Comprehensive Psychoneuroendocrinology 16 (2023) 100206

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.sciencedirect.com/journal/comprehensive-psychoneuroendocrinology

Monitoring oxytocin signaling in the brain: More than a love story

Inga D. Neumann

Department of Behavioural and Molecular Neurobiology, Regensburg Centre of Neurosciences, University of Regensburg, Regensburg, Germany

ARTICLE INFO

Keywords:
History
Neuropeptides
Push-pull
Microdialysis
East Germany
Social behaviour

ABSTRACT

More than any other neuropeptide, oxytocin (OXT) is attracting the attention of neurobiologists, psychologists, psychiatrists, evolutionary biologists and even economists. It is often called a "love hormone" due to its many prosocial functions described in vertebrates including mammals and humans, especially its ability to support "bonding behaviour". Oxytocin plays an important role in female reproduction, as it promotes labour during parturition, enables milk ejection in lactation and is essential for related reproductive behaviours. Therefore, it particularly attracts the interest of many female researchers. In this short narrative review I was invited to provide a personal overview on my scientific journey closely linked to my research on the brain OXT system and the adventures associated with starting my research career behind the Iron Curtain.

1. Introduction

My personal odyssey started in the German Democratic Republic (GDR, also simply called East Germany), where I was a student of Biology at the Karl-Marx-University in Leipzig. During my diploma thesis, I started to monitor the release of the neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) in the rat brain during relevant physiological stimuli using push-pull perfusion and, some years later during my PhD period, microdialysis approaches. Later, I have continued to use these and complementary other methods to focus on the functional impact of OXT release in distinct brain regions under healthy conditions, including during suckling, birth, mating or exposure to stress, and in rodent models of psychopathology. With the help of a variety of increasingly sophisticated behavioural paradigms along with pharmacological, genetic and pharmacogenetic, and optogenetic approaches to manipulate central OXT release, we were able to reveal multiple relationships between OXT and social behaviors, such as aggression, maternal behaviour, social preference behaviour and social fear. This scientific journey, with OXT as my major companion, has continued with more recent studies linking intraneuronal signaling cascades to nonapeptide-receptor interactions, the impact of OXT receptor mutations on the receptor's functionality, intracellular interactions with small non-coding RNAs, and the role of oxytocinergic neurocircuits in social interactions. I consider it a huge privilege to dedicate my scientific energy to such an amazing, evolutionary conserved and somehow mysterious molecule, central to all aspects of sociability.

2. The OXT and AVP systems

The closely related nonapeptides OXT and AVP are synthesized as neurophysin-containing pro-hormones in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, which are central components of the hypothalamo-neurohypophysial system (HNS) in mammals. After axonal transport to the neurohypophysis, OXT and AVP are stored in terminal vesicles and secreted into the blood stream as neurohormones in response to appropriate stimulation. Hemorrhage and osmotic stimulation are major stimuli for the secretion of AVP, which exerts antidiuretic actions at the kidney, whereas birth-related stimuli, and suckling in the lactating female, but also exposure to various kinds of stressors, induce OXT secretion into the blood. The long-known physiological functions of OXT as a circulating hormone, such as the uterine-contracting effect as well as the induction of milk ejection from the mammary gland, have originally been revealed by application of pituitary extracts [1–3] (for more historical details see Refs. [4,5]). The effect of OXT to promote labour and to speed up the mammalian birth process was the basis for the name oxytocin (Greek: ὀξύς, oxys, and τόκος, tokos, meaning "quick birth"), generally used today. However, according to the Nobel laureate Roger Guillemin, the spelling "oxytocin," may have been a "mistaken translation", and the correct name should be "ocytocin" [6].

In the 1970s, extrahypothalamic pathways in the brain, which were neurophysin-positive and, thus, potentially oxytocinergic or vasopressinergic, had been identified in The Netherlands [7,8] and - independently - in East Germany [9]. The discovery of these neurophysin-positive intracerebral pathways, along with the

E-mail address: inga.neumann@ur.de.

https://doi.org/10.1016/j.cpnec.2023.100206
Received 13 June 2023; Received in revised form 31 August 2023; Accepted 1 September 2023
Available online 6 September 2023

description of first central effects of synthetic AVP and OXT on learning and memory [10,11], and on maternal behaviour [12], respectively, sparked an enormous scientific interest in the brain nonapeptide systems and their capacity to modulate multiple behavioural and physiological functions, which remains until today.

Thus, many details of the complex brain OXT and AVP systems have been revealed until today, but their detailed description goes beyond the scope of this small review, the more as they have been extensively reviewed [5,13–15]. In brief, the brain OXT and AVP systems consist mainly of hypothalamic magnocellular and parvocellular neurons, which synthesize either OXT or AVP and have distinct electrophysiological properties, and their widespread intracerebral fiber networks. Whereas OXT seems to be exclusively expressed in the hypothalamus, AVP neurons have also be identified in other brain regions of the limbic system. OXT and AVP neurons respond to various physiological, social or stressful stimuli with neurohormone secretion into the blood, and - partly independent, partly coordinated - with neuropeptide release within the brain. Such central release may occur from different neuronal compartments including dendrites, cell soma and axons. Further, OXT receptors (OXTR) and AVP (V1A) receptors are expressed in neurons and astrocytes of many brain regions. Distinct receptor-coupled intracellular signaling pathways enable multiple facets of cellular responses.

3. The origin of our research on oxytocin and vasopressin

A vision begins with the right idea being born at the right time. In the 1980-ies, we did not know whether the neuropeptides OXT and AVP were released within the brain under physiological conditions, in parallel to their well-known secretion as hormones into the blood stream. Thus, scientists began to monitor the release of these neuropeptides within the brain. Among them was my mentor and later PhD supervisor, Rainer Landgraf. Rainer succeeded in developing highly sensitive radioimmunoassays for the quantification of the nonapeptides AVP and OXT. Using selective extraction in combination with extremely specific and sensitive antibodies, he eventually succeeded in detecting unbound, (i.e. bioavailable) OXT and AVP in the lower pg range [16,17]. These tools were critical to our ability to study the dynamics of release and the functions of OXT and AVP.

4. The back story on OXT research in Leipzig, East Germany

This achievement was not originally driven by purely scientific interest, but rather also by political interests of the governmental institution, where Rainer Landgraf worked in Leipzig - the German High School for Physical Culture and Sports (DHFK). During the Cold War various countries used international sporting events, especially the Olympic Games, to compete and show their dominance. The tiny GDR, with only 17 million inhabitants, was always among the three leading countries in medal rankings, competing successfully against the US and Soviet Union teams in swimming, athletics, weightlifting, or rowing and canoeing, among others. German discipline was not the only reason for their dominance, as children were often selected at a young age based on their physical potential for athletics. The implementation of excellent training facilities further enabled this overwhelming success, especially among women's teams. Consequent government-driven and goal-directed biomedical research, combined with pharmaceutical applications, resulted in the development of effective (however, risky) strategies to improve physical fitness, muscle growth, and endurance. Thus, international competition, for instance in the swimming pool, was also a scientific and technological competition among the Western and Eastern block countries. This competition also contributed to the development of strong scientific programs.

The neurohormones AVP and OXT became subjects of interest, as it was discovered that their blood concentrations increased in response to physical and mental exertion, e.g., in marathon runners or high-level chess players experiencing cognitive challenges [16]. A plethora of cognitive, emotional, and social effects of the use of synthetic OXT or AVP have been detailed in numerous research articles and journals. However, to my knowledge, even to the present not a single study has reported the improvement of physical fitness nor muscle strength by either nonapeptide. Of interest to the unique functions of OXT and AVP, nobody has examined the potential social effects in team players. Do professional soccer players seek to increase their team spirit by secretly sniffing OXT in the cabin together? Do choir singers inhale OXT alongside their conductor behind the curtain prior to performance?

Although the governmental interest in OXT and AVP dropped in the GDR, the potential to quantify tiny nonapeptide amounts had been established, allowing for independent research behind the Iron Curtain. This is important to note, as sensitive assays for AVP and OXT had also been established around this time in other (Western) countries including Canada [18,19], The Netherlands [20], France [21,22], the UK [23,24], and the USA [25].

