Behavioural Brain Research 261 (2014) 315–322

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Local oxytocin expression and oxytocin receptor binding in the male rat brain is associated with aggressiveness

Federica Calcagnoli[a,*], Sietse F. de Boer[a], Daniela I. Beiderbeck[b], Monika Althaus[c], Jaap M. Koolhaas[a], Inga D. Neumann[b]

[a] Department of Behavioral Physiology, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands
[b] Department of Behavioral and Molecular Neuroscience, University of Regensburg, 93053 Regensburg, Germany
[c] Child and Adolescent Psychiatry, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

HIGHLIGHTS

- Excessively aggressive rats show diminished OXT transcription level in the PVN.
- Excessively aggressive rats exhibit higher OXTR binding in the CeA and BNST.
- Variations in OXTergic system are associated with phenotypical expressed aggression.

ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form
18 December 2013
Accepted 21 December 2013
Available online 7 January 2014

Keywords:
Oxytocin mRNA
Oxytocin receptor binding
Aggressiveness
Paraventricular nucleus
Central amygdala
Bed nucleus of the stria terminalis

ABSTRACT

We recently demonstrated in male wild-type Groningen rats that enhancing brain oxytocin (OXT) levels acutely produces marked pro-social explorative and anti-aggressive effects. Moreover, these pharmacologically-induced changes are moderated by the individual's aggressive phenotype, suggesting an inverse relationship between aggressiveness and tonic endogenous OXT signaling properties. Aim of the present study was to verify the hypothesis that variations in OXT expression and/or OXT receptor (OXTR) binding in selected brain regions are associated with different levels or forms of aggression. To this end, male resident wild-type Groningen rats that repeatedly contested and dominated intruder conspecifics were categorized as being low aggressive, highly aggressive or excessively aggressive. Their brains were subsequently collected and quantified for OXT mRNA expression and OXTR binding levels. Our results showed that OXT mRNA expression in the hypothalamic paraventricular nucleus (PVN), but not in the supraoptic nucleus (SON), negatively correlates with the level of offensiveness. In particular, the excessively aggressive group showed a significantly lower OXT mRNA expression in the PVN as compared to both low and highly aggressive groups. Further, the excessively aggressive animals showed the highest OXTR binding in the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST). These findings demonstrate that male rats with excessively high levels and abnormal forms of aggressive behavior have diminished OXT transcription and enhanced OXTR binding capacities in specific nodes of the social behavioral brain circuitry.

1. Introduction

The neuropeptide oxytocin (OXT) is known to influence a variety of socio-emotional behaviors including parental care and affiliation, bonding and conflict behavior, in-group cooperation and out-group competition, social learning and recognition [1–5]. OXT is believed to exert this important role by modulating the neuronal activity within several brain regions implicated in the regulation of these social behaviors like, for example, the amygdala, septum, hypothalamus, hippocampus, and brain stem [6–8].

From the clinical literature, there are data suggesting that different plasma levels of OXT are related to individual differences in social skills and interactions. Although human plasma OXT only poorly reflects central OXTergic neurotransmission [9], higher plasma OXT levels have been associated with trust [10], positive parenting style [11], and high social engagement [12]. Two studies in human adults have shown that cerebrospinal fluid (CSF) OXT levels are diminished after childhood abuse and are negatively correlated with suicidal (auto-aggressive) behavior [13,14]. Finally, infants with higher CSF OXT levels appear to actively seek parental social interaction for soothing, and also have a greater interest in

* Corresponding author. Tel.: +31 50 363 2337; fax: +31 50 363 2331.
E-mail address: f.calcagnoli@rug.nl (F. Calcagnoli).

0166-4328/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.12.050

social interaction at 6 months of age [15]. Moreover, Lee and colleagues have reported a negative correlation between both CSF and plasma OXT levels and the life history of aggression in male subjects with conduct disorder. Finally, specific polymorphisms of the OXTR gene have been found to be associated with a high frequency of disruptive behaviors and temper outbursts in young boys [16–19].

These clinical results are in agreement with the preponderance of preclinical studies indicating that diminished OXT receptor (OXTR) binding in various rat brain regions is associated with impaired social functioning after poor social rearing conditions [20] or after early life stress [21] in several species. Moreover, activating the brain OXTergic system by pharmacological manipulation was shown to promote affiliative and attachment behaviors [2,22,23]. In contrast, however, a number of studies have also shown decreased social interaction and increased aggression with high levels of OXT or OXTR [24–26]. Therefore, species and individual differences, as well as brain region specificity have to be critically considered when linking the OXTergic activity and social behaviors. Among other animals, the monogamous prairie voles (*Microtus ochrogaster*) that display higher levels of parental care, affiliation, and pair bonding as compared to the solitary montane voles (*Microtus montanus*) also exhibit a higher density of OXTRs in the ventral tegmental area, nucleus accumbens, and caudate putamen [27,28]; but lower density in the lateral septum (LS) [29]. In contrast with the virtually asocial cape mole-rats (*Georychus capensis*), naked mole-rats (*Heterocephalus glaber*) represent the pinnacle of sociality for their remarkable high level of social cohesion, tolerance, and cooperation in burrowing, foraging, and defending the colony [30]. These animals also show greater OXTR binding in the nucleus accumbens, central (CeA) and medial amygdala, and bed nucleus of the stria terminalis (BNST) [31]. Two studies have further shown that CSF OXT level is higher in more social bonnet macaques as compared to pigtail macaques, and that it is positively associated with affiliation in rhesus macaques [32,33]. In addition, young peer-reared rhesus monkeys were found to display aberrant social behaviors; as a group, these monkeys have lower CSF OXT levels over the course of development when compared to maternally reared controls [33]. Linfoot and colleagues have reported higher levels of OXT mRNA in the magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of low burying male rats [34]. Defensive burying behavior is a typical proactive coping strategy in rodents that vigorously displace bedding material towards a variety of noxious stimuli that pose a near and immediate threat, such as a wall-mounted electrified shock-prod [35]. Several studies have demonstrated a close link between levels of shock-prod burying and levels of offensive aggression in a resident-intruder test indicating that low levels of burying behavior are characteristic for rodents with a low level of aggression [36,37].

