlation analyses with all available 58692 gene probes in the Allen Human Brain Atlas and each of the three oxytocin pathway gene probes are summarized in Table 1 (full details in Table S3), with the top 10 strongest positive and top 10 strongest negative correlations. Histograms of the correlations between the three oxytocin pathway mRNA probes and all other available mRNA probes are presented in Figure 4.

Cognitive correlates. Decoding mental states meta-analytically from mRNA maps via quantitative reverse inference revealed that *OXTR* and *CD38* mRNA expression maps were most highly correlated with functional imaging maps with motivation/dopaminergic topics such as “reward”, “anticipation”, and “dopaminergic”, and emotion-processing topics (Figure 2B, Table S4). Examination of these patterns revealed that the *CD38* and *OXTR* mRNA maps were most associated with motivation-related topics whereas the *OXT* mRNA map was most associated with

emotion processing-related topics. Other topic categories with strong associations included pain and learning.

Discussion

The anatomical distribution of gene expression in the brain is heterogeneous and highly coordinated (37). Whereas dynamic alterations in gene expression are abundant in response to environmental demands, and are critically involved in range of cognitive functions, learning and diseases (38, 39), the basic organization likely partly reflects the evolutionary conserved modular layout of the brain (40). Indeed, gene-gene co-expression patterns form specific genetic signatures in the brain, representing distinct tissues, biological systems and associations with various brain disorders (37), and likely reflect the potential of complex and differential gene-gene interactions with implications for brain disorders and mental health. Here, we leverage the unique human brain mRNA expression library from Allen Brain Atlas to show that mRNA reflecting specific genes in the oxytocin pathway are highly expressed in central and temporal brain structures, along with the olfactory region. We also show reduced expression of *OXTR* and *CD38* in the cerebellum, consistent with prior animal research (41). Oxytocin pathway genes showed considerable co-expression of *DRD2* and *CHRM4* mRNA, providing evidence for putative interactions between dopaminergic and cholinergic systems with the central oxytocin pathways. Exploratory analysis across 58692 RNA probes revealed strong positive spatial correlations between oxytocin pathway mRNA expression and mRNA associated with metabolic functions (*GLUD1*, *GLUD2*, *C12orf39*, *SIM1*), and strong negative correlations with *GABRD, CAMKK2, PAK7, KCNJ3, KCNK1,* and *RTN4R*, which have been associated with autism (42-44) and schizophrenia (45-49). Lastly,

quantitative reverse inference via meta-analysis of a corpus of 11,406 neuroimaging studies revealed that the distribution of the central genetic oxytocin pathway is most strongly associated with terms related to motivation and emotion, supporting the gene-system interactions suggested by the gene expression patterns. Together, these findings are consistent with a role of oxytocin in the processing of social stimuli and corroborate emerging evidence for interactions between the oxytocin, cholinergic (19), and dopaminergic systems (24), and for oxytocin's role in metabolic regulation (7).

Compelling animal evidence suggests that direct central oxytocin administration influences social behavior and cognition via action on central oxytocin receptors (50). In these animals, oxytocin receptors are located in regions that are crucial for social behavior and the processing of social cues (18). Our data reveals striking similarities with central oxytocin pathway mRNA distribution observed in rodents (14, 15) and non-human primates (51). Given these between-species similarities in oxytocin receptor distribution, we suggest that the lack of robust effects of oxytocin administration in human clinical studies are most likely due to issues surrounding intranasal oxytocin administration methods which may constrain the potential availability of central oxytocin for oxytocin receptor binding (52, 53).

Along with the oxytocin pathway expression, we also investigated co-expression with dopaminergic and cholinergic receptor networks in the brain. Our results indicate that brain regions with increased expression of oxytocin pathway mRNA also have increased expression of the dopamine D2 receptor. Moreover, reverse inference of our generated oxytocin pathway mRNA maps on cognition via large-scale fMRI meta-analysis demonstrate that these maps closely correspond with brain regions underpinning for motivational processes. Altogether, our data

provides mRNA evidence that oxytocin exerts its effects synergistically with the dopaminergic network (24). Indeed, evidence points to a bidirectional relationship between central oxytocin and dopaminergic functioning. For instance, the oxytocin and dopaminergic systems work in concert to promote rodent maternal behaviors (54) and central D2 receptors have been shown to modulate social functioning in prairie voles (55, 56). There is also evidence of direct dopaminergic fibers to the supraoptic nucleus and the paraventricular nucleus (57), consistent with demonstrations that dopamine may stimulate oxytocin release in the hypothalamus (58, 59). This is also consistent with reported deficits in dopaminergic signaling in schizophrenia (60) and autism (61). The muscarinic cholinergic system may also work with the oxytocin system to boost attention (62) and to assist with the release of oxytocin (63). Moreover, the muscarinic cholinergic system is also thought to play a role in the cognitive dysfunction observed in schizophrenia (64).