5. My scientific journey – a bumpy start

As a student of Biology and Animal Physiology at the Faculty of Biosciences of the Karl-Marx-University in Leipzig, East Germany, I was offered a Diploma project (an equivalent of the Master thesis) in the small Landgraf lab in 1985. Working with the lab technician, I sought to establish a technique that would allow us to monitor the central release of AVP and OXT in response to a physiological stimulus, known as push-pull perfusion. Unfortunately, the project was postponed before it even started due to the birth of my first son, and consequently, one full year of maternity leave. Without access to the internet during my leave, I was left completely undisturbed; no emails had to be answered and no important papers were sent for reading.

When I returned to the lab in 1986, I began to "re-invent" the push-pull perfusion method in freely behaving rats; this technique had already been established in cat, sheep, monkey, and rat brains in several "Western" labs [18,22,26–29]. Western products were inaccessible, so we had to build our own, home-made concentric cannula systems, which consisted of an inner push and an outer pull stainless steel cannula (Fig. 1).

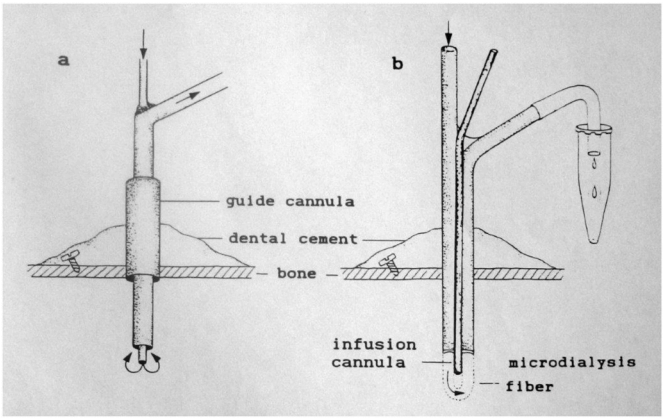

Fig. 1. Principle of push-pull perfusion (a) and microdialysis (b). a) The concentric push-pull cannula device is inserted into the guide cannula implanted above the brain target region. The arrows indicate the direction of flow of the perfusion fluid at a constant rate (20 µl/min). The push cannula extends the pull cannula allowing a direct washout of surrounding extracelluluar fluid (ECF) and tissue at the tip. b) The U-shaped microdialysis probe allows continuous flow of perfusion fluid (3 µl/min) via in parallel inflow and outflow tubings connected via a bent, U-shaped semipermeable microdialysis fiber. The semiparmeable membrane allows diffusion of substances in the surrounding ECF into the microdialysis sample. Visible is also an attached infusion cannula glued in between the U of the membrane allowing local drug delivery (so-called triple probe). (The figure was drawn by hand. From PhD thesis Neumann I, 1992, University of Leipzig.)

The major challenge was the *in vivo* implantation of the pull cannula in rats within a defined brain region of interest. Anesthesia was introduced using ether from a bottle, a cotton swab, and a plastic cap for the rat's nose. Due to the lack of a stereotaxic frame, the coordinates found in the rat brain atlas [30] were approximated via Bregma using a divider and a ruler. Without a stereotaxic device, a stable hand was needed to keep the forceps holding the pull cannula in the right position (either straight or with an angle in the case of the mediolateral septum to prevent sagittal bleeding) until the dental cement solidified. A dental drill, dental cement, and a glue-like substance, "adhesor", were donated by a friend working in the field of dentistry. Not being allowed to visit a Western lab, where the method had already been well established, in addition to an inability to access relevant publications caused us to initially overlook the use of miniature screws for fixing the pull cannula to the bone. As a result, many animals lost their implanted pull cannula during the first 2 days of postsurgical rest.

The push-pull perfusion is based on the assumption that outflow of artificial cerebrospinal fluid (aCSF) at a certain speed (3 µl/min) remains constant via the 1-mm extending tip of the push cannula and in complete balance with the backflow via the pull cannula (Fig. 1). This technique allows a tiny, targeted wash of extracellular fluid at the tip of the push-pull cannula system, which can be collected for quantification of locally released neuroactive substances within the target region upon backflow. In order to ensure an identical flow rate of outflow (push) and backflow (pull), a perfusion pump with 2 syringes mounted in opposite directions was constructed with a push and pull syringe connected to the implanted push and pull cannulas, respectively. The infusion pump was self-made by our mechanics. Although the procedure was often disrupted due to an imbalance of pushed and pulled volumes of aCSF and, consequently, tissue blockage at the push-pull tip, we were able to sample our first 30-min perfusates in freely moving rats.

With the previously described technical problems, and a 1-year old son (who was often sick due to substantial air pollution in Leipzig during the first year in childcare) I experienced quite a lot of frustration over many months of my Diploma project. But the topic was too exciting to give up. The atmosphere in the lab, which was slowly growing in size thanks to joining Diploma students, was relaxed and humorous, but also creative, inspiring and motivating. Among the students were Mario Engelmann, who focused on AVP effects on memory, especially on social memory, and established the social discrimination paradigm [31,32], Mike Ludwig, who studied AVP release within the SON during direct osmotic stimulation [33] and continued to characterize dendritic release of AVP within the SON later, and Thomas Horn, who was challenged by establishing push-pull perfusion in the Nucleus of the solitary tract, at this time. Also, Carsten Wotjak joined the lab as a Diploma student [34] and continued his research at the Max-Planck-Institute of Psychiatry in Munich later. All of them have started their successful neurobiological career in the lab in Leipzig, and most of them are still prominent in the neuropeptide field today.

I remember very well the day that the radioimmunoassay provided the first results from 5 freely moving rats. We observed a rise in AVP content in push-pull perfusates sampled within the dorsal hippocampus during intraperitoneal application of a hypertonic saline solution. AVP, also known as antidiuretic hormone (ADH), is an important regulator of blood pressure as well as water and salt homeostasis. We were not the first to show that AVP is released in the brain during osmotic stimulation [22,29,35], but we could build on these initial observations by demonstrating a simultaneous increase in OXT release both in the medio-lateral septum and dorsal hippocampus [36].

Lab and office space was extremely limited, and all procedures including surgery, behavioural and perfusion experiments, histology, construction of push-pull devices, or preparation of sampling tubes (Fig. 2) were performed in one room, were I also had my desk. However, the lab progressed technically. The implantation procedure became increasingly reliable thanks to the gift of a stereotaxic apparatus from the veterinary department and of small screws for fixation from a watchmaker. I decided to move on with a PhD in that lab. This decision was also inspired by my first scientific meeting in Budapest in 1988, where I was able to meet prominent members of the British Neuroendocrine Group. This led not only to a very fruitful collaboration with John Russell, from Edinburgh, but also to a long-lasting friendship. It was also at that International Brain Research Organization (IBRO) conference, where I learned about the method of intracerebral microdialysis, which - in comparison with push-pull perfusion – causes less tissue damage due to the coverage of the perfusion site by the semi-permeable dialysis membrane. Microdialysis was established in our lab soon thereafter [33,37]. Our U-shaped microdialysis probes (Fig. 1) are self-constructed, with a microdialysis membrane obtained from a defective artificial kidney of a local hospital, and have proved themselves in our hands until today both in rats and mice [38,39]. These probes work reliably with a relatively large dialyzing surface (and also happen to be extremely affordable). However, there is a dramatically low relative neuropeptide recovery in the microdialysis samples (2–3% recovery via microdialysis compared to 20% in push-pull perfusates). Only the availability of sensitive radioimmunoassays for both non-apeptides [16], and the fact that neuropeptide concentrations in the extracellular fluid, for example of the SON, are in the 10^{-9} M range, i.e., about 100–1000 times higher than in plasma [40], allowed for the successful application of microdialysis. Thus, we first monitored intracerebral neuropeptide release using microdialysis in the hypothalamic

Fig. 2. Impressions from the Leipzig lab in 1989. In the one available lab all experiments were performed including brain cutting and histology, stereotaxic and other surgeries, push-pull perfusions and microdialysis, and behavioural experiments. The upper picture shows Mike Ludwig at the left preparing a microdialysis experiment and the author at the right. The lower picture shows the author sorting push-pull sampling tubes. The back on the right site belongs to Mario Engelmann performing stereotaxic surgery right beside the author's desk at the window.

