

ADOLESCENT NEUROCHEMICAL PLASTICITY OF
OXYTOCIN RECEPTORS IN THE BED NUCLEUS
OF THE STRIA TERMINALIS

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Abstract

Adolescence has become an intriguing key period in neurodevelopment when different types of neuroplasticity occur. The bed nucleus of the stria terminalis (BNST) is a part of the limbic system involved in the generation of anxiety, control of social behaviour, and aggression. Because of the increasing frequency of these conditions in adolescence, we set it as a goal to investigate the BNST for neurochemical plasticity during that period. The oxytocin receptor was studied due to its known function in neurodevelopment, anxiety-related behaviours, and social activity. Its expression in preadolescent 20-days-old rats both male and female was compared with adult 60-days-old rats from both sexes too. We found that during adolescence there was a significant down-regulation of these receptors in some subnuclei of BNST. The expression of oxytocin receptors did not differ between sexes. Our results suggest that such a down-regulation in the pubertal period could have effects on the aggressive behaviour and the shifts in the social conduct. The observed neuroplasticity can serve as a fundamental paradigm for further pharmacological research.

Key words: bed nucleus of the stria terminalis, adolescence, oxytocin receptors, Wistar rat

Introduction. The bed nucleus of the stria terminalis (BNST) functions as a key limbic structure belonging to the extended amygdala [1]. The nucleus is a

complex structure that consist of 12–18 subnuclei [1] which makes it a challenging paradigm for investigation. It is believed to be involved in the genesis of general anxiety, social anxiety, and addiction [2]. The nucleus also serves as an integrating entity between mood, behaviour, and autonomic responses [3], making it an intriguing region for neuroscience experiments. On the other hand, oxytocin and its receptors (OXTR) have been implied in the processing of social information and the regulation of different behavioural modes [4]. The abundance of OXTR [1,5], the existent sex differences [6], and the adolescent dynamics [7] about which there are scarce data, require a clarification of the distribution and age-related neuroplasticity of this receptor in the BNST.

Therefore, we set as a goal of our study to investigate the expression of OXTR by immunohistochemistry in two subnuclei of BNST, i.e. the medial anterior (BNSTMA) and lateral posterior (BNSTLP).

Material and methods. We used 24 Wistar rats of both sexes distributed equally between males and females. They were divided into 20-days-old ($n = 12$) (preadolescent) and 60-days-old ($n = 12$) (postadolescent, adult) rats. All experiments were in keeping with the ethical guidelines of the EU Directive 2010/63/EU for the protection of animals used for scientific purposes, and with a permit No. 374 by the Bulgarian Food Safety Agency for working with experimental animals. The procedures were specified so as to avoid any suffering of the animals. The latter were anesthetized intraperitoneally with Pentobarbital (SupelcoTM, 35 mg/kg), and then each was transcardially perfused with phosphate-buffered saline, followed by a 4% formaldehyde solution. After that, the brains were taken out, and put in the fixative for 12 h at 4°C. Then they were processed for paraffin embedding. Afterwards, they were cut at 6 µm thick sections and mounted on poly-L-lysine (Sigma-Aldrich[®]) covered glass slides. Immunohistochemistry was performed, using a kit with a DAB-detection system (PolyQ Stain 2 step detection system goat anti-mouse/rabbit HRP, Peroxidase quench, DAB kit; quartet GmbH). The immunohistochemistry procedure was begun by blockage of the endogenous peroxidase for 10 min, followed by antigen retrieval using citrate buffer at 9°C for 20 min. Then, the primary rabbit polyclonal antibody (OXTR, dilution 1:300, Elabscience,) was applied and the slides were incubated overnight at 4°C in a humidified chamber. Thereafter, an incubation with the secondary antibody in the kit was performed for 30 min. The visualization of the reaction was with DAB as a chromogen. All the slides were proceeded simultaneously to standardize the results.

The images were obtained with a scanning microscope acquisition system (Olympus VS-ASW Image Acquisition software). Photomicrographs were taken from the BNSTMA and BNSTLP, both from the left and right sides to increase the number of measured neurons. Then the images were turned into black and white, and measured the intensity of grey in the grayscale (from 0 – black to 255 – white). At least 30 neurons from each subnucleus were assessed. We performed

the Kolmogorov–Smirnov test of normality. All the obtained data were not significantly different from the normal distribution; accordingly, we carried out the Student’s *t*-test to compare the means of the compared groups. After evaluating the *p*-value, if $p < 0.05$, there was a statistically significant difference between the means of the grayscale intensity of the compared groups.