These data strongly support the general idea that inter-individual differences in social skills and aggression may be closely related to individual differences in brain OXTergic neurotransmission. Indeed, in our previous studies we have found that the behavioral response of male rats to exogenous OXTergic manipulation was moderated by their individual baseline aggression score. Greater OXT-induced anti-aggressive and pro-social effects were observed in animals with higher baseline levels of aggression, whereas pro-aggressive and anti-social effects occurred after treatment with a selective OXTR antagonist in the least aggressive animals only [38].

Based on these findings, we hypothesized an inverse relationship between aggressiveness and brain OXTergic activity. The current experiments were principally aimed to verify this hypothesis. The wide variability in intermale offensive behavior in wild-type Groningen rats reaching up to excessive and abnormal levels of aggression allowed us to associate quantitative and qualitative variations in aggression [39] with the activity of brain OXTergic system. We define offensive aggression as "excessive", when the frequency and/or duration of the aggressive acts are out of proportion to the causes and the representative threat of the target; while "abnormal" aggression refers to a qualitative connotation for offensive display such as attack of female or anesthetized conspecifics, or of vulnerable body parts [39]. Therefore, our experimental design included groups of male wild-type Groningen rats that developed excessive and abnormal forms of aggressive behavior upon repeated winning aggressive contests, in addition to normally low and high aggressive animals. OXT mRNA expression and OXTR binding were chosen as neurobiological parameters reflecting the activity of the brain OXTergic system. OXT mRNA levels were assessed in both the PVN and SON as the two main hypothalamic sites of OXT synthesis and release of central OXT [7,40,41]. In addition, OXTR binding was quantified in the LS, CeA, and BNST as part of the social behavior network. Within these regions high local density of OXTRs [42,43] and local effects of OXT on socio-emotional behaviors [9,21,44–46], including the discrimination of biologically relevant social cues [47,48] and the acquisition of appropriate social skills [49], were described.

In summary, in this study we aimed at revealing the potential link between the individual variation in intermale offensive aggressive behavior including its escalation into excessive and abnormal forms of aggression and some functional properties of the endogenous OXTergic system including OXT mRNA expression and local OXTR binding.

2. Material and methods

2.1. Animals and housing conditions

Young adult male wild-type Groningen rats (*Rattus norvegicus*) ($N = 21$) were used as experimental subjects. This strain of rats descended from pairs of wild-trapped individuals that were outbred under conventionalized conditions for over 35 generations now in our laboratory. Throughout the experimental period, animals were held under standard conditions (12:12 h light–dark photoperiod, lights off at 13:00 h; ambient temperature $21 \pm 2\,^{\circ}C$; humidity $50 \pm 5\%$) with *ad lib*. access to food (Hope Farms, RMH-B) and water. After the age of 120 days, rats (body weight 350–400 g), previously housed with 5 non-sibling conspecifics in macrolon cages ($55 \times 34 \times 20$ cm), were then housed in observation cages ($80 \times 55 \times 50$ cm), together with an oviduct-ligated but gonadally-intact female to avoid social isolation, in order to allow normal sexual activity and to stimulate territorial behavior. As compared to commonly used laboratory strains of rats, this strain of rats expresses a more varied ethogram when socially challenged and a higher level of offensive behavior in conflicting/hostile context [50]. All experimental and behavioral procedures were approved by the Animal Ethics Committee on Care and Use of Laboratory Animals (DEC 5824) of Groningen University and were conducted in agreement with Dutch laws (Wet op de Dierproeven 1996) and European regulations (Guideline 86/609/EEC).

2.2. Behavioral characterization and selection criteria of the experimental groups

After one week of habituation in the observation cage, each resident was repeatedly exposed to an unfamiliar male intruder Wistar rat (Harlan Laboratories, Horst, NL; body weight 300–350 g) for 10 times, evenly distributed over a period of 1 month, in order to allow the development of potentially escalated and abnormal forms of aggression [51]. Each resident encountered a different intruder every time. The lower weight and general docility of the Wistar intruders guaranteed the expression of dominance from