We performed an exploratory correlational analysis between oxytocin pathway mRNA and 58692 other mRNA probes. There are several relationships worth noting in the context of psychiatric illness. First, *OXTR* is highly co-expressed with Neurotensin receptor gene (*NTSR2*), which has been found to regulate ethanol consumption in mice (65), and Glutamate dehydrogenase 1 and 2 (*GLUD1*, *GLUD2*), which are involved in glutamate recycling and insulin secretion. Second, *CD38* is highly co-expressed with *NTSR2*, *GLUD1*, and Spexin (*C12orf39*), with the latter associated with weight regulation (66). Third, *OXT* is highly co-expressed with *AVP*, and Single-minded homolog 1 (*SIM1*), which may regulate feeding behavior (67). Altogether, these results are consistent with emerging evidence that the oxytocin system may play a role in the metabolic dysfunction often observed in severe mental illnesses (7). Strikingly, the strongest negative correlation across 58692 mRNA

probes for both *OXTR* and *OXT* were found for the gamma-aminobutyric acid type A receptor delta subunit (*GABRD*) gene, which has been associated with autism (42, 43), mood disorders (68), and schizophrenia (69). Another strong negative correlation was *CAMKK2*, which has been identified as a schizophrenia susceptibility gene (45).

Our data revealed high expression of oxytocin pathway mRNA in the olfactory region, which is consistent with animal research (e.g., 70). Indeed, social olfactory deficits in mice without the oxytocin gene (71) are rescued with injection of oxytocin into rat olfactory bulbs (72). Increased mRNA in the human olfactory region may be vestigial, as olfaction is not as important for human conspecific identification compared to most other mammals due to species specialization, however, intranasal oxytocin has been shown to improve olfactory detection performance in schizophrenia (73), and intranasal oxytocin is thought to enter the brain via the olfactory bulbs (74).

There are three important limitations worth noting. First, given the nature of human tissue collection for mRNA studies, the sample size was small. However, the relatively small amount of measurement error for the mRNA probes is consistent with high precision. Second, there was only 1 female in the sample, which prohibited an examination of sex differences of mRNA distribution. However, evidence suggests there is limited sex differences in *OXTR* expression in humans (75). Third, our reverse inference approach is better suited to identify broad domains, such as “emotion”, rather than more specific domains.

Comprehensive clinical trials need to demonstrate engagement of drug targets, such as *OXTR* occupancy reflected by regional brain activity changes (11). Without precise targets, it is unclear whether null effects are due to an inefficacious

drug or misidentified drug targets. By identifying accurate oxytocin pathway targets in the human brain, our study provides an oxytocin treatment target map that can facilitate efforts to better understand treatment efficacy and the most effective treatment dose. Using this map, research can move towards studying dose-dependent effects on neural targets rich in oxytocin receptors (e.g., 76).

Analysis of data from the Allen Brain Atlas database provides a unique opportunity to map the central oxytocin system and explore co-expression with other systems. Altogether, these results provide a proof-of-principle demonstration of corresponding cognitive and gene expression patterns of a neuropeptide pathway involved in complex human and animal behaviors, and identify brain regions and cognitive processing targets for future oxytocin trials (11).

Acknowledgements. This research was supported by an Excellence Grant from the Novo Nordisk Foundation (NNF16OC0019856 to D.S.Q), the Research Council of Norway (223273, 249795), KG Jebsen Stiftelsen and the South-Eastern Norway Regional Health Authority (2014097, 2015073).

References

1. Insel TR (2010) The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron* 65(6):768-779.

2. Domes G, Heinrichs M, Michel A, Berger C, & Herpertz SC (2007) Oxytocin Improves “Mind-Reading” in Humans. *Biol. Psychiatry* 61(6):731-733.

3. Guastella AJ, Mitchell PB, & Dadds MR (2008) Oxytocin increases gaze to the eye region of human faces. *Biol. Psychiatry* 63(1):3-5.

4. Marsh AA, Henry HY, Pine DS, & Blair R (2010) Oxytocin improves specific recognition of positive facial expressions. *Psychopharmacology* 209(3):225-232.

5. Feldman R, Monakhov M, Pratt M, & Ebstein RP (2016) Oxytocin pathway genes: evolutionary ancient system impacting on human affiliation, sociality, and psychopathology. *Biol. Psychiatry* 79(3):174-184.

6. Blevins JE & Baskin DG (2015) Translational and therapeutic potential of oxytocin as an anti-obesity strategy: Insights from rodents, nonhuman primates and humans. *Physiol. Behav.* 152:438-449.

7. Quintana DS, Dieset I, Elvsåshagen T, Westlye LT, & Andreassen OA (2017) Oxytocin system dysfunction as a common mechanism underlying metabolic syndrome and psychiatric symptoms in schizophrenia and bipolar disorders. *Front. Neuroendocrinol.* 45:1-10.

