Comenius University in Bratislava

Faculty of Mathematics, Physics and Informatics

Oxytocin effects on neurons isolated from dopaminergic brain regions crucial in social cognition

Master's thesis

Bratislava 2025 **Bc. Dalibor Putala**

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Master's thesis

Study program: Cognitive Science

Institute of supervision: Department of Neuroscience, Institute of Experimental Endocrinology, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, and the Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava

Supervisor: doc. RNDr. Ján Bakoš, PhD.

Bratislava 2025 **Bc. Dalibor Putala**

Comenius University Bratislava
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THESIS ASSIGNMENT

Name and Surname:	Bc. Dalibor Putala
Study programme:	Cognitive Science (Single degree study, master II. deg., full time form)
Field of Study:	Computer Science
Type of Thesis:	Diploma Thesis
Language of Thesis:	English
Secondary language:	Slovak

Title: The effects of oxytocin on neurons isolated from dopaminergic brain regions involved in social cognition

Annotation: Oxytocin is a neuropeptide well-known for its peripheral and behavioral effects, produced in the hypothalamus and distributed throughout the brain, including the midbrain and striatum, where it influences neurotransmission. Oxytocin plays a role in brain development, neurogenesis, and the growth and morphology of neurons.

Aim: Examine the effect of oxytocin on the outcomes of transient silencing of the Shank3 gene in primary dopaminergic neuronal striatal and midbrain cell cultures, which represent cells from regions involved in social cognition. Focus on (a) neurite arborization and length, and (b) the intensity of fluorescence signals of selected synaptic parameters (neuroligin 3, D2 receptor). Analyze images of neurons obtained from cytological samples using fluorescence microscopy.

Literature:
Bakos, J., Srancikova, A., Havranek, T., & Bacova, Z. (2018). Molecular Mechanisms of Oxytocin Signaling at the Synaptic Connection. Neural Plasticity, 2018, 1–9.
Havranek, T., Bacova, Z., & Bakos, J. (2024). Oxytocin, GABA, and dopamine interplay in autism. Endocrine Regulations, 58(1), 105–114.
Pavăl, D. (2017). A Dopamine Hypothesis of Autism Spectrum Disorder. In Developmental Neuroscience (Vol. 39, Issue 5, pp. 355–360). S. Karger AG.

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Assigned:	19.02.2024
Approved:	03.03.2024

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ZADANIE ZÁVEREČNEJ PRÁCE

Meno a priezvisko študenta: Bc. Dalibor Putala
Študijný program: kognitívna veda (Jednoodborové štúdium, magisterský II. st., denná forma)
Študijný odbor: informatika
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Názov: The effects of oxytocin on neurons isolated from dopaminergic brain regions involved in social cognition
Vplyv oxytocínu na neuróny izolované z dopaminergných oblastí mozgu súvisiacich s reguláciou sociálnych funkcií

Anotácia: Oxytocín je neuropeptid dobre známy pre svoje periférne a behaviorálne účinky, produkovaný v hypotalame a distribuovaný v mozgu, vrátane stredného mozgu a striata, kde ovplyvňuje neurotransmisiu. Oxytocín hrá úlohu pri vývoji mozgu, neurogenéze a raste a morfológii neurónov.

Cieľ: Preskúmajte vplyv oxytocínu na dôsledky silencingu Shank3 génu v primárnych neuronálnych bunkových kultúrach izolovaných z dopamínergných oblastí striata a stredného mozgu experimentálneho zvieraťa, ktoré predstavujú oblasti mozgu zapojené do sociálnej kognície. Hodnotené budú 1) arborizácia a dĺžka neuritov a 2) intenzita fluorescenčných signálov vybraných synaptických parametrov (neuroligín 3, D2 receptor).

Literatúra: Bakos, J., Srancikova, A., Havranek, T., & Bacova, Z. (2018). Molecular Mechanisms of Oxytocin Signaling at the Synaptic Connection. Neural Plasticity, 2018, 1–9.
Havranek, T., Bacova, Z., & Bakos, J. (2024). Oxytocin, GABA, and dopamine interplay in autism. Endocrine Regulations, 58(1), 105–114.
Pavăl, D. (2017). A Dopamine Hypothesis of Autism Spectrum Disorder. In Developmental Neuroscience (Vol. 39, Issue 5, pp. 355–360). S. Karger AG.

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Dátum zadania: 19.02.2024

Dátum schválenia: 03.03.2024

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vedúci práce

Acknowledgments

The thesis is a part of other studies coming from the Department of Neuroscience Biomedical Research Center, Slovak Academy of Science, and from the Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava which systematically investigate oxytocin's impacts on brain development and its changes in neurodevelopmental disorders. Mutations and/or modifications in the *SHANK3* gene are a major focus in this area in this department and it is also the topic of currently funded projects: Characterization of excitatory and inhibitory neurons in the brain areas relevant for the development of social behavior in the autism-related model, APVV-21-0189.

I would like to express my gratitude to my supervisor doc. RNDr. Ján Bakoš, PhD. for his academical guidance, time and friendly, yet professional approach. I am also thankful to Mgr. Tomáš Havránek, PhD. for his assistance with image acquisition and analysis, and to Mgr. Zuzana Bačová, PhD. for her support with statistics and laboratory work. Additionally, I would like to thank to Mgr. Denisa Mihalj for her help with laboratory work. The friendly and supportive atmosphere within this research group greatly contributed to the completion of my master's thesis.

Abstract

Bc. Dalibor Putala: Oxytocin effects on neurons isolated from dopaminergic brain regions crucial in social cognition

Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, and Department of Neuroscience, Institute of Experimental Endocrinology, Biomedical Research Centre, Slovak Academy of Sciences, and Institute of Physiology, Faculty of Medicine, Comenius University in Bratislava. Master's thesis 2025, 66 pages

Oxytocin is a neuropeptide well-known for its peripheral and behavioral effects. It plays an important role in regulating dopaminergic brain regions, essential for reward, cognition, and motivation. Dysfunctions in these regions have been linked to neurodevelopmental disorders, including autism spectrum disorder (ASD). Their development and function are influenced by genetic variations, particularly by genes encoding synaptic scaffolding ("linking") proteins, such as SHANK3 (SH3 and multiple ankyrin repeat containing domains 3). These proteins maintain neuronal structure and synaptic integrity by regulating cytoskeletal organization. Impairments in SHANK3 and related proteins have been associated with synaptic deficits, which are considered a common underlying mechanism in certain subsets of ASD. Oxytocin may affect the regulation of neuronal cell morphology in dopaminergic brain regions, impacting certain cell types like inhibitory GABAergic neurons, especially in a condition of SHANK3 deficiency. This study aims to investigate the impact of oxytocin on the consequences of transient *Shank3* gene silencing in primary neuronal cultures derived from a striatal and midbrain neonatal rat dopaminergic regions. Specifically, the study evaluates 1) neurite arborization and length, and 2) the intensity of fluorescence signals of selected synaptic parameters (neuroligin 3, D2 receptor). The main hypothesis is that oxytocin will elongate neurites, increase their number, and simultaneously enhance the intensity of the observed signals, thereby compensating for the deficits caused by *Shank3* downregulation.

The results show that oxytocin did not stimulate neurite outgrowth in striatal or midbrain primary neuronal cells under control conditions. However, a significant increase in neurite length was observed in striatal neurons when oxytocin was combined with *Shank3* gene silencing. The key finding is that *Shank3* gene silencing reduces intersections near the nucleus in GABAergic neurons, with variability in striatal and midbrain neurons, with only minor effects of oxytocin on these arborizations. Oxytocin notably affected D2R levels, decreasing intensity in the control group and slightly increasing it in the silenced group. These differential

effects of oxytocin, depending on SHANK3 presence, suggest a complex interaction in regulating neuronal function, with distinct mechanisms in cells unaffected by silencing. It is likely that oxytocin impacts neurite growth, but this effect varies with brain region, receptor density, and developmental stage, which needs further investigation.

Abstrakt

Bc. Dalibor Putala: Vplyv oxytocínu na neuróny izolované z dopaminergických oblastí mozgu kľúčových pre sociálnu kogníciu

Fakulta Matematiky, Fyziky a Informatiky, Univerzita Komenského v Bratislave a Oddelenie Neurovied, Ústav experimentálnej endokrinológie, Biomedicínske centrum SAV, Bratislava a Fyziologický ústav, Lekárska fakulta, Univerzita Komenského v Bratislave. Diplomová práca 2025, 66 strán

Neuropeptid oxytocín je známy svojimi periférnymi a behaviorálnymi účinkami. Zohráva dôležitú úlohu pri regulácii dopaminergických oblastí mozgu, ktoré sú zapojené do riadenia procesov odmeny, motivácie a kognície. Dysfunkcie v dopaminergických oblastiach sú spojené s neurovývojovými poruchami, vrátane poruchy autistického spektra (ASD). Ich vývoj a funkcia sú ovplyvnené genetickými faktormi, najmä mutáciami v génoch kódujúcich synaptické, tzv. skafoldové proteíny, ako napríklad SHANK3 (SH3 domain and ankyrin repeat containing 3). Tieto proteíny sa podieľajú na tvorbe synapsií a organizácii cytoskeletu. Poškodenie SHANK3 a príbuzných proteínov je spojené so synaptickými deficitmi, ktoré sa považujú za jeden z mechanizmov prejavu a symptomatológii niektorých typov ASD. Oxytocín môže ovplyvniť reguláciu morfológie neuronálnych buniek v dopaminergických oblastiach mozgu, s vplyvom na určité typy buniek, ako sú inhibičné GABAergické neuróny, najmä v podmienkach SHANK3 deficiencie. Cieľom tejto diplomovej práce je preskúmať vplyv oxytocínu na dôsledky „silencingu“ *Shank3* génu v primárnych neuronálnych bunkových kultúrach izolovaných z dopaminergichých oblastí striata a stredného mozgu neonatálneho potkana. Práca konkrétne hodnotila 1) arborizáciu a dĺžku neuritov a 2) intenzitu fluorescenčných signálov vybraných synaptických parametrov (neuroligín 3, D2 receptor). Hlavnou hypotézou bolo, že oxytocín predĺži neurity, zvýši ich počet a súčasne zvýši intenzitu pozorovaných signálov, čím by kompenzoval deficity spôsobené nedostatkom SHANK3.

Ukázalo sa, že oxytocín nestimuloval rast neuritov v primárnych neuronálnych bunkách striata ani stredného mozgu v kontrolných podmienkach. Významné zvýšenie dĺžky neuritov bolo pozorované v striatálnych neurónoch, až pri kombinácii oxytocínu a „silencingu“ *Shank3*. Dôležitým zistením je, že „silencing“ *Shank3* znížil počet neuritov/vetvenie najmä v blízkosti jadra v GABAergických neurónoch s relatívne veľkou variabilitou pri striatálnych neurónoch a neurónoch stredného mozgu a iba malými účinkami oxytocínu na tieto arborizácie. Oxytocín výrazne ovplyvnil hladinu D2R iba v striatálnych bunkách, kde znížil intenzitu v kontrolnej

skupine a mierne ju zvýšil v podmienkach „silencingu“. Rozdielne účinky oxytocínu v závislosti od prítomnosti SHANK3 naznačujú odlišné mechanizmy v jednotlivých bunkách. Je pravdepodobné, že oxytocín ovplyvňuje rast neuritov, ale tento účinok sa líši v závislosti od oblasti mozgu, hustoty receptorov a vývinového štádia, čo si vyžaduje ďalší výskum.

Abbreviations

ADHD – attention deficit hyperactivity disorder

ASD – autism spectrum disorder

DA – dopamine

DIV – day *in vitro*

DR – dopamine receptor

GABA – γ-aminobutyric acid

GAD – glutamate decarboxylase

MAP – microtubule associated protein

mRNA – messenger RNA

MSN – medium spiny neurons

NAc – nucleus accumbens

NGF – nerve growth factor

NLGN – neuroligin

OXT – oxytocin

OXTR – oxytocin receptor

PBS – phosphate-buffered saline

PVN – paraventricular nucleus

SHANK – SH3 and multiple ankyrin repeat containing domains

siRNA – small interfering RNA

SN – substantia nigra

VTA – ventral tegmental area

Introduction

To elucidate behavior, it is essential to examine multiple levels of analysis, from molecular mechanisms to systemic connections. The fundamental principles of behavior are rooted in molecular processes, where various neurotransmitters and hormones interact with distinct receptors and proteins. Higher organizational levels show significant importance in the density of specific proteins and cells in different brain regions. Moreover, the interconnection of these regions plays a crucial role. Ultimately, the complexity of behavior arises from a confluence of internal and external factors, interacting with the organizational levels mentioned above. There exist significant species-specific differences, yet animal models continue to play a crucial role in developing hypotheses about human behavior. This research aims to acknowledge the complexity of the interplay and to investigate various levels to gain a deeper understanding of the underlying mechanisms driving behavior. Among the numerous factors influencing these mechanisms, oxytocin plays an important role.

Oxytocin is a well-studied neuropeptide synthesized in the hypothalamus. First effects of oxytocin were described at the beginning of the 20th century as uterine-contracting properties. Its structure was described 50 years later. For long time the research focused on its peripheral roles in reproduction. Over the past several decades, research has focused more on the effects on the brain. While extensively studied for its behavioral effects, and its impact on brain development, specific cellular mechanisms of behavioral influence remain less understood with emerging studies in the last few years (Bakos et al., 2018; Knoop et al., 2022). It is being explored both as a potential explanation for social variability in typically developing individuals and as a contributor to, or therapeutic target for, social deficits observed in neurodevelopmental disorders such as autism spectrum disorder (ASD) (Havranek et al., 2024).

Moreover, oxytocin plays a crucial role in regulating dopaminergic brain regions, which are essential for processes such as reward, cognition, and motivation (Klein et al., 2019). Dysfunctions in these regions have been linked to neurodevelopmental disorders, including ASD (Pavăl, 2017). Research indicates that their development and function are influenced by genetic variations (Blum et al., 2024), particularly in genes encoding synaptic scaffolding ("linking") proteins like SH3 and multiple ankyrin repeat domains (SHANK) proteins. These proteins maintain neuronal structure and synaptic integrity by regulating cytoskeletal organization. Impairments in SHANK3 and related proteins have been associated with synaptic deficits, which are considered a common underlying mechanism in certain subsets of ASD (Monteiro & Feng, 2017).

Furthermore, synaptic connections are essential for the proper and complex functioning of communication in the brain. They are strongly impacted by the morphology of the neurons, including the shape and complexity of neurites, as well as the composition and structure of synaptic proteins, such as SHANK proteins. During development, neurons undergo extensive morphological changes (Chklovskii, 2004; Colón-Ramos, 2009). They dynamically adjust in response to experience and learning even throughout the life and show different sensitivity in specific periods (Citri & Malenka, 2008). Understanding mechanisms regulating neuronal morphology is crucial for comprehending how synaptic plasticity and cognitive function are achieved. Disruptions in these mechanisms can contribute to a range of cognitive and social impairments, as well as neurological and neurodevelopmental disorders. Understanding of these processes might lead not to only comprehension but also to relevant therapeutical targets of various disorders.

This work's interdisciplinarity lies in the combination of biochemical, neuroscientific, and socio-behavioral aspects, including behavioral neuroendocrinology and social cognition. From a methodological perspective, the work covers interdisciplinarity by integrating multiple approaches. It combines animal models exhibiting autistic symptoms, *in vitro* isolation of primary neuronal cells, molecular and cellular techniques such as gene silencing, and confocal microscopy of cytological samples. Additionally, it employs specialized software for tracking and evaluating neuron morphology and immunofluorescence signal intensity.

1. Literature overview

1.1. Oxytocin

The first neuropeptide to have its structure described was oxytocin (OXT). It consists of nine amino acids (Du Vigneaud et al., 1953). It is synthesized in neurons of the paraventricular (PVN), supraoptic, and accessory nuclei of the hypothalamus along with arginine-vasopressin. There are two subtypes of OXT neurons. Magnocellular and parvocellular cells which differ in morphology, OXT synthesis, and electrophysiological properties (Luther & Tasker, 2000; Xiao et al., 2017). The neurons project to the posterior, and anterior pituitary, as well as other brain regions (Baribeau & Anagnostou, 2015). Anatomical mapping with OXT neuron-specific viruses uncovered the presence of long-range axonal projections of hypothalamic OXT neurons throughout the brain (Knobloch et al., 2012). Some of the OXT is transported into the dendrites of neurons from which it can act on surrounding tissue. Dendritic release is limited to the main sites of OXT synthesis (Brown et al., 2020; Neumann, 2007).

1.1.1. Oxytocin modulation

The effect of OXT is mediated by its receptor (OXTR) (Figure 1). The receptor is part of G protein-coupled receptor family (Kimura et al., 1992). OXT has also a low affinity to vasopressin receptors (Tribollet et al., 1988). Chapter **1.1.5. Oxytocin effects on behavior (Manipulating OXT)** introduces OXT's behavioral effects, but to establish a foundational understanding within the molecular mechanisms Froemke et al. describes three modes of oxytocinergic neuromodulation (Figure 2). First, second, and third-order modulation, while all starts through OXTR (Froemke & Young, 2024).

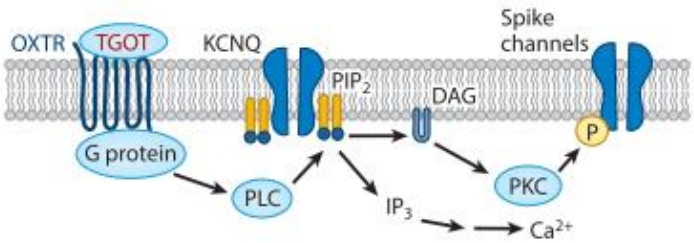

Figure 1 – The suggested metabolic pathway of oxytocin receptor signaling influencing spike shape and excitability (Froemke & Young, 2024) OXTR – oxytocin receptor; TGOT – oxytocin antagonist [Thr 4, Gly 7]-oxytocin; KCNQ- M-type K+ channel; PIP_2- phosphatidylinositol-4,5-bisphosphate; PLC – phospholipase C; DAG – diacylglycerol; IP3- inositol 1,4,5-trisphosphate; PKC – protein kinase C; P – phosphorylation

First-order neuromodulation of OXT shows direct enhancement of neuronal excitability by modulating ion channel activity in the membrane, thereby influencing synaptic transmission (Jo et al., 1998). For example, CA2 pyramidal neurons in the hippocampus, essential for social recognition memory (Hitti & Siegelbaum, 2014), were found to depolarize after OXTR

activation in whole-cell recordings from hippocampal brain slices. Furthermore, OXT can modulate changes of spike shape and threshold of CA2 cells (Tirko et al., 2018). In some cases, OXT can lower the neuronal excitability, suggesting a bidirectional communication between pre- and postsynaptic regions (Kombian et al., 2002).

Second-order neuromodulation acts on inhibitory interneurons rather than directly on excitatory neurons. Synaptic inhibition seems to be a major mode of oxytocinergic modulation in the brain and offers fine-tuned control over the neural activity. It can selectively amplify specific inputs while maintaining broader circuit stability. This allows OXT to boost cortical signal-to-noise ratio by reducing background activity and enhancing information transfer, therefore improving brain information processing. This was shown in rat hippocampal cells where OXT activated parvalbumin-positive fast-spiking neurons, which are a subset of GABAergic neurons, and influenced firing patterns of the pyramidal neurons (Owen et al., 2013).

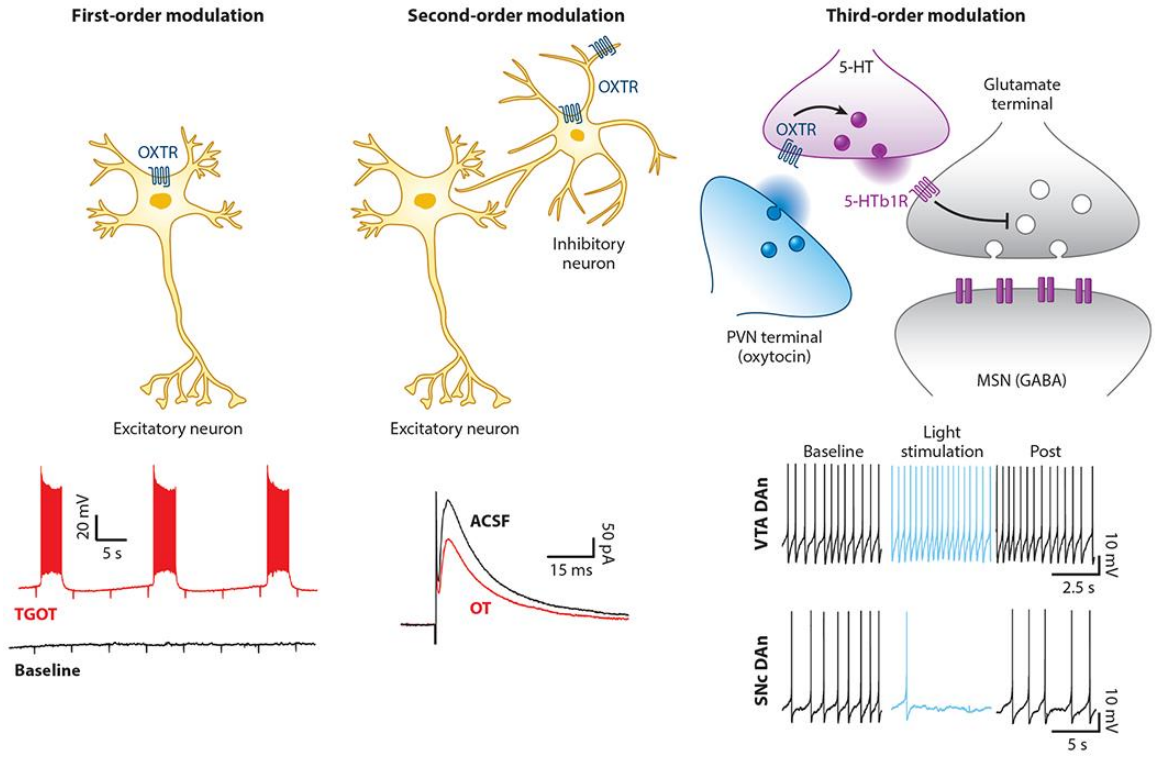

Figure 2 – Suggested types of oxytocin modulation. First-order modulation, oxytocin directly depolarizes principal excitatory (Left). Second-order modulation, oxytocin reduces inhibitory transmission by either increasing spontaneous firing or impairing γ-aminobutyric acid (GABA) release (Middle). Third-order modulation, oxytocin acts on other modulation system (Top right) and oxytocin increases ventral tegmental area dopamine neuron (VTA DAn) firing but decreases subsantia nigra pars compacta dopamine neuron firing (SNc Dan) (Right bottom). OT – oxytocin; OXTR – oxytocin receptor; TGOT – [Thr4, Gly7]-oxytocin; ACSF – artificial cerebrospinal fluid; PVN – paraventricular nucleus; MSN – medium spiny neuron; 5-HT – 5-hydroxytryptamine; 5-HTb1R – 5-hydroxytryptamine 1B receptor. (Froemke & Young, 2024).