SON [33] and PVN [37], but later also in other neuropeptide target regions, such as the amygdala or septum [38,41,42].

6. OXT release in the female brain during reproduction

My interest to study the release of OXT within the brain during female reproduction was stimulated by fascinating findings of OXT being able to trigger maternal behaviour in rats [12], but also by the impressive results of Keith Kendrick and Berry Keverne, who showed that OXT is released within the olfactory bulb during vaginocervical stimulation in ewes [43]. Moreover, they demonstrated that the experience of birth and the release of OXT or hormonal priming are required for selective maternal attachment to an offspring in ewes [35]. Together, these findings implicated stimulated OXT release within the brain during birth and suckling, especially in regions involved in the regulation of social behaviours.

Thus, my own research became focused on monitoring OXT (as well as AVP for comparison) release during physiological stimuli, such as suckling and birth. The discovery of OXT release in the septum and dorsal hippocampus in the freely moving lactating rat during ongoing maternal behaviour and suckling [44] were fascinating moments for me. The figures for this paper as well as all previous ones were, of course, drawn by hand using ink, with pre-printed letters and numbers glued onto axes and graphs, although why I decided to draw a 3-dimensional graph remains my secret (Fig. 3).

Studying parturition proved to be more challenging. Because rat dams do not display a preferential time of the day when delivering their pups, we spent many late to early hours in the lab hoping to observe and perform push-pull perfusions during the process of ongoing birth. Sometimes we were successful, but the rat dams would often give birth after I had finally left the lab. On top of that, I remember that our electrical supply had a knack for repeated malfunctioning, which disrupted ongoing intracerebral microperfusions during birth - incredibly bad timing!

Nevertheless, we continued to conduct the protocol, step by step, until a sufficient number of samples were collected and analysed. Our data showed a substantial rise in local neuropeptide release within the ventral (OXT and AVP) and medial (AVP) septum on days 20/21 of late pregnancy; and neuropeptide release remained elevated during ongoing birth without further increase [45]. Thus, we could not detect an association between the increase in local OXT (or AVP) and pup delivery, indicating that the release of OXT as neuromodulator/neurotransmitter within the limbic brain may occur independently of peripheral secretion of OXT as neurohormone into the blood. Such release may rather prepare the maternal brain for the major behavioural and physiological adaptations that we, and others, identified later on [12,38,46–50]. Based on the finding that the activity of hypothalamic OXT neurons and OXT secretion into blood is strongly inhibited by endogenous opioids

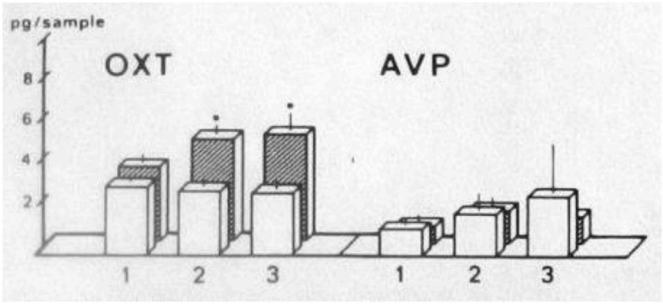

Fig. 3. Oxytocin (OXT) and arginine vasopressin (AVP) content in three consecutive 30-min push-pull perfusates sampled from the septum of lactating rats before (1), during (2) and after (3) a 30-min suckling period (hatched columns) or in presence of a single pup (white columns). Mean +SEM, n = 5–7 (from: [44]; hand-drawn figure).

peripartum [51,52], we hypothesized that opioid inhibition was the probable cause of the observed lack of a detectable rise in local OXT release during the delivery process. In collaboration with John Russell, who repeatedly visited us in Leipzig behind the Iron curtain, we could indeed show that the opioid antagonist naloxone disinhibited local OXT (but not AVP) release in parturient rats within the medio-lateral septum and dorsal hippocampus [53,54]. This finding indicated that similar opioid mechanisms were involved in regulating peripheral and central release patterns.

In order to monitor OXT release within the hypothalamic nuclei of origin, i.e., in the SON and PVN, we decided to capitalize on the advantages of microdialysis. We found a rise in OXT, but not AVP, content in microdialysates sampled from the SON and PVN both during birth and suckling in lactating rats. Pharmacological manipulation by adding drugs to the perfusion fluid (called retrodialysis) provided evidence of local neuropeptide release from intact neuronal structures and excluded the possibility that OXT from blood significantly contaminated the microdialysis samples [37]. These microdialysis experiments remain to this day the only studies describing OXT release within selected brain regions during ongoing parturition. Although the parturition process is a biologically fundamental and philosophically exciting physiological event, many mysteries regarding the contribution of brain OXT persist. It is not clear whether modern students will be willing to watch late pregnant animals for hours during the day and night in order to further shed light on these mysteries.

Our early results in combination with those from other labs [22,29,35,43] demonstrate stimulus-specific, peptide-specific, and region-dependent release of OXT and AVP within the brain during female reproduction. These findings revealed a missing puzzle piece in neuropeptide research. When secreted as a classical neurohormone from the neurohypophysis into the blood stream, OXT is essential for physiological functions during birth (promotion of labour) and suckling (milk ejection). However, when OXT is released in the brain at these times, it facilitates the onset of maternal behaviour and its fine-tuned regulation. Furthermore, OXT released within the hypothalamic SON and PVN peripartum stimulates local glial-neuronal plasticity [55]. Glial retraction from OXT neurons, and local OXT are essential for synchronicity among hypothalamic OXT neurons during the milk ejection reflex in the lactating animal [21] (and possibly also during birth, but this has never been demonstrated), which causes pulsatile OXT secretion into the blood [56]. OXT release within the brain, which is induced by birth- and pup-related stimuli, also contributes to various other behavioural and physiological adaptations of the maternal brain in preparation of motherhood in the context of emotionality and stress coping, which were discovered later [46,47,57,58] (for review see Refs. [48,49]). A detailed knowledge of OXT pathways, neuropeptide release patterns, and the distribution of its receptor within the brain (Fig. 4) remain to the present fundamental to the interpretation of the many behavioural effects caused by endogenous or synthetic OXT (Fig. 5).

7. Science in a world of political changes

In the late 1980s, specifically in 1989, scientific research often had to give place to endless political discussions about national and international events: Evidence for fake elections in the GDR in May 1989, opening of the Hungarian border to Austria in August, weekly “monday demonstrations” in Leipzig and many other cities in the GDR demanding political changes, and the sudden, unexpected opening of the German-German border on November 9th were real and life-touching events that changed the political, and later scientific landscape in the GDR. We worked at the Karl-Marx-University in a socialistic country. A politically incorrect statement at the Department meeting on monday morning (which always started with a political report of the week’s political events), or participation in any demonstrations could easily mean the end of your contract as a PhD student. This threatening atmosphere and reports of dismissed students from other faculties were always present.