Results. The results clearly showed that the immunohistochemical reaction against OXTR was more intense in preadolescent animals than in adults in both the investigated subnuclei of BNST (Fig. 1). We did not find any sex differences in the expression of OXTR in either age groups. In particular, the immunoreaction was localised predominantly on the periphery of the perikarya which complies with the membrane localisation of the receptor. After performing the statistical analysis we concluded that the observed adolescent decrease in OXTR expression levels was statistically significant in both of the tested regions (Fig. 2, 3). The means of grayscale intensity, the SD and Kolmogorov–Smirnov criteria of OXTR expression levels, together with the *t*-value and *p*-value of the compared groups are shown in Table 1.

T a b l e 1

Statistical data are represented for different groups of animals and both sub-nuclei of BNST

BNST nucleus	Experimental group	Mean	SD	K-S criteria
BNSTMA	20-days males	137.66	13.30	0.15262
	20-days females	139.64	10.10	0.08413
	60-days males	168.50	6.18	0.09911
	60-days females	166.07	7.37	0.09391
BNSTLP	20-days males	144.10	9.09	0.07215
	20-days females	147.21	6.85	0.0764
	60-days males	161.10	9.11	0.19579
	60-days females	162.39	7.95	0.12618
BNST nucleus	Compared groups		<i>t</i> -value	<i>p</i> -value
BNSTMA	20-days males/60-days males		−11.54771	< 0.00001
	20-days females/60-days females		−11.57439	< 0.00001
	20-days males/20-days females		−0.6544	0.515397
	60-days males/60-days females		1.38637	0.170942
BNSTLP	20-days males/60-days males		−7.23339	< 0.00001
	20-days females/60-days females		−7.92365	< 0.00001
	20-days males/20-days females		−1.49374	0.140665
	60-days males/60-days females		−0.58425	0.561321

Discussion. BNST is a limbic region rich in OXTR [5]. Our knowledge of the oxytocin system and OXTR plasticity may be crucial for understanding a lot of behavioural disorders. It is well documented that OXTR plays a role

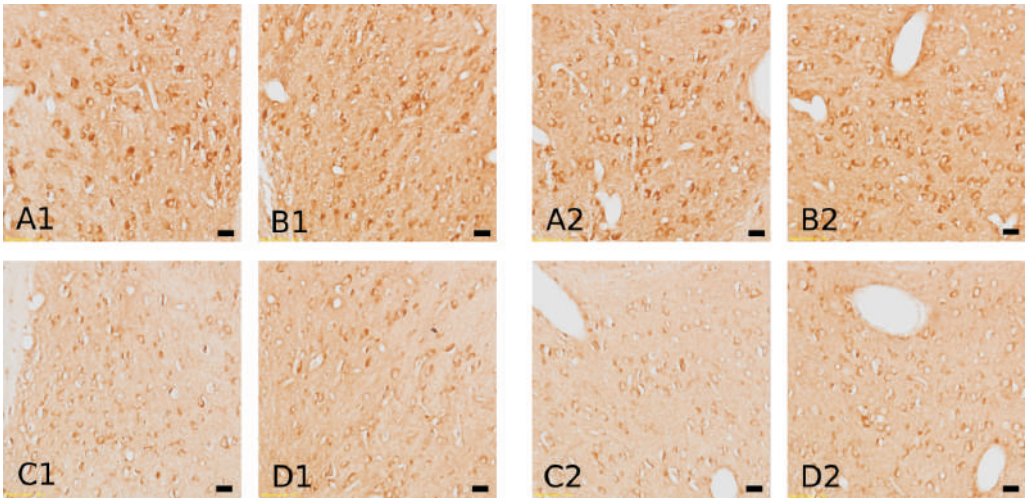


Fig. 1. Photomicrographs from the studied subnuclei of BNST (BNSTMA in photos labelled with number 1, and the BNSTLP in those with number 2), showing the expression of OXTR. In all of the photomicrographs, (A) represents preadolescent male animals, (B) preadolescent female, (C) postadolescent male, and (D) postadolescent female. In all the images, the immunolabelling of OXTR is clearly more intense in the preadolescent animals (A) and (B), than in the postadolescent (C) and (D). Scale bar = 20 μ m