Third-order neuromodulation or metamodulation refers to the modulation of modulation, where OXT influences other neuromodulatory systems and their effects on neural circuits. OXT can

indirectly affect gene expression, excitability, synaptic transmission, and overall neural computations (Jurek & Neumann, 2018). For instance, OXT fibers innervate various brain regions, including the nucleus accumbens (NAc) and the midbrain dopaminergic centers, where OXT can modulate the activity of other neurotransmitter systems, such as serotonin and dopamine (DA) (Dölen et al., 2013; Xiao et al., 2017). Electrophysiological recordings of DA neurons revealed that both the application of OXT and optogenetic stimulation of oxytocinergic terminals heightened spontaneous firing of DA neurons in the ventral tegmental area (VTA) but inhibited the spontaneous firing of DA neurons in the substantia nigra (Xiao et al., 2017). This interaction suggests that OXT does not act in isolation but can enhance or inhibit the effects of other neuromodulators, potentially influencing the development and connectivity of neurons.

Understanding these pathways can provide insights into how OXT may facilitate or alter neurodevelopmental processes, particularly in the context of social behavior and bonding, which are often influenced by the structural and functional properties of neurons in these regions.

1.1.2. Oxytocin's role in alterations of synaptic connections

Synaptic plasticity is a crucial property of every neuronal connection. It involves structural and functional alterations of synaptic regions occurring during development and throughout life. Bakos et al. reviewed various mechanisms of OXT on these alterations (Bakos et al., 2018). They consist of 1) Modulation of synaptic activity. This is done by regulating ion channels or by more complex modulations described in the previous chapter. 2) Impact on neurogenesis. OXT has been shown to stimulate the neurogenesis of the cells in the hippocampus (Lin et al., 2017), even under stress conditions in adult rats, suggesting a protective role of OXT (Leuner et al., 2012). 3) Changes in synapse numbers. Cell cultures from OXTR knockout mice showed an increase in the number of excitatory presynapses and a decrease in inhibitory presynapses, suggesting a role of OXT in balancing excitatory and inhibitory synaptic connections (Ripamonti et al., 2017). 4) Cytoskeletal regulation. OXT promotes the formation of cytoskeletal networks in brain cells (Lestanova et al., 2016a; Wang & Hatton, 2006). It also regulates the expression of proteins associated with synaptic scaffolding ("structuring"), such as postsynaptic density protein 95 (Miyazaki et al., 2016) and proteins from the SHANK family (Zatkova et al., 2018), which are critical for maintaining synaptic structure. All the mechanisms mentioned above are part of the pre- and postsynaptic modulation and the effects themselves depend on the cell type and the region of the brain (Bakos et al., 2018).

1.1.3. Oxytocin receptor's spatiotemporal distribution

Even though the number of OXT neurons remains similar from gestation to adulthood (Quintana & Guastella, 2020), there are spatiotemporal differences in the distribution of the OXTR, that might affect the developmental, behavioral, and functional aspects (Rokicki et al., 2022). They differ also between different sexes and species (Freeman et al., 2020; Rogers Flattery et al., 2022). A study from 1992 showed contrasting patterns of OXTR distribution in monogamous prairie vole and polygamous montane vole. They believe the distribution of the receptors influences these variations in behavior (Insel & Shapiro, 1992), which is also hypothesized in newer studies (Rokicki et al., 2022; Young & Zhang, 2021).

The temporal difference might represent stages of development related to survival and reproduction connected with unique behavioral changes. For instance, in individuals diagnosed with mood disorders, OXTR expression is elevated in the dorsolateral prefrontal cortex (Lee, Sheskier, et al., 2018), whereas it is diminished in the temporal cortex of those diagnosed with schizophrenia (Uhrig et al., 2016).

In humans generally (Figure 3), OXTR expression in the brain increases significantly just before birth, reaching its highest levels during early childhood. OXTR expression also shows a general increase during late adulthood compared to the average across the lifespan. Regional analyses revealed heightened OXTR expression in the striatum during the third trimester, as well as a second peak observed throughout adolescence and adulthood (Rokicki et al., 2022). Males demonstrated a more pronounced early childhood peak in OXTR expression, along with greater regional differentiation in brain expression patterns. Expression in the mediodorsal nucleus of the thalamus was increased during childhood, which is connected to the regulation of attention and memory in association with the prefrontal cortex. In females, the strongest peak of OXTR expression was observed around birth, with a smaller peak in childhood and a dip during adolescence. The most expressed OXTR region in females was the cerebral cortex during adulthood (Rokicki et al., 2022). These differences may reveal dissimilar critical periods between sexes for experience-dependent development mediated by OXT signaling (Hammock, 2015).

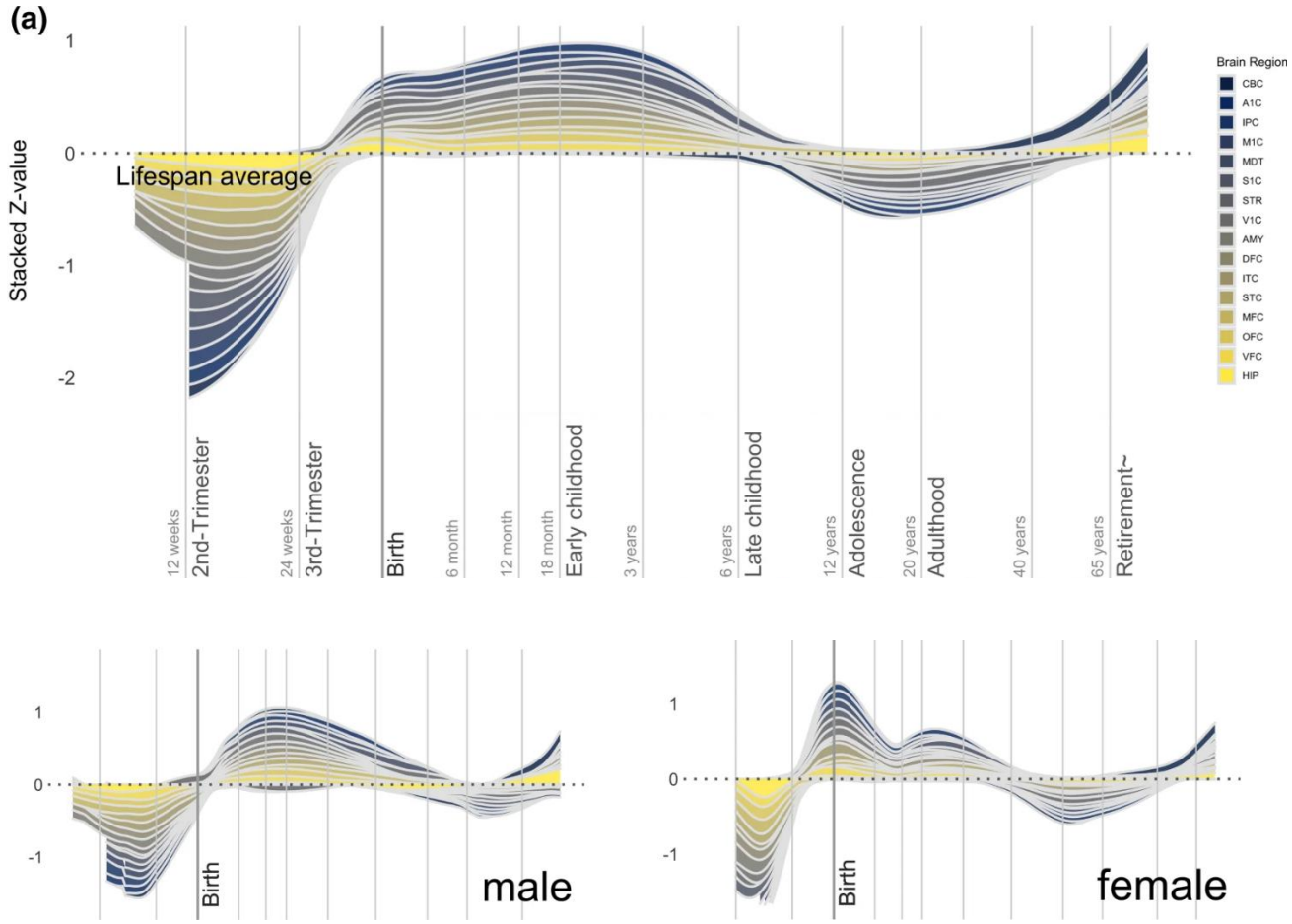

Figure 3 – Spatiotemporal distribution of oxytocin receptor in humans. Ribbon plots depict normalized gene expression across sixteen brain regions, stacked and scaled relative to the lifespan average. Time is presented on a log10 scale. Separate plots for males and females, also normalized to their respective lifespan averages. CBC – cerebellar cortex; A1C – primary auditory cortex; IPC – inferior parietal cortex; M1C – primary motor cortex; MDT – mediodorsal nucleus of the thalamus; S1C – primary somatosensory cortex; STR – striatum; V1C – primary visual cortex; AMY – amygdala; DFC – dorsolateral prefrontal cortex; ITC – inferolateral temporal cortex; STC – caudal superior temporal cortex; MFC – medial prefrontal cortex; OFC – orbital frontal cortex; VFC – ventrolateral prefrontal cortex; HIP – hippocampus (adapted from Rokicki et al., 2022).

Furthermore, the distribution of OXTR expression showed a strong correlation with dopaminergic gene expression and closely aligned with neural activity patterns associated with anticipatory, appetitive, and aversive mental states, as well as homeostatic regulation. The strong spatiotemporal correlation between OXTR and D2 receptor gene expression (Rokicki et al., 2022) suggests that OXT signaling works synergistically with dopaminergic signaling to support learning throughout development, especially during critical developmental periods (Love, 2014; Rokicki et al., 2022).

1.1.4. Stimuli triggering oxytocin release (Measuring OXT)

It is essential to distinguish between peripheral and intracerebral release in the context of stimuli-inducing release of OXT. Peripheral secretion can be quantified by measurements of blood, saliva, or urine OXT levels. The most common stimuli triggering OXT secretion into the periphery are parturition-related events (Douglas et al., 2002), lactation (Higuchi et al., 1986), mating and sexual stimulation (Carmichael et al., 1987), and stress-related stimuli (Lang et al., 1983). According to Jurek and Neumann, all the mentioned stimuli also activated OXT release in the brain (Jurek & Neumann, 2018). Over the past decades, OXT levels have been evaluated in various extracellular fluids mentioned above, including non-peripheral cerebrospinal fluid. Nevertheless, the accuracy and reliability of these measurements remain subjects of considerable debate (MacLean et al., 2019; Tabak et al., 2023). The most prevalent method is measuring OXT levels in blood plasma. Although radioimmunoassays were traditionally used (Bakos et al., 2007), ELISA assays are now more frequent (Husarova et al., 2016; Mora-Jensen et al., 2024). The main method of direct intracerebral OXT measurement is microdialysis, focusing on extracellular fluid of selected brain regions (Grossmann et al., 2024; Kendrick, 1990). This method involves surgically implanting a probe to a selected region, in which local extracellular fluid is able to diffuse from areas of higher concentration to areas of lower concentration. It has relatively low temporal resolution (>1min) (Landgraf & Neumann, 2004). OXT release independent of peripheral secretion was showed during emotional stress and social defeat (Engelmann et al., 1999). Stress-free social stimuli, such as social discrimination test, led to an increase of OXT level in the extracellular fluid during retrieval (Lukas et al., 2013). In non-social study OXT levels in the posterior pituitary in the brain were decreased while plasma levels have not been altered after prolonged voluntary exercise in rats (Bakos et al., 2007).

1.1.5. Oxytocin effects on behavior (Manipulating OXT)

Different methods of OXT level manipulation are used to study the direct effect of OXT on physiological or behavioral aspects. One of the first combined effects of OXT and arginine-vasopressin on behavior was described in rodents in connection to learning and memory (De Wled, 1965), sexual (Melin & Kihlström, 1963), and maternal behavior (Pedersen & Prange, 1979). Furthermore, the pro-social (Uvnäs-Moberg, 1998b), anxiolytic, and anti-stress effects (Uvnäs-Moberg, 1998a) of OXT led to increased interest in research since the 1990s. With even higher interest after a study of OXT effect on human behavior after intranasal application

(Heinrichs et al., 2003). Most studies are performed on rodents, sheep, and voles and non-human primates. However, since 2003, human social behavioral studies have grown every year (Jurek & Neumann, 2018). The intranasal and intravenous administration brings mixed results, which increase concerns about missing evidence on precise mechanism on the influence of brain activity (Kendrick et al., 2018). These concerns are supported by the fact that only a very small fraction of OXT could potentially get through the blood-brain barrier (Mens et al., 1983), although a higher amount is able to get to the cerebrospinal fluid (Lee et al., 2018). Majority of human studies measure only blood levels of OXT which do not have to mirror brain levels. According to Kendrick et al. the most reasonable conclusion of intranasal OXT mechanism on reported behavior is through yet unspecified direct central and indirect peripherally mediated actions (Kendrick et al., 2018). So far, one research group found limited evidence suggesting specific mechanism of OXT crossing the blood brain barrier (Higashida et al., 2024).

OXT has been found to influence responses to social stimuli by increasing the salience of social cues. In an earlier study on voles, OXT release into the ventral striatum promoted affiliative social behavior (Liu & Wang, 2003). During normal behavior, rodents usually explore novel stimuli introduced by unfamiliar rodent. This behavior is decreased over repeated exposure, which is described as social memory. Studies on mice lacking OXT gene show impaired social memory/recognition which was recovered by OXT treatment before the social encounter started. OXT antagonist had similar effect on the social memory as OXT knockout mice. On the other hand, the OXT knockout mice did not have deficits in spatial memory or non-social olfactory memory (Ferguson et al., 2000). More recent study conducted with wireless optogenetic headset, which enabled movement of rodents in a semi-natural environment, showed that prolonged PVN OXT neurons stimulation increased both prosocial and agonistic behavior (Anpilov et al., 2020). This study further supports notion of OXT being social salience modulator depending on the social context rather than only having prosocial properties. Bartz et al. added an interactionist perspective which does not rely only on general social contextual and situational factors but also on dispositional (internal) factors (Bartz et al., 2011). Specific types of stimuli may vary in value and salience for different individuals, especially humans, same as their expectations regarding the meaning of the stimuli. These psychological factors, along personality traits, contribute to individual differences in behavior within a given context (Mischel & Shoda, 1995) and should be taken into consideration in evaluating the effects of OXT on humans. To show clearer picture, there exist also other hypotheses of OXT effects such as Social-approach/withdrawal hypothesis (Kemp & Guastella, 2011), or a newer General

approach-avoidance hypothesis of OXT, which considers not only social but also non-social effects of OXT (Harari-Dahan & Bernstein, 2014).

1.2. Dopaminergic system

Dopamine (DA) is an organic compound from the catecholamine group. It acts as a neurotransmitter that plays a pivotal role in the functioning of the central and peripheral nervous system. It is also a precursor to norepinephrine and epinephrine. DA is primarily synthesized in dopaminergic neurons, with the main regions of synthesis located in the substantia nigra within the basal ganglia, the ventral tegmental area (VTA) in the midbrain, and the hypothalamus. As a neurotransmitter DA facilitates communication between neurons influencing physiological and psychological processes (Juárez Olguín et al., 2016). DA neurons show three main patterns of activity: an inactive - hyperpolarized firing pattern, a burst or 'phasic' mode, and tonic – single-spike firing mode (Lodge & Grace, 2011).

DA exerts its effects through dopamine receptors (DR). There are five subtypes of DR ranging from 1 to 5. They are classified as D1-class receptors (D1R, D5R) and D2-class receptors (D2R, D3R, D4R) and are used as well-established pharmacological targets of numerous disorders (Beaulieu et al., 2014). DR signaling pathways are mediated through G protein-dependent and -independent mechanisms. D1-class receptors are positively coupled to G proteins and facilitate excitatory neurotransmission (Keeler et al., 2014). They have lower affinity which makes them more sensitive to phasic changes than to changes in tonic firing (Dreyer et al., 2010). D2-class receptors are negatively coupled to G proteins which provides inhibitory feedback in dopaminergic circuits (Keeler et al., 2014). They have a greater affinity, ranging from 10- to 100-fold, and therefore are more sensitive to changes in tonic DA levels (Dreyer et al., 2010; Martel & Gatti-McArthur, 2020).

DR subtypes create complexes in form of homo or heterodimers *in vivo.* The authors believe that original classification of DRs signaling mechanism is not sufficient due to the complexity of heterocomplexes (Martel & Gatti-McArthur, 2020). In the NAc D1- and D2-class receptors work in cooperativity as heteromers in the same neuronal population influencing motivation and decisional processes (Hamid et al., 2015; Martel & Gatti-McArthur, 2020).

1.2.1. Dopaminergic pathways

There are four dopaminergic pathways, each known for different effects on brain and organism (Chen et al., 2024; Xu & Yang, 2022).

- Mesocortical pathway connecting the VTA and the cortex associated with cognitive functions.
- Mesolimbic pathway connecting the VTA and the nucleus accumbens (NAc) in the ventral striatum associated with reward and motivation.
- Tuberoinfundibular pathway connecting the hypothalamic nuclei and the pituitary associated with endocrine regulation.
- Nigrostriatal pathway connecting the substantia nigra (SN) and the striatum associated with motion control (M. O. Klein et al., 2019).

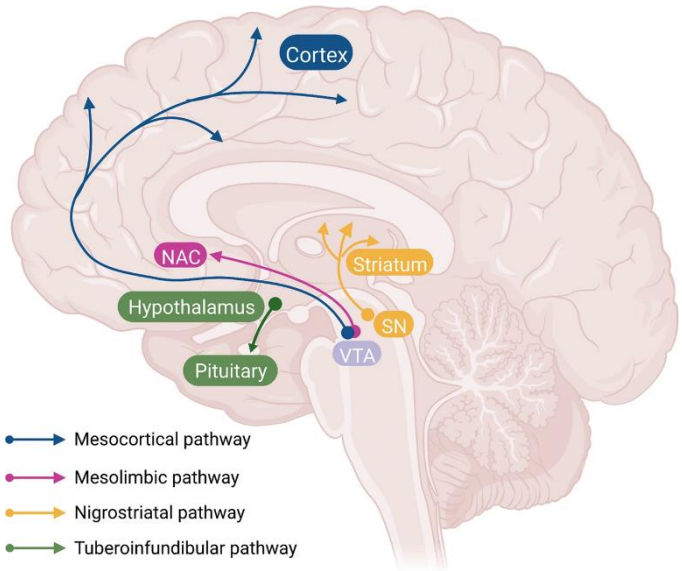

Figure 4 – Dopaminergic pathways. NAC – Nucleus accumbens; VTA – ventral tegmental area; SN – substantia nigra (Xu & Yang, 2022)

1.2.2. Mesolimbic pathway

It is a pathway crucial for positive reward and appetitive-motivated behavior. It reinforces behaviors linked to positive outcomes, including activities like eating, social interactions, and substance use (Wise, 2004). Additionally, it can be activated by negative stimuli and stress, which might be connected to an overall state of increased behavioral arousal linked to "seeking safety" in various emotional situations, whether positive or negative (Ikemoto & Panksepp, 1999a). Some studies suggest that the interplay of mesolimbic and mesocortical pathway plays an important role in the determination of personality traits, such as extraversion (Depue & Collins, 1999; DeYoung, 2013), and openness (Passamonti et al., 2015).

The reinforcement DA learning hypothesis connected to this pathway is described as first reacting to primary reward which is later adapted to a signal of a sooner reward-producing stimuli, not the reward itself. The signal is sent to postsynaptic neurons by short pulse release of DA. DA release acts as a teaching signal in reinforcement learning. This mechanism enhances learning by altering synaptic transmission and modifying approach behavior by providing reward information before the behavior occurs (Schultz, 1998).

Earlier research on responses in midbrain dopamine neurons to behavioral trigger signals showed specific patterns of activation and deactivation of DA neurons, with set latencies between bursts of impulses and moderate changes during the execution of the behavioral reaction. According to W. Schultz, DA neurons subserve a specific mechanism related to the behavioral reactivity of the organism (Schultz, 1986). The author described DA neurons showing activation-depression response to novel stimuli or closely resembling reward event similar for all different stimuli. They occur in 60 to 80% of neurons in groups A8 (dorsal to lateral SN), A9 (pars compacta of SN), and A10 (medial VTA to SN) (Dahlstroem & Fuxe, 1964; Schultz, 2001). According to this, there is a relatively homogenous population signal of DA neurons that varies based on the response magnitude and the proportion of activated neurons (Schultz, 2001).

More recent research indicates that distinct populations of DA neurons are specialized for different aspects of motivational control (Bromberg-Martin et al., 2010; Cho et al., 2021; Matsumoto & Hikosaka, 2009). According to this hypothesis, motivational value-coding DA neurons encode a complete prediction error signal, representing rewarding and aversive events in opposite directions (activation and inhibition). These neurons are located primarily in the VTA or subsantia nigra pars compacta (SNc) and include those projecting to the lateral NAc. They are crucial for behaviors related to seeking, evaluation, and value-based learning (de Jong et al., 2019; Matsumoto & Hikosaka, 2009). In contrast, motivational salience-coding DA neurons have a weaker responses to neutral stimuli but are activated by both rewarding and aversive stimuli. They are found in the lateral SNc and include VTA cells projecting to the amygdala (Lutas et al., 2019; Matsumoto & Hikosaka, 2009). This emphasizes their role in detecting and signaling the importance of significant environmental cues. Beyond their phasic signaling roles in encoding motivational value and salience, most of the DA neurons can exhibit burst responses to various sensory events that are unrelated to rewarding or aversive experiences. These responses highlight their broader involvement in processing diverse environmental stimuli (Bromberg-Martin et al., 2010).