8. Davis MC*, et al.* (2013) Effects of single dose intranasal oxytocin on social cognition in schizophrenia. *Schizophr. Res.* 147(2):393-397.

9. Yatawara C, Einfeld S, Hickie I, Davenport T, & Guastella A (2015) The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. *Mol. Psychiatry*.

10. Alvares GA, Quintana DS, & Whitehouse AJ (2016) Beyond the hype and hope: critical considerations for intranasal oxytocin research in autism spectrum disorder.

11. Insel TR (2016) Translating oxytocin neuroscience to the clinic: A National Institute of Mental Health perspective. *Biol. Psychiatry* 79(3):153–154.

12. Young LJ, Muns S, Wang Z, & Insel TR (1997) Changes in oxytocin receptor mRNA in rat brain during pregnancy and the effects of estrogen and interleukin‐6. *J. Neuroendocrinol.* 9(11):859-865.

13. Vaccari C, Lolait SJ, & Ostrowski NL (1998) Comparative Distribution of Vasopressin V1b and Oxytocin Receptor Messenger Ribonucleic Acids in Brain. *Endocrinology* 139(12):5015-5033.

14. Yoshimura R*, et al.* (1993) Localization of oxytocin receptor messenger ribonucleic acid in the rat brain. *Endocrinology* 133(3):1239-1246.

15. Adan R*, et al.* (1995) Rat oxytocin receptor in brain, pituitary, mammary gland, and uterus: partial sequence and immunocytochemical localization. *Endocrinology* 136(9):4022-4028.

16. Keebaugh AC & Young LJ (2011) Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. *Horm. Behav.* 60(5):498-504.

17. Keebaugh AC, Barrett CE, Laprairie JL, Jenkins JJ, & Young LJ (2015) RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. *Soc. Neurosci.* 10(5):561-570.

18. Insel TR & Shapiro LE (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl. Acad. Sci.* 89(13):5981-5985.

19. Loup F, Tribollet E, Dubois-Dauphin M, & Dreifuss J (1991) Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. *Brain Res.* 555(2):220-232.

20. Uhrig S*, et al.* (2016) Reduced oxytocin receptor gene expression and binding sites in different brain regions in schizophrenia: A post-mortem study. *Schizophr. Res.*

21. Jin D*, et al.* (2007) CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* 446(7131):41-45.

22. Sausville E, Carney D, & Battey J (1985) The human vasopressin gene is linked to the oxytocin gene and is selectively expressed in a cultured lung cancer cell line. *J. Biol. Chem.* 260(18):10236-10241.

23. Dal Monte O*, et al.* (2017) Oxytocin under opioid antagonism leads to supralinear enhancement of social attention. *Proc. Natl. Acad. Sci.* 114(20):5247-5252.

24. Love TM (2014) Oxytocin, motivation and the role of dopamine. *Pharmacology Biochemistry and Behavior* 119:49-60.

25. Noble EP (2003) D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 116(1):103-125.

26. Aragona BJ*, et al.* (2006) Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat. Neurosci.* 9(1):133-139.

27. Smeltzer MD, Curtis JT, Aragona BJ, & Wang Z (2006) Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. *Neurosci. Lett.* 394(2):146-151.

28. Scarr E, Sundram S, Keriakous D, & Dean B (2007) Altered hippocampal muscarinic M4, but not M1, receptor expression from subjects with schizophrenia. *Biol. Psychiatry* 61(10):1161-1170.

29. Okada K*, et al.* (2014) Enhanced flexibility of place discrimination learning by targeting striatal cholinergic interneurons. *Nat. Commun.* 5.

30. Koshimizu H, Leiter LM, & Miyakawa T (2012) M 4 muscarinic receptor knockout mice display abnormal social behavior and decreased prepulse inhibition. *Mol. Brain* 5(1):10.

31. Grilli M*, et al.* (2008) Release‐enhancing pre‐synaptic muscarinic and nicotinic receptors co‐exist and interact on dopaminergic nerve endings of rat nucleus accumbens. *J. Neurochem.* 105(6):2205-2213.

32. Cho MM, DeVries AC, Williams JR, & Carter CS (1999) The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). *Behav. Neurosci.* 113(5):1071.

33. Bielsky IF, Hu S-B, Szegda KL, Westphal H, & Young LJ (2004) Profound impairment in social recognition and reduction in anxiety-like

behavior in vasopressin V1a receptor knockout mice. *Neuropsychopharmacology* 29(3):483.

34. Caldwell HK, Lee H-J, Macbeth AH, & Young WS (2008) Vasopressin: behavioral roles of an "original" neuropeptide. *Prog. Neurobiol.* 84(1):1-24.

