CD38 Regulates oxytocin secretion and complex social behavior

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Summary

The peptide hormone oxytocin plays a critical role in regulating affiliative behaviors including mating, pair-bond formation, maternal/parenting behavior, social recognition, separation distress and other aspects of attachment. Jin and colleagues[1] recently reported intriguing findings that *CD38*, a transmembrane receptor with ADP-ribosyl cyclase activity, plays a critical role in maternal nurturing behavior and social recognition by regulating oxytocin secretion. This research may have implications for understanding disorders marked by deficits in social cognition and social functioning, including autism, social anxiety disorder, borderline personality disorder and schizophrenia. *BioEssays* 29:837–841, 2007. © 2007 Wiley Periodicals, Inc.

Key Word: oxytocin

Introduction

The mental and physical benefits of close relationships have been well-documented: Over two decades of research has shown that close relationships are associated with improved physical and mental well-being[2,3] and, conversely, that the failure to achieve closeness is associated with increased risk for mental and physical illness, and suicide.[4–6] Moreover, deficits in attachment and/or the regulation of social behavior are a key feature of a number of psychiatric disorders, including autism, social anxiety disorder, borderline personality disorder and schizophrenia. Given the importance of close bonds in healthy and abnormal behavior, researchers have been interested in understanding the neurobiological factors involved in the formation and maintenance of social bonds.

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Abbreviations: OT, oxytocin; AVP, arginine vasopressin; CNS, central nervous system; KO, knock-out; *Oxtr/OXTR*, mouse/human oxytocin receptor gene; *Oxt* oxytocin gene; OT, receptor; CSF, cerebrospinal fluid; cADPR, cyclic ADP-ribose; NAADP, nicotinic acid adenine dinucleotide phosphate; ASD, autism spectrum disorders; SNPs, single nucleotide polymorphisms.

The neurobiology of affliation: oxytocin and arginine vasopressin

Studies, mainly with rodents and non-human primates, point to the role of oxytocin (OT) in the regulation of affiliative behaviors including mating, pair-bond formation, maternal/parenting behavior, social recognition, separation distress and other aspects of attachment. A structurally similar peptide, arginine vasopressin (AVP), is also involved in affiliative behaviors but, as we discuss below, OT release in the central nervous system (CNS) is uniquely regulated by at least one protein, CD38. As the latter molecule is the focus of this review, we will confine ourselves to a discussion of OT on behavior at this time. OT is a nine-amino-acid peptide that is synthesized in magnocellular neurons in the paraventricular and supraoptic nucleus of the hypothalamus. OT is secreted into peripheral circulation from axon terminals in the posterior pituitary, facilitating uterine contractions during parturition and milk-ejection during nursing.[7] OT is also secreted into the brain from dendrites of hypothalamic neurons to limbic sites, including the hippocampus, amygdala, striatum and nucleus accumbens, and to nuclei in the mid- and hindbrain,[8] it is thought that OT is involved in the regulation of social behaviors through its neuromodulatory role in the CNS (Figure 1).[9-11]

The role of OT in social attachment is supported by animal studies investigating selective and enduring adult–adult pair bonds and maternal behavior, and especially by studies comparing prairie and montane and meadow voles.[10,12,13] Prairie voles form long-term pair bonds and tend to be biparental, whereas montane voles do not form long-term pair bonds, do not display biparental care, and generally show little interest in social contact. Studies suggest that OT is involved in the social behavior displayed by prairie voles. Centrally administered OT facilitates partner preference formation in female prairie voles in the absence of mating,[14,15] whereas OT antagonists given to female prairie voles before mating blocks partner preference formation.[14] Moreover, OT