Within computational models of reinforcement learning, Gershman et al. merge classical reinforcement DA learning hypothesis connected to reward prediction error (Schultz, 1998) and DA control of uncertainty-guided exploration behavior into common reinforcement learning framework. This framework brings into consideration also the value of the states (Gershman & Uchida, 2019). Dabney et al. inspired by recent AI research on distributional reinforcement learning also extend classical reinforcement DA learning theory. They hypothesize that the brain represents possible future rewards as a probability distribution rather than a single mean. This represents multiple future outcomes simultaneously and in parallel. Similar to the hypothesis of action-value neurons described later in subchapter **1.2.3. Anatomy of mesolimbic pathway.** The authors support it with *in vivo* recordings from the mouse VTA (Dabney et al., 2020).

Back in connection with neuroscientific and psychological view, dysregulation of dopamine signaling has been shown to play an important role in many neurological diseases, such as Parkinson's, Huntington's disease, major depression disorder, ADHD, schizophrenia (Chen et al., 2024). Furthermore, the mesolimbic pathway has been linked to social deficits observed in neurodevelopmental disorders such as autism spectrum disorder (ASD) (Manduca et al., 2016; Supekar et al., 2018) with emerging dopamine hypothesis of ASD (Pavăl, 2017). By focusing on this pathway, one might explore how alterations in DA signaling may contribute to the social challenges faced by individuals with these disorders.

1.2.3. Anatomy of the mesolimbic pathway

1.2.3.1. Midbrain - VTA

The VTA is a part of the midbrain which projects its neurons into the prefrontal cortex and the NAc, but also to other important regions such as amygdala (Loughlin & Fallon, 1983), hippocampus (Gasbarri et al., 1997), and olfactory bulb (Fallon & Moore, 1978, Klein et al., 2019). Both mesocortical and mesolimbic pathways start here. Due to the overlap of dopaminergic neurons in this region and their interplay, the pathway is sometimes referred to as mesocorticolimbic pathway (Wise, 2004). This proximity allows for integrated processing of reward-related information and cognitive control, enabling the brain to balance immediate rewards with long-term goals. The dopaminergic neurons serve as key targets of oxytocinergic regulation. Neurons producing OXT in the hypothalamus project directly to the VTA (Numan & Smith, 1984). A review by Morales and Margolis shows accumulating evidence for the activation of VTA dopaminergic neurons by unpredicted reward, which shifts their activation after learning to cues predicting the reward and decrease in firing when the expected reward is

omitted. Similar applies for aversive stimuli either for excitation or inhibition of VTA dopaminergic neurons. Laterodorsal tegmentum nucleus neurons have excitatory connections to VTA dopaminergic neurons which project to the lateral shell of the NAc, which seem to be particulary involved in motivated behavior (Morales & Margolis, 2017).

1.2.3.2. Striatum - NAc

The NAc has two main regions, the shell and the core, with both having different connections. It receives afferent inputs from regions throughout the brain and plays an important role in motor control and reward-related functions and is considered a regulator of motivational drive. DA in the NAc is released primarily by neurons located in the VTA (Ikemoto & Panksepp, 1999). The main cell type in the striatum is the medium spiny neuron (MSN), which releases γ-aminobutyric acid (GABA) at its synaptic terminals (Báez-Mendoza & Schultz, 2013).

Activation of D2R in the NAc leads to enhanced motivation in mice, while inhibition leads to decreased motivation (Soares-Cunha et al., 2016). The reward-related view is supported by self-administration of D-amphetamine in rats (Phillips et al., 1994), which increases extracellular DA within the NAc. The same applies for DA reuptake blocker (nomifensine) administration into the NAc (Carlezon et al., 1995). It was connected to the preference of a drug-paired environment with microinjections into the NAc (Phillips et al., 1994). The same drug-paired preference of environment did not occur if the microinjections were administered into different DA terminal regions (Carr & White, 1986). Direct injection of DA into the NAC in rodent models also showed increase in locomotor activity, which is an important component of exploration and spontaneous activity (Mogenson & Nielsen, 1984).

More recent research shows that the striatum seems to be connected to the evaluation of different stimuli in reward-related function. Action-value neurons can track the value of actions independent of the performed action. By tracking the value of more actions, the organism can decide to focus on the action with highest value or to explore new options. It seems that both social information and value converge in the striatum (Báez-Mendoza & Schultz, 2013). Klein and Platt found neurons primarily in the medial striatum selectively signaling social information, while a non-overlapping population of neurons signaled information about fluid reward. This shows that information about social context and information about nutritive reward are encoded by independent neurons in the striatum. According to the authors, striatal neurons do not necessarily signal value, but a small neuronal population could encode specific information about context, cues, outcomes, or combinations. They believe that reward is not the best construct to describe the activity of the striatal neurons due to their robust encoding of

social stimuli. This kind of stimuli is connected to complex approach and avoidance behaviors which is reflected in the activity of individual neurons (Klein & Platt, 2013).

Striatum importance in social evaluation is supported by more studies. A study on macaque monkeys revealed higher valuation of images of dominant monkeys in comparison with subordinate monkeys, even if they got lower liquid reward (Deaner et al., 2005). Human fMRI study showed there is higher activity in the ventral striatum when winning a lottery in public vs winning the same amount in private (Bault et al., 2011). These results support the involvement of the striatum in evaluating reward within social context.

1.2.4. Abnormalities in behavior in connection to mesolimbic pathway

Similarly to OXTR, DRs exert different spatiotemporal distribution in rat brain, with selective elimination of excess DRs during puberty in the caudate-putamen and the NAc (Tarazi & Baldessarini, 2000). Bendersky et al. focused on a review of the long-term impacts of post-weaning social isolation in rodents on the NAc function. Both D1R and D2R expression peaks in adolescence and differs between sexes. It is shown that stress in adulthood has effect on synaptic plasticity in the NAc and on protein and gene expression of molecules connected to synaptogenesis. Social isolation increases aversion- and reward-evoked dopamine release into the NAc. The results reviewed by authors suggest hypersensitivity of dopaminergic neurons to an environmental stimulus after isolation rearing (Bendersky et al., 2021).

It seems that possibly sex-specific behavioral phenotypes induced by social isolation are connected to cellular changes within the NAc. These changes are connected to different gene and protein expression which leads to changes in synaptic plasticity, and therefore to possible impairments and pathological alterations. Stress during adolescence could alter developmental trajectory connected to DR expression and binding in the NAc. This mechanism is connected to second messenger cascade of D1R and D2R (Figure 5). In general, there is many behavioral and circuitry studies but much less focusing on the molecular level itself. The authors conclude that the adolescent social isolation impact on gene/protein expression is sex-, species-, isolation type, and stimulus-specific (Bendersky et al., 2021). These results similarly to OXT mentioned before, suggest different critical periods of development which might have different impact on the alterations in synaptic biology and therefore on the behavior itself.

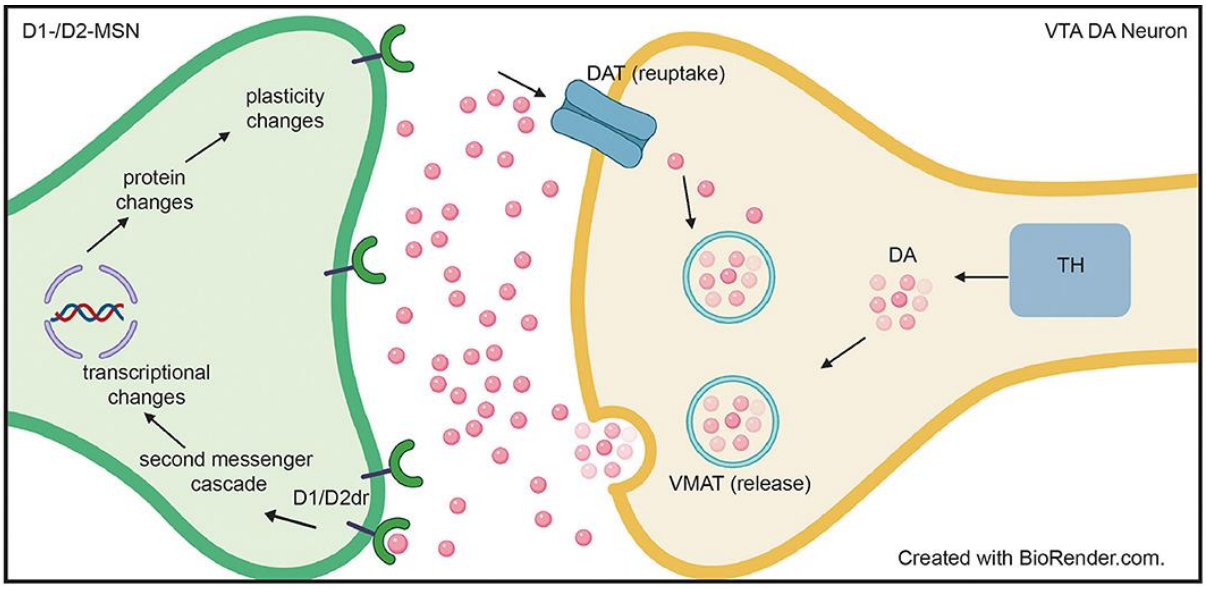

Figure 5 – Synaptic alteration mechanism possibly induced by social isolation. Post-synaptic neuron in nucleus accumbens in green; presynaptic ventral tegmental area (VTA) neuron in yellow; TH- Tyrosine hydroxylase; VMAT – vesicular monoamine transporter; DAT – dopamine transporter; DA – dopamine; D1/D2dr – dopamine receptor 1 and dopamine receptor 2; D1-/D2-MSN – medium spiny neurons of dopamine 1 and 2 receptors (Bendersky et al., 2021).

The behavioral effects of dopaminergic pathways are not driven solely by DA but arise from a complex interplay with other neurotransmitter systems. DA is viewed as slow neurotransmitter which affects fast neurotransmitters such as GABA and glutamate. The interactions are crucial for shaping complex behaviors. One of the suggested mechanisms is the DR alteration in the phosphorylation of ionotropic receptors (Beaulieu et al., 2014). Furthermore, glutamatergic, GABAergic, and also cholinergic terminals are implicated in regulation through D1- and D2-class receptors. The receptors might alter, positively or negatively, the release of mentioned neurotransmitters and act on the activity of these neurons (Tritsch & Sabatini, 2012). For example, D2-class receptor decreases release of glutamate on MSNs in the striatum (W. Wang et al., 2012), moreover DA fosters the activation but also deactivation of MSNs depending on the type of the receptor and type of the MSN (Gerfen & Surmeier, 2011). Additionaly, accumulating evidence shows that generating motivated behavior might be partially mediated by GABA release from VTA projection neurons, independent of DA. Selective activation of specific fibers in the VTA elicits glutamate exertion, which leads to increased firing of NAc-projecting DA neurons (Morales & Margolis, 2017), which shows that the interaction can be reciprocal. The overexpression of D2R in the striatum shows a deficit in inhibitory GABA-mediated transmission (Li et al., 2011). There is many more evidence supporting the interplay (Tritsch & Sabatini, 2012). The mentioned examples emphasize the complexity of the interactions. Havranek et al. reviewed complex interplay of OXT, GABA, and dopamine in

connection to deficits in social behavior in ASD (Havranek et al., 2024). OXT seems to play an important role in the modulation of dopaminergic regions.

1.3. Oxytocin influence on dopaminergic activity

DA and OXT are known to interact with one another. Both share functional effects, primarily on social behavior among other functions, such as yawning, grooming, and mating (Y. Liu & Wang, 2003). They are both related to reward and pleasure, and both versions of knockout and deficient rodents have impaired social behavior. Dopaminergic neurons influence OXT release and similarly, OXT influences levels of DA in various regions (Petersson & Uvnäs-Moberg, 2024).

Xiao et al. was among the first complex studies anatomically and also functionally characterizing hypothalamic oxytocinergic projections to midbrain DA neurons in mice, focusing mostly on the VTA and SNc. OXT acts differently on these two regions. The projections to the VTA originate primarily from parvocellular neurons of the PVN. OXT projections to the VTA and SNc are mostly separate. OXT in the VTA directly increases the fire rate of DA neurons, while in the SNc causes a decrease of the firing rate. Mice OXT-sensing receptors are on both DA and GABA neurons in the VTA, which does not apply for SNc DA neurons. Further results of this study suggest that OXT may act on DA and GABA neurons in the VTA both via OXTR and vasopressin receptor (Xiao et al., 2017).

OXTR and DR were found to form, among homomers, also combined dimeric complexes D2R-OXTR in the NAc in the ventral striatum and the caudate putamen in the dorsal striatum which combine their effect and alter the signalling pathways. OXT increased maximal binding capacity for D2R antagonist in accumbal membrane indicating allosteric receptor-receptor interaction (Romero-Fernandez et al., 2013). The formation of dimers was later validated by other study (de la Mora et al., 2016). The authors believe these heterocomplexes seem to play a significant role in social behavior (Borroto-Escuela et al., 2022) and might bring a novel view on the interaction between DA and OXT.

Regarding the social effects of the interplay, OXT plays a crucial role in pair bonding in connection with dopaminergic system. In monogamous prairie voles, higher OXTR density in the prefrontal cortex and NAc has been observed (Insel & Shapiro, 1992). Furthermore, studies have shown that OXTR activation in these regions is crucial for pair bond formation in female prairie voles (Young et al., 2001). Injecting OXT into the NAc is connected to pair bonding in female prairie voles which is achieved through the interaction with D2R (Liu & Wang, 2003).

Administration of OXTR antagonist into anterior cingulate cortex (an important part of the limbic system) leads to a decrease in social behavior (Burkett et al., 2016). Furthermore, administration of dopaminergic antagonist into NAc leads to diminishing of the effect of OXT on partner preference in female praire voles (Liu & Wang, 2003). In maternal behavior, OXT release in the hypothalamus stimulates dopaminergic activity in the VTA and increase the dopamine signal in the NAc, promoting maternal behaviors like pup retrieval and nursing (Shahrokh et al., 2010). Furthermore, stimulation of D1R within the NAc or the medial preoptic promotes the onset of maternal behavior in rats (Stolzenberg et al., 2007). Together the coordination of these systems among other functions underly social cognition. Potentially, neonatal period might be crucial development time for epigenetic mechanism responsive to experiences, leading to sexually dimorphic consequences of adult social behavior (Bales et al., 2007; Skuse & Gallagher, 2009).

1.4. Neuronal morphological factors

Neurite formation is crucial for the development of axons and dendrites (Sainath & Gallo, 2014). Axons and dendrites create collaterals (Rockland, 2018), varicosities (Gu, 2021; Rollenhagen & Lübke, 2013), dendritic spines (Sala & Segal, 2014), and branches (Lanoue & Cooper, 2019). They represent the shape, structure, and in the end also connectivity of the neuron. These are important aspects of neuronal function essential for establishing functional neuronal networks. Alterations can lead to cognitive disfunctions, which can be seen in many brain disorders (Lin & Koleske, 2010; Martínez-Cerdeño, 2017).

The interplay between proteins of cytoskeletal structure is vital for neurite formation. One of the crucial part of the cytoskeleton are microtubules (Sainath & Gallo, 2014). These cytoskeletal polymers are made of tubulin proteins. They are highly dynamic and can move within cytoskeletal structure through further de/polymerization. They are involved in wide range of cellular processes, including maintaining cell shape, intracellular transport, mitosis, and cell signalling (Desai & Mitchison, 1997). Furthermore, microtubule-associated proteins (MAPs) are diverse group of proteins interacting with microtubules to regulate their stability, organization, and function. MAP1 and MAP2 stabilize microtubules by binding along their length. MAPs can be used as selective markers for brain development and cell type. The MAP2 is localized mostly on the dendritic compartment and can be used as a biomarker of matured neuronal cells (Huber & Matus, 1984; Mandelkow & Mandelkow, 1995).

The precise regulation of neurite morphology is crucial for optimal synaptic function and plasticity. The complexity of the regulation among others includes adhesion molecules

(Kozlova et al., 2020), growth factors (Park & Poo, 2013), and growing evidence also show neuropeptides such as OXT as an important factor. Lestanova et al. found that OXT increases neurite length *in vitro* in SH-SY5Y neuron-like cells (human neuroblastoma cell line), while no change was observed in the presence of OXT antagonist (Lestanova et al., 2016). Neonatal alterations in OXT levels led to enhanced gene expression of *Map2* and nestin (other cytoskeletal protein) in the right hippocampus of adolescent rat. A neuropeptide involved in the regulation of growth called nerve growth factor (NGF) was also expressed in higher concentrations but only in the male hippocampus (Bakos et al., 2014).

1.5. Crucial synaptic proteins

The formation and maintenance of synaptic connections, along with synaptic plasticity, are essential for proper brain function and homeostasis. The functional characteristics of a synapse are primarily determined by the molecular composition of its synaptic proteins. Impairments in any component of synaptic connections can lead to various behavioral disorders. Understanding these components from the molecular to behavioral level is crucial for relevant therapeutic targets and understanding of neurotypical and impaired brain functioning.

1.5.1. SHANK protein family

Proteins regulating signal transduction and targeting are often built around one or more central scaffold ("linking") proteins. SH3 and multiple ankyrin repeat domains (SHANK) family is a group of scaffolding proteins at postsynaptic sites (Figure 6) (Sheng & Kim, 2000). They play an important role in the synaptic development and function, influencing molecular neuronal composition. There are three known members (SHANK1, SHANK2, and SHANK3). Mutations in all members have been associated with neurodevelopmental disorders, especially ASD (Berkel et al., 2010; Durand et al., 2007; Sato et al., 2012). *SHANK3* mutation was the first one discovered in connection to ASD. The disruption at the genetic level in humans is associated with Phelan McDermid syndrome, characterized by intellectual disability, absent or delayed speech, neonatal hypotonia, and attention deficits (Harony-Nicolas et al., 2017).

*Shank*3 mutant mice exhibit repetitive grooming and deficits in social interaction. The authors found defects at striatal synapses and cortico-striatal circuits (Peça et al., 2011). *Shank3* deficiency alters neurite number and length in primary hippocampal neurons *in vitro*, which were reversed by OXT treatment (Reichova et al., 2020). Furthermore, the behavioral and electrophysiological deficits of *Shank3* knockout rats in Harony-Nicolas et al. study were rescued by administration of OXT (Harony-Nicolas et al., 2017).

Interestingly *Shank3* knock-in mouse model, where it is possible to re-express *Shank3* gene during adulthood, led to improvements in synaptic composition, spine density and neural function in the striatum. Furthermore, some of the behavioral impairments were rescued, such as social interaction deficit, and repetitive grooming. This shows some level of plasticity also in the adult brain (Mei et al., 2016).

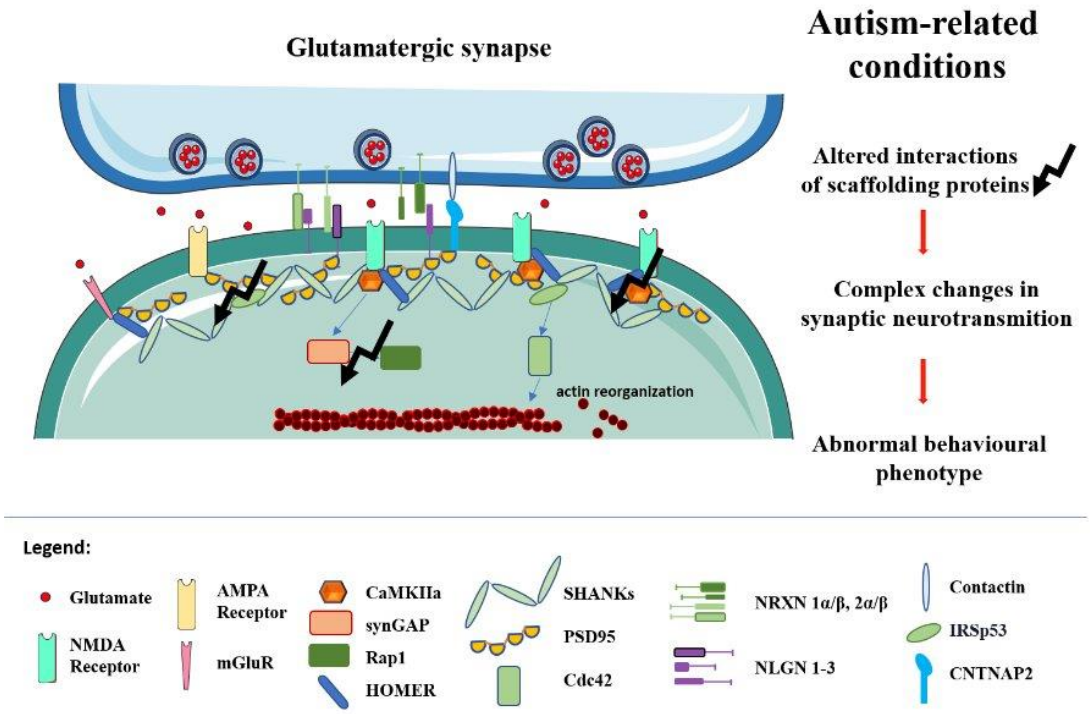

Figure 6 – Representation of glutamatergic synaptic proteins. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor; Calmodulin-dependent protein kinase II-alpha (CaMKIIα); Cell division control protein 42 (Cdc42); contactin-associated protein-like 2 (CNTNAP2); Insulin receptor substrate p53 (IRSp53); N-methyl-D-aspartate receptor (NMDA) receptor; metabotropic glutamate receptor 1 and 5 (mGluR); Postsynaptic density protein 95 (PSD-95); Neurexins (NRXNs); Neuroligins (NLGNs); synaptic Ras GTPase-activating protein (synGAP); Ras-proximate-1 or Ras-related protein 1 (Rap1); SH3 and multiple ankyrin repeat domains proteins (SHANKs) (Meliskova et al., 2021).

1.5.2. Neuroligin protein family

The structure of synapse is formed with a help of cell adhesion molecules. They hold the pre- and post-synaptic neuron in proximity. Neuroligins are postsynaptic adhesion molecules which interact in synaptic gap with presynaptic adhesion molecules called neurexins (Figure 6). These connections could be described as trans-synaptic bridges. There are five members of the neuroligin family. NLGN1, NLGN2, NLGN3, and NLGN4, which are shared among mammals, along with an additional homolog found on the human Y chromosome (Liu et al., 2022). NLGN1 has been found at excitatory glutamatergic synapses (Song et al., 1999), NLGN2 at inhibitory GABAergic synapses (Varoqueaux et al., 2004), and NLGN3 on both synapses

(Budreck & Scheiffele, 2007). NLGN4 is located differently in humans and rodents (Hoon et al., 2011).