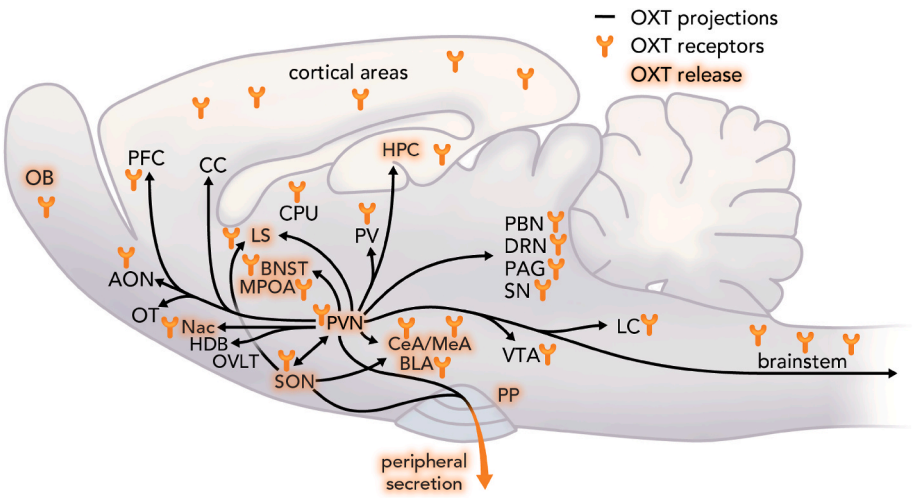

Fig. 4. The complexity of the brain oxytocin (OXT) system has been revealed in many laboratories worldwide in the last 30 years including the presence of OXT projections, OXT receptors and sites of OXT release (from: Grinevich & Neumann [59]).

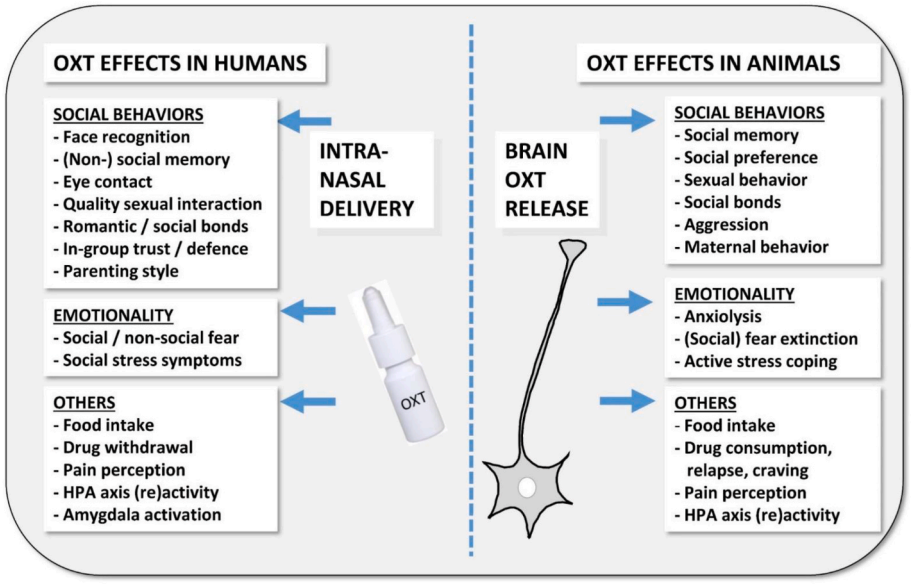

Fig. 5. The neuromodulator oxytocin (OXT) regulates multiple behaviours and physiological functions after its release within distinct brain regions, as revealed by manipulation of the endogenous OXT system in laboratory animals (right). In humans, intranasal application of OXT has been shown to affect comparable social, emotional and other behaviours (from: Jurek & Neumann [5]).

To return to the world of science from time to time, and to focus on a molecule released within the brain during reproductive events was like meditation for the mind during these stirring times.

After the break down of the political system in East Germany and the fall of the German Wall, we suddenly gained the ability to travel to conferences in the West and meet colleagues we only knew from scientific literature. This opened the door to an entirely new world - a friendly, but competitive international scientific community. It also allowed me to move to the esteemed laboratory of Quentin Pittman, at the University of Calgary in Canada, as a postdoc right after defending my PhD thesis. I was equipped with a solid stipend provided by the Human Frontiers Science Organization Programme (HFSOP), my 6-year old son, and a miserable level of English I had learned in school. Working for two years in an extremely stimulating and supportive scientific environment, and living in the Western world for the first time with all its possibilities, temptations, and challenges was an overwhelming, yet exciting and unforgettable experience.

During this time, I continued to study the functional significance of OXT release within the SON using either antisense oligonucleotides to prevent OXT synthesis or a peptidergic OXT receptor antagonist, which was always kindly provided by our good friend Maurice Manning (Toledo, USA). During ongoing microdialysis, the OXT receptor antagonist was directly infused into the SON using a so-called triple probe with a 31-gauge infusion cannula attached to the U-shaped microdialysis probe (Fig. 1b). We could show that local OXT release during parturition and OXT receptor-mediated actions exert a positive feedback on OXT release within the SON, which is essential for regulating the temporal patterns of the birth process. The OXT receptor antagonist also impaired various facets of maternal behaviour [56,60], but whether this was due to blocked local OXT actions or inhibition of centrally projecting OXT neurons remained open.

In the Pittman lab, I also learned that *in vivo* electrophysiology on OXT neurons of the SON in lactating rats (previously treated with antisense oligonucleotides bilaterally within the SON to interrupt local

OXT synthesis) is an extremely challenging task ([61]). Unfortunately, despite excellent supervision by Quentin, this method did not seem suitable for a researcher, who has a son that would enter the lab after school and needed to be accompanied home at 4.30 p.m. latest - which is usually when you have just hit your first neuron with the hope of more to come!

8. Physiological significance of brain OXT

With an unbroken fascination for brain neuropeptides, their intracerebral release patterns and functions, I continued to search for an opportunity to continue in this direction. In this period, I was fortunately offered a postdoc position at the Max-Planck-Institute for Psychiatry in Munich. Our lab and others were able to link together the low levels of anxiety and of hormonal stress responses identified in pregnant and lactating rats [46,62,63] (for review see Refs. [48,49]) with the high activity of the brain OXT system identified peripartum [47,64,65]. We could also reveal that OXT exerts anxiolytic effects, modulates stress coping behaviour and inhibits the responsiveness of the hypothalamo-pituitary adrenal (HPA) axis, in males and females independent of reproduction (Fig. 5) [42,66,67].

9. Gaining scientific independence

In the 1990s, general research performed at the Max-Planck-Institute focused, among others, on neuropeptides including AVP and corticotropin releasing factor (CRF). Because these neuropeptides exert anxiogenic effects, their brain systems became targets for methods to treat anxiety and depression disorders. Due to practical reasons, all experiments in the context of psychopathologies were performed on male rodents at that time. Somehow, I joined the institute as an outsider working on a female hormone and its actions in the female brain in the context of female physiological events at this prestigious institute. To increase my local standing and scientific acceptance, I agreed to also study specific HPA axis responses in aged male rats (24 months old, up to 1.2kg body weight) [68]. However, I never gave up my OXT focus and found my scientific niche in this competitive environment. I was fortunate and had been selected as a recipient of the prestigious Heisenberg stipend by the German Research Council (DFG) in 1997. Although this means that I was capable to provide my own research funding, I had never been offered the status of an independent junior group leader, which would have significantly increased my chances for professorship elsewhere.

I had only gained complete scientific independence after becoming full professor of Neurobiology and Animal Physiology at the University of Regensburg in 2001. My personal research playground provided many opportunities to continue studying the role of OXT in the context of emotionality, anxiety, and depression-related behaviours. These include various aspects of social interactions under healthy conditions and using animal models of socio-emotional dysfunctions, such as early life stress, chronic psychosocial stress, or social fear. However, the start in Regensburg was challenging: I was creating a new Department with technicians and researchers taken over from my predecessor (working on olfaction in mosquitos), organizing the reconstruction of animal, surgery and molecular labs, while establishing a new home for the family and managing one small child as well as a teenager. On top of that: A full semester lecture series on Animal Physiology had to be prepared using powerpoint for the first time and starting one week after arrival at the university. I recall this as almost unbearable. But somehow, you can rely on your energy depot in times when you need it most.

Obtaining an external grant was essential for future research ambitions. Applying for those was extremely frustrating and without success at the beginning. A study performed by Wennerås and Wold in Sweden and published in *Nature* [69], demonstrated that women scientists need 2.7 fold more publications or research impact factors than men to be similarly acknowledged by grant reviewers for their scientific excellence. Being the first full professor of the Faculty of Biology and Preclinical Medicine of this Bavarian University, I felt the pressure to work harder to reach similar goals compared to my colleagues. In the end, gaining the financial independence to perform each project I aimed for was worth every effort, every day and every weekend invested in grant writing. The freedom to even discuss, initiate, and perform "fun" projects involving local students and colleagues as subjects allowed particularly enjoyable and rewarding moments. For example, we were able to study changes in salivary OXT before, during and after running or sexual activity [17], or in the context of solo versus choir singing [70].