35. Yarkoni T, Poldrack RA, Nichols TE, Van Essen DC, & Wager TD (2011) Large-scale automated synthesis of human functional neuroimaging data. *Nat. Methods* 8(8):665-670.

36. Avants BB*, et al.* (2011) A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* 54(3):2033-2044.

37. Hawrylycz M*, et al.* (2015) Canonical genetic signatures of the adult human brain. *Nat. Neurosci.* 18(12):1832-1844.

38. Jaenisch R & Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* 33:245-254.

39. Rampon C*, et al.* (2000) Effects of environmental enrichment on gene expression in the brain. *Proc. Natl. Acad. Sci.* 97(23):12880-12884.

40. Richiardi J*, et al.* (2015) Correlated gene expression supports synchronous activity in brain networks. *Science* 348(6240):1241-1244.

41. Hashimoto H, Fukui K, Noto T, Nakakima T, & Kato N (1985) Distribution of vasopressin and oxytocin in rat brain. *Endocrinol. Jpn.* 32(1):89-97.

42. Sesarini CV*, et al.* (2015) Association between GABA (A) receptor subunit polymorphisms and autism spectrum disorder (ASD). *Psychiatry Res.* 229(1):580-582.

43. Sesarini CV*, et al.* (2014) Evidence for interaction between markers in GABA (A) receptor subunit genes in an Argentinean autism spectrum disorder population. *Autism Res.* 7(1):162-166.

44. Hussman JP*, et al.* (2011) A noise-reduction GWAS analysis implicates altered regulation of neurite outgrowth and guidance in autism. *Molecular autism* 2(1):1.

45. Luo X*, et al.* (2014) Convergent lines of evidence support CAMKK2 as a schizophrenia susceptibility gene. *Mol. Psychiatry* 19(7):774-783.

46. Morris DW*, et al.* (2014) An inherited duplication at the gene p21 Protein-Activated Kinase 7 (PAK7) is a risk factor for psychosis. *Hum. Mol. Genet.* 23(12):3316-3326.

47. Budel S*, et al.* (2008) Genetic variants of Nogo-66 receptor with possible association to schizophrenia block myelin inhibition of axon growth. *J. Neurosci.* 28(49):13161-13172.

48. Yamada K*, et al.* (2012) Association study of the KCNJ3 gene as a susceptibility candidate for schizophrenia in the Chinese population. *Hum. Genet.* 131(3):443-451.

49. Mistry M, Gillis J, & Pavlidis P (2013) Genome-wide expression profiling of schizophrenia using a large combined cohort. *Mol. Psychiatry* 18(2):215-225.

50. Ross HE & Young LJ (2009) Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front. Neuroendocrinol.* 30(4):534-547.

51. Sofroniew M, Weindl A, Schrell U, & Wetzstein R (1980) Immunohistochemistry of vasopressin, oxytocin and neurophysin in the

hypothalamus and extrahypothalamic regions of the human and primate brain. *Acta Histochem. Suppl.* 24:79-95.

52. Guastella AJ*, et al.* (2013) Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology* 38(5):612-625.

53. Quintana DS, Guastella AJ, Westlye LT, & Andreassen OA (2016) The promise and pitfalls of intranasally administering psychopharmacological agents for the treatment of psychiatric disorders. *Mol. Psychiatry* 21(1):29-38.

54. Pedersen CA, Caldwell JD, Walker C, Ayers G, & Mason GA (1994) Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behav. Neurosci.* 108(6):1163.

55. Gingrich B, Liu Y, Cascio C, Wang Z, & Insel TR (2000) Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (Microtus ochrogaster). *Behav. Neurosci.* 114(1):173.

56. Wang Z*, et al.* (1999) Dopamine D2 receptor-mediated regulation of partner preferences in female prairie voles (Microtus ochrogaster): a mechanism for pair bonding? *Behav. Neurosci.* 113(3):602.

57. Lindvall O, Björklund A, & Skagerberg G (1984) Selective histochemical demonstration of dopamine terminal systems in rat di-and telecephalon: new evidence for dopaminergic innervation of hypothalamic neurosecretory nuclei. *Brain Res.* 306(1):19-30.

58. Mason W (1983) Excitation by dopamine of putative oxytocinergic neurones in the rat supraoptic nucleus in vitro: evidence for two classes of continuously firing neurones. *Brain Res.* 267(1):113-121.

59. Moos F & Richard P (1982) Excitatory effect of dopamine on oxytocin and vasopressin reflex releases in the rat. *Brain Res.* 241(2):249-260.

60. Stopper CM & Floresco SB (2015) Dopaminergic circuitry and risk/reward decision making: implications for schizophrenia. *Schizophr. Bull.* 41(1):9-14.

61. Hettinger JA*, et al.* (2012) DRD2 and PPP1R1B (DARPP-32) polymorphisms independently confer increased risk for autism spectrum disorders and additively predict affected status in male-only affected sib-pair families. *Behav. Brain Funct.* 8(1):19.