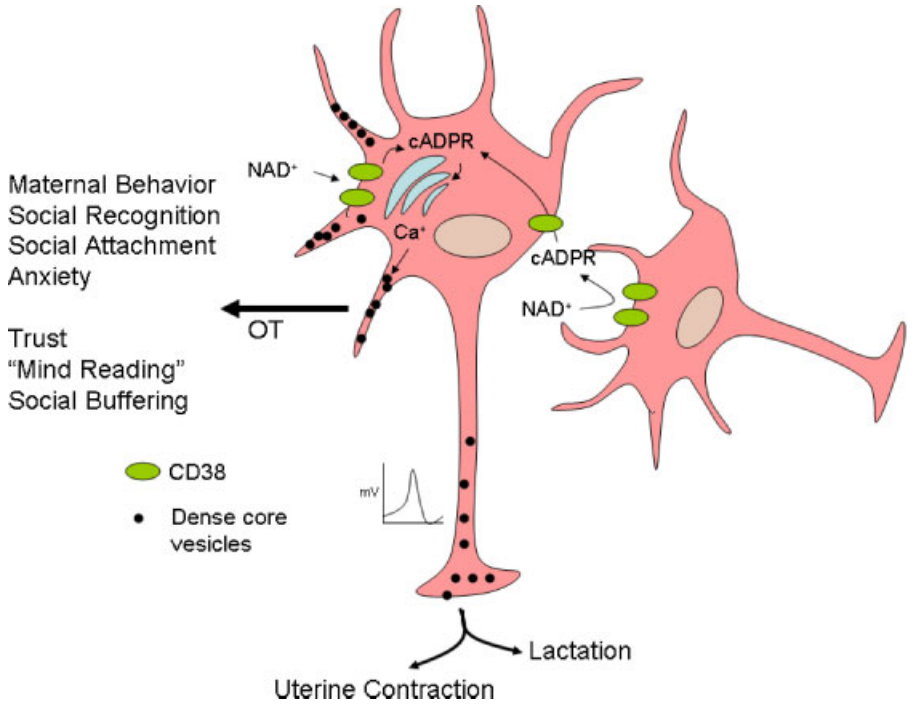

Figure 1. Schematic depicting[40] the proposed role of *CD38* in regulating oxytocin release and social behavior. This figure was published in Neuron, 54, L. J. Young. Regulating the social brain: A new role for CD38. p. 355. Copyright Elsevier (2007).

administered peripherally to developing prairie voles influences social behavior into adulthood (for review see Ref. (16)). Interestingly, research suggests that it is not the overall expression of OT but differences in the distribution of OT receptors that underlie social behavior differences in the voles.[17–21] Prairie voles have high levels of OT receptors in the prelimbic cortex and nucleus accumbens—regions implicated in reinforcement and conditioning—whereas montane voles have few receptors in these regions and more receptors in the lateral septum.

Studies of maternal behavior also support the role of OT in social affiliation. Centrally administered OT facilitates maternal behavior in female nulliparious rats, who typically find pups to be aversive and actively avoid them.[22] Conversely, centrally administered OT antagonists inhibit the initiation, but not the maintenance of maternal behavior.[9] Studies of sheep—one of the few species to display selective maternal care—also implicate OT in maternal behavior.[13] Vaginocervical stimulation induces maternal behavior in steroid primed, non-pregnant ewe[23] and acceptance of a foreign lamb.[24] Intracerebroventricular (ICV) injection of OT also facilitates acceptance of a foreign lamb, even in non-pregnant ewe.[25,26] Finally, OT is involved in maternal behavior in female and juvenile prairie voles. Olazabal and Young[27] found that time spent crouching over pups was positively correlated with the density of OT receptor binding in the nucleus accumbens in juvenile prairie voles, and the administration of an OT antagonist in the nucleus accumbens blocked maternal behavior in adult female prairie voles.[28]

Social memory plays an important role in social behavior, and OT has also been implicated in this process. Low doses of OT, administered centrally or peripherally, facilitate social recognition in rodents,[29] and centrally administered OT antagonists disrupt social memory in female rats.[30] Moreover, mice with a null mutation in the gene coding for OT production (OT knock-out (KO) mice) fail to recognize a novel mouse of the same species (conspecific) over repeated exposures;[31,32] this social recognition deficit, however, can be rescued by administering a single injection of OT directly into the CNS prior to the initial encounter.[33]

A caveat regarding the significance of behaviors observed in knock-out animals is that the use of different mouse species, different technologies for disrupting the gene or different behavioral paradigms, can lead to spurious findings. Therefore, Crawley and colleagues[34] recently tested two separate mouse OT KO strains using the same procedures at two independent sites and have unequivocally shown that lack of OT does not alter general prosocial behavior in the mutant, nor does it appear to confer an anxiety-related phenotype. Rather, lack of OT appears to selectively impair the ability to remember having previously met a novel mouse. In addition to impaired social memory, Takayanagi and colleagues found that OT receptor

(Otxr) KO dams display impaired maternal nurturing, and pups exhibit fewer ultrasonic vocalizations when removed from the nest.[35] Interestingly, these authors noted that ligand Oxt$^{-/-}$ males from Oxt$^{-/-}$ dams—but not from Oxt$^{+/-}$ dams—showed similar high levels of aggression to the Oxtr$^{-/-}$ mice indicating that maternal exposure to OT in utero may be important for the development of aggressive behavior in males.