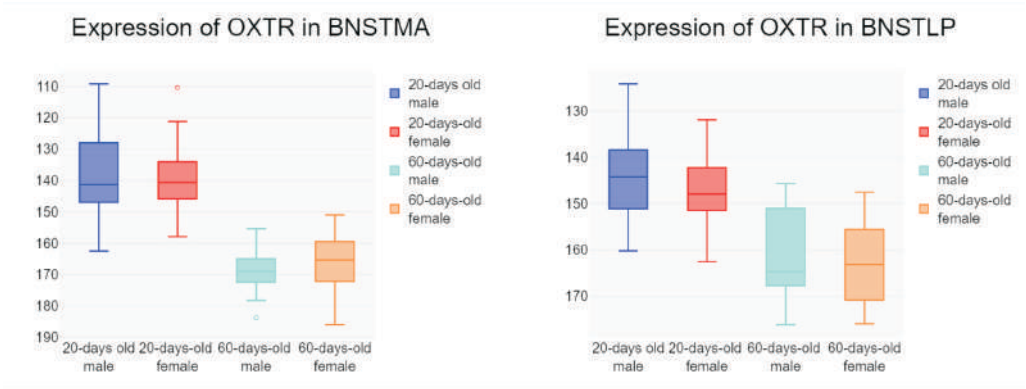


Fig. 2. Panel image, representing Box and Whiskers diagrams of the statistical data from the BNST subnuclei. There is a clearly significant adolescent decrease in the expression of OXTR, obtained via grayscale intensity

in the neurogenesis in the hippocampus [8], and also in the hypothalamus [4] in the early postnatal period. Therefore, all the impacts on the system during this stage can incur long-lasting effects [4, 9]. Moreover, there is evidence about the different distributional patterns and dynamics of OXTR [9, 10]. In particular the influence of oxytocin is not limited to the perinatal period. In addition, it has been shown that it could be a significant factor during the adolescent development [11, 12], when a great deal of neuroplasticity occurs in different brain regions [13].

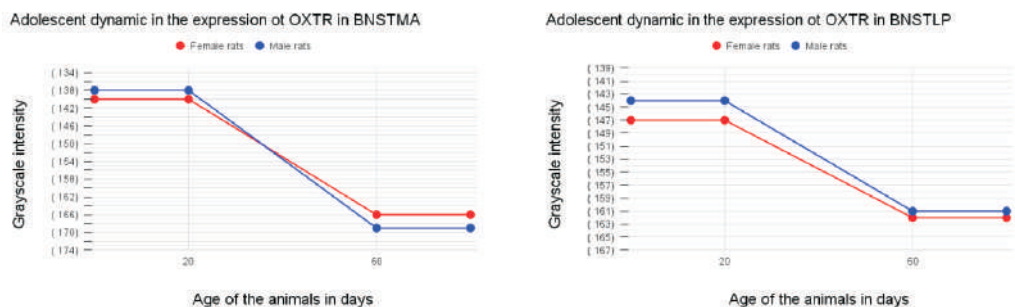


Fig. 3. Panel image showing linear diagrams of the means of grayscale intensity representing OXTR immunostaining strength. There is a visible decrease during the adolescent period

Therefore various changes in the oxytocin-OXTR system can be expected at this time. Moreover, it is known that the changes that happen during puberty could be long-lasting too [14]. BALES and PERKEYBILE [9] state that during development there is a down-regulation of OXTR in a lot of brain regions. It has also been shown that in humans there is a down-regulation of OXTR with aging in the brain [15]. Such a down-regulation in the adolescent period is reported by IMAIZUMI et al. [12] as well. The effect of oxytocin via its OXTR is generally anxiolytic, although there may be variations in the different age periods [16]. Conversely some authors claim that oxytocin via its receptors in BNST exerts antisocial and anxiogenic effects [17]. Moreover there is evidence for the mitigation of aggressive behaviour after the activation of OXTR in BNST [18]. Such a downregulation in the adolescent period reported by us in this study could explain the emerging aggressive behaviour in teenagers [19]. In spite of the differing data on the main effects of oxytocin, the conclusion made by VAIDYANATHAN and HAMMOCK [20] is ingenious. The authors claim that the regulation of OXTR is regional and species-specific, and it serves to adapt the behaviour-responsible regions in the brain to the social environment. Our results on the adolescent downregulation of OXTR in BNST are consistent with the existing literature about it in different brain regions. Such a phenomenon could explain the emerging aggressive behaviour in adolescence, and also the shifts in social conduct during the period.

The data obtained in this study provide novel information about the adolescent neuroplasticity of OXTR in BNST. Nonetheless, they do not provide evidence on the sex differences in the nucleus. Therefore BNST could possibly be excluded as a specific region and cannot at present be associated with sexual differences in anxiety disorders with which OXTR are usually linked. It can be inferred that the decrease in OXTR in BNST is a cause for the growing frequency of aggressive behaviour in puberty, and also the changes in social approaches. Thus, our understanding of the overall oxytocin receptor dynamics in the adolescent period could be fundamental for future experimental paradigms.

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