62. Demeter E & Sarter M (2013) Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology* 64:294-304.

63. Jankowski M*, et al.* (2000) Oxytocin and its receptors are synthesized in the rat vasculature. *Proc. Natl. Acad. Sci.* 97(11):6207-6211.

64. Carruthers SP, Gurvich CT, & Rossell SL (2015) The muscarinic system, cognition and schizophrenia. *Neurosci. Biobehav. Rev.* 55:393-402.

65. Lee MR, Hinton DJ, Unal SS, Richelson E, & Choi DS (2011) Increased ethanol consumption and preference in mice lacking neurotensin receptor type 2. *Alcoholism: Clinical and Experimental Research* 35(1):99-107.

66. Walewski JL*, et al.* (2014) Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet‐induced obesity. *Obesity* 22(7):1643-1652.

67. Michaud JL*, et al.* (2001) Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. *Hum. Mol. Genet.* 10(14):1465-1473.

68. Feng Y*, et al.* (2010) Association of the GABRD gene and childhood‐onset mood disorders. *Genes, Brain and Behavior* 9(6):668-672.

69. Hashimoto T*, et al.* (2008) Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am. J. Psychiatry* 165(4):479-489.

70. Levy F, Kendrick K, Goode J, Guevara-Guzman R, & Keverne E (1995) Oxytocin and vasopressin release in the olfactory bulb of parturient ewes: changes with maternal experience and effects on acetylcholine, γ-aminobutyric acid, glutamate and noradrenaline release. *Brain Res.* 669(2):197-206.

71. Ferguson JN*, et al.* (2000) Social amnesia in mice lacking the oxytocin gene. *Nat. Genet.* 25(3):284-288.

72. Dluzen DE, Muraoka S, Engelmann M, & Landgraf R (1998) The effects of infusion of arginine vasopressin, oxytocin, or their antagonists into the olfactory bulb upon social recognition responses in male rats. *Peptides* 19(6):999-1005.

73. Lee MR*, et al.* (2013) Effects of adjunctive intranasal oxytocin on olfactory identification and clinical symptoms in schizophrenia: results from a randomized double blind placebo controlled pilot study. *Schizophr. Res.* 145(1):110-115.

74. Quintana DS, Alvares GA, Hickie IB, & Guastella AJ (2015) Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neurosci. Biobehav. Rev.* 49:182-192.

75. Dumais KM & Veenema AH (2016) Vasopressin and oxytocin receptor systems in the brain: sex differences and sex-specific regulation of social behavior. *Front. Neuroendocrinol.* 40:1-23.

76. Quintana DS*, et al.* (2016) Low dose intranasal oxytocin delivered with Breath Powered device dampens amygdala response to emotional stimuli: A peripheral effect-controlled within-subjects randomized dose-response fMRI trial *Psychoneuroendocrinology* 69:180-188.