10. An example of our research approach: brain OXT in response to mating in male and female rats

One of the first behavioural studies on OXT (or neurohypophysial extract) suggested the involvement of this peptide in sexual behaviour in fish [71]; this was later confirmed in mammals [72,73]. As studied in humans and male rats, the OXT system is responsive to sexual stimulation, reflected by increased activation of hypothalamic OXT neurons in rodents and elevated secretion into the bloodstream during orgasm in humans [17,74,75]. These findings implicated sexual stimulation is a physiological stimulus for the male OXT system. However, whether OXT was also released in the male brain and might have the potential to exert positive effects, e.g., on emotionality, was unknown. Thus, we performed a microdialysis study during mating in male rats and found elevated OXT release within the hypothalamic PVN, which began to rise in the presence of a primed, receptive female and peaked during sexual interaction and mating [76]. Interestingly, males also displayed lower levels of anxiety-related behaviour up to 4 h after mating, which was found to be the consequence of mating-induced OXT release [76].

Compared to males, studying mating-associated consequences on emotionality in females was more complex [77]. Females need to be steroid-primed to become receptive, however, receptive, estrus females were found to be generally less anxious in comparison with non-primed ones. Surprisingly, mating significantly increased their level of anxiety, which was evaluated 30 min after mating on an elevated plus maze. After experimental adaptation and using a paced-mating design, in which the female can determine the time point of mating, the anxiogenic effect of mating disappeared. Only under these paced-mating conditions we detected an increased release of OXT within the PVN [77]. Overall, this is an interesting finding by itself, as in all breeding facilities worldwide, females seem to be exposed to highly stressful, forced mating conditions.

Some indication for elevated OXT release was also found in the Nucleus accumbens of female prarie voles during mating [78]. In this socially monogamous species, brain OXT is important for both partner preference and pair-bonding [79] (for review see Refs. [80,81]).

11. Ongoing OXT research

Today, it is still fascinating and a challenge to contribute to a growing body of global scientific evidence that brain OXT exerts a plethora of behavioural effects (for review see Refs. [5,82]). For example, OXT significantly contributes to the fine-tuned regulation of brain regions, which are part of the social network, thus modulating sexual behaviour [83] (for review see Ref. [84]), pair bonding and the consequences of partner loss [85] (for review see Ref. [86]), various subtle aspects of maternal behaviour [50,87] (for review see Ref. [88]), empathy and consolation [89], aggression both in male and female rats [39,90,91], defensive aggression in lactation [92,93], social interactions including social preference [94,95], and social stress-induced social avoidance [38,50,94,96–99] (Fig. 5).

Another line of my research efforts was focused on finding the underlying mechanisms behind OXT activity at the molecular and cellular levels. Thus, we and others studied intraneuronal signaling cascades linked to the OXT receptor in a behavioural context [100–103] (for

review see Refs. [5,15]). We began to ask ourselves: What molecular events are triggered by OXT in OXT receptor-expressing target neurons? What happens on that level, when OXT is applied chronically [104–107], or when the cells express an OXT receptor mutant [108]? Does OXT also modulate interactions with neuronal non-coding RNA, such as microRNA [109]. A plethora of open questions remains regarding OXT-associated effects and their underlying mechanisms. Impressively, innovative experimental approaches, such as optogenetics [95,110], pharmacogenetics [38,111], or other viral approaches [50,85,87,112] including biosensors to detect tiny amounts of local OXT release are rapidly used and made available to the scientific community by colleagues in the OXT family. As a result, we are able to highly specifically manipulate the activity of OXT neurons and their central projections to reveal neuronal connectivities and downstream targets of OXT in the context of socio-emotional behaviours. This growing, worldwide OXT family enabled collaborations with colleagues in Calgary, Edinburgh, Hershey, Bordeaux, Atlanta, Sydney, Toledo, Innsbruck, Japan, Amsterdam, Paris, and within Germany. Overall, I feel very privileged that I have consistently been able to dedicate my scientific work to reveal the many secrets of the fascinating molecule known as oxytocin.

