Combining Nissl Staining and Digital Densitometry to Accurately Quantify Oxytocin Receptors in the Human Substantia Nigra

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I. Introduction

- Oxytocin (OT) has been shown to be vital to social function in animals and may play a role in social deficits in individuals with Autism Spectrum Disorder (ASD)
- Autoradiography has been used to show the presence of oxytocin receptors (OXTR) in the human substantia nigra (SN)
- The SN is composed of two subsections: the dopaminergic pars compacta and the GABAergic pars reticulata
- Localization of OXTR within one subdivision of the SN or the other influences how we interpret the function of OT in that area
- Goals of the current study:
  1. To anatomically inform the analysis of OXTR density in the SN of postmortem human brain specimens
  2. To evaluate the effect of sex and ASD on SN OXTR

Figure 1 – Representative autoradiograms showing the process of creating OXTR-specific binding for subsequent quantification. A: total binding, B: non-specific binding, C: Subtraction of B from A, producing OXTR-specific binding.

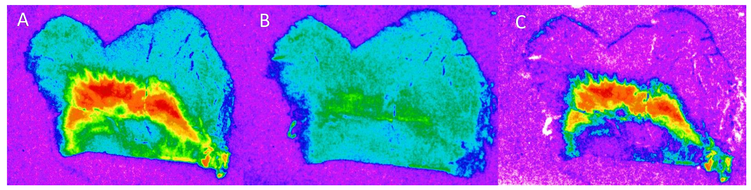
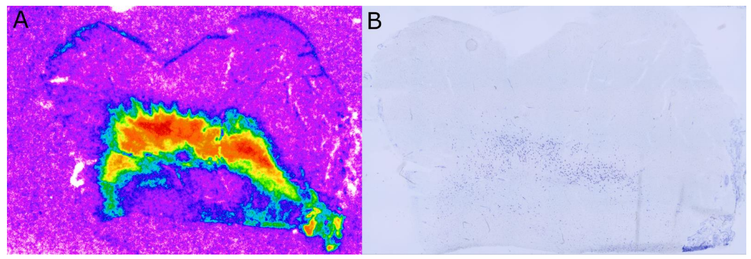


Figure 2 – Alignment of OXTR-specific binding (A) with counterstained tissue (B) for pars compacta quantification



II. Methods

- Unfixed, frozen blocks of de-identified human brain tissue provided by the University of Maryland Brain and Tissue Bank, which is a Brain and Tissue Repository of the NIH NeuroBioBank
- n=30 (8 typically developing males, 7 typically developing females, 8 males with ASD, 7 females with ASD)
- Brain blocks stored at -80°C, brought to -20°C and sectioned at 20µm on a cryostat
- OXTR autoradiography was performed using a commercially-available OXTR radioligand[1]
- To determine non-specific binding, adjacent slides were co-incubated with an OXTR antagonist (Figure 1B)
- Non-specific binding was subtracted from total binding to produce OXTR-specific binding autoradiograms (Figure 1C)
- Tissue sections that were previously processed for OXTR autoradiography were Nissl stained; this counterstaining technique also reveals dopaminergic neurons in the SN[2]
- Receptor binding density was measured using the MCID Digital Densitometry System; specifically:
  - Autoradiograms showing OXTR-specific binding (Figure 2B) were placed side by side with the corresponding Nissl-stained tissue sections (Figure 2A)
  - Dopaminergic pars compacta was outlined in a neuroanatomically informed way using the Nissl-stained sections as a reference.
- To determine the effects of sex and ASD on pars compacta OXTR density, we ran a two-way ANOVA. We found a main effect of sex and an interaction effect between sex and ASD.

III. Results

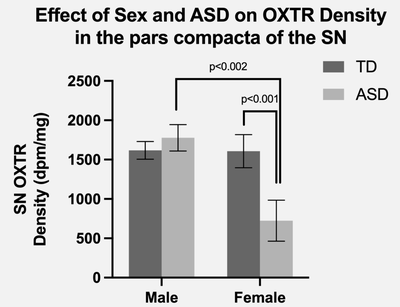


Figure 3 – Specimens from females with ASD had significantly reduced OXTR density in the pars compacta when compared to both males with ASD ($p<0.002$) and to typically developing (TD) females ($p<0.001$).

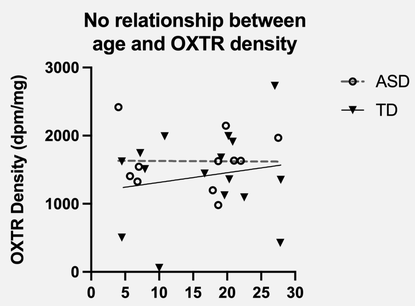


Figure 4 – There was no significant effect of age on OXTR density in either ASD or typically developing individuals across all specimens; this confirms that age is not a confounding factor in our analysis.

IV. Conclusions & Future Directions

- Females with ASD have reduced OXTR density in the pars compacta of the SN compared to unaffected females and compared to males with ASD.
- This result may be related to known sex differences in ASD symptomology: Females with ASD internalize their symptoms leading to anxiety, depression, and other emotional symptoms not seen as frequently in males with ASD[3].
- Our results support and extend previous evidence[1] that the ASD brain exhibits differences in OXTR levels in several regions.
- Future directions include measuring OXTR mRNA levels in adjacent sections from the same samples to see if this effect is driven by differences in gene expression.

1. Freeman, S.M., Palumbo, M.C., Lawrence, R.H. et al. Effect of age and autism spectrum disorder on oxytocin receptor density in the human basal forebrain and midbrain. *Transl Psychiatry* 8, 257 (2018). ; **2.** Domesick, V., Stinus, L., & Paskevich, P. (1983). The cytology of dopaminergic and nondopaminergic neurons in the substantia nigra and ventral tegmental area of the rat: A light- and electron-microscopic study. Neuroscience, 8(4), 743-765. ; **3.** Werling, D. M. & Geschwind, D. H. (2013). Sex differences in autism spectrum disorders. Current Opinion in Neurology, 26(2), 146-153.