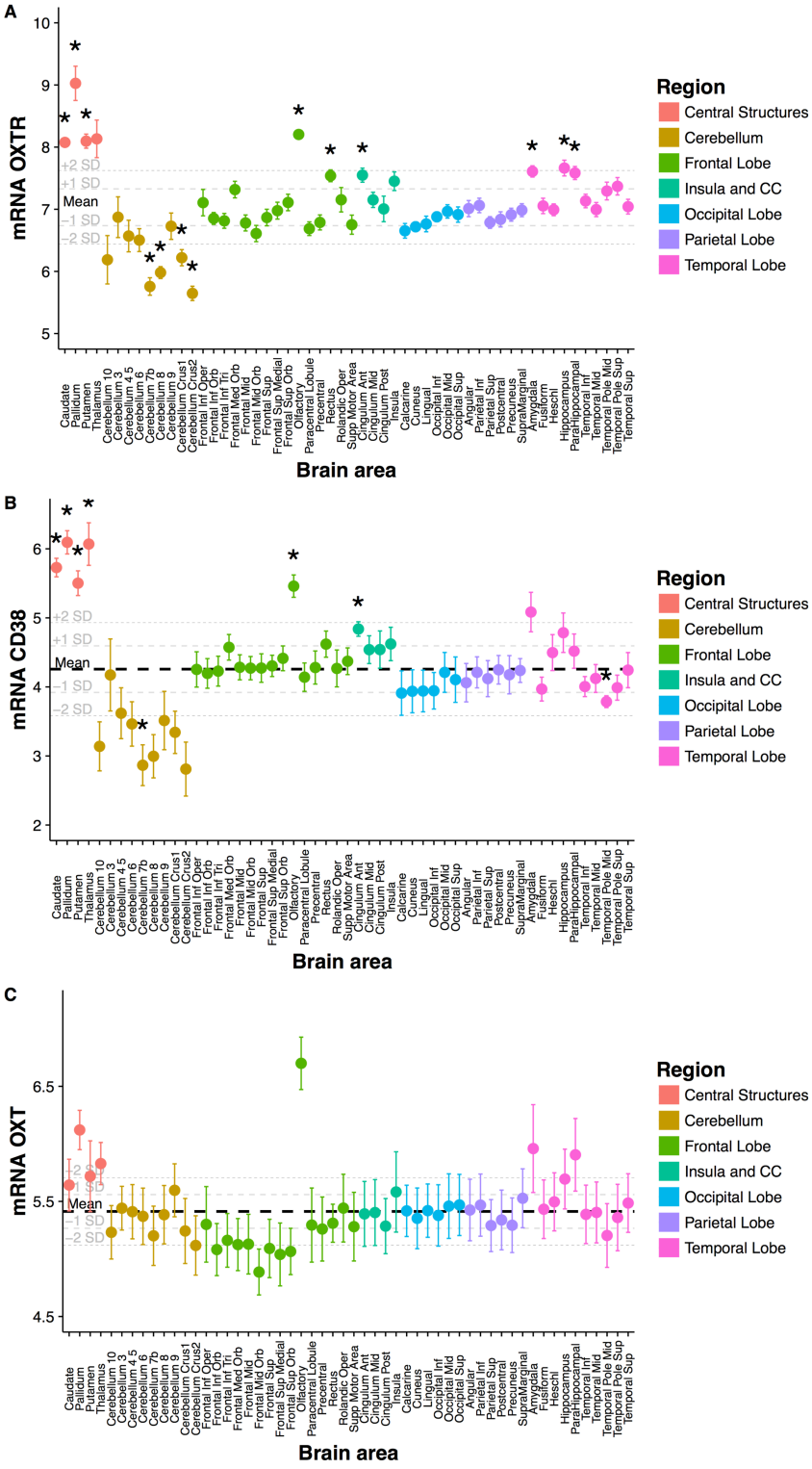

Figure 1. Central gene expression for *OXTR* (A), CD38 (B), and *OXT* (C). Each point represents mean expression with standard errors for a given brain region. The bolded dashed lines represent whole-brain mean expressions. One sample t-tests were performed to assess which regions expressed mRNA to a significantly greater degree compared to average expression across the brain. * $p < 0.05$ (FDR corrected for 54 tests).

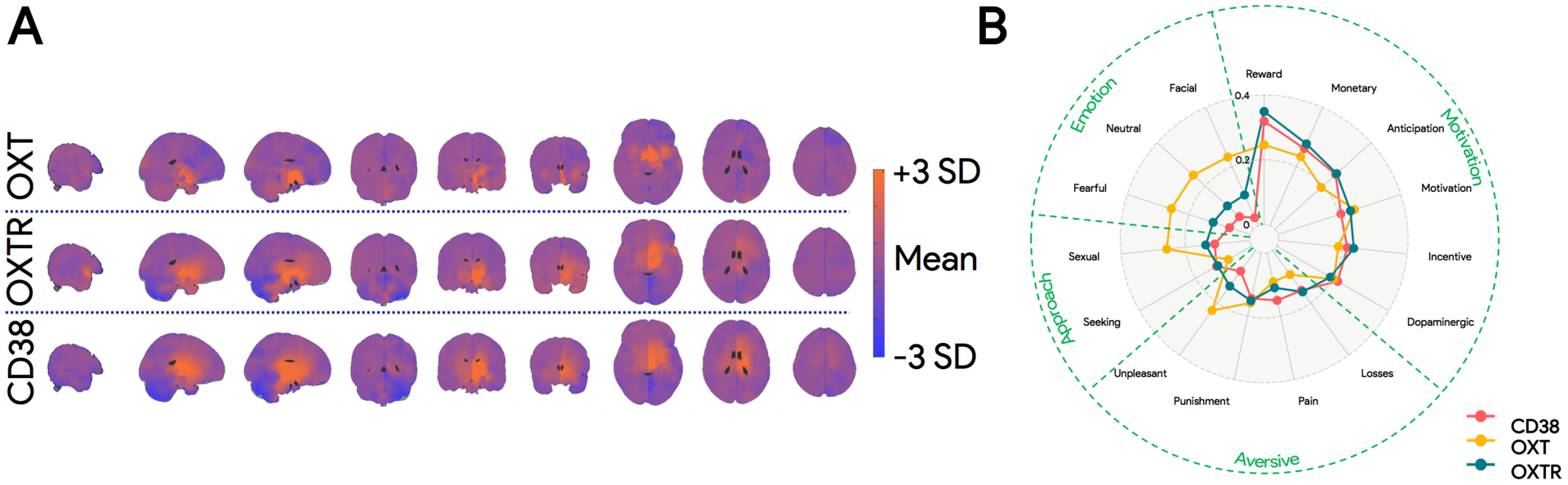

Figure 2. Whole-brain gene expression maps for the oxytocin pathway and their cognitive correlates. Whole-brain gene expression maps are presented for *OXT*, *OXTR* and *CD38* (A). A large-scale meta-analysis of functional neuroimaging using the NeuroSynth framework was performed to infer mental state correlates of oxytocin pathway gene expression, with plot units representing Pearson's *r* (B). SD = Standard deviation.