Nlgn3 mutant mice show altered behavior, such as repetitive behavior (Rothwell et al., 2014). Social reward and social novelty responses are reduced in *Nlgn3* mutant mice in connection to VTA DA neurons (Bariselli et al., 2018). *Nlgn3* knockout mice exhibit changes in oxytocinergic signaling. (Hörnberg et al., 2020). Neonatal *in vitro* OXT treatment reduces *Nlgn3* expression of CA3 hippocampal area neurons (Filova et al., 2020). On the other hand, the OXT treatment on primary cerebellar cells and SH-SY5Y cells shows increased messenger RNA (mRNA) levels of *Nlgn3* (Zatkova et al., 2019). *SHANK3* deficiency in SH-SY5Y led to decrease in expression levels of neurexins and NLGN3 (Reichova et al., 2020).

1.5.3. Dopamine type 2 receptor

D2R, as mentioned in the chapter **1.2. Dopaminergic system,** is crucial for proper function of dopaminergic signaling. It is mostly associated with inhibitory activity of neurons and are found on both pre- and postsynaptic terminals (C. De Mei et al., 2009). Most D2-class receptors are located on non-DA neurons, with the highest density in the striatum, including the NAc and the olfactory tubercle (Beaulieu & Gainetdinov, 2011; Bouthenet et al., 1991).

Majority of studies connected to the behavior and D2R focus on motor action and addiction (Dobbs et al., 2016; Puighermanal et al., 2020). Social interactions often involve rewarding experiences, and D2R signaling is part of encoding and reinforcing these rewards. D2-class receptor antagonist injected into NAc blocks the formation of partner preference in female prairie voles, while the agonist increases the preference in the absence of mating (Gingrich et al., 2000).

Dysregulation of D2-class receptor is implicated in ASD and schizophrenia where social cognitive deficits are a prominent feature (Hettinger et al., 2012; Seeman, 2006). Lower extrastriatal availability of D2-class receptors was shown in participants with ASD. The authors correlate the low D2/D3R density with socio-communication challenges in ASD (Zürcher et al., 2021). They support their findings with other studies, such as Hettinger et al. which showed increased likelihood of ASD diagnosis with polymorphism in *D2R* gene and its association between social interaction and communication scores in The Autism Diagnostic Interview – Revised (ADI-R) (Hettinger et al., 2012). On the other hand, study in 2021 by Liu et al. did not show correlation of the severity of symptoms with single nucleotide polymorphism in *D2R* gene

(Liu et al., 2021). Therefore, impairments in *D2R* does not have to automatically lead to social deficits.

1.6. Aims

The aim of this work is to examine the effect of oxytocin on the outcomes of transient silencing (explained in the chapter **2.2.3. Shank3 gene silencing and oxytocin treatment**) of the *Shank3* gene in primary dopaminergic neuronal striatal and midbrain cell cultures, which represent cells from regions involved in social cognition. Cells were isolated from neonatal rats on the day of birth. Specifically, the study focuses on 1) neurite arborization and length, and 2) the intensity of fluorescence signals of selected synaptic parameters (neuroligin 3, D2 receptor). This will be achieved by analyzing images of neurons obtained from cytological samples using fluorescence microscopy.

The main hypothesis is that oxytocin will elongate neurites, increase their number, and simultaneously enhance the intensity of the observed signals, thereby compensating for the deficits caused by *Shank3* downregulation.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Name	Manufacturer	Product number
Poly-D-Lysine hydrobromide	Sigma-Aldrich, Germany	P6407
DNase 1	Roche, Switzerland	11284932001
FBS	Pan-Biotech, UK	P40-37500
B27	Gibco, USA	17504-044
RPMI 1640	Gibco, USA	21875-034
Neurobasal A	Gibco, USA	10888-022
Trypsin	Gibco, USA	15090-046
ATB (penicillin, streptomycin)	Gibco, USA	15140-122
Shank3 siRNA	Dharmacon, USA	L-080173-02
DAPI	Thermo Fisher Scientific, USA	D1306
Fluoromount-G	Thermo Fisher Scientific, USA	00-4958-02

2.1.2. Solutions

Isolation	
Solution A	0.1% (w/v) trypsin
	0.1 mg/ml DNase I
	1x HBSS
Solution B	RPMI 1640
	10% FBS
HBSS	53.33 mM KCl
	4.41 mM KH2PO4
	1379.31 mM NaCl
	3.36 mM Na2HPO4-7H20
	55.56 mM D-Glucose
	0.25 mM Phenol Red

Cultivation	
Cultivation medium A	Neurobasal A
	1 % ATB
	2 mM L-glutamine
	2% supplement B27
Cultivation medium B	Neurobasal A
	2 mM L-glutamine
	2% supplement B27
Cultivation medium C	Neurobasal A
	1 % ATB
	2 mM L-glutamine
Immunocytochemistry	
ICC blocking solution	3% (v/v) normal goat serum
	0.1% Triton X-100

2.1.3. Antibodies

Name	**Host species**	**Dilution**	**Product number**
Anti-MAP2	Mouse	1:500	M4403; Sigma-Aldrich, Germany
Anti-MAP2	Rabbit	1:500	M3696; Sigma-Aldrich, Germany
Anti-GAD65/67	Rabbit	1:500	Ab11070; Abcam, UK
Anti-D2	Rabbit	1:500	376203; Synaptic systems, Germany
Anti-NLGN3	Mouse	1:500	129311; Synaptic systems, Germany

Table 1 – Primary antibodies

Name	**Host species**	**Dilution**	**Product number**
anti-rabbit Alexa Fluor 555	Goat	1:500	A21428; Sigma-Aldrich, Germany
anti-mouse Alexa Fluor 448	Goat	1:500	A11001; Sigma-Aldrich, Germany

Table 2- Fluorescent secondary antibodies

2.2. Methods

2.2.1. Wistar rats

Pregnant Wistar rats were obtained from Charles River Laboratories (Germany) and kept under standard laboratory conditions (average temperature 22±2 °C, 55±10% humidity, 12:12 hour light/dark cycle with lights on 06:00-18:00 h) with access to pellet diet and tap water *ad libitum*. The experimental procedures were approved by The State Veterinary and Food Administration of the Slovak Republic (5578/2023 and 5467-3/2023-220), conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (N.R.C., 1996) and the European Communities Council Directive of September 22nd, 2010 (2010/ 63/EU, 74). The experimental protocols were approved by the Institutional Ethical Committee Guidelines for Animal Research.

2.2.2. Primary neuronal cell isolation

Primary neuronal cells were isolated according to a protocol described by Reichova et al., 2020. The newborn rats were euthanized shortly after birth to allow for the dissection and isolation of specific brain regions bilaterally, in this case striatum and midbrain. The isolated tissue went through enzymatic treatment in 1.5 ml tubes. The treatment was done at 37 °C for 20 minutes in solution A (with antibiotics). The dissociation process was stopped by removing solution A and adding solution B (without antibiotics) (2 x 5 min at 37 °C). Cells were resuspended and at density of 0.8 x 10^5/ml plated onto 24-well plate. The wells contained cover slips coated with 10 µg/ml poly-D-lysine containing 10% FBS. The cells were incubated for 3 hours in solution B at 37 °C and 5% CO2. The medium was exchanged for cultivation medium A. After 5 days in vitro (DIV5) 50% of medium was exchanged for a fresh one.

2.2.3. *Shank3* gene silencing and oxytocin treatment

Transient silencing was done by introducing specific small interference RNA (siRNA) for the *Shank3* gene using lipid transfection. This method enables transfer of siRNA into the cytoplasm of the host cell through a vehicle. The siRNA acts on mRNA by complementary binding to it. This temporarily blocks the translation of SHANK3 protein. The siRNAs sequences of the complementary part of the rat *Shank3* gene were: GCAAGUUCGAUGUGGGCGA, GGAUAGUGCUCGGCGCAGA, CCACUCGGGAAGAGCGGAA, AGGUAAACGGAGUGAACGU.

The experimental groups consisted of:

1) control group incubated with vehicle only
2) control group incubated with oxytocin
3) si*Shank3* group
4) si*Shank3* group incubated with oxytocin

5 μM solution of siRNA and transfect reagent DharmaFect (vehicle) were transferred into two separate tubes. Both solutions were 20x diluted with cultivation medium C (without B27), stirred and incubated for 5 minutes at RT. Both tubes were mixed together and incubated for another 20 minutes. The solution was diluted in ratio 1:5 using cultivation medium B and mixed with primary cell culture with/without 1 μM OXT on DIV5 and cultivated for 48 hours.

2.2.4. Immunocytochemistry

Primary neuronal cells were fixed and stained according to a protocol described by Reichova et al. (2020). The primary striatal/midbrain cells were fixed on DIV7 for 20 minutes at room temperature (RT) with 4% paraformaldehyde (pH 7,4). They were washed 2 times with cold phosphate-buffered saline (PBS), and the unspecific binding spots were blocked in ICC blocking solution for 30 min at RT. The solution was removed, and the cells were stained for 2 hours at RT with primary antibodies (Table 1 - for MAP2, neuroligin, glutamate decarboxylase - GAD, and D2R) diluted in ICC blocking solution. The cells were washed 3 times with PBS and incubated for 1 hour at RT by corresponding secondary antibodies (Table 2) diluted in PBS. Nuclei were stained for 1 minute by 300 nM DAPI. The final step involved rinsing the cover slips with PBS (2x) and mounting them onto a microscope slide using Fluoromount-G (Bukatová, 2018).

2.2.5. Neuronal cell morphology

Microscopy images for further evaluation were taken on a microscope Olympus BX63 with 20x magnification. Only cells with sufficient signal quality and containing MAP2 were considered for the evaluation, as MAP2 was used as a neuron marker. A subgroup of GABAergic neurons was identified by GAD. If neurons were fragmented or their neurites intersected with others without knowing the origin, they were excluded from imaging. Full-frame pictures were taken from 2 cover slips for every experimental group (on average 27 pictures per group). The arborization was quantified by Sholl analysis using open-source Image J/Fiji software (Cloarec et al., 2019). The software measured the number of intersections of neurites with circular lines

starting in the middle of the nucleus and repeating every 1 µm up to 150 µm. The cytomorphological analysis was conducted after isolating MAP2-corresponding staining color in the software (Figure 7).

The length of the longest neurite was measured in the same software after isolating MAP2-corresponding staining color by following the longest neurite by hand starting in the middle of nucleus.

Quantitative analysis of synaptic protein levels (D2R, NLGN3) was performed using ImageJ/Fiji software. For each neuron, three neurite segments with the size of 20 × 5 µm were selected: two from regions closest to the soma, and one from a continuation of one of the neurites (Figure 8). The fluorescence intensity of NLGN3 and D2R was expressed as relative intensity in percentage in comparison with control.

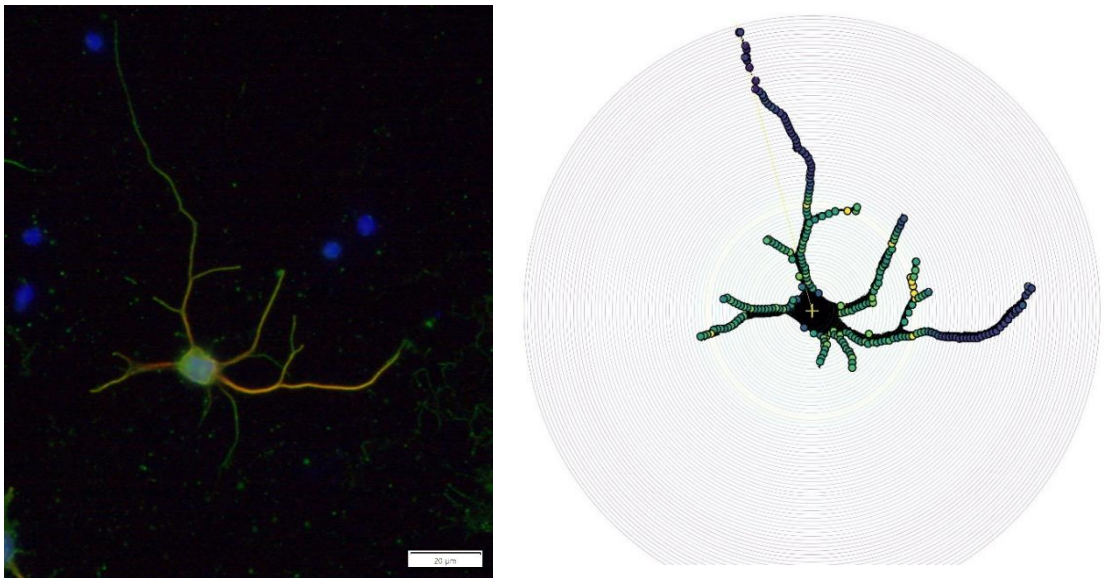

Figure 7 – Representative image of Sholl analysis. Striatal neuron- vehicle control group; (Left) Original image with scale = 20 µm taken on microscope Olympus BX63, colors: blue – nucleus, green – neuroligin 3, red – microtubule associated protein 2 (MAP2); (Right) Isolated MAP2 staining color with circular lines starting in the middle of the nucleus for further Sholl analysis.

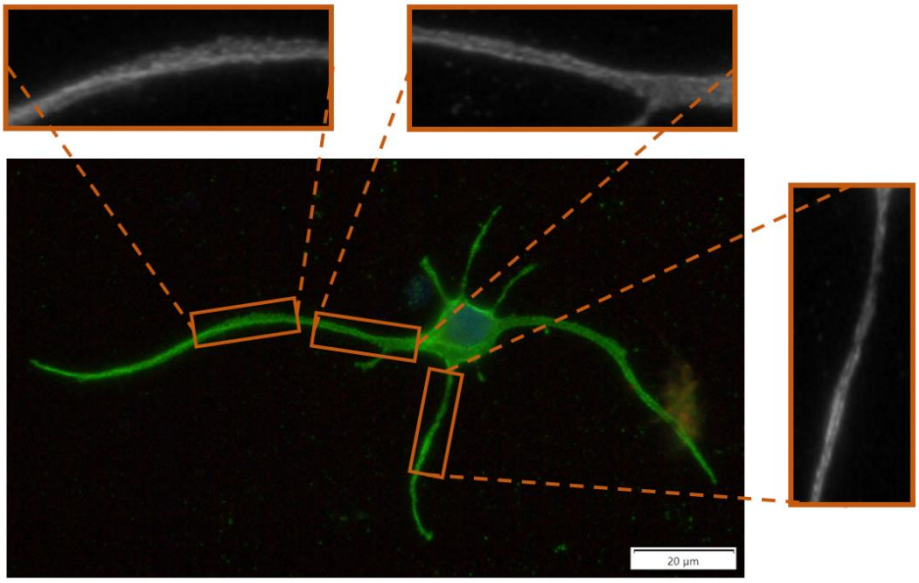

Figure 8 – Representative image of segment selection for quantitative analysis of synaptic protein level. Midbrain neuron- vehicle control group; (Bottom left) Original image with scale = 20 µm taken on microscope Olympus BX63, colors: blue – nucleus, green – neuroligin 3, red – microtubule associated protein 2; (Upper and right) Selected neuronal segments after isolating neuroligin 3 staining color.

2.2.6. Statistics

GraphPad version 10.3.1. was used for statistical analysis. Interquartile range method was applied to identify outliers which were excluded from further evaluation. For Scholl analysis, Two-way ANOVA (factors: Treatment, Distance) was employed, followed by Tukey's *post hoc* test. Differences between longest neurites were assessed using two-way ANOVA (factors: Oxytocin, Silencing) and Tukey's multiple comparisons test. The same approach was applied to analyze the intensity of D2R and NLGN3. Results are presented as mean ± SEM. Threshold for statistical significance was set to $p < 0.05$.

3. Results

The morphological changes in striatal and midbrain neuronal cells, following *Shank3* gene silencing and OXT treatment, were evaluated by measuring the length of the longest neurite and conducting Sholl analysis to assess neurite arborization. The results for both striatal and midbrain cells are divided into two parts: 1) GAD positive neurons, and 2) NLGN3 and D2R positive neurons. GAD positive neurons show GABAergic neurons, while NLGN3 and D2R do not show specific subgroups of neurons and therefore are pooled together. Significance of measured factors is shown in the text, as well as in the figures.

3.1. Morphology of striatal neurons

3.1.1. Neurite length

The length of the longest neurite in GAD-positive striatal neurons did not differ following *Shank3* silencing or after OXT treatment (Figure 9).

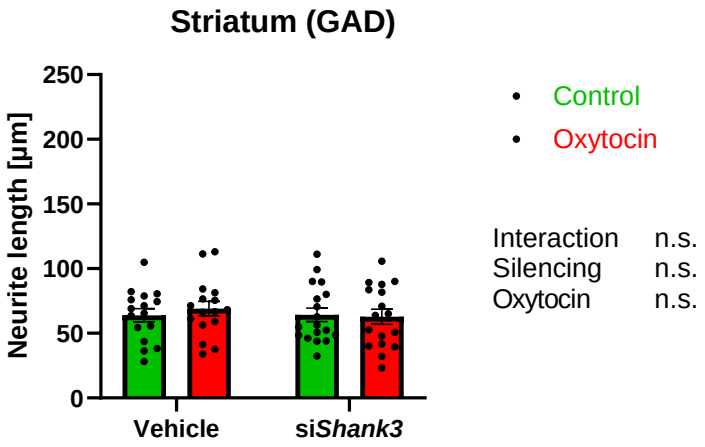

Figure 9 – The neurite length of GAD positive striatal neurons. n = 17-20 per group; GAD- Glutamate decarboxylase; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

In the combined group of neurons positive for NLGN3 and D2R, a two-way ANOVA revealed significant differences for the factor OXT treatment ($F(1, 133) = 4.924$, $p \leq 0.05$) and the interaction between OXT and *Shank3* silencing ($F(1, 133) = 4.091$, $p \leq 0.05$). Factor *Shank3* silencing was not significant ($F(1, 133) = 1.457$, $p = 0.2296$). Tukey's post hoc test indicated a significant increase in neurite length in the si*Shank3* group following OXT treatment ($p \leq 0.05$, Figure 10).

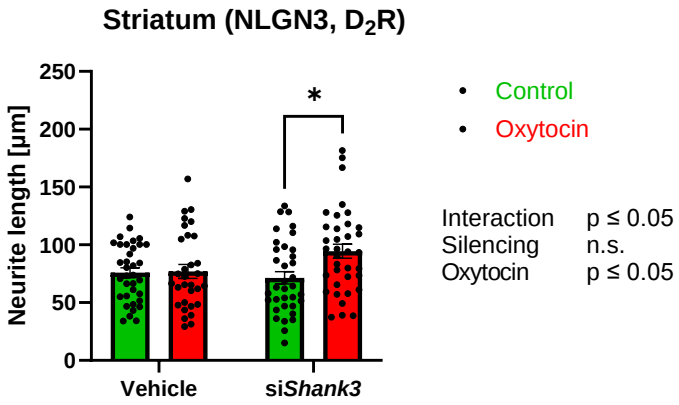

Figure 10 – The neurite length of NLGN3 and D2R positive neurons. n = 31-36 per group; NLGN3 – neuroligin 3; D2R- dopamine type 2 receptor; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

3.1.2. Neurite arborization

The arborization was measured by the number of intersection of neurites with circular lines starting in the middle of nucleus. Number of these intersections significantly decreases ($p \leq 0.001$) with growing distance from the nucleus which applies for GAD-positive (Figure 11) as well as pooled NLGN3 and D2R positive neurons (Figure 12).

For GAD-positive neurons, a two-way ANOVA revealed significant effects for factors oxytocin treatment and distance from the nucleus: interaction ($F(447, 10050) = 0.7186$, n.s.), distance ($F(149, 10050) = 82.30$, $p < 0.001$), and oxytocin treatment ($F(3, 10050) = 8.691$, $p < 0.001$). Tukey's post hoc test showed significant decreases in intersection numbers at 13 µm and 17 µm following oxytocin treatment ($p < 0.001$), and at 23, 25, and 27 µm due to *Shank3* silencing ($p < 0.001$). A notable effect was observed when comparing oxytocin treatment with its combination with si*Shank3*, as a significant decrease was seen at 46-47 µm from the nucleus compared to *Shank3* silencing only. Overall, these results suggest a lower number of intersections in shorter neurites in response to both *Shank3* silencing and oxytocin treatment, with an even greater reduction when combined with si*Shank3*. When only comparing two groups, vehicle control vs si*Shank3* (Figure 11 B) two-way ANOVA showed significant effects for distance and silencing treatment: interaction ($F(149, 5100) = 0.794$, n.s.), distance ($F(149, 5100) = 49.57$, $p < 0.001$), and silencing treatment ($F(1, 5100) = 21.09$, $p < 0.001$). si*Shank3* with oxytocin treatment vs vehicle control showed significant result for distance and oxytocin treatment (Figure 11 C): interaction ($F(149, 4950) = 1.042$, n.s.), distance ($F(149, 4950) = 38.27$, $p<0.001$), and oxytocin treatment ($F(1, 4950) = 10.91$, $p = 0.001$).

Nevertheless, a two-way ANOVA for all neurons (MAP2 positive, either NLGN or D2R, so called pooled) for factors oxytocin treatment and distance from the nucleus revealed significant effects: interaction ($F(447, 20250) = 1.123$, $p < 0.05$), distance ($F(149, 20250) = 168.7$, $p < 0.001$), and oxytocin treatment ($F(3, 20250) = 91.74$, $p < 0.001$). Tukey's post hoc test showed significant decrease in intersection numbers at 21-30 µm following oxytocin treatment ($p < 0.005$), and at 29-52 µm due to *Shank3* silencing in combination with oxytocin treatment, though not continuously ($p < 0.001$). These results indicate that the number of intersections in shorter neurites decreased in response to both *Shank3* silencing and oxytocin treatment, but the combination of these influences did not produce any additional effect. In two-group comparison, vehicle control vs si*Shank3* group (Figure 12 B) two-way ANOVA showed significant effects for distance and silencing treatment: interaction ($F(149, 10500) = 0.3805$, n.s.), distance ($F(149, 10500) = 95.82$, $p < 0.001$), and silencing treatment ($F(1, 10500) = 2.151$, n.s.). si*Shank3* with oxytocin treatment vs vehicle control (Figure 12 C) showed significant effects for distance and treatment: interaction ($F(149, 10500) = 1.135$, n.s.), distance ($F(149, 10500) = 107.3$, $p < 0.001$), and oxytocin treatment ($F(1, 10500) = 75.24$, $p < 0.001$).