Recent research suggests that OT may also be implicated in human social behavior. For example, compared to placebo, intranasally administered OT was shown to promote trust among male volunteers playing an investment game,[36] and to reduce amygdala activity in response to fearful stimuli.[37] Finally, intranasal OT was shown to facilitate the ability to infer the mental status of others, known as "theory of mind";[38] notably, impaired theory of mind is a classic characteristic of persons with autism.

In sum, research suggests that OT plays an important role in the regulation of social and affiliative behavior; however, as Insel[10] and others note, the regulation of social behavior is likely a complex process involving a number of players. Germane to the topic of our review, Jin and colleagues recently discovered that mouse knock-outs for the *CD38* gene show low plasma and cerebrospinal fluid (CSF) levels of OT—but not AVP—and deficits in social memory and maternal behavior reminiscent of *Oxtr*$^{-/-}$ and *Oxt*$^{-/-}$ mice.

CD38: A new player in the neurobiology of affiliation

Recent research by Jin and colleagues[1] suggests that the *CD38* gene plays a critical role in regulating social behavior by regulating OT secretion. *CD38* is highly expressed in the hypothalamic region but also in haematopoietic or blood related tissues and the pancreas. It is a transmembrane receptor with ADP-ribosyl cyclase activity which catalyses the formation of the second messenger molecules, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), that send further signals throughout the cell by mobilizing calcium. The results of Jin et al's study show that the product of the *CD38* gene regulates depolarization-induced OT secretion from soma and axon terminals of hypothalamic neurons into various brain regions, with profound effects on maternal behavior and social memory in rodents.[1]

The effects of *CD38* on OT secretion and social behavior was determined by making a knock-out mouse model and investigating maternal nurturing in females and social recognition/amnesia in males. Maternal behavior was examined by observing postpartum dams whose 6- to 14-day-old pups were returned to them after a 10-minute separation; the pups were placed in different corners of the cage and maternal nurturing was measured by latency to retrieve pups and time spent crouching over pups. Normal mothers quickly retrieved the first pup and crouched over it, and then quickly retrieved the other pups and crouched over them to warm and nurse them. By contrast, *CD38* mutant mothers took much longer to approach their pups and spent less time crouching over them. These findings are consistent with research by Takayanagi et al. showing maternal nurturing impairments in OT-receptor deficient mice.[35] However, there is one notable difference: Whereas *Oxt* or *Oxtr* KO female mice completely fail to lactate, resulting in pup mortality and the need to cross foster pups to a wild-type to insure survival,[35,39] there was no disruption of lactation in the *CD38* KO mice despite a 30% reduction of OT in the CSF. Moreover, Takayanagi et al. found that *Oxt*$^{-/-}$ females showed normal maternal behavior despite their inability to lactate when compared with *Oxtr*$^{-/-}$ females. Thus, it is possible that decreased OT secretion in the *CD38*$^{-/-}$ dams may not be the only factor contributing to maternal behavior disruption and that *CD38* may influence other systems involved in maternal behavior.

Social deficits were also observed in male *CD38* KO mice, specifically, memory deficits for a novel conspecific. Rodents primarily rely on olfaction and pheromonal cues to learn about a novel rodent, and duration of olfactory investigation of a conspecific over repeated encounters is a standard way of assessing social recognition in rodents. In this paradigm, the experimental rodent is introduced to a novel rodent and duration of olfactory investigation is measured; the novel rodent is taken out of the cage and reintroduced after a period of time; in an intact system the duration of olfactory investigation should go down and, indeed, *CD38*$^{+/+}$ mice showed a significant decline in time spent investigating a female on subsequent presentations. By contrast, the *CD38*$^{-/-}$ mice showed no attenuation in the duration of investigation of the female on subsequent presentations. Importantly, this failure to attenuate in the *CD38*$^{-/-}$ mice could not be accounted for by more general deficits in olfaction or habituation; nor could it be accounted for by more global deficits in cognitive function. Again, these findings are remarkably similar to those observed by Young and colleagues described earlier with respect to social memory deficits in *Oxt* or *Oxtr* receptor KO mice.[35]

Jin and colleagues then investigated the mediating role that OT may play in the social deficits displayed by *CD38* KO mice. *CD38* KO mice had consistently low levels of plasma and CSF OT, but not AVP. Interestingly, hypothalamic and pituitary stores of OT in secretory vesicles were elevated, suggesting that OT release was selectively impaired in the *CD38* mutants. Additionally, hypothalamic levels of cADPR were markedly decreased in the *CD38* mutants, indicating that ADP-ribosyl cyclase activity is also greatly reduced in the hypothalamus due to the absence of *CD38*.