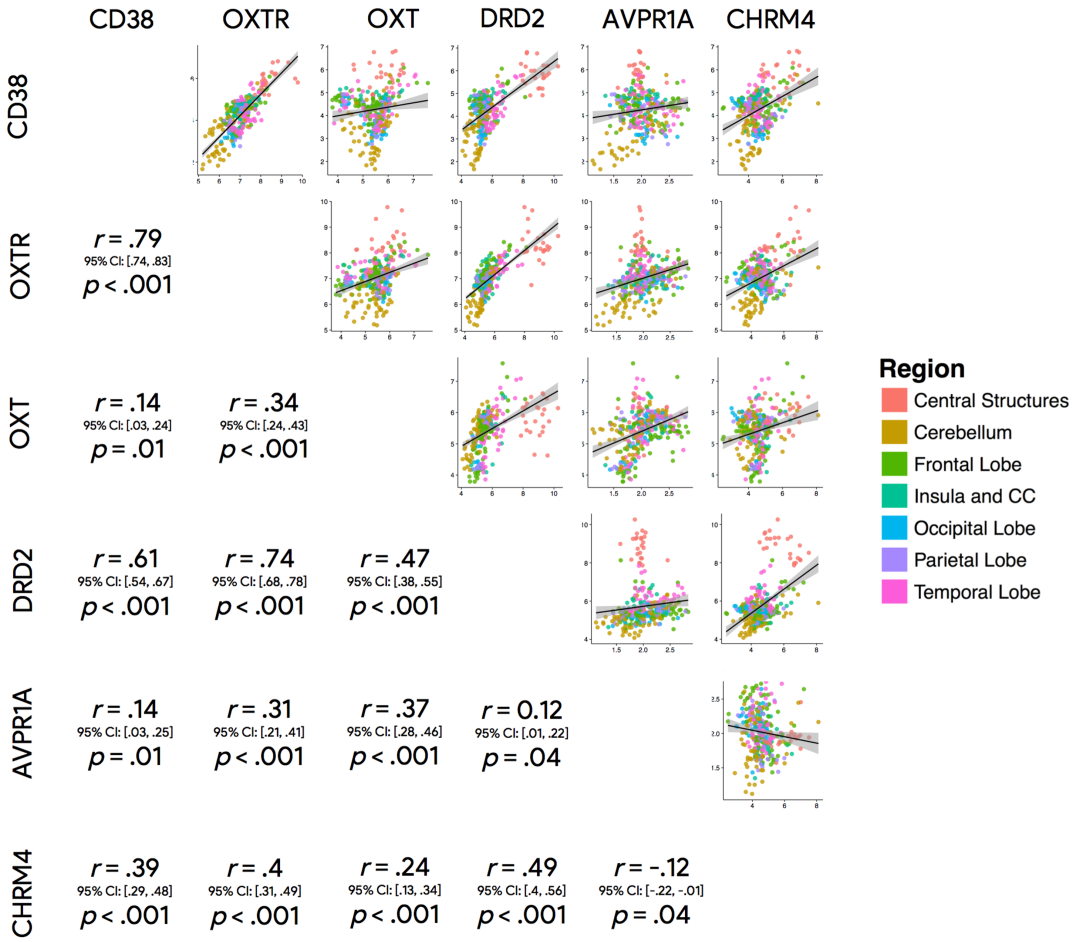

Figure 3. Correlations between central gene expression of *OXTR, CD38, OXT, AVPR1A, DRD2*, and *CHRM4*. Each scatterplot point represents a location in the left hemisphere of the brain, with 54 regions in total. *P*-values are FDR corrected for multiple tests. See Figure 1 for a list of locations.