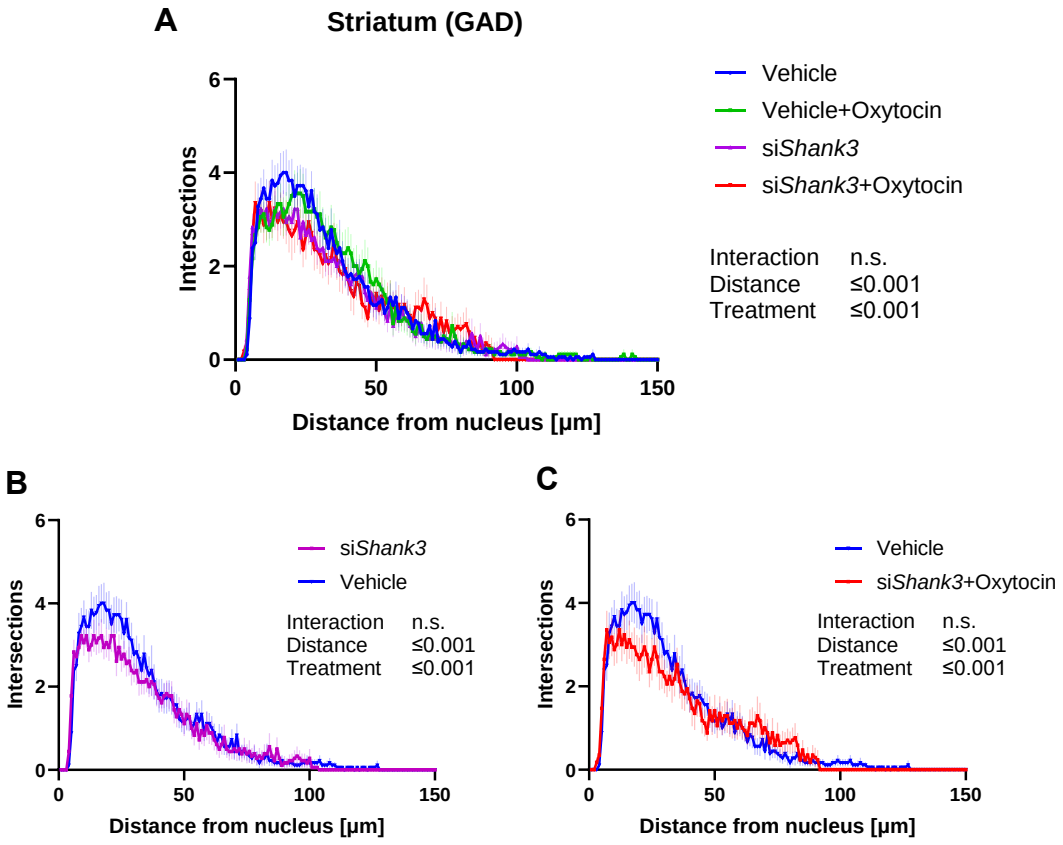

Figure 11 – Arborization in striatal GAD positive neurons. n (neurons) = 17-20 per group; GAD- Glutamate decarboxylase; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

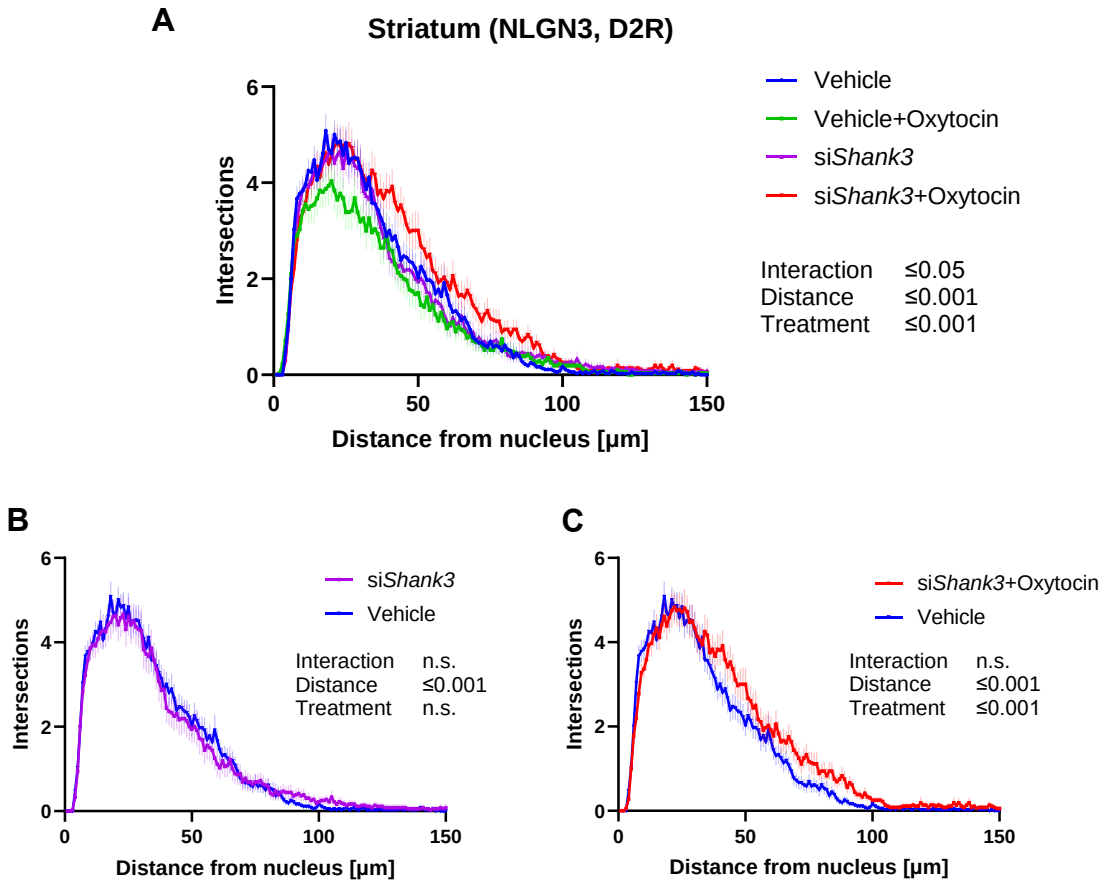

Figure 12 – Arborization in striatal NLGN3 and D2R positive neurons. n = 31-36 per group; NLGN3- neuroligin 3; D2R- dopamine type 2 receptor; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

3.2. Morphology of midbrain neurons

3.2.1. Neurite length

Different trends were observed in neurons isolated from the midbrain (Figure 13 and figure 14). The length of the longest neurite in GAD-positive midbrain neurons varied across groups (Figure 13). A two-way ANOVA revealed significant differences for the factor *Shank3* silencing ($F(1, 50) = 5.019$, $p < 0.05$) and no significance for the factor oxytocin treatment ($F(1, 50) = 3.298$, $p = 0.0754$), and interaction ($F(1, 50) = 0.4352$, n.s.). Although Tukey's post hoc test showed no relevant differences, the main effects suggest *Shank3* silencing had a reducing impact on neurite length, which OXT did not significantly alter. These effects were not observed in the pooled neurons positive for NLGN and D2R (Figure 14).

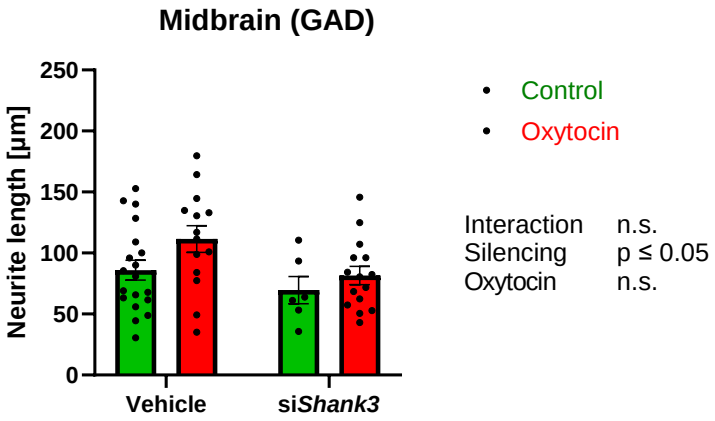

Figure 13 – The neurite length of midbrain GAD positive neurons. n = 6-19 per group; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

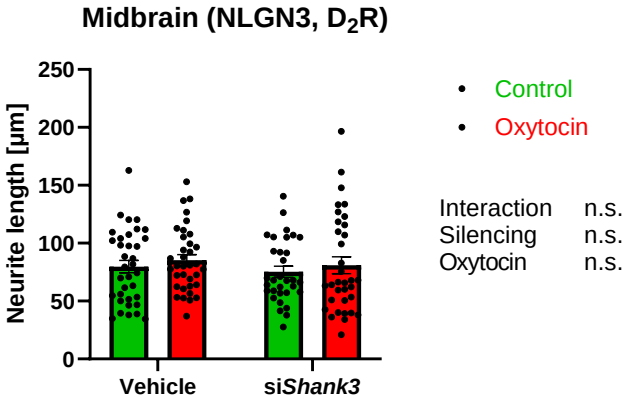

Figure 14 – The neurite length of midbrain NLGN3 and D2R positive neurons. n = 32-35 per group, NLGN3 – protein neuroligin 3, D2R- dopamine type 2 receptor; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

3.2.2. Neurite arborization

Regarding neurite arborization in neurons isolated from the midbrain, distinct differences were observed compared to striatal neurons, particularly in GAD-positive neurons (Figure 15). ANOVA revealed significant effects for factors oxytocin treatment and distance from the nucleus: interaction ($F(447, 7500) = 0.7194$, n.s.), distance ($F(149, 7500) = 26.32$, $p < 0.001$), and oxytocin treatment ($F(3, 7500) = 108.3$, $p < 0.001$). Tukey's post hoc test showed a significant increase in intersection numbers at 34 µm from the nucleus in the OXT-treated group compared to the vehicle control ($p<0.05$). Additionally, significant decrease was observed at 58 µm in the group treated with OXT and *Shank3* silencing, compared to the group with only *Shank3* silencing ($p<0.05$). In two-group comparison, vehicle control vs si*Shank3* (Figure 15

B) two-way ANOVA showed significant effects for factors distance and treatment: interaction ($F(149, 3300) = 0.5857$, n.s.), distance ($F(149, 3300) = 12.95$, $p < 0.001$), and silencing treatment ($F(1, 3300) = 104.3$, $p < 0.001$). si*Shank3* vs vehicle control (Figure 15 C) showed significant result only for distance: interaction ($F(149, 4650) = 0.8474$, n.s.), distance ($F(149, 4650) = 22.95$, $p < 0.001$), and treatment ($F(1, 4650) = 0.4574$, n.s.). These results suggest that *Shank3* gene silencing generally reduces the number of intersections, though not markedly at specific distances from the nucleus. OXT's effect has significant variability at different distances and partially rescuing effects of silencing as shown by non-significant difference between treatment in control group vs OXT with silencing group.

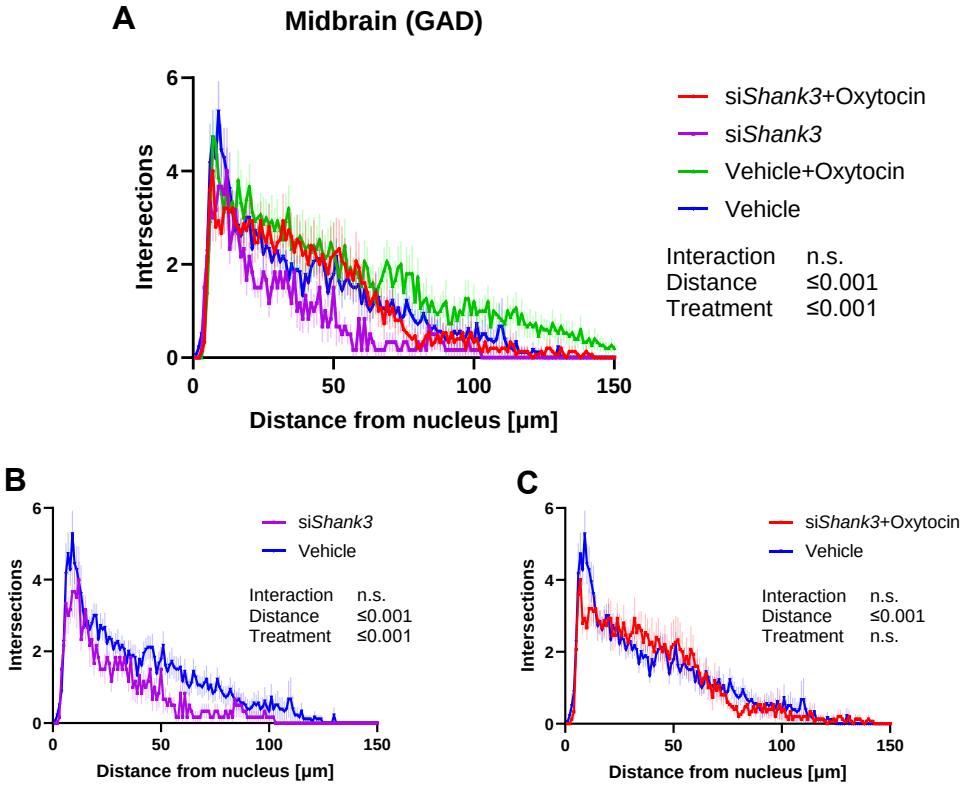

Figure 15 – Arborization in midbrain GAD positive neurons. n = 6-19 per group; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

Similar, slightly different trends were observed in all neurons isolated from the midbrain (Figure 16). Two-way ANOVA showed significant effects for factors oxytocin treatment and distance from the nucleus: interaction ($F(447, 20250) = 0.8843$, $p = 0.9609$, n.s.), distance ($F(149, 20250) = 94.02$, $p < 0.001$), and oxytocin treatment ($F(3, 20250) = 138.8$, $p < 0.001$). Tukey's post hoc test showed a reduction mostly in intersections at 10-16, 19-20, and 27-32 µm from the nucleus in the si*Shank3* group compared to control ($p < 0.05$). These results, like those for GAD-positive neurons, show that *Shank3* silencing primarily reduced intersections at shorter

distances from the nucleus, though not consistently across all distances, without apparent additional or compensatory effect of OXT. When comparing two groups, vehicle control vs si*Shank3* (Figure 16 B) two-way ANOVA showed significant effects for distance and treatment: interaction ($F(149, 10050) = 1.134$, n.s.), distance ($F(149, 10050) = 43.86$, $p < 0.001$), and treatment ($F(1, 10050) = 251.7$, $p < 0.001$). si*Shank3* with oxytocin treatment vs vehicle control (Figure 16 C) showed significant effects for all categories: interaction ($F(149, 10200) = 1.517$, $p < 0.001$), distance ($F(149, 10200) = 53.31$, $p < 0.001$), and treatment ($F(1, 10200) = 309.8$, $p < 0.001$).

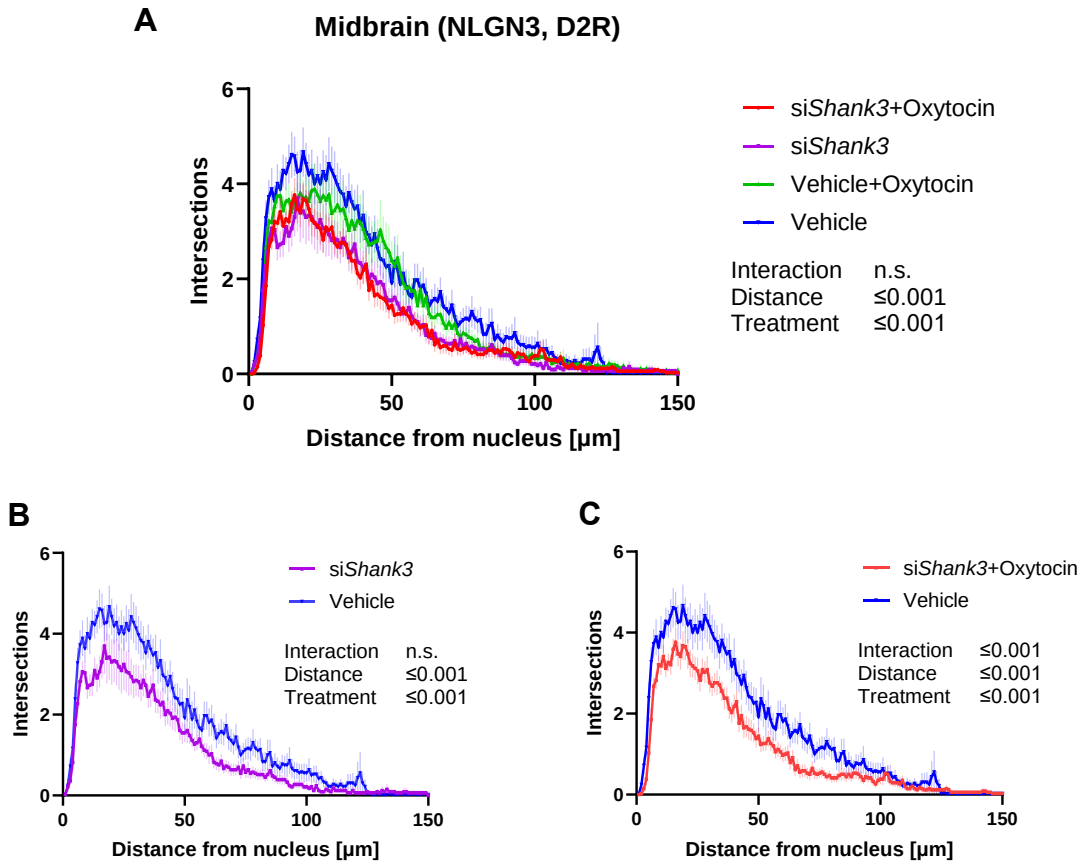

Figure 16 – Arborization in midbrain NLGN3 and D2R positive neurons. n = 32-35 per group; NLGN3 – neuroligin 3; D2R- dopamine type 2 receptor; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

3.3. Immunofluorescence signal intensity

Immunofluorescence signals for dopamine receptors (D2R) and neuroligin 3 (NLGN3) were evaluated, with the hypothesis that *Shank3* silencing would reduce these signals and OXT would compensate these effects. Two-way ANOVA for *Shank3* silencing and oxytocin treatment showed significant changes for D2R signals: interaction ($F(1, 186) = 98.73$, $p < 0.001$), silencing ($F(1, 186) = 31.32$, $p < 0.001$), and oxytocin treatment ($F(1, 186) = 94.99$, p

< 0.001). Tukey's post hoc test revealed differences among groups, indicating *Shank3* silencing reduced D2R signal intensity, unaffected by OXT. OXT alone also lowered D2R levels in control group (Figure 17 A). Similarly, signals for NLGN3 were analyzed, showing less pronounced differences. Two-way ANOVA for main factors showed these results: interaction ($F(1, 180) = 3.233$, n.s.), silencing ($F(1, 180) = 25.99$, $p < 0.001$), and oxytocin treatment ($F(1, 180) = 8.506$, $p < 0.01$). Tukey's post hoc test showed no change for *Shank3* silencing alone but revealed that combining oxytocin treatment with *Shank3* silencing resulted in a lower NLGN3 signal compared to silencing alone. Additionally, OXT decreased signal intensity in the *Shank3*-silenced group compared to the control group (Figure 17 B).

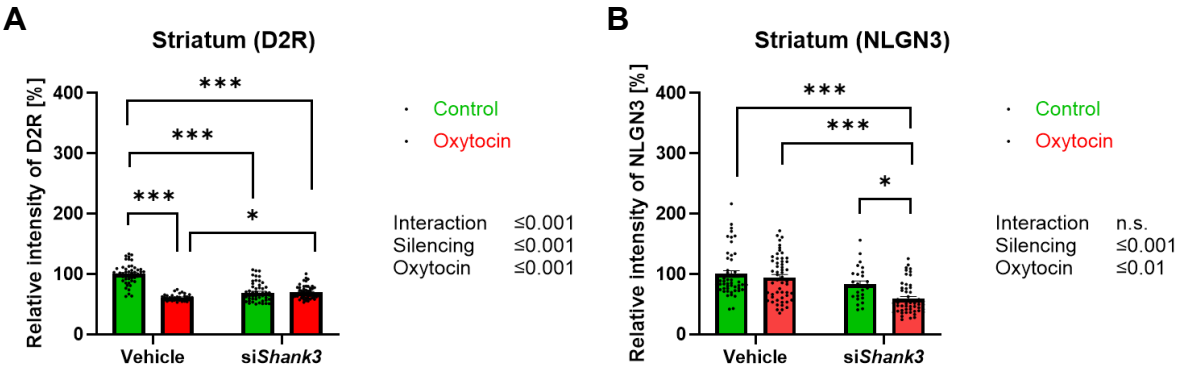

Figure 17 – Relative immunofluorescence signal intensity for dopamine type 2 receptor (D2R) [A] and neuroligin 3 (NLGN3) [B] in striatal neurons. n of D2R segments per group = 35-54; n of NLGN3 segments per group = 28-54; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

For D2R and NLGN3 immunofluorescence in midbrain neurons, OXT showed no effect, with significant differences only for D2R. Two-way ANOVA results: interaction ($F(1, 175) = 61.71$, n.s.), silencing ($F(1, 175) = 4.802$, $p < 0.05$), and oxytocin treatment ($F(1, 175) = 1.451$, n.s.). Tukey's post hoc test showed that OXT increased D2R immunofluorescence signal levels in the control group but decreased them with *Shank3* silencing. *Shank3* silencing alone increased D2R signal levels (Figure 18 A).