Finally, Jin et al. showed that maternal nurturing and social memory deficits in *CD38* mutant mice could be rescued by replacing OT via peripheral injection or direct injection into the

third ventricle. Social deficits could also be rescued by injecting a virus engineered to express *CD38* into the brain. Specifically, two weeks after injection, infected cells in the hypothalamus and posterior pituitary started to express *CD38* in the mutant mouse, returning OT levels and the behavioral deficits to control levels and greatly restoring cADPR activity. In fact, application of cADPR to wild-type neurons increased OT release in control but not KO neurons. However, permeabilization of the membrane to permit cADPR entry into *CD38* KO neurons increased OT release indicating that cADPR is essential for mediating OT secretion. In his commentary, Young[40] noted that the majority of neurons infected with the virus expressing *CD38* were actually peri-ventricular and not OT neurons, implying that *CD38* can have distant effects on OT secretion. He also pointed out that *CD38* is more frequently found not on but adjacent to OT neurons in the hypothalamus, suggesting that the effects of *CD38* on OT release may rely on the release of other signaling substances that communicate with OT neurons.

Implications for disorders marked by social functioning deficits

Based on their findings, Jin et al. suggest that altered *CD38* function and subsequent dysregulated OT secretion may underlie the social behavior deficits that characterize such disorders as autism. Indeed, researchers have hypothesized that OT may be implicated in autism given that deficits in social interaction and repetitive behaviors are core features of autism, and that OT is involved in the regulation of affiliative and repetitive behaviors.[11,41–48]

Supporting this idea, significantly lower plasma OT levels have been found in children with autism compared to age-matched controls.[49] Moreover, these children showed higher OT precursor levels, and an increased ratio of OT precursor to OT, suggesting that the way OT is processed in the brain may be altered in autism.[50] Another study, however, found higher OT plasma levels in adults with ASD compared to controls.[51] The reason for this discrepancy is unclear, but development may be a factor.

Genetic associations between the OT receptor gene and autism have also been observed. A recent genome-wide linkage screen looking for overlap between a predominantly Caucasian sample collected in the USA (AGRE consortium) and ethnically distinct Finnish families found evidence for linkage to 3p24–26, a region containing the *OXTR* gene.[52] Association of two single nucleotide polymorphisms (SNPs) at this locus has also been reported in a sample of 195 Han Chinese cases.[53] Finally, a recent small study of 57 Caucasian autism cases found evidence of over-transmission of one of these SNPs; however, it was the opposite allele from that observed in the Chinese study.[54] Much larger samples, on the order of $>$1000 subjects, will probably be needed to determine whether there truly is a genetic association between the OT receptor and autism. However, given the biological evidence described previously, it is of interest that a recent microarray-based gene expression study found altered *OXTR* expression in lymphocytes from subjects with Prader-Willi syndrome, a disorder characterized by obsessive behaviors in common with autism.[55]

Finally, Hollander and colleagues have used a laboratory challenge methodology to examine the functional role of OT in autism with respect to repetitive behaviors and social cognition.[41,48] Adults with ASD underwent two challenge procedures in which OT or placebo was administered via intravenous infusion over a 4-hour period; OT infusion resulted in a significant reduction in repetitive behaviors,[41] and also facilitated social information processing in ASD patients.[48] These findings suggest that OT may have potential therapeutic value in the treatment of core ASD symptoms; however, one challenge in using OT as a treatment for ASD concerns issues related to brain penetration.[47] Specifically, although Hollander et al. observed behavioral changes, it is unclear whether peripherally administered OT passes the blood–brain barrier and, if so, in what quantities. In this regard, it is noteworthy that Jin et al. found that maternal nurturing and social memory deficits in *CD38* mutant mice could be rescued by peripheral injection of OT. Similarly, another study found that peripherally administered OT produced anxiolytic effects in male mice, suggesting that peripherally administered OT passes the blood–brain barrier, albeit in small amounts.[56] Clearly more research is needed to determine how subcutaneous injections of OT could increase CSF OT concentrations; for example, as Neumann[57] noted, one possibility is that because neuronal stores of OT are overfull in *CD38*$^{-/-}$ mice, even small amounts of additional OT could trigger significant OT release. Nonetheless, these findings suggest that peripheral rescue may be a viable treatment modality.