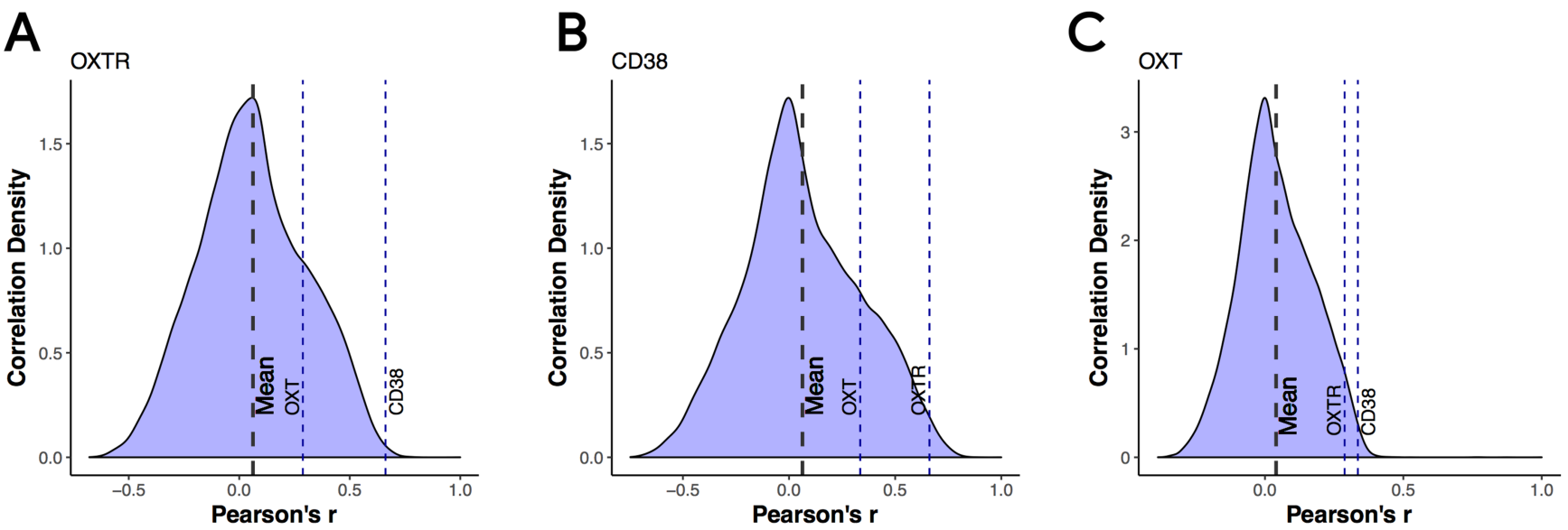

Figure 4. Distributions of correlations between oxytocin pathway genes and all other mRNA probes in the Allen Human Brain Atlas. Histograms are presented for *OXTR* (A), *CD38* (B), and *OXT* (C). Each histogram visualises the correlation between the target oxytocin pathway mRNA probe and all other mRNA probes (n = 58691). For each oxytocin pathway gene, the position of the other two oxytocin pathway genes is shown.

Table 1. Top correlations between oxytocin pathway probes and all remaining probes

	OXTR		CD38		OXT	
	Gene	*r*	Gene	*r*	Gene	*r*
Top 10 positive	LUZP2	0.804	CD38	0.891	OXT [a]	0.866
	NTSR2	0.797	NTSR2	0.837	OXT [b]	0.778
	THBS4	0.794	LIX1 (probe 1)	0.830	AVP	0.773
	GLUD2	0.793	SLC14A1	0.829	AVP	0.765
	GLUD1	0.782	PSAT1	0.806	SIM1	0.513
	HEYL	0.776	C12orf39	0.806	OTP	0.482
	LUZP2	0.752	AQP4	0.804	FEZF1	0.467
	ZC3HAV1	0.748	GLUD1	0.799	TMEM114	0.444
	A_32_P232413	0.741	LIX1	0.798	ECEL1	0.442
	ISOC1	0.738	RARRES3	0.796	CUST_1286_PI416379584	0.433
Top 10 negative	RTN4R	-0.632	ATP4A	-0.689	A_24_P554040	-0.323
	PAK7	-0.634	KCNJ3	-0.697	ITPKA	-0.323
	SLC6A7	-0.640	MICAL2 [d]	-0.698	KCNK1	-0.324
	KIAA1456	-0.641	MICAL2 [e]	-0.705	GPR22	-0.324
	KCNJ3	-0.646	NEUROD1 [f]	-0.708	CAMKK2 [h]	-0.330
	CAMKK2 [c]	-0.651	ZNF238	-0.710	CA7	-0.333
	CHRD	-0.659	A_32_P203232	-0.718	ZNF385B	-0.335
	A_32_P98423	-0.663	NEUROD1 [g]	-0.718	CAMKK2	-0.344
	ZNF238	-0.668	A_32_P85131	-0.720	ADCY1	-0.351
	GABRD	-0.679	CHRD	-0.727	GABRD	-0.351

Note. Some genes had more than one probe feature in the top 10 positive or negative correlations (e.g., *OXT*). Full probe identification numbers available in Table S3. [a] Probe no. A_24_P382579, [b] Probe no. CUST_497_PI416408490, [c] Probe no. A_23_P408830, [d] Probe no. A_23_P13442, [e] Probe no. A_23_P24843, [f] Probe no. CUST_14143_PI416261804, [g] Probe no. A_23_P209484, [h] Probe no. A_32_P210390.