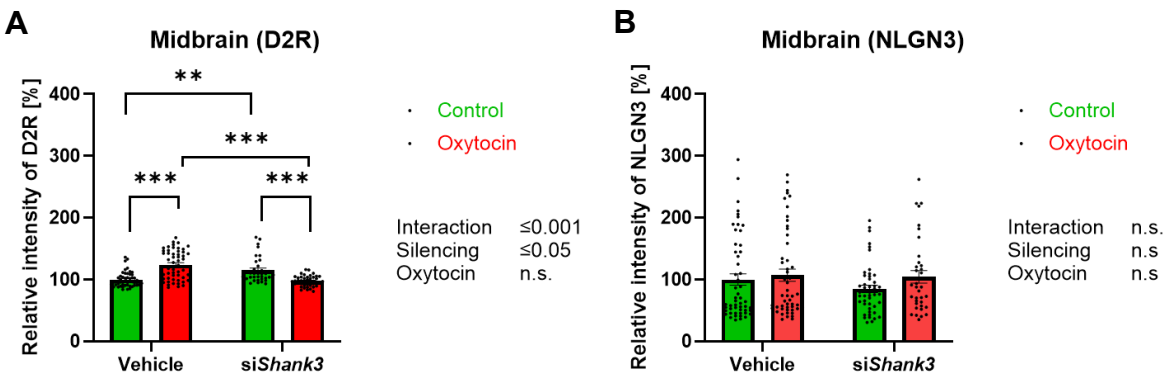

Figure 18 – Relative immunofluorescence signal intensity for dopamine type 2 receptor (D2R) [A] and neuroligin 3 (NLGN3) [B] in midbrain neurons. n of D2R segments per group = 34-54; n of NLGN3 segments per group = 36-51; si*Shank*3 – silencing of SH3 and multiple ankyrin repeat domains 3 gene; n.s. – non-significant.

4. Discussion

This study investigates the effects of oxytocin treatment on the morphology and specific protein expression (neuroligin 3, D2 receptor) of striatal and midbrain neurons after *Shank3* gene silencing in primary neuronal culture (*in vitro*).

Our results demonstrate that OXT influence the morphology of the neurons. Furthermore, it suggests that the effects vary depending on the type of the neuron and the specific morphological parameter measured. Contrary to our hypothesis, OXT did not enhance neuronal morphology or protein expression in every group. Instead, its effects were context-dependent, sometimes compensating for *Shank3* silencing effects, leaving them unchanged, or even supporting the effects in certain scenarios.

4.1. Effects of silencing

Shank3 silencing similarly showed different alterations depending on the region and cell type. Comparable results were found in 2018 study on region-specific *Shank3* mutant mice. At the systemic level, disruption of SHANK3 could be specific for different neuronal cortical and striatal circuits, especially in inhibitory GABAergic neurons (Bey et al., 2018). For instance, human postmortem samples from autistic patients showed fewer cortical GABAergic interneurons and altered branching patterns, which correlate with core autism symptoms like repetitive behaviors and motor stereotypies (repetitive movements/sounds) (Dufour et al., 2023).

In our results, the silencing showed significant effects on neurite length only in GAD-positive (GABAergic) midbrain neurons. Furthermore, the effects on the arborization were significant in both midbrain and striatum group of GAD-positive neurons, particularly at shorter distances from the nucleus (up to 50 μm) indicating impaired connectivity. This might suggest SHANK3's critical role in maintaining the structural complexity of GABAergic neurons which is consistent with findings mentioned above and by Bacova et al. in primary neuronal cultures isolated from midbrain of *Shank3* deficient mice, where a decrease in the distance of neurites was found in GABAergic primary neurons isolated from the midbrain region (Bačová et al., 2024).

On the other hand, a different result was observed in a study on striatal MSN (primarily GABAergic neurons), where an increase in the complexity of dendritic arborization in *Shank3* deficient mice was found (Peça et al., 2011). These differences can be caused by the fact that in this work we used a temporary reduction of the gene expression, while in the case of *Shank3*

deficient mice it is a permanent knockout. Hypothetically, the cell could use different mechanism depending on the level of SHANK3 available. Similiarly, other study showed that reducing *SHANK3* using CRISPR-Cas9 technology on isolated ASD patient stem cells iPSCs (induced pluripotent stem cells) leads to hyperdifferentiation (Thibaudeau et al., 2024), which was not observed in this work, possibly due to differences in neuronal cell types. Moreover, short-term *SHANK3* silencing in HEK293 (human embryonic kidney) cells also affected cell motility, cytoskeletal proteins and GTPases (Lilja et al., 2017), which represent another possible factors in changes in the growth of the neurites in this work as well. Conversely, *SHANK3* deficient human iPSCs in another study showed significant impairments in soma size, growth cone area of the neurite (the tip of the developing neurite), and neurite development (Huang et al., 2019). Although not entirely identical, this work observed a decrease in the number of intersections at short distances from the nucleus, in both GABAergic neurons isolated from dopaminergic brain regions. But within NLGN3 and D2R-positive pooled neurons only in the midbrain.

Shank3 silencing likely affect not just neuronal shape, but also its connections and synapse formation. In striatal neurons, *Shank3* silencing decreased D2R immunofluorescence, suggesting disrupted receptor trafficking or expression. This reduction aligns with abnormalities and decreased postsynaptic endings needed for functional synapses. A similar concept was demonstrated by Arons et al., 2012. Other study measuring gene expression of D2R in striatal neurons isolated from *Shank3* deficient 21-day old mice did not find a significant difference compared to the wild type (Bacova et al., 2024). In our results, a contrasting increase in D2R signal was observed in midbrain neurons, pointing to region-specific compensatory mechanisms or regulatory differences in receptor pathways. Distinct effects might also suggest age-dependent mechanism of the neurons, as neurons in this work were isolated from neonatal rats.

4.2. Effects of oxytocin

Hypothetically if OXT would be able to prolong the neurites in midbrain neurons, it could support the growth of the projections into different regions of the brain, which could improve connectivity in a SHANK3-impaired brain and strengthen mesolimbic pathway connections.

We did not observe such an effect on the length of the neurites. A significant difference after OXT treatment was observable only in striatal NLGN3/D2R pooled neurons where OXT in combination with *Shank3* silencing increased longest neurites. The difference seems to be stronger due to few cells which did not qualify as outliers (Figure 10).

OXT exerted a complex and distance-dependent effect on the neurite arborization. Contrary to our initial hypothesis, OXT reduced arborization in striatal GAD-positive neurons, particularly in shorter neurites. Interestingly, in NLGN3/D2R-positive neurons, *Shank3* silencing alone did not significantly affect arborization. However, when combined with OXT, it increased arborization, surpassing levels observed in the control group (Figure 12 C).

In midbrain GAD-positive neurons, OXT treatment increased the arborization at a shorter distance from the nucleus. However, when combined with *Shank3* silencing, OXT reduced arborization at a greater distance. Altogether OXT appears to partially compensate for the altered arborization in this group at specific distances caused by silencing alone, as there was no general significant difference between the combined treatment and the vehicle control (Figure 15 C). In NLGN3/D2R-positive group OXT seem to lower arborization at shorter distance in vehicle control group but did not appear to further provide any significant additional or compensatory impact (Figure 16), highlighting its differential impact based on cell type and treatment context.

In other studies, the application of OXT extended the neurites, mainly in studies where SH-SY5Y neurons were used, but also primary neurons isolated from the hippocampus, and brain stem (Lestanova et al., 2016b; Reichova et al., 2020; Zhu et al., 2021). In this work, the only clear stimulatory effect of OXT on neurite growth was observed in the longest neurite in striatal NLGN3/D2R neurons silenced for the *Shank3*. It is possible that this effect is specific for dopaminergic neurons since it was not observed in GABAergic neurons. Similar effect was seen after silencing and OXT treatment on arborization of striatal NLGN3/D2R pooled group and partially on GAD-silenced and OXT-treated midbrain group.

The trend in the stimulatory effect of OXT on the growth of neurites, or on the remodeling of the cytoskeleton, was also found in studies that used other cell models than mentioned above. For example, the stimulatory effect of OXT on the growth of neurites in so-called short-term scenario was observed in hair follicle stem cells (Pandamooz et al., 2023). It should be noted that a recent study also describes that OXT induces neurite retraction in hypothalamic H32 cells (Meyer et al., 2018). The authors explain the short-term shortening of neurites under the influence of OXT by reduced phosphorylation of the transcription factor MEF-2A (protein that regulates the expression of genes). This mechanism could also be the case for neurons isolated from the striatum or midbrain in this work. Since MEF-2A was not measured in the present work, this hypothesis needs a verification.

The fact is that the effects of OXT are cell-specific, depending on the concentration of OXTR at a given moment in the neuronal cells, and at the same time they also depend on the dose of OXT present (Zatkova et al., 2019). In this context, OXT can affect various neuron precursors and stem cells but also various other tissues (Pampanella et al., 2024). Moreover, some studies indicate that OXT activates receptors at varying distances from its release (Busnelli & Chini, 2017).

Within the impact of OXT on protein intensity, OXT significantly altered only D2R intensity in midbrain neurons. OXT increased D2R levels in control group but surprisingly decreased them in *Shank3* silenced neurons, suggesting that the presence or absence of SHANK3 modulates OXT influence on D2R expression. In striatal neurons, OXT had lowering effect on the fluorescence intensity of D2R in both control and silencing, while in NLGN3 group only when combined with the silencing. This again shows a potential influence of SHANK3 protein on OXT effects. Even though we did not observe direct effect of OXT alone on the NLGN3 fluorescence intensity, study by Hornberg et al. found effect of *Nlgn3* knockout mice on OXT signaling in the VTA (Hörnberg et al., 2020), suggesting potential indirect interactions.

Our findings demonstrate that the effects of *Shank3* silencing and OXT treatment vary significantly depending on the type of the neuron and the specific morphological parameter measured. This variability underscores the importance of considering regional and cellular specificity when studying neurobiological processes. Distinct effect of OXT on different types of neurons might positively and also negatively influence the morphology, therefore potentially connectivity of the cells and regions.

Furthermore, our results align with the scope of an allostatic theory of OXT (allostasis - ability to maintain stability through change) proposed by Quintana and Guastella, where OXT modulates both social and non-social cues, cognition, and behavior by regulating underlying processes to maintain stability in a changing environment. According to the theory, OXTR's spatiotemporal quality and OXT's release pattern determine these diverse physiological and behavioral effects. Moreover, these qualities dynamically adjust across developmental stages adapting to internal and environmental pressures (Quintana & Guastella, 2020). If we would extend this theory, molecular mechanisms of OXT would differ according to actual situation of the cell, for example *Shank3* silencing, and therefore act on it accordingly. In other words, OXT would start different mechanism of action as an answer to distinct alterations depending on the brain region and cell type in order to maintain stability through change.

4.3. Limitations

Although molecular mechanisms provide valuable insights into the biological underpinnings of behavior, it is not possible to conclude specific behavioral effects at this level of analysis, unless they are directly tested. Experimental paradigms that directly assess behavior are crucial for understanding the full impact of these molecular processes. Furthermore, the use of *in vitro* models does not fully replicate the complex *in vivo* environment and its interactions with other brain regions.

Important to note is that the specific subtype or subset of NLGN3- and D2R-positive neurons remains unclear. Both proteins can be present on different subtypes and can therefore have different mechanism of OXT action, if any. Similar thing applies for *Shank3* silencing where the protein can have slightly different role depending on its surrounding protein structure and cell-type specific mechanisms. This might explain why in some groups the silencing did not alter morphology.

Transfection and further silencing of the gene is not always fully efficient, and it is time dependent. The translation of mRNA could continue to create SHANK3 protein from the gene if the siRNA would not be available anymore. Furthermore, the silencing is targeting only parts of the genetic information which should disable the function of the protein. There is a possibility that part of the protein would still create bonds with other molecules and partially fulfill its function. This fact could also influence the effect of OXT.

4.4. Future perspectives

Future studies should further involve measurements of specific cell-type biomarkers (such as GAD for GABAergic neurons in this study) and of OXTRs to assess the direct effect of OXT on different cell types. This could be later expanded by either molecular studies to elucidate specific mechanisms of OXT action or focus on higher level of analysis to connect molecular findings with behavioral assessments. Computational modelling could be used to predict the effects.

Conclusions

The stimulatory effect of OXT on the growth of neurites in striatal and midbrain neurons was generally not confirmed, except for the lengthening of the neurites in striatal neurons in conditions of *Shank3* gene silencing.

The impact of *Shank3* silencing on neurite branching was significant, shown by a reduction in the shorter extensions in GABAergic and other neurons isolated from the striatum and midbrain. However, this effect was quite variable. OXT did not substantially influence neurite branching.

Significant effects were also observed on the immunofluorescence of D2R and NLGN3 signals, which were reduced in a condition of *Shank3* silencing in striatal neurons. OXT notably affected D2R levels in striatal neurons, decreasing intensity in the control group and slightly increasing it in the silenced group, with an additional negative effect in silenced striatal NLGN3-positive neurons. This reduction did not occur in the neurons isolated from the midbrain.

Further investigation into the effects of OXT and its interaction with *Shank3* silencing is needed, particularly concerning the density of OXTRs and specific brain structures and their developmental stages.

This study is crucial for systematically evaluating neuronal morphology and the immunofluorescence of selected synaptic proteins, which may be useful for other students and experts focused on neuritogenesis and neurogenesis. Furthermore, it provides a basis for subsequent research with either molecular, computational, or behavioral focus. Additionally, future studies on the etiology of ASD should consider the effects of OXT both *in vitro* and *in vivo*, especially in relation to behavioral evaluations and cognitive aspects of neurodevelopmental disorders.

Bibliography

Anpilov, S., Shemesh, Y., Eren, N., Harony-Nicolas, H., Benjamin, A., Dine, J., Oliveira, V. E. M., Forkosh, O., Karamihalev, S., Hüttl, R. E., Feldman, N., Berger, R., Dagan, A., Chen, G., Neumann, I. D., Wagner, S., Yizhar, O., & Chen, A. (2020). Wireless Optogenetic Stimulation of Oxytocin Neurons in a Semi-natural Setup Dynamically Elevates Both Pro-social and Agonistic Behaviors. *Neuron*, *107*(4), 644-655.e7. https://doi.org/10.1016/j.neuron.2020.05.028

Arons, M. H., Thynne, C. J., Grabrucker, A. M., Li, D., Schoen, M., Cheyne, J. E., Boeckers, T. M., Montgomery, J. M., & Garner, C. C. (2012). Autism-Associated Mutations in ProSAP2/Shank3 Impair Synaptic Transmission and Neurexin–Neuroligin-Mediated Transsynaptic Signaling. *The Journal of Neuroscience*, *32*(43), 14966–14978. https://doi.org/10.1523/JNEUROSCI.2215-12.2012

Bacova, Z., Havranek, T., Mihalj, D., Borbelyova, V., Kostrubanicova, K., Kramarova, M., Ostatnikova, D., & Bakos, J. (2024). Reduced Neurite Arborization in Primary Dopaminergic Neurons in Autism-Like Shank3B-Deficient Mice. *Molecular Neurobiology*. https://doi.org/10.1007/s12035-024-04652-0

Bačová, Z., Jurkovičová-Tarabová, B., Havránek, T., Mihalj, D., Borbélyová, V., Pirnik, Z., Mravec, B., Ostatníková, D., & Bakoš, J. (2024). Shank3 deficiency alters midbrain GABAergic neuron morphology, GABAergic markers and synaptic activity in primary striatal neurons. *Molecular Brain*, *17*(1), 71. https://doi.org/10.1186/s13041-024-01145-y

Báez-Mendoza, R., & Schultz, W. (2013). The role of the striatum in social behavior. *Frontiers in Neuroscience*, *7*(7 DEC). https://doi.org/10.3389/fnins.2013.00233

Bakos, J., Hlavacova, N., Makatsori, A., Tybitanclova, K., Zorad, S., Hinghofer-Szalkay, H., Johansson, B. B., & Jezova, D. (2007). Oxytocin levels in the posterior pituitary and in the heart are modified by voluntary wheel running. *Regulatory Peptides*, *139*(1–3), 96–101. https://doi.org/10.1016/j.regpep.2006.10.011

Bakos, J., Lestanova, Z., Strbak, V., Havranek, T., & Bacova, Z. (2014). Neonatal manipulation of oxytocin prevents lipopolysaccharide-induced decrease in gene expression of growth factors in two developmental stages of the female rat. *Neuropeptides*, *48*(5), 281–286. https://doi.org/10.1016/j.npep.2014.06.004

Bakos, J., Srancikova, A., Havranek, T., & Bacova, Z. (2018). Molecular Mechanisms of Oxytocin Signaling at the Synaptic Connection. *Neural Plasticity*, *2018*, 1–9. https://doi.org/10.1155/2018/4864107

Bales, K. L., Plotsky, P. M., Young, L. J., Lim, M. M., Grotte, N., Ferrer, E., & Carter, C. S. (2007). Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. *Neuroscience*, *144*(1), 38–45. https://doi.org/10.1016/j.neuroscience.2006.09.009

Baribeau, D. A., & Anagnostou, E. (2015). Oxytocin and vasopressin: Linking pituitary neuropeptides and their receptors to social neurocircuits. In *Frontiers in Neuroscience* (Vol. 9, Issue SEP). Frontiers Media S.A. https://doi.org/10.3389/fnins.2015.00335

Bariselli, S., Hörnberg, H., Prévost-Solié, C., Musardo, S., Hatstatt-Burklé, L., Scheiffele, P., & Bellone, C. (2018). Role of VTA dopamine neurons and neuroligin 3 in sociability traits related to nonfamiliar conspecific interaction. *Nature Communications*, *9*(1), 3173. https://doi.org/10.1038/s41467-018-05382-3

Bartz, J. A., Zaki, J., Bolger, N., & Ochsner, K. N. (2011). Social effects of oxytocin in humans: Context and person matter. In *Trends in Cognitive Sciences* (Vol. 15, Issue 7, pp. 301–309). https://doi.org/10.1016/j.tics.2011.05.002

Bault, N., Joffily, M., Rustichini, A., & Coricelli, G. (2011). Medial prefrontal cortex and striatum mediate the influence of social comparison on the decision process. *Proceedings of the National Academy of Sciences*, *108*(38), 16044–16049. https://doi.org/10.1073/pnas.1100892108

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. In *Pharmacological Reviews* (Vol. 63, Issue 1, pp. 182–217). https://doi.org/10.1124/pr.110.002642

Beaulieu, J.-M., Espinoza, S., & Gainetdinov, R. R. (2014). *NC-IUPHAR REVIEW*. https://doi.org/10.1111/bph.12445/abstract

Bendersky, C. J., Milian, A. A., Andrus, M. D., De La Torre, U., & Walker, D. M. (2021). Long-Term Impacts of Post-weaning Social Isolation on Nucleus Accumbens Function. In *Frontiers in Psychiatry* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fpsyt.2021.745406

Berkel, S., Marshall, C. R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D., Bonin, M., Riess, A., Engels, H., Sprengel, R., Scherer, S. W., & Rappold, G. A. (2010). Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nature Genetics*, *42*(6), 489–491. https://doi.org/10.1038/ng.589

Bey, A. L., Wang, X., Yan, H., Kim, N., Passman, R. L., Yang, Y., Cao, X., Towers, A. J., Hulbert, S. W., Duffney, L. J., Gaidis, E., Rodriguiz, R. M., Wetsel, W. C., Yin, H. H., & Jiang, Y. H. (2018). Brain region-specific disruption of Shank3 in mice reveals a dissociation for cortical and striatal circuits in autism-related behaviors. *Translational Psychiatry*, *8*(1). https://doi.org/10.1038/s41398-018-0142-6

Blum, K., Bowirrat, A., Sunder, K., Thanos, P. K., Hanna, C., Gold, M. S., Dennen, C. A., Elman, I., Murphy, K. T., & Makale, M. T. (2024). Dopamine Dysregulation in Reward and Autism Spectrum Disorder. In *Brain Sciences* (Vol. 14, Issue 7). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/brainsci14070733

Borroto-Escuela, D. O., Cuesta-Marti, C., Lopez-Salas, A., Chruścicka-Smaga, B., Crespo-Ramírez, M., Tesoro-Cruz, E., Palacios-Lagunas, D. A., Perez de la Mora, M., Schellekens, H., & Fuxe, K. (2022). The oxytocin receptor represents a key hub in the GPCR heteroreceptor network: potential relevance for brain and behavior. In *Frontiers in*

Molecular Neuroscience (Vol. 15). Frontiers Media S.A. https://doi.org/10.3389/fnmol.2022.1055344

Bouthenet, M.-L., Souil, E., Martres, M.-P., Sokoloff, P., Giros, B., & Schwartz, J.-C. (1991). Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. *Brain Research*, *564*(2), 203–219. https://doi.org/10.1016/0006-8993(91)91456-B

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. In *Neuron* (Vol. 68, Issue 5, pp. 815–834). https://doi.org/10.1016/j.neuron.2010.11.022

Brown, C. H., Ludwig, M., Tasker, J. G., & Stern, J. E. (2020). Somato-dendritic vasopressin and oxytocin secretion in endocrine and autonomic regulation. *Journal of Neuroendocrinology*, *32*(6). https://doi.org/10.1111/jne.12856

Budreck, E. C., & Scheiffele, P. (2007). Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. *European Journal of Neuroscience*, *26*(7), 1738–1748. https://doi.org/10.1111/j.1460-9568.2007.05842.x

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B. M., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science*, *351*(6271), 375–378. https://doi.org/10.1126/science.aac4785

Busnelli, M., & Chini, B. (2017). *Molecular Basis of Oxytocin Receptor Signalling in the Brain: What We Know and What We Need to Know* (pp. 3–29). https://doi.org/10.1007/7854_2017_6

Carlezon, W. A., Devine, D. P., & Wise, R. A. (1995). Habit-forming actions of nomifensine in nucleus accumbens. *Psychopharmacology*, *122*(2), 194–197. https://doi.org/10.1007/BF02246095

CARMICHAEL, M. S., HUMBERT, R., DIXEN, J., PALMISANO, G., GREENLEAF, W., & DAVIDSON, J. M. (1987). Plasma Oxytocin Increases in the Human Sexual Response*. *The Journal of Clinical Endocrinology & Metabolism*, *64*(1), 27–31. https://doi.org/10.1210/jcem-64-1-27

Carr, GeoffreyD., & White, NormanM. (1986). Anatomical disassociation of amphetamine's rewarding and aversive effects: An intracranial microinjection study. *Psychopharmacology*, *89*(3). https://doi.org/10.1007/BF00174372