Complex behavioral genetics: *CD38* and type II diabetes

In addition to its possible role in OT secretion, cADPR also mobilizes calcium from pancreatic islet cells to affect insulin release. As *CD38* is both responsible for the formation and hydrolysis of cADPR, one group hypothesized that it might affect insulin secretion. Yagui et al.[58] screened 31 Japanese patients with familial type II diabetes and found a functional polymorphism, Arg^{140}Trp, in exon 3 that reduced expression of the enzyme activity (ADP-ribosyl clyclase and cADPR hydrolase) by roughly 50% in COS-7 cells. This mutation was found in 4 of 31 cases and 0 of 95 controls.[58] Interestingly, one case inherited both the exon 3 mutation and an additional Type II diabetes predisposing variant in the beta cell/liver glucose transporter gene (*GLUT2*) from her mother, as well as another *GLUT2* Type II diabetes associated variant, presumably from the father. It would be interesting to note if the mother and daughter had some sort of autism related phenotype as Jin

et al. failed to rescue the *CD38*$^{-/-}$ mouse social memory deficit using a virus engineered to make the mutant Arg^{140}Trp exon 3 protein. Of course, it is not known whether the other 30 diabetes patients also carried additional unknown modifier variants that, combined with the *CD38* mutation, would predispose them to diabetes type II, or whether reduced activity of *CD38* alone would predispose to diabetes type II. Thus it is possible that reduced plasma OT levels might be observed in subjects carrying the exon 3 missense mutation, or another functional variant in the gene, who do not have Type II diabetes. The autism community will most certainly be screening this gene for other functional variants that could affect enzyme activity and calcium-signaling induced OT secretion. To our knowledge, no epidemiological link has ever been established between type II diabetes and autism.

Conclusion

Research by Jin and colleagues sheds light on the molecular and cellular mechanisms underlying complex social behavior, and on the role that OT plays in this process. This research has potential implications for understanding disorders marked by deficits in social functioning and cognition, including autism. In particular, we and others[40] conjecture that individual differences in social cognitive expertise may be related to variation in *CD38* activity and that *CD38* may be a fruitful candidate in understanding how early adversity affects social behavior in adulthood.