Funding

Over the years, I appreciated financial support by multiple research funding agencies including German Research Foundation (DFG), German Academic Exchange Programme (DAAD), International Brain Research Organisation (IBRO), Human Frontiers Science Organisation Programme (HFSOP), Alberta Heritage Foundation, BMBF (Germany), and European Union.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H.H. Dale, On some physiological actions of ergot, J. Physiol. 34 (1906) 163–206.
[2] I. Ott, J.C. Scott, The action of glandular extracts upon the contractions of the uterus, J. Exp. Med. 11 (1909) 326–330.
[3] E.A. Schafer, K.F. MacKenzie, The Action of animal extracts on milk secretion, Proc. R. Soc. B 84 (1911).
[4] G. Leng, R. Pineda, N. Sabatier, M. Ludwig, 60 years of neuroendocrinology: the posterior pituitary, from Geoffrey Harris to our present understanding, J. Endocrinol. 226 (2015) T173–T185.
[5] B. Jurek, I.D. Neumann, The oxytocin receptor: from intracellular signaling to behavior, Physiol. Rev. 98 (2018) 1805–1908.
[6] C.S. Carter, Oxytocin and love: myths, metaphors and mysteries, Compr. Psychoneuroendocrinol. 9 (2022), 100107.
[7] R.M. Buijs, Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat, Cell Tissue Res. 192 (1978) 423–435.
[8] R.M. Buijs, D.F. Swaab, Immuno-electron microscopical demonstration of vasopressin and oxytocin synapses in the limbic system of the rat, Cell Tissue Res. 204 (1979) 355–365.
[9] G. Sterba, in: F. Knowles, L. Vollrath (Eds.), Ascending neurosecretory pathways of the peptidergic type, Neurosecretion — the Final Neuroendocrine Pathway: VI International Symposium on Neurosecretion, London 1973, Springer Berlin Heidelberg, Berlin, Heidelberg, 1974, pp. 38–47.
[10] B. Bohus, D. De Wied, Inhibitory and facilitatory effect of two related peptides on extinction of avoidance behavior, Science (New York, N.Y 153 (1966) 318–320.
[11] D. De Wied, Long term effect of vasopressin on the maintenance of a conditioned avoidance response in rats, Nature 232 (1971) 58–60.
[12] C.A. Pedersen, A.J. Prange Jr., Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 6661–6665.
[13] R. Landgraf, The involvement of the vasopressin system in stress-related disorders, CNS Neurol. Disord.: Drug Targets 5 (2006) 167–179.
[14] I.D. Neumann, R. Landgraf, Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors, Trends Neurosci. 35 (2012) 649–659.
[15] V. Grinevich, H.S. Knobloch-Bollmann, M. Eliava, M. Busnelli, B. Chini, Assembling the Puzzle: Pathways of Oxytocin Signaling in the Brain, Biol. Psychiatry 79 (2016) 155–164.
[16] R. Landgraf, Simultaneous measurement of arginine vasopressin and oxytocin in plasma and neurohypophyses by radioimmunoassay, Endokrinologie 78 (1981) 191–204.
[17] T.R. de Jong, R. Menon, A. Bludau, T. Grund, V. Biermeier, S.M. Klampfl, B. Jurek, O.J. Bosch, J. Hellhammer, I.D. Neumann, Salivary oxytocin concentrations in response to running, sexual self-stimulation, breastfeeding and the TSST: the Regensburg Oxytocin Challenge (ROC) study, Psychoneuroendocrinology 62 (2015) 381–388.
[18] K.E. Cooper, N.W. Kasting, K. Lederis, W.L. Veale, Evidence supporting a role for endogenous vasopressin in natural suppression of fever in the sheep, J. Physiol. 295 (1979) 33–45.
[19] N.W. Kasting, Simultaneous and independent release of vasopressin and oxytocin in the rat, Can. J. Physiol. Pharmacol. 66 (1988) 22–26.
[20] J. Dogterom, F.G. Snijdewint, P. Pevet, R.M. Buijs, On the presence of neuropeptides in the mammalian pineal gland and subcommissural organ, Prog. Brain Res. 52 (1979) 465–470.
[21] F. Moos, M.J. Freund-Mercier, Y. Guerne, J.M. Guerne, M.E. Stoeckel, P. Richard, Release of oxytocin and vasopressin by magnocellular nuclei in vitro: specific facilitatory effect of oxytocin on its own release, J. Endocrinol. 102 (1984) 63–72.
[22] J. Demotes-Mainard, J. Chauveau, F. Rodriguez, J.D. Vincent, D.A. Poulain, Septal release of vasopressin in response to osmotic, hypovolemic and electrical stimulation in rats, Brain Res. 381 (1986) 314–321.
[23] E.L. Sheldrick, A.P. Flint, Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in sheep, Prostaglandins 22 (1981) 631–636.
[24] K.M. Kendrick, E.B. Keverne, B.A. Baldwin, D.F. Sharman, Cerebrospinal fluid levels of acetylcholinesterase, monoamines and oxytocin during labour, parturition, vaginocervical stimulation, lamb separation and suckling in sheep, Neuroendocrinology 44 (1986) 149–156.
[25] T. Hattori, G.M. So, M. Morris, P.M. Hutchins, D.K. Sundberg, Effect of central hypertonic stimulation on plasma atrial natriuretic peptide and oxytocin in conscious rats, Regul. Pept. 23 (1988) 271–278.
[26] R.D. Myers, T.L. Yaksh, Thermoregulation around a new set-point' established in the monkey by altering the ratio of sodium to calcium ions within the hypothalamus, J. Physiol. 218 (1971) 609–633.
[27] G.M. Tyce, T.L. Yaksh, Monoamine release from cat spinal cord by somatic stimuli: an intrinsic modulatory system, J. Physiol. 314 (1981) 513–529.
[28] T.V. Bliss, R.M. Douglas, M.L. Errington, M.A. Lynch, Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats, J. Physiol. 377 (1986) 391–408.
[29] F. Moos, D.A. Poulain, F. Rodriguez, Y. Guerne, J.D. Vincent, P. Richard, Release of oxytocin within the supraoptic nucleus during the milk ejection reflex in rats, Exp. Brain Res. 76 (1989) 593–602.
[30] G. Paxinos, C.R. Watson, The Rat Brain in Stereotaxic Coordinates, fourth ed., Academic press, New York, 1998.
[31] M. Engelmann, M. Ludwig, R. Landgraf, Microdialysis administration of vasopressin and vasopressin antagonists into the septum during pole-jumping behavior in rats, Behav. Neural. Biol. 58 (1992) 51–57.
[32] M. Engelmann, C.T. Wotjak, R. Landgraf, Social discrimination procedure: an alternative method to investigate juvenile recognition abilities in rats, Physiol. Behav. 58 (1995) 315–321.
[33] R. Landgraf, M. Ludwig, Vasopressin release within the supraoptic and paraventricular nuclei of the rat brain: osmotic stimulation via microdialysis, Brain Res. 558 (1991) 191–196.
[34] C.T. Wotjak, M. Ludwig, R. Landgraf, Vasopressin facilitates its own release within the rat supraoptic nucleus in vivo, Neuroreport 5 (1994) 1181–1184.
[35] K.M. Kendrick, E.B. Keverne, C. Chapman, B.A. Baldwin, Intracranial dialysis measurement of oxytocin, monoamine and uric acid release from the olfactory bulb and substantia nigra of sheep during parturition, suckling, separation from lambs and eating, Brain Res. 439 (1988) 1–10.
[36] R. Landgraf, I. Neumann, H. Schwarzberg, Central and peripheral release of vasopressin and oxytocin in the conscious rat after osmotic stimulation, Brain Res. 457 (1988) 219–225.
[37] I. Neumann, J.A. Russell, R. Landgraf, Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: a microdialysis study, Neuroscience 53 (1993) 65–75.
[38] R. Menon, T. Grund, I. Zoicas, F. Althammer, D. Fiedler, V. Biermeier, O.J. Bosch, Y. Hiraoka, K. Nishimori, M. Eliava, V. Grinevich, I.D. Neumann, Oxytocin signaling in the lateral septum prevents social fear during lactation, Curr. Biol. 28 (2018), 1066-1078 e1066.
[39] V.E.M. Oliveira, M. Lukas, H.N. Wolf, E. Durante, A. Lorenz, A.L. Mayer, A. Bludau, O.J. Bosch, V. Grinevich, V. Egger, T.R. de Jong, I.D. Neumann, Oxytocin and vasopressin within the ventral and dorsal lateral septum modulate aggression in female rats, Nat. Commun. 12 (2021) 2900.
[40] R. Landgraf, I.D. Neumann, Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication, Front. Neuroendocrinol. 25 (2004) 150–176.
[41] K. Ebner, C.T. Wotjak, R. Landgraf, M. Engelmann, A single social defeat experience selectively stimulates the release of oxytocin, but not vasopressin, within the septal brain area of male rats, Brain Res. 872 (2000) 87–92.
[42] K. Ebner, O.J. Bosch, S.A. Kromer, N. Singewald, I.D. Neumann, Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids, Neuropsychopharmacology 30 (2005) 223–230.