Chen, H., Li, J., Huang, Z., Fan, X., Wang, X., Chen, X., Guo, H., Liu, H., Li, S., Yu, S., Li, H., Huang, X., Ma, X., Deng, X., Wang, C., & Liu, Y. (2024). Dopaminergic system and neurons: Role in multiple neurological diseases. In *Neuropharmacology* (Vol. 260). Elsevier Ltd. https://doi.org/10.1016/j.neuropharm.2024.110133

Chklovskii, D. B. (2004). Synaptic Connectivity and Neuronal Morphology. *Neuron*, *43*(5), 609–617. https://doi.org/10.1016/j.neuron.2004.08.012

Cho, J. R., Chen, X., Kahan, A., Robinson, J. E., Wagenaar, D. A., & Gradinaru, V. (2021). Dorsal raphe dopamine neurons signal motivational salience dependent on internal state,

expectation, and behavioral context. *Journal of Neuroscience*, *41*(12), 2645–2655. https://doi.org/10.1523/JNEUROSCI.2690-20.2021

Citri, A., & Malenka, R. C. (2008). Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacology*, *33*(1), 18–41. https://doi.org/10.1038/sj.npp.1301559

Colón-Ramos, D. A. (2009). *Chapter 2 Synapse Formation in Developing Neural Circuits* (pp. 53–79). https://doi.org/10.1016/S0070-2153(09)01202-2

Dabney, W., Kurth-Nelson, Z., Uchida, N., Starkweather, C. K., Hassabis, D., Munos, R., & Botvinick, M. (2020). A distributional code for value in dopamine-based reinforcement learning. *Nature*, *577*(7792), 671–675. https://doi.org/10.1038/s41586-019-1924-6

DAHLSTROEM, A., & FUXE, K. (1964). EVIDENCE FOR THE EXISTENCE OF MONOAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM. I. DEMONSTRATION OF MONOAMINES IN THE CELL BODIES OF BRAIN STEM NEURONS. *Acta Physiologica Scandinavica. Supplementum*, SUPPL 232:1-55.

de Jong, J. W., Afjei, S. A., Pollak Dorocic, I., Peck, J. R., Liu, C., Kim, C. K., Tian, L., Deisseroth, K., & Lammel, S. (2019). A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System. *Neuron*, *101*(1), 133-151.e7. https://doi.org/10.1016/j.neuron.2018.11.005

de la Mora, M. P., Pérez-Carrera, D., Crespo-Ramírez, M., Tarakanov, A., Fuxe, K., & Borroto-Escuela, D. O. (2016). Signaling in dopamine D2 receptor-oxytocin receptor heterocomplexes and its relevance for the anxiolytic effects of dopamine and oxytocin interactions in the amygdala of the rat. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1862*(11), 2075–2085. https://doi.org/10.1016/j.bbadis.2016.07.004

De Mei, C., Ramos, M., Iitaka, C., & Borrelli, E. (2009). Getting specialized: presynaptic and postsynaptic dopamine D2 receptors. *Current Opinion in Pharmacology*, *9*(1), 53–58. https://doi.org/10.1016/j.coph.2008.12.002

De Wled, D. (1965). The influence of the posterior and inter-mediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. *International Journal of Neuropharmacology*, *4*(3), 157–167. https://doi.org/10.1016/0028-3908(65)90005-5

Deaner, R. O., Khera, A. V., & Platt, M. L. (2005). Monkeys Pay Per View: Adaptive Valuation of Social Images by Rhesus Macaques. *Current Biology*, *15*(6), 543–548. https://doi.org/10.1016/j.cub.2005.01.044

Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: Dopamine, facilitation of incentive motivation, and extraversion. In *Behavioral and Brain Sciences* (Vol. 22, Issue 3, pp. 491–517). https://doi.org/10.1017/S0140525X99002046

Desai, A., & Mitchison, T. J. (1997). MICROTUBULE POLYMERIZATION DYNAMICS. In *Annu. Rev. Cell Dev. Biol* (Vol. 13). www.annualreviews.org

Dobbs, L. K., Kaplan, A. R., Lemos, J. C., Matsui, A., Rubinstein, M., & Alvarez, V. A. (2016). Dopamine Regulation of Lateral Inhibition between Striatal Neurons Gates the Stimulant

Actions of Cocaine. *Neuron*, *90*(5), 1100–1113. https://doi.org/10.1016/j.neuron.2016.04.031

Dölen, G., Darvishzadeh, A., Huang, K. W., & Malenka, R. C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature*, *501*(7466), 179–184. https://doi.org/10.1038/nature12518

Douglas, A., Leng, G., & Russell, J. (2002). The importance of oxytocin mechanisms in the control of mouse parturition. *Reproduction*, *123*(4), 543–552. https://doi.org/10.1530/rep.0.1230543

Dreyer, J. K., Herrik, K. F., Berg, R. W., & Hounsgaard, J. D. (2010). Influence of Phasic and Tonic Dopamine Release on Receptor Activation. *The Journal of Neuroscience*, *30*(42), 14273–14283. https://doi.org/10.1523/JNEUROSCI.1894-10.2010

DU VIGNEAUD, V., RESSLER, C., & TRIPPETT, S. (1953). The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. *The Journal of Biological Chemistry*, *205*(2), 949–957. https://doi.org/10.1016/s0021-9258(18)49238-1

Dufour, B. D., McBride, E., Bartley, T., Juarez, P., & Martínez-Cerdeño, V. (2023). Distinct patterns of GABAergic interneuron pathology in autism are associated with intellectual impairment and stereotypic behaviors. *Autism*, *27*(6), 1730–1745. https://doi.org/10.1177/13623613231154053

Durand, C. M., Betancur, C., Boeckers, T. M., Bockmann, J., Chaste, P., Fauchereau, F., Nygren, G., Rastam, M., Gillberg, I. C., Anckarsäter, H., Sponheim, E., Goubran-Botros, H., Delorme, R., Chabane, N., Mouren-Simeoni, M.-C., de Mas, P., Bieth, E., Rogé, B., Héron, D., … Bourgeron, T. (2007). Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nature Genetics*, *39*(1), 25–27. https://doi.org/10.1038/ng1933

Engelmann, Ebner, Landgraf, Holsboer, & Wotjak. (1999). Emotional Stress Triggers Intrahypothalamic But Not Peripheral Release of Oxytocin in Male Rats. *Journal of Neuroendocrinology*, *11*(11), 867–872. https://doi.org/10.1046/j.1365-2826.1999.00403.x

Fallon, J. H., & Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain III. Olfactory bulb, anterior olfactory nuclei, olfactory tubercle and piriform cortex. *Journal of Comparative Neurology*, *180*(3), 533–544. https://doi.org/10.1002/cne.901800309

Filova, B., Reichova, A., Zatkova, M., Srancikova, A., Bukatova, S., Bacova, Z., & Bakos, J. (2020). Expression of synaptic proteins in the hippocampus is modulated by neonatal oxytocin treatment. *Neuroscience Letters*, *725*, 134912. https://doi.org/10.1016/j.neulet.2020.134912

Freeman, A. R., Aulino, E. A., Caldwell, H. K., & Ophir, A. G. (2020). Comparison of the distribution of oxytocin and vasopressin 1a receptors in rodents reveals conserved and derived patterns of nonapeptide evolution. *Journal of Neuroendocrinology*, *32*(4). https://doi.org/10.1111/jne.12828

Froemke, R. C., & Young, L. J. (2024). Oxytocin, Neural Plasticity, and Social Behavior. *Annual Review of Neuroscience Downloaded from Www.Annualreviews.Org. Guest*, *58*, 5. https://doi.org/10.1146/annurev-neuro-102320

Gasbarri, A., Sulli, A., & Packard, M. G. (1997). The dopaminergic mesencephalic projections to the hippocampal formation in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *21*(1), 1–22. https://doi.org/10.1016/S0278-5846(96)00157-1

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of Striatal Projection Systems by Dopamine. *Annual Review of Neuroscience*, *34*(1), 441–466. https://doi.org/10.1146/annurev-neuro-061010-113641

Gershman, S. J., & Uchida, N. (2019). Believing in dopamine. *Nature Reviews Neuroscience*, *20*(11), 703–714. https://doi.org/10.1038/s41583-019-0220-7

Gingrich, B., Liu, Y., Cascio, C., Wang, Z., & Insel, T. R. (2000). Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (Microtus ochrogaster). *Behavioral Neuroscience*, *114*(1), 173–183. https://doi.org/10.1037/0735-7044.114.1.173

Grossmann, C. P., Sommer, C., Fahliogullari, I. B., Neumann, I. D., & Menon, R. (2024). Mating-induced release of oxytocin in the mouse lateral septum: Implications for social fear extinction. *Psychoneuroendocrinology*, *166*, 107083. https://doi.org/10.1016/j.psyneuen.2024.107083

Gu, C. (2021). Rapid and Reversible Development of Axonal Varicosities: A New Form of Neural Plasticity. *Frontiers in Molecular Neuroscience*, *14*. https://doi.org/10.3389/fnmol.2021.610857

Hamid, A. A., Pettibone, J. R., Mabrouk, O. S., Hetrick, V. L., Schmidt, R., Vander Weele, C. M., Kennedy, R. T., Aragona, B. J., & Berke, J. D. (2015). Mesolimbic dopamine signals the value of work. *Nature Neuroscience*, *19*(1), 117–126. https://doi.org/10.1038/nn.4173

Hammock, E. A. D. (2015). Developmental perspectives on oxytocin and vasopressin. In *Neuropsychopharmacology* (Vol. 40, Issue 1, pp. 24–42). Nature Publishing Group. https://doi.org/10.1038/npp.2014.120

Harari-Dahan, O., & Bernstein, A. (2014). A general approach - avoidance hypothesis of Oxytocin: Accounting for social and non-social effects of oxytocin. In *Neuroscience and Biobehavioral Reviews* (Vol. 47, pp. 506–519). Elsevier Ltd. https://doi.org/10.1016/j.neubiorev.2014.10.007

Harony-Nicolas, H., Kay, M., Hoffmann, J. du, Klein, M. E., Bozdagi-Gunal, O., Riad, M., Daskalakis, N. P., Sonar, S., Castillo, P. E., Hof, P. R., Shapiro, M. L., Baxter, M. G., Wagner, S., & Buxbaum, J. D. (2017). Oxytocin improves behavioral and electrophysiological deficits in a novel Shank3-deficient rat. *ELife*, *6*. https://doi.org/10.7554/eLife.18904

Havranek, T., Bacova, Z., & Bakos, J. (2024). Oxytocin, GABA, and dopamine interplay in autism. *Endocrine Regulations*, *58*(1), 105–114. https://doi.org/10.2478/enr-2024-0012

Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry*, *54*(12), 1389–1398. https://doi.org/10.1016/S0006-3223(03)00465-7

Hettinger, J. A., Liu, X., Hudson, M. L., Lee, A., Cohen, I. L., Michaelis, R. C., Schwartz, C. E., Lewis, S. M., & Holden, J. J. (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. *Behavioral and Brain Functions*, *8*(1), 19. https://doi.org/10.1186/1744-9081-8-19

Higashida, H., Oshima, Y., & Yamamoto, Y. (2024). Oxytocin transported from the blood across the blood-brain barrier by receptor for advanced glycation end-products (RAGE) affects brain function related to social behavior. *Peptides*, *178*, 171230. https://doi.org/10.1016/j.peptides.2024.171230

Higuchi, T., Tadokoro, Y., Honda, K., & Negoro, H. (1986). Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *Journal of Endocrinology*, *110*(2), 251–256. https://doi.org/10.1677/joe.0.1100251

Hitti, F. L., & Siegelbaum, S. A. (2014). The hippocampal CA2 region is essential for social memory. *Nature*, *508*(1), 88–92. https://doi.org/10.1038/nature13028

Hoon, M., Soykan, T., Falkenburger, B., Hammer, M., Patrizi, A., Schmidt, K.-F., Sassoè-Pognetto, M., Löwel, S., Moser, T., Taschenberger, H., Brose, N., & Varoqueaux, F. (2011). Neuroligin-4 is localized to glycinergic postsynapses and regulates inhibition in the retina. *Proceedings of the National Academy of Sciences*, *108*(7), 3053–3058. https://doi.org/10.1073/pnas.1006946108

Hörnberg, H., Pérez-Garci, E., Schreiner, D., Hatstatt-Burklé, L., Magara, F., Baudouin, S., Matter, A., Nacro, K., Pecho-Vrieseling, E., & Scheiffele, P. (2020). Rescue of oxytocin response and social behaviour in a mouse model of autism. *Nature*, *584*(7820), 252–256. https://doi.org/10.1038/s41586-020-2563-7

Huang, G., Chen, S., Chen, X., Zheng, J., Xu, Z., Doostparast Torshizi, A., Gong, S., Chen, Q., Ma, X., Yu, J., Zhou, L., Qiu, S., Wang, K., & Shi, L. (2019). Uncovering the Functional Link Between SHANK3 Deletions and Deficiency in Neurodevelopment Using iPSC-Derived Human Neurons. *Frontiers in Neuroanatomy*, *13*. https://doi.org/10.3389/fnana.2019.00023

Huber, G., & Matus, A. (1984). Differences in the cellular distributions of two microtubule-associated proteins, MAP1 and MAP2, in rat brain. *The Journal of Neuroscience*, *4*(1), 151–160. https://doi.org/10.1523/JNEUROSCI.04-01-00151.1984

Husarova, V. M., Lakatosova, S., Pivovarciova, A., Babinska, K., Bakos, J., Durdiakova, J., Kubranska, A., Ondrejka, I., & Ostatnikova, D. (2016). Plasma Oxytocin in Children with Autism and Its Correlations with Behavioral Parameters in Children and Parents. *Psychiatry Investigation*, *13*(2), 174. https://doi.org/10.4306/pi.2016.13.2.174

Ikemoto, S., & Panksepp, J. (1999a). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews*, *31*(1), 6–41. https://doi.org/10.1016/S0165-0173(99)00023-5

Ikemoto, S., & Panksepp, J. (1999b). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews*, *31*(1), 6–41. https://doi.org/10.1016/S0165-0173(99)00023-5

Insel, T. R., & Shapiro, L. E. (1992). *Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles (microtine/affiliation/parental behavior/amygdala/septum)* (Vol. 89). https://www.pnas.org

Jo, Y.-H., Stoeckel, M.-E., Freund-Mercier, M.-J., & My Schlichter, R. (1998). Oxytocin Modulates Glutamatergic Synaptic Transmission between Cultured Neonatal Spinal Cord Dorsal Horn Neurons. In *The Journal of Neuroscience* (Vol. 18, Issue 7).

Juárez Olguín, H., Calderón Guzmán, D., Hernández García, E., & Barragán Mejía, G. (2016). The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *2016*(1). https://doi.org/10.1155/2016/9730467

Jurek, B., & Neumann, I. D. (2018). THE OXYTOCIN RECEPTOR: FROM INTRACELLULAR SIGNALING TO BEHAVIOR. *Physiol Rev*, *98*, 1805–1908. https://doi.org/10.1152/physrev.00031.2017.-The

Keeler, J. F., Pretsell, D. O., & Robbins, T. W. (2014). Functional implications of dopamine D1 vs. D2 receptors: A "prepare and select" model of the striatal direct vs. indirect pathways. In *Neuroscience* (Vol. 282, pp. 156–175). Elsevier Ltd. https://doi.org/10.1016/j.neuroscience.2014.07.021

Kemp, A. H., & Guastella, A. J. (2011). The role of oxytocin in human affect: A novel hypothesis. In *Current Directions in Psychological Science* (Vol. 20, Issue 4, pp. 222–231). https://doi.org/10.1177/0963721411417547

Kendrick, K. M. (1990). Microdialysis measurement of in vivo neuropeptide release. *Journal of Neuroscience Methods*, *34*(1–3), 35–46. https://doi.org/10.1016/0165-0270(90)90040-M

Kendrick, K. M., Guastella, A. J., & Becker, B. (2018). Overview of human oxytocin research. In *Current Topics in Behavioral Neurosciences* (Vol. 35, pp. 321–348). Springer Verlag. https://doi.org/10.1007/7854_2017_19

Kimura, T., Tanizawa, O., Mori, K., Brownstein, M. J., & Okayama, H. (1992). Structure and expression of a human oxytocin receptor. *Nature*, *356*(6369), 526–529. https://doi.org/10.1038/356526a0

Klein, J. T., & Platt, M. L. (2013). Social information signaling by neurons in primate striatum. *Current Biology*, *23*(8), 691–696. https://doi.org/10.1016/j.cub.2013.03.022

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., & Correa, R. G. (2019). Dopamine: Functions, Signaling, and Association with Neurological Diseases. In *Cellular and Molecular Neurobiology* (Vol. 39, Issue 1, pp. 31–59). Springer New York LLC. https://doi.org/10.1007/s10571-018-0632-3

Knobloch, H. S., Charlet, A., Hoffmann, L. C., Eliava, M., Khrulev, S., Cetin, A. H., Osten, P., Schwarz, M. K., Seeburg, P. H., Stoop, R., & Grinevich, V. (2012). Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron*, *73*(3), 553–566. https://doi.org/10.1016/j.neuron.2011.11.030

Knoop, M., Possovre, M. L., Jacquens, A., Charlet, A., Baud, O., & Darbon, P. (2022). The Role of Oxytocin in Abnormal Brain Development: Effect on Glial Cells and Neuroinflammation. In *Cells* (Vol. 11, Issue 23). MDPI. https://doi.org/10.3390/cells11233899

Kombian, S. B., Hirasawa, M., Mouginot, D., & Pittman, Q. J. (2002). *Chapter 18 Modulation of synaptic transmission by oxytocin and vasopressin in the supraoptic nucleus* (pp. 235–246). https://doi.org/10.1016/S0079-6123(02)39020-4

Kozlova, I., Sah, S., Keable, R., Leshchyns'ka, I., Janitz, M., & Sytnyk, V. (2020). Cell Adhesion Molecules and Protein Synthesis Regulation in Neurons. *Frontiers in Molecular Neuroscience*, *13*. https://doi.org/10.3389/fnmol.2020.592126

Landgraf, R., & Neumann, I. D. (2004). Vasopressin and oxytocin release within the brain: A dynamic concept of multiple and variable modes of neuropeptide communication. *Frontiers in Neuroendocrinology*, *25*(3–4), 150–176. https://doi.org/10.1016/j.yfrne.2004.05.001

Lang, R. E., Heil, J. W. E., Ganten, D., Hermann, K., Unger, T., & Rascher, W. (1983). Oxytocin Unlike Vasopressin Is a Stress Hormone in the Rat. *Neuroendocrinology*, *37*(4), 314–316. https://doi.org/10.1159/000123566

Lanoue, V., & Cooper, H. M. (2019). Branching mechanisms shaping dendrite architecture. *Developmental Biology*, *451*(1), 16–24. https://doi.org/10.1016/j.ydbio.2018.12.005

Lee, M. R., Scheidweiler, K. B., Diao, X. X., Akhlaghi, F., Cummins, A., Huestis, M. A., Leggio, L., & Averbeck, B. B. (2018). Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay. *Molecular Psychiatry*, *23*(1), 115–122. https://doi.org/10.1038/mp.2017.27

Lee, M. R., Sheskier, M. B., Farokhnia, M., Feng, N., Marenco, S., Lipska, B. K., & Leggio, L. (2018). Oxytocin receptor mRNA expression in dorsolateral prefrontal cortex in major psychiatric disorders: A human post-mortem study. *Psychoneuroendocrinology*, *96*, 143–147. https://doi.org/10.1016/j.psyneuen.2018.05.039

Lestanova, Z., Bacova, Z., Kiss, A., Havranek, T., Strbak, V., & Bakos, J. (2016a). Oxytocin Increases Neurite Length and Expression of Cytoskeletal Proteins Associated with Neuronal Growth. *Journal of Molecular Neuroscience*, *59*(2), 184–192. https://doi.org/10.1007/s12031-015-0664-9

Lestanova, Z., Bacova, Z., Kiss, A., Havranek, T., Strbak, V., & Bakos, J. (2016b). Oxytocin Increases Neurite Length and Expression of Cytoskeletal Proteins Associated with Neuronal Growth. *Journal of Molecular Neuroscience*, *59*(2), 184–192. https://doi.org/10.1007/s12031-015-0664-9

Leuner, B., Caponiti, J. M., & Gould, E. (2012). Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus*, *22*(4), 861–868. https://doi.org/10.1002/hipo.20947

Li, Y.-C., Kellendonk, C., Simpson, E. H., Kandel, E. R., & Gao, W.-J. (2011). D2 receptor overexpression in the striatum leads to a deficit in inhibitory transmission and dopamine

sensitivity in mouse prefrontal cortex. *Proceedings of the National Academy of Sciences*, *108*(29), 12107–12112. https://doi.org/10.1073/pnas.1109718108

Lilja, J., Zacharchenko, T., Georgiadou, M., Jacquemet, G., Franceschi, N. D., Peuhu, E., Hamidi, H., Pouwels, J., Martens, V., Nia, F. H., Beifuss, M., Boeckers, T., Kreienkamp, H.-J., Barsukov, I. L., & Ivaska, J. (2017). SHANK proteins limit integrin activation by directly interacting with Rap1 and R-Ras. *Nature Cell Biology*, *19*(4), 292–305. https://doi.org/10.1038/ncb3487

Lin, Y. C., & Koleske, A. J. (2010). Mechanisms of synapse and dendrite maintenance and their disruption in psychiatric and neurodegenerative disorders. In *Annual Review of Neuroscience* (Vol. 33, pp. 349–378). https://doi.org/10.1146/annurev-neuro-060909-153204

Lin, Y.-T., Chen, C.-C., Huang, C.-C., Nishimori, K., & Hsu, K.-S. (2017). Oxytocin stimulates hippocampal neurogenesis via oxytocin receptor expressed in CA3 pyramidal neurons. *Nature Communications*, *8*(1), 537. https://doi.org/10.1038/s41467-017-00675-5

Liu, J., Fu, H., Kong, J., Yu, H., & Zhang, Z. (2021). Association between autism spectrum disorder and polymorphisms in genes encoding serotine and dopamine receptors. *Metabolic Brain Disease*, *36*(5), 865–870. https://doi.org/10.1007/s11011-021-00699-3

Liu, X., Hua, F., Yang, D., Lin, Y., Zhang, L., Ying, J., Sheng, H., & Wang, X. (2022). Roles of neuroligins in central nervous system development: focus on glial neuroligins and neuron neuroligins. In *Journal of Translational Medicine* (Vol. 20, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12967-022-03625-y

Liu, Y., & Wang, Z. X. (2003). Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. *Neuroscience*, *121*(3), 537–544. https://doi.org/10.1016/S0306-4522(03)00555-4

Lodge, D. J., & Grace, A. A. (2011). Developmental pathology, dopamine, stress and schizophrenia. *International Journal of Developmental Neuroscience*, *29*(3), 207–213. https://doi.org/10.1016/j.ijdevneu.2010.08.002

Loughlin, S. E., & Fallon, J. H. (1983). Dopaminergic and non-dopaminergic projections to amygdala from substantia nigra and ventral tegmental area. *Brain Research*, *262*(2), 334–338. https://doi.org/10.1016/0006-8993(83)91029-6

Love, T. M. (2014). Oxytocin, motivation and the role of dopamine. In *Pharmacology Biochemistry and Behavior* (Vol. 119, pp. 49–60). Elsevier Inc. https://doi.org/10.1016/j.pbb.2013.06.011

Lukas, M., Toth, I., Veenema, A. H., & Neumann, I. D. (2013). Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. *Psychoneuroendocrinology*, *38*(6), 916–926. https://doi.org/10.1016/j.psyneuen.2012.09.018

Lutas, A., Kucukdereli, H., Alturkistani, O., Carty, C., Sugden, A. U., Fernando, K., Diaz, V., Flores-Maldonado, V., & Andermann, M. L. (2019). State-specific gating of salient cues

by midbrain dopaminergic input to basal amygdala. *Nature Neuroscience*, *22*(11), 1820–1833. https://doi.org/10.1038/s41593-019-0506-0

Luther, J. A., & Tasker, J. G. (2000). Voltage-gated currents distinguish parvocellular from magnocellular neurones in the rat hypothalamic paraventricular nucleus. *The Journal of Physiology*, *523*(1), 193–209. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00193.x

MacLean, E. L., Wilson, S. R., Martin, W. L., Davis, J. M., Nazarloo, H. P., & Carter, C. S. (2019). Challenges for measuring oxytocin: The blind men and the elephant? In *Psychoneuroendocrinology* (Vol. 107, pp. 225–231). Elsevier Ltd. https://doi.org/10.1016/j.psyneuen.2019.05.018

Mandelkow, E., & Mandelkow, E.-M. (1995). Microtubules and microtubule-associated proteins. In *Current Opinion in Cell Biology* (Vol. 7).

Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L. J. M. J., & Trezza, V. (2016). Dopaminergic neurotransmission in the nucleus accumbens modulates social play behavior in rats. *Neuropsychopharmacology*, *41*(9), 2215–2223. https://doi.org/10.1038/npp.2016.22

Martel, J. C., & Gatti-McArthur, S. (2020). Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia. In *Frontiers in Pharmacology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fphar.2020.01003

Martínez-Cerdeño, V. (2017). Dendrite and spine modifications in autism and related neurodevelopmental disorders in patients and animal models. *Developmental Neurobiology*, *77*(4), 393–404. https://doi.org/10.1002/dneu.22417

Matsumoto, M., & Hikosaka, O. (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature*, *459*(7248), 837–841. https://doi.org/10.1038/nature08028

Mei, Y., Monteiro, P., Zhou, Y., Kim, J. A., Gao, X., Fu, Z., & Feng, G. (2016). Adult restoration of Shank3 expression rescues selective autistic-like phenotypes. *Nature*, *530*(7591), 481–484. https://doi.org/10.1038/nature16971

Melin, P., & Kihlström, J. E. (1963). Influence of Oxytocin on Sexual Behavior in Male Rabbits. *Endocrinology*, *73*(4), 433–435. https://doi.org/10.1210/endo-73-4-433

Meliskova, V., Havranek, T., Bacova, Z., & Bakos, J. (2021). The role of selected postsynaptic scaffolding proteins at glutamatergic synapses in autism-related animal models. *Journal of Integrative Neuroscience*, *20*(4). https://doi.org/10.31083/j.jin2004106

Meyer, M., Berger, I., Winter, J., & Jurek, B. (2018). Oxytocin alters the morphology of hypothalamic neurons via the transcription factor myocyte enhancer factor 2A (MEF-2A). *Molecular and Cellular Endocrinology*, *477*, 156–162. https://doi.org/10.1016/j.mce.2018.06.013

Mischel, W., & Shoda, Y. (1995). A cognitive-affective system theory of personality: Reconceptualizing situations, dispositions, dynamics, and invariance in personality structure. *Psychological Review*, *102*(2), 246–268. https://doi.org/10.1037/0033-295X.102.2.246

Miyazaki, S., Hiraoka, Y., Hidema, S., & Nishimori, K. (2016). Prenatal minocycline treatment alters synaptic protein expression, and rescues reduced mother call rate in oxytocin receptor-knockout mice. *Biochemical and Biophysical Research Communications*, *472*(2), 319–323. https://doi.org/10.1016/j.bbrc.2016.02.109

Mogenson, G. J., & Nielsen, M. (1984). Neuropharmacological evidence to suggest that the nucleus accumbens and subpallidal region contribute to exploratory locomotion. *Behavioral and Neural Biology*, *42*(1), 52–60. https://doi.org/10.1016/S0163-1047(84)90424-2

Monteiro, P., & Feng, G. (2017). SHANK proteins: Roles at the synapse and in autism spectrum disorder. In *Nature Reviews Neuroscience* (Vol. 18, Issue 3, pp. 147–157). Nature Publishing Group. https://doi.org/10.1038/nrn.2016.183

Mora-Jensen, A.-R. C., Clemmensen, L. K. H., Grønberg, M. G., Lebowitz, E. R., Quintana, D. S., Jørgensen, N. R., Larsen, C. S., Bak, L. K., Christensen, G. L., Pretzmann, L., Uhre, V., Christensen, S. H., Uhre, C., Korsbjerg, N. L. J., Thoustrup, C. L., Hagstrøm, J., Ritter, M., Plessen, K. J., Pagsberg, A. K., & Lønfeldt, N. N. (2024). The association between salivary oxytocin, age, and puberty in children with and without OCD. *Scientific Reports*, *14*(1), 28693. https://doi.org/10.1038/s41598-024-80194-8

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. In *Nature Reviews Neuroscience* (Vol. 18, Issue 2, pp. 73–85). Nature Publishing Group. https://doi.org/10.1038/nrn.2016.165

Neumann, I. D. (2007). Stimuli and consequences of dendritic release of oxytocin within the brain. *Biochemical Society Transactions*, *35*(5), 1252–1257. https://doi.org/10.1042/BST0351252

Numan, M., & Smith, H. G. (1984). Maternal behavior in rats: Evidence for the involvement of preoptic projections to the ventral tegmental area. *Behavioral Neuroscience*, *98*(4), 712–727. https://doi.org/10.1037/0735-7044.98.4.712

Owen, S. F., Tuncdemir, S. N., Bader, P. L., Tirko, N. N., Fishell, G., & Tsien, R. W. (2013). Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature*, *500*(7463), 458–462. https://doi.org/10.1038/nature12330

Pampanella, L., Petrocelli, G., Forcellini, F., Cruciani, S., Ventura, C., Abruzzo, P. M., Facchin, F., & Canaider, S. (2024). Oxytocin, the Love Hormone, in Stem Cell Differentiation. *Current Issues in Molecular Biology*, *46*(11), 12012–12036. https://doi.org/10.3390/cimb46110713

Pandamooz, S., Salehi, M. S., Jurek, B., Meinung, C.-P., Azarpira, N., Dianatpour, M., & Neumann, I. D. (2023). Oxytocin Receptor Expression in Hair Follicle Stem Cells: A Promising Model for Biological and Therapeutic Discovery in Neuropsychiatric Disorders. *Stem Cell Reviews and Reports*, *19*(7), 2510–2524. https://doi.org/10.1007/s12015-023-10603-4

Park, H., & Poo, M. (2013). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience*, *14*(1), 7–23. https://doi.org/10.1038/nrn3379

Passamonti, L., Terracciano, A., Riccelli, R., Donzuso, G., Cerasa, A., Vaccaro, MG., Novellino, F., Fera, F., & Quattrone, A. (2015). Increased functional connectivity within mesocortical networks in open people. *NeuroImage*, *104*, 301–309. https://doi.org/10.1016/j.neuroimage.2014.09.017

Pavăl, D. (2017). A Dopamine Hypothesis of Autism Spectrum Disorder. In *Developmental Neuroscience* (Vol. 39, Issue 5, pp. 355–360). S. Karger AG. https://doi.org/10.1159/000478725

Peça, J., Feliciano, C., Ting, J. T., Wang, W., Wells, M. F., Venkatraman, T. N., Lascola, C. D., Fu, Z., & Feng, G. (2011). Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*, *472*(7344), 437–442. https://doi.org/10.1038/nature09965

Pedersen, C. A., & Prange, A. J. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proceedings of the National Academy of Sciences*, *76*(12), 6661–6665. https://doi.org/10.1073/pnas.76.12.6661

Phillips, G. D., Robbins, T. W., & Everitt, B. J. (1994). Bilateral intra-accumbens self-administration ofd-amphetamine: Antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology*, *114*(3), 477–485. https://doi.org/10.1007/BF02249339

Puighermanal, E., Castell, L., Esteve-Codina, A., Melser, S., Kaganovsky, K., Zussy, C., Boubaker-Vitre, J., Gut, M., Rialle, S., Kellendonk, C., Sanz, E., Quintana, A., Marsicano, G., Martin, M., Rubinstein, M., Girault, J.-A., Ding, J. B., & Valjent, E. (2020). Functional and molecular heterogeneity of D2R neurons along dorsal ventral axis in the striatum. *Nature Communications*, *11*(1), 1957. https://doi.org/10.1038/s41467-020-15716-9

Quintana, D. S., & Guastella, A. J. (2020). An Allostatic Theory of Oxytocin. In *Trends in Cognitive Sciences* (Vol. 24, Issue 7, pp. 515–528). Elsevier Ltd. https://doi.org/10.1016/j.tics.2020.03.008

Reichova, A., Bacova, Z., Bukatova, S., Kokavcova, M., Meliskova, V., Frimmel, K., Ostatnikova, D., & Bakos, J. (2020). Abnormal neuronal morphology and altered synaptic proteins are restored by oxytocin in autism-related SHANK3 deficient model. *Molecular and Cellular Endocrinology*, *518*. https://doi.org/10.1016/j.mce.2020.110924

Ripamonti, S., Ambrozkiewicz, M. C., Guzzi, F., Gravati, M., Biella, G., Bormuth, I., Hammer, M., Tuffy, L. P., Sigler, A., Kawabe, H., Nishimori, K., Toselli, M., Brose, N., Parenti, M., & Rhee, J. (2017). Transient oxytocin signaling primes the development and function of excitatory hippocampal neurons. *ELife*, *6*. https://doi.org/10.7554/eLife.22466

Rockland, K. S. (2018). Axon Collaterals and Brain States. *Frontiers in Systems Neuroscience*, *12*. https://doi.org/10.3389/fnsys.2018.00032

Rogers Flattery, C. N., Coppeto, D. J., Inoue, K., Rilling, J. K., Preuss, T. M., & Young, L. J. (2022). Distribution of brain oxytocin and vasopressin V1a receptors in chimpanzees (Pan troglodytes): comparison with humans and other primate species. *Brain Structure and Function*, *227*(5), 1907–1919. https://doi.org/10.1007/s00429-021-02369-7

Rokicki, J., Kaufmann, T., de Lange, A. M. G., van der Meer, D., Bahrami, S., Sartorius, A. M., Haukvik, U. K., Steen, N. E., Schwarz, E., Stein, D. J., Nærland, T., Andreassen, O. A., Westlye, L. T., & Quintana, D. S. (2022). Oxytocin receptor expression patterns in the

human brain across development. *Neuropsychopharmacology*, *47*(8), 1550–1560. https://doi.org/10.1038/s41386-022-01305-5

Rollenhagen, A., & Lübke, J. H. R. (2013). Dendrites: A Key Structural Element of Neurons. In *Neuroscience in the 21st Century* (pp. 179–217). Springer New York. https://doi.org/10.1007/978-1-4614-1997-6_11

Romero-Fernandez, W., Borroto-Escuela, D. O., Agnati, L. F., & Fuxe, K. (2013). Evidence for the existence of dopamine d2-oxytocin receptor heteromers in the ventral and dorsal striatum with facilitatory receptor-receptor interactions. In *Molecular Psychiatry* (Vol. 18, Issue 8, pp. 849–850). https://doi.org/10.1038/mp.2012.103

Rothwell, P. E., Fuccillo, M. V., Maxeiner, S., Hayton, S. J., Gokce, O., Lim, B. K., Fowler, S. C., Malenka, R. C., & Südhof, T. C. (2014). Autism-Associated Neuroligin-3 Mutations Commonly Impair Striatal Circuits to Boost Repetitive Behaviors. *Cell*, *158*(1), 198–212. https://doi.org/10.1016/j.cell.2014.04.045

Sainath, R., & Gallo, G. (2014). Cytoskeletal and signaling mechanisms of neurite formation. In *Cell and Tissue Research* (Vol. 359, Issue 1, pp. 267–278). Springer Verlag. https://doi.org/10.1007/s00441-014-1955-0

Sala, C., & Segal, M. (2014). Dendritic Spines: The Locus of Structural and Functional Plasticity. *Physiological Reviews*, *94*(1), 141–188. https://doi.org/10.1152/physrev.00012.2013

Sato, D., Lionel, A. C., Leblond, C. S., Prasad, A., Pinto, D., Walker, S., O'Connor, I., Russell, C., Drmic, I. E., Hamdan, F. F., Michaud, J. L., Endris, V., Roeth, R., Delorme, R., Huguet, G., Leboyer, M., Rastam, M., Gillberg, C., Lathrop, M., … Scherer, S. W. (2012). SHANK1 Deletions in Males with Autism Spectrum Disorder. *The American Journal of Human Genetics*, *90*(5), 879–887. https://doi.org/10.1016/j.ajhg.2012.03.017

Schultz, W. (1986). Responses of Midbrain Dopamine Neurons to Behavioral Trigger Stimuli in the Monkey. In *huRNALoF NEUR~PH~SIOKIGY* (Vol. 56, Issue 5).

Schultz, W. (1998). Predictive Reward Signal of Dopamine Neurons. *Journal of Neurophysiology*, *80*(1), 1–27. https://doi.org/10.1152/jn.1998.80.1.1

Schultz, W. (2001). Reward signaling by dopamine neurons. In *Neuroscientist* (Vol. 7, Issue 4, pp. 293–302). SAGE Publications Inc. https://doi.org/10.1177/107385840100700406

Seeman, P. (2006). Targeting the dopamine D $_2$ receptor in schizophrenia. *Expert Opinion on Therapeutic Targets*, *10*(4), 515–531. https://doi.org/10.1517/14728222.10.4.515

Shahrokh, D. K., Zhang, T.-Y., Diorio, J., Gratton, A., & Meaney, M. J. (2010). Oxytocin-Dopamine Interactions Mediate Variations in Maternal Behavior in the Rat. *Endocrinology*, *151*(5), 2276–2286. https://doi.org/10.1210/en.2009-1271

Sheng, M., & Kim, E. (2000). The Shank family of scaffold proteins. *Journal of Cell Science*, *113*(11), 1851–1856. https://doi.org/10.1242/jcs.113.11.1851

Skuse, D. H., & Gallagher, L. (2009). Dopaminergic-neuropeptide interactions in the social brain. In *Trends in Cognitive Sciences* (Vol. 13, Issue 1, pp. 27–35). https://doi.org/10.1016/j.tics.2008.09.007

Soares-Cunha, C., Coimbra, B., David-Pereira, A., Borges, S., Pinto, L., Costa, P., Sousa, N., & Rodrigues, A. J. (2016). Activation of D2 dopamine receptor-expressing neurons in the nucleus accumbens increases motivation. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms11829

Song, J.-Y., Ichtchenko, K., Südhof, T. C., & Brose, N. (1999). Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proceedings of the National Academy of Sciences*, *96*(3), 1100–1105. https://doi.org/10.1073/pnas.96.3.1100

Stanislava Bukatová. (2018). *Neuroendocrine mechanisms of neuronal cells differentiation* [Dissertation thesis]. Comenius University in Bratislava.

Stolzenberg, D. S., McKenna, J. B., Keough, S., Hancock, R., Numan, M. J., & Numan, M. (2007). Dopamine D_1 receptor stimulation of the nucleus accumbens or the medial preoptic area promotes the onset of maternal behavior in pregnancy-terminated rats. *Behavioral Neuroscience*, *121*(5), 907–919. https://doi.org/10.1037/0735-7044.121.5.907

Supekar, K., Kochalka, J., Schaer, M., Wakeman, H., Qin, S., Padmanabhan, A., & Menon, V. (2018). Deficits in mesolimbic reward pathway underlie social interaction impairments in children with autism. *Brain*, *141*(9), 2795–2805. https://doi.org/10.1093/brain/awy191

Tabak, B. A., Leng, G., Szeto, A., Parker, K. J., Verbalis, J. G., Ziegler, T. E., Lee, M. R., Neumann, I. D., & Mendez, A. J. (2023). Advances in human oxytocin measurement: challenges and proposed solutions. In *Molecular Psychiatry* (Vol. 28, Issue 1, pp. 127–140). Springer Nature. https://doi.org/10.1038/s41380-022-01719-z

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D_1, D_2 and D_4 receptors in rat forebrain. *International Journal of Developmental Neuroscience*, *18*(1), 29–37. https://doi.org/10.1016/S0736-5748(99)00108-2

Tirko, N. N., Eyring, K. W., Carcea, I., Mitre, M., Chao, M. V., Froemke, R. C., & Tsien, R. W. (2018). Oxytocin Transforms Firing Mode of CA2 Hippocampal Neurons. *Neuron*, *100*(3), 593-608.e3. https://doi.org/10.1016/j.neuron.2018.09.008

Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M., & Dreifuss, J. J. (1988). Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. *Brain Research*, *442*(1), 105–118. https://doi.org/10.1016/0006-8993(88)91437-0

Tritsch, N. X., & Sabatini, B. L. (2012). Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. In *Neuron* (Vol. 76, Issue 1, pp. 33–50). https://doi.org/10.1016/j.neuron.2012.09.023

Uhrig, S., Hirth, N., Broccoli, L., von Wilmsdorff, M., Bauer, M., Sommer, C., Zink, M., Steiner, J., Frodl, T., Malchow, B., Falkai, P., Spanagel, R., Hansson, A. C., & Schmitt, A. (2016). Reduced oxytocin receptor gene expression and binding sites in different brain regions in schizophrenia: A post-mortem study. *Schizophrenia Research*, *177*(1–3), 59–66. https://doi.org/10.1016/j.schres.2016.04.019

Varoqueaux, F., Jamain, S., & Brose, N. (2004). Neuroligin 2 is exclusively localized to inhibitory synapses. *European Journal of Cell Biology*, *83*(9), 449–456. https://doi.org/10.1078/0171-9335-00410

Wang, W., Dever, D., Lowe, J., Storey, G. P., Bhansali, A., Eck, E. K., Nitulescu, I., Weimer, J., & Bamford, N. S. (2012). Regulation of prefrontal excitatory neurotransmission by dopamine in the nucleus accumbens core. *The Journal of Physiology*, *590*(16), 3743–3769. https://doi.org/10.1113/jphysiol.2012.235200

Wang, Y.-F., & Hatton, G. I. (2006). Mechanisms Underlying Oxytocin-Induced Excitation of Supraoptic Neurons: Prostaglandin Mediation of Actin Polymerization. *Journal of Neurophysiology*, *95*(6), 3933–3947. https://doi.org/10.1152/jn.01267.2005

Wise, R. A. (2004). Dopamine, learning and motivation. In *Nature Reviews Neuroscience* (Vol. 5, Issue 6, pp. 483–494). Nature Publishing Group. https://doi.org/10.1038/nrn1406

Xiao, L., Priest, M. F., Nasenbeny, J., Lu, T., & Kozorovitskiy, Y. (2017). Biased Oxytocinergic Modulation of Midbrain Dopamine Systems. *Neuron*, *95*(2), 368-384.e5. https://doi.org/10.1016/j.neuron.2017.06.003

Xu, H., & Yang, F. (2022). The interplay of dopamine metabolism abnormalities and mitochondrial defects in the pathogenesis of schizophrenia. In *Translational Psychiatry* (Vol. 12, Issue 1). Springer Nature. https://doi.org/10.1038/s41398-022-02233-0

Young, L. J., & Zhang, Q. (2021). On the Origins of Diversity in Social Behavior. *Japanese Journal of Animal Psychology*, *71*(1), 45–61. https://doi.org/10.2502/janip.71.1.4

Zatkova, M., Reichova, A., Bacova, Z., & Bakos, J. (2019). Activation of the Oxytocin Receptor Modulates the Expression of Synaptic Adhesion Molecules in a Cell-Specific Manner. *Journal of Molecular Neuroscience*, *68*(2), 171–180. https://doi.org/10.1007/s12031-019-01296-x

Zatkova, M., Reichova, A., Bacova, Z., Strbak, V., Kiss, A., & Bakos, J. (2018). Neurite Outgrowth Stimulated by Oxytocin Is Modulated by Inhibition of the Calcium Voltage-Gated Channels. *Cellular and Molecular Neurobiology*, *38*(1), 371–378. https://doi.org/10.1007/s10571-017-0503-3

Zhu, J., Li, Y., Liang, J., Li, J., Huang, K., Li, J., & Liu, C. (2021). The neuroprotective effect of oxytocin on vincristine-induced neurotoxicity in mice. *Toxicology Letters*, *340*, 67–76. https://doi.org/10.1016/j.toxlet.2021.01.008

Zürcher, N. R., Walsh, E. C., Phillips, R. D., Cernasov, P. M., Tseng, C.-E. J., Dharanikota, A., Smith, E., Li, Z., Kinard, J. L., Bizzell, J. C., Greene, R. K., Dillon, D., Pizzagalli, D. A., Izquierdo-Garcia, D., Truong, K., Lalush, D., Hooker, J. M., & Dichter, G. S. (2021). A simultaneous [11C]raclopride positron emission tomography and functional magnetic resonance imaging investigation of striatal dopamine binding in autism. *Translational Psychiatry*, *11*(1), 33. https://doi.org/10.1038/s41398-020-01170-0