References

1. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, et al. 2007. Nature 446: 41–45.
2. House JS, Landis KR, Umberson D. 1988. Science 241:540–545.
3. Kiecolt-Glaser JK, Newton TL. 2001. Psychol Bull 127:472–503.
4. Bowlby J. 1969. Attachment and loss: Vol. 1. Attachment New York: Basic Books.
5. Lynch JJ. 1979. The broken heart: The medical consequences of loneliness New York: Basic Books.
6. Trout DL. 1980. Suicide and Life-Threatening Behavior 10:10–23.
7. Burbach JP, Young LJ, Russell J. 2006. Oxytocin: Synthesis, Secretion, and Reproductive Functions. In: Neill JD editor Knobil and Neill's Physiology of Reproduction. Elsevier. pp 3055–3128.
8. Sofroniew MV. 1983. Prog Brain Res 60:101–114.
9. Insel TR. 1992. Psychoneuroendocrinology 17:3–35.
10. Insel TR. 1997. Am J Psychiatry 154:726–735.
11. Insel TR, O'Brien DJ, Leckman JF. 1999. Biol Psychiatry 45:145–157.
12. Carter CS, DeVries AC, Getz LL. 1995. Neurosci Biobehav Rev 19:303–314.
13. Insel TR, Young LJ. 2001. Nat Rev Neurosci 2:129–136.
14. Insel TR, Hulihan TJ. 1995. Behav Neurosci 109:782–789.
15. Williams JR, Insel TR, Harbaugh CR, Carter CS. 1994. J Neuroendocrinol 6:247–250.
16. Carter CS. 2003. Physiol Behav 79:383–397.
17. Insel TR, Wang ZX, Ferris CF. 1994. J Neurosci 14:5381–5392.
18. Young LJ, Huot B, Nilsen R, Wang Z, Insel TR. 1996. J Neuroendocrinol 8:777–783.
19. Insel TR, Shapiro LE. 1992. Proc Natl Acad Sci USA 89:5981–5985.
20. Young LJ, Winslow JT, Nilsen R, Insel TR. 1997. Behav Neurosci 111: 599–605.
21. Wang Z, Zhou L, Hulihan TJ, Insel TR. 1996. J Comp Neurol 366:726–737.
22. Pedersen CA, Ascher JA, Monroe YL, Prange AJ Jr. 1982. Science 216: 648–650.
23. Keverne EB, Levy F, Poindron P, Lindsay DR. 1983. Science 219:81–83.
24. Kendrick KM, Levy F, Keverne EB. 1991. Physiol Behav 50:595–600.
25. Kendrick KM, Keverne EB, Baldwin BA. 1987. Neuroendocrinology 46:56–61.
26. Keverne EB, Kendrick KM. 1992. Ann N Y Acad Sci 652:83–101.
27. Olazabal DE, Young LJ. 2006. Horm Behav 49:681–687.
28. Olazabal DE, Young LJ. 2006. Neuroscience.
29. Popik P, Vetulani J, van Ree JM. 1992. Psychopharmacology (Berl) 106:71–74.
30. Engelmann M, Ebner K, Wotjak CT, Landgraf R. 1998. Behav Brain Res 90:89–94.
31. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, et al. 2000. Nat Genet 25:284–288.
32. Choleris E, Gustafsson JA, Korach KS, Muglia LJ, Pfaff DW, et al. 2003. Proc Natl Acad Sci USA 100:6192–6197.
33. Ferguson JN, Aldag JM, Insel TR, Young LJ. 2001. J Neurosci 21:8278–8285.
34. Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, et al. 2007. Neuropeptides 41:145–163.
35. Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, et al. 2005. Proc Natl Acad Sci USA 102:16096–16101.
36. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. 2005. Nature 435:673–676.
37. Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, et al. 2005. J Neurosci 25:11489–11493.
38. Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC. 2006. Biol Psychiatry.
39. Nishimori K, Young LJ, Guo Q, Wang Z, Insel TR, et al. 1996. Proc Natl Acad Sci USA 93:11699–11704.
40. Young LJ. 2007. Neuron 54:353–356.
41. Hollander E, Novotny S, Hanratty M, Yaffe R, DeCaria CM, et al. 2003. Neuropsychopharmacology 28:193–198.
42. Lim MM, Bielsky IF, Young LJ. 2005. Int J Dev Neurosci 23:235–243.
43. McCarthy MM, Altemus M. 1997. Mol Med Today 3:269–275.
44. Modahl C, Fein D, Waterhouse L, Newton N. 1992. J Autism Dev Disord 22:449–451.
45. Waterhouse L, Fein D, Modahl C. 1996. Psychol Rev 103:457–489.
46. Panksepp J. 1992. Ann N Y Acad Sci 652:243–252.
47. Bartz JA, Hollander E. 2006. Horm Behav 50:518–528.
48. Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, et al. 2007. Biol Psychiatry 61:498–503.
49. Modahl C, Green L, Fein D, Morris M, Waterhouse L, et al. 1998. Biol Psychiatry 43:270–277.
50. Green L, Fein D, Modahl C, Feinstein C, Waterhouse L, et al. 2001. Biol Psychiatry 50:609–613.
51. Jansen LM, Gispen-de Wied CC, Wiegant VM, Westenberg HG, Lahuis BE, et al. 2006. J Autism Dev Disord 36:891–899.
52. Ylisaukko-oja T, Alarcon M, Cantor RM, Auranen M, Vanhala R, et al. 2006. Ann Neurol 59:145–155.
53. Wu S, Jia M, Ruan Y, Liu J, Guo Y, et al. 2005. Biol Psychiatry 58:74–77.
54. Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, et al. 2007. Neuroscience Letters.
55. Bittel DC, Kibiryeva N, McNulty SG, Driscoll DJ, Butler MG, et al. 2007. Am J Med Genet A 143:422–429.
56. Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, et al. 2006. Psychopharmacology (Berl) 185:218–225.
57. Neumann ID. 2007. Cell Metab 5:231–233.
58. Yagui K, Shimada F, Mimura M, Hashimoto N, Suzuki Y, et al. 1998. Diabetologia 41:1024–1028.