[43] K.M. Kendrick, E.B. Keverne, C. Chapman, B.A. Baldwin, Microdialysis measurement of oxytocin, aspartate, gamma-aminobutyric acid and glutamate release from the olfactory bulb of the sheep during vaginocervical stimulation, Brain Res. 442 (1988) 171–174.
[44] I. Neumann, R. Landgraf, Septal and hippocampal release of oxytocin, but not vasopressin, in the conscious lactating rat during suckling, J. Neuroendocrinol. 1 (1989) 305–308.
[45] R. Landgraf, I. Neumann, Q.J. Pittman, Septal and hippocampal release of vasopressin and oxytocin during late pregnancy and parturition in the rat, Neuroendocrinology 54 (1991) 378–383.
[46] I.D. Neumann, H.A. Johnstone, M. Hatzinger, G. Liebsch, M. Shipston, J. A. Russell, R. Landgraf, A.J. Douglas, Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes, J. Physiol. 508 (Pt 1) (1998) 289–300.
[47] I.D. Neumann, L. Torner, A. Wigger, Brain oxytocin: differential inhibition of neuroendocrine stress responses and anxiety-related behaviour in virgin, pregnant and lactating rats, Neuroscience 95 (2000) 567–575.
[48] P.J. Brunton, J.A. Russell, The expectant brain: adapting for motherhood, Nat. Rev. Neurosci. 9 (2008) 11–25.
[49] D.A. Slattery, I.D. Neumann, No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain, J. Physiol. 586 (2008) 377–385.
[50] B.J. Marlin, M. Mitre, A. D'Amour J, M.V. Chao, R.C. Froemke, Oxytocin enables maternal behaviour by balancing cortical inhibition, Nature 520 (2015) 499–504.
[51] R.J. Bicknell, G. Leng, J.A. Russell, R.G. Dyer, S. Mansfield, B.G. Zhao, Hypothalamic opioid mechanisms controlling oxytocin neurones during parturition, Brain Res. Bull. 20 (1988) 743–749.
[52] A.J. Douglas, S. Dye, G. Leng, J.A. Russell, R.J. Bicknell, Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat, J. Neuroendocrinol. 5 (1993) 307–314.
[53] I. Neumann, J.A. Russell, B. Wolff, R. Landgraf, Naloxone increases the release of oxytocin, but not vasopressin, within limbic brain areas of conscious parturient rats: a push-pull perfusion study, Neuroendocrinology 54 (1991) 545–551.
[54] I. Neumann, J.A. Russell, R. Landgraf, Endogenous opioids regulate intracerebral oxytocin release during parturition in a region-specific manner, Prog. Brain Res. 91 (1992) 55–58.
[55] D.T. Theodosis, D.B. Chapman, C. Montagnese, D.A. Poulain, J.F. Morris, Structural plasticity in the hypothalamic supraoptic nucleus at lactation affects oxytocin-, but not vasopressin-secreting neurones, Neuroscience 17 (1986) 661–678.
[56] I. Neumann, E. Koehler, R. Landgraf, J. Summy-Long, An oxytocin receptor antagonist infused into the supraoptic nucleus attenuates intranuclear and peripheral release of oxytocin during suckling in conscious rats, Endocrinology 134 (1994) 141–148.
[57] R.J. Windle, S. Wood, N. Shanks, P. Perks, G.L. Conde, A.P. da Costa, C.D. Ingram, S.L. Lightman, Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity, J. Neuroendocrinol. 9 (1997) 407–414.
[58] M. Altemus, L.S. Redwine, Y.M. Leong, C.A. Frye, S.W. Porges, C.S. Carter, Responses to laboratory psychosocial stress in postpartum women, Psychosom. Med. 63 (2001) 814–821.
[59] V. Grinevich, I.D. Neumann, Brain oxytocin: how puzzle stones from animal studies translate into psychiatry, Mol. Psychiatr. 26 (2021) 265–279.
[60] I. Neumann, A.J. Douglas, Q.J. Pittman, J.A. Russell, R. Landgraf, Oxytocin released within the supraoptic nucleus of the rat brain by positive feedback action is involved in parturition-related events, J. Neuroendocrinol. 8 (1996) 227–233.
[61] I. Neumann, Q.J. Pittman, R. Landgraf, Release of oxytocin within the supraoptic nucleus. Mechanisms, physiological significance and antisense targeting, Adv. Exp. Med. Biol. 395 (1995) 173–183. PMID: 8713962.
[62] J.M. Stern, L. Goldman, S. Levine, Pituitary-adrenal responsiveness during lactation in rats, Neuroendocrinology 12 (1973) 179–191.
[63] A. Wigger, P. Lorscher, I. Oehler, M.E. Keck, T. Naruo, I.D. Neumann, Nonresponsiveness of the rat hypothalamo-pituitary-adrenocortical axis to parturition-related events: inhibitory action of endogenous opioids, Endocrinology 140 (1999) 2843–2849.
[64] I.D. Neumann, S.A. Kromer, N. Toschi, K. Ebner, Brain oxytocin inhibits the (re) activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions, Regul. Pept. 96 (2000) 31–38.
[65] A.J. Douglas, P.J. Brunton, O.J. Bosch, J.A. Russell, I.D. Neumann, Neuroendocrine responses to stress in mice: hyporesponsiveness in pregnancy and parturition, Endocrinology 144 (2003) 5268–5276.
[66] I.D. Neumann, A. Wigger, L. Torner, F. Holsboer, R. Landgraf, Brain oxytocin inhibits basal and stress-induced activity of the hypothalamo-pituitary-adrenal axis in male and female rats: partial action within the paraventricular nucleus, J. Neuroendocrinol. 12 (2000) 235–243.
[67] R.J. Windle, Y.M. Kershaw, N. Shanks, S.A. Wood, S.L. Lightman, C.D. Ingram, Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of hypothalamo-pituitary-adrenal activity, J. Neurosci. 24 (2004) 2974–2982.
[68] M.E. Keck, M. Hatzinger, C.T. Wotjak, R. Landgraf, F. Holsboer, I.D. Neumann, Ageing alters intrahypothalamic release patterns of vasopressin and oxytocin in rats, Eur. J. Neurosci. 12 (2000) 1487–1494.
[69] C. Wenneras, A. Wold, Nepotism and sexism in peer-review, Nature 387 (1997) 341–343.
[70] T.M. Schladt, G.C. Nordmann, R. Emilius, B.M. Kudielka, T.R. de Jong, I. D. Neumann, Choir versus solo singing: effects on mood, and salivary oxytocin and cortisol concentrations, Front. Hum. Neurosci. 11 (2017) 430.
[71] A.E. Wilhelmi, G.E. Pickford, W.H. Sawyer, Initiation of the spawning reflex response in Fundulus by the administration of fish and mammalian neurohypophysial preparations and synthetic oxytocin, Endocrinology 57 (1955) 243–252.
[72] K. Mahalati, K. Okanoya, D.M. Witt, C.S. Carter, Oxytocin inhibits male sexual behavior in prairie voles, Pharmacol. Biochem. Behav. 39 (1991) 219–222.
[73] A. Argiolas, M.R. Melis, The role of oxytocin and the paraventricular nucleus in the sexual behaviour of male mammals, Physiol. Behav. 83 (2004) 309–317.
[74] M.D. Stoneham, B.J. Everitt, S. Hansen, S.L. Lightman, K. Todd, Oxytocin and sexual behaviour in the male rat and rabbit, J. Endocrinol. 107 (1985) 97–106.
[75] M.S. Carmichael, R. Humbert, J. Dixen, G. Palmisano, W. Greenleaf, J. M. Davidson, Plasma oxytocin increases in the human sexual response, J. Clin. Endocrinol. Metabol. 64 (1987) 27–31.
[76] M. Waldherr, I.D. Neumann, Centrally released oxytocin mediates mating-induced anxiolysis in male rats, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 16681–16684.
[77] K.D. Nyuyki, M. Waldherr, S. Baeuml, I.D. Neumann, Yes, I am ready now: differential effects of paced versus unpaced mating on anxiety and central oxytocin release in female rats, PLoS One 6 (2011), e23599.
[78] H.E. Ross, C.D. Cole, Y. Smith, I.D. Neumann, R. Landgraf, A.Z. Murphy, L. J. Young, Characterization of the oxytocin system regulating affiliative behavior in female prairie voles, Neuroscience 162 (2009) 892–903.
[79] D.M. Witt, C.S. Carter, D.M. Walton, Central and peripheral effects of oxytocin administration in prairie voles (Microtus ochrogaster), Pharmacol. Biochem. Behav. 37 (1990) 63–69.
[80] Z.R. Donaldson, L.J. Young, Oxytocin, vasopressin, and the neurogenetics of sociality, Science (New York, N.Y 322 (2008) 900–904.
[81] C.S. Carter, The oxytocin-vasopressin pathway in the context of love and fear, Front. Endocrinol. 8 (2017) 356.
[82] R. Menon, I.D. Neumann. Detection, processing, and reinforcement of social cues by the brain oxytocin system. Nat. Rev. Neurosci. 2023 in press.
[83] M.M. McCarthy, S.P. Kleopoulos, C.V. Mobbs, D.W. Pfaff, Infusion of antisense oligodeoxynucleotides to the oxytocin receptor in the ventromedial hypothalamus reduces estrogen-induced sexual receptivity and oxytocin receptor binding in the female rat, Neuroendocrinology 59 (1994) 432–440.
[84] M.R. Melis, A. Argiolas, Oxytocin, erectile function and sexual behavior: last discoveries and possible advances, Int. J. Mol. Sci. 22 (2021).
[85] O.J. Bosch, J. Dabrowska, M.E. Modi, Z.V. Johnson, A.C. Keebaugh, C.E. Barrett, T.H. Ahern, J. Guo, V. Grinevich, D.G. Rainnie, I.D. Neumann, L.J. Young, Oxytocin in the nucleus accumbens shell reverses CRFR2-evoked passive stress-coping after partner loss in monogamous male prairie voles, Psychoneuroendocrinology 64 (2016) 66–78.
[86] H. Walum, L.J. Young, The neural mechanisms and circuitry of the pair bond, Nat. Rev. Neurosci. 19 (2018) 643–654.
[87] L.L. Oettl, N. Ravi, M. Schneider, M.F. Scheller, P. Schneider, M. Mitre, M. da Silva Gouveia, R.C. Froemke, M.V. Chao, W.S. Young, A. Meyer-Lindenberg, V. Grinevich, R. Shusterman, W. Kelsch, Oxytocin enhances social recognition by modulating cortical control of early olfactory processing, Neuron 90 (2016) 609–621.
[88] R.C. Froemke, L.J. Young, Oxytocin, neural plasticity, and social behavior, Annu. Rev. Neurosci. 44 (2021) 359–381.
[89] J.P. Burkett, E. Andari, Z.V. Johnson, D.C. Curry, F.B. de Waal, L.J. Young, Oxytocin-dependent consolation behavior in rodents, Science (New York, N.Y 351 (2016) 375–378.
[90] T.R. de Jong, D.I. Beiderbeck, I.D. Neumann, Measuring virgin female aggression in the female intruder test (FIT): effects of oxytocin, estrous cycle, and anxiety, PLoS One 9 (2014), e91701.
[91] V.E.M. Oliveira, T.R. de Jong, I.D. Neumann, Synthetic oxytocin and vasopressin act within the central amygdala to exacerbate aggression in female Wistar rats, Front. Neurosci. 16 (2022), 906617.
[92] O.J. Bosch, S.L. Meddle, D.I. Beiderbeck, A.J. Douglas, I.D. Neumann, Brain oxytocin correlates with maternal aggression: link to anxiety, J. Neurosci. 25 (2005) 6807–6815.
[93] O.J. Bosch, I.D. Neumann, Brain vasopressin is an important regulator of maternal behavior independent of dams' trait anxiety, Proc. Natl. Acad. Sci. U.S. A. 105 (2008) 17139–17144.
[94] M. Lukas, I. Toth, S.O. Reber, D.A. Slattery, A.H. Veenema, I.D. Neumann, The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice, Neuropsychopharmacology 36 (2011) 2159–2168.
[95] S. Anpilov, Y. Shemesh, N. Eren, H. Harony-Nicolas, A. Benjamin, J. Dine, V.E. M. Oliveira, O. Forkosh, S. Karamihalev, R.E. Huttl, N. Feldman, R. Berger, A. Dagan, G. Chen, I.D. Neumann, S. Wagner, O. Yizhar, A. Chen, Wireless optogenetic stimulation of oxytocin neurons in a semi-natural setup dynamically elevates both pro-social and agonistic behaviors, Neuron 107 (2020) 644–655 e647.
[96] A.J. Grippo, D.M. Trahanas, R.R. Zimmerman 2nd, S.W. Porges, C.S. Carter, Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation, Psychoneuroendocrinology 34 (2009) 1542–1553.
[97] Y.F. Guzman, N.C. Tronson, V. Jovasevic, K. Sato, A.L. Guedea, H. Mizukami, K. Nishimori, J. Radulovic, Fear-enhancing effects of septal oxytocin receptors, Nat. Neurosci. 16 (2013) 1185–1187.
[98] I. Zoicas, D.A. Slattery, I.D. Neumann, Brain oxytocin in social fear conditioning and its extinction: involvement of the lateral septum, Neuropsychopharmacology 39 (2014) 3027–3035.
[99] N. Duque-Wilckens, L.Y. Torres, S. Yokoyama, V.A. Minie, A.M. Tran, S. P. Petkova, R. Hao, S. Ramos-Maciel, R.A. Rios, K. Jackson, F.J. Flores-Ramirez,

I. Garcia-Carachure, P.A. Pesavento, S.D. Iniguez, V. Grinevich, B.C. Trainor, Extrahypothalamic oxytocin neurons drive stress-induced social vigilance and avoidance, Proc. Natl. Acad. Sci. U.S.A. 117 (2020) 26406–26413.

[100] A. Blume, O.J. Bosch, S. Miklos, L. Torner, L. Wales, M. Waldherr, I.D. Neumann, Oxytocin reduces anxiety via ERK1/2 activation: local effect within the rat hypothalamic paraventricular nucleus, Eur. J. Neurosci. 27 (2008) 1947–1956.

[101] E.H. van den Burg, I.D. Neumann, Bridging the gap between GPCR activation and behaviour: oxytocin and prolactin signalling in the hypothalamus, J. Mol. Neurosci. 43 (2011) 200–208.

[102] B. Jurek, D.A. Slattery, R. Maloumby, K. Hillerer, S. Koszinowski, I.D. Neumann, E.H. van den Burg, Differential contribution of hypothalamic MAPK activity to anxiety-like behaviour in virgin and lactating rats, PLoS One 7 (2012), e37060.

[103] M. Busnelli, E. Peverelli, G. Mantovani, A. Spada, B. Chini, Deciphering the specific role of G(alphai/o) isoforms: functional selective oxytocin ligands and somatostatin SST5 receptor mutants, Biochem. Soc. Trans. 41 (2013) 166–171.

[104] J. Winter, M. Meyer, I. Berger, M. Royer, M. Bianchi, K. Kuffner, S. Peters, S. Stang, D. Langgartner, F. Hartmann, A.K. Schmidtner, S.O. Reber, O.J. Bosch, A. Bludau, D.A. Slattery, E.H. van den Burg, B. Jurek, I.D. Neumann, Chronic oxytocin-driven alternative splicing of Crfr2alpha induces anxiety, Mol. Psychiatry. (2021), https://doi.org/10.1038/s41380-021-01141-x.

[105] K.L. Bales, A.M. Perkeybile, O.G. Conley, M.H. Lee, C.D. Guoynes, G.M. Downing, C.R. Yun, M. Solomon, S. Jacob, S.P. Mendoza, Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles, Biol. Psychiatr. 74 (2013) 180–188.

[106] H. Huang, C. Michetti, M. Busnelli, F. Manago, S. Sannino, D. Scheggia, L. Giancardo, D. Sona, V. Murino, B. Chini, M.L. Scattoni, F. Papaleo, Chronic and acute intranasal oxytocin produce divergent social effects in mice, Neuropsychopharmacology 39 (2014) 1102–1114.

[107] S. Peters, D.A. Slattery, N. Uschold-Schmidt, S.O. Reber, I.D. Neumann, Dose-dependent effects of chronic central infusion of oxytocin on anxiety, oxytocin receptor binding and stress-related parameters in mice, Psychoneuroendocrinology 42 (2014) 225–236.

[108] M. Meyer, B. Jurek, M. Alfonso-Prieto, R. Ribeiro, V.M. Milenkovic, J. Winter, P. Hoffmann, C.H. Wetzel, A. Giorgetti, P. Carloni, I.D. Neumann, Structure-function relationships of the disease-linked A218T oxytocin receptor variant, Mol. Psychiatr. 27 (2022) 907–917.

[109] M. Royer, B. Pai, R. Menon, A. Bludau, K. Gryksa, R.B. Perry, I. Ulitsky, G. Meister, I.D. Neumann, Nuclear localization of Meg3-ex10 in mice lateral septum after social fear extinction, Mol. Psychiatr. 27 (2022) 3925.

[110] H.S. Knobloch, A. Charlet, L.C. Hoffmann, M. Eliava, S. Khrulev, A.H. Cetin, P. Osten, M.K. Schwarz, P.H. Seeburg, R. Stoop, V. Grinevich, Evoked axonal oxytocin release in the central amygdala attenuates fear response, Neuron 73 (2012) 553–566.

[111] T. Grund, S. Goyon, Y. Li, M. Eliava, H. Liu, A. Charlet, V. Grinevich, I. D. Neumann, Neuropeptide S activates paraventricular oxytocin neurons to induce anxiolysis, J. Neurosci. 37 (2017) 12214–12225.

[112] M. Eliava, M. Melchior, H.S. Knobloch-Bollmann, J. Wahis, M. da Silva Gouveia, Y. Tang, A.C. Ciobanu, R. Triana Del Rio, L.C. Roth, F. Althammer, V. Chavant, Y. Goumon, T. Gruber, N. Petit-Demouliere, M. Busnelli, B. Chini, L.L. Tan, M. Mitre, R.C. Froemke, M.V. Chao, G. Giese, R. Sprengel, R. Kuner, P. Poisbeau, P.H. Seeburg, R. Stoop, A. Charlet, V. Grinevich, A new population of parvocellular oxytocin neurons controlling magnocellular neuron activity and inflammatory pain processing, Neuron 89 (2016) 1291–1304.