Neural mechanisms of female aggression: **Implications on the oxytocin and vasopressin systems**

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SUMMARY

Aggression is defined as a social behavior that has the intention of physically harming a conspecific. In nature, aggressive behavior emerges whenever an individuum, engages in conflict over essential resources for its survival, such as food, water, territory, and mating partners. Thus, aggression may act as an evolutive force controlling populational levels and keeping hierarchy. However, in humans, when expressed out-of-context and in exacerbated manner aggressive behavior becomes disruptive constituting a severe burden on society. This is especially evident in the excessive as well as pathological levels of aggression expressed by individuals suffering from conduct disorder (CD) in childhood and anti-social personality disorder (ASPD) in adulthood. As violent aggressive behavior causes serious damage not only to the victims but also to the perpetrators, scientists have worked throughout the last decades to understand the neurobiological underpinnings of escalated aggression. In order to do so, several rodent models have been established to study aggressive behavior, using mostly males as model organisms whereas females have been rarely studied. Nevertheless, recent evidence shows that women and girls may develop ASPD and CD just like men and boys, respectively. Additionally, there are some new indications that the neurobiology of aggression might be sex-specific in humans and rodents. Thus, new animal models are necessary to understand the neural mechanisms of female aggression.

In my thesis, I first established and utilized two rat models of female aggression based on different etiologic strategies, namely post-weaning social isolation (PWSI) was used as a model of early life stress-induced aggression whereas a combination of social isolation and aggressive training was used to enhance aggression in an ethological setting in adult rats. These models allowed me to investigate the role of the

brain oxytocin (OXT) and vasopressin (AVP) systems on aggressive behavior. Both neuropeptides are known to regulate social including aggressive behavior in males and lactating females.

Although aggression is a naturally occurring behavior in animals and humans, exacerbated aggressive behavior may also emerge as a maladaptive response to stress. Especially, early life adverse experiences are known to evoke violent aggressive behavior. Thus, in chapter 2 post-weaning social isolation (PWSI) was used as a reliable model of early life stress-induced (ELS) aggression in order to compare its effects on male and female aggression as well as on the endogenous OXT and AVP systems. My results show that males and females displayed similar levels of aggression independent of the housing conditions and that PWSI increased aggression in both male and female Wistar rats. However, abnormal aggression was displayed in a sex-dependent manner, i.e. females exhibited elevated aggression towards juveniles, whereas males tended to show more attack bites and attacks towards vulnerable body parts. In addition, PWSI also impaired social discrimination in both sexes. From a neurobiological point of view, PWSI decreased OXTmRNA in the paraventricular nucleus of the hypothalamus (PVN) and OXT receptor (OXTR) binding in the nucleus *accumbens* (NAcc), independent of the sex. Regarding the AVP system, I have found that PWSI rats showed decreased AVP 1a receptor (V1aR) binding in the dentate gyrus (DG) and lateral hypothalamus (LH) independent of sex. However, the anterior part of the BNST was affected by PWSI in a sex-dependent manner, i.e. in control conditions, females exhibited higher V1aR binding than males in this region, but after PWSI females had lower V1aR binding than males. Thus, my data supports PWSI as a reliable rat model to instigate exaggerated as well as abnormal aggression not only in males but also in females. In addition, OXTRs in the

NAcc and V1aR in the BNSTa, DG, and LH may play a role in the link between PWSI and aggression in rats.

In chapter 3, in order to specifically investigate the role of OXT and AVP on female aggression, social isolation, as well as successive encounters with a same-sex and unknown conspecific (aggression training) (IST), were used to enhance the mild levels os aggression displayed by group-housed (GH) and non-trained females. In comparison to low aggressive GH controls, highly aggressive IST females exhibited elevated levels of OXT and reduced levels of AVP in both CSF and LS in response to a female intruder test (FIT). Furthermore, both OXTR and V1aR binding were decreased in the ventral (vLS) and dorsal (dLS) portion of the LS of IST rats, respectively. Manipulation of both neuropeptide systems using a combination of neuropharmacological, chemo- and optogenetic approaches resulted in dramatic changes in aggression. Elevating OXT availability either centrally or in the vLS of GH rats enhanced aggression. Accordingly, blockade of OXTR, via OXTR antagonist, either centrally or in the vLS decreased aggressive display in IST rats. Regarding the AVP system, synthetic AVP administrated either locally in the dLS or centrally (intracerebroventricular) decreased aggression in IST rats.

Due to the fact that OXT and AVP effects appear to be region- and receptor-specific, i.e. OXT acted via OXTR in the vLS and AVP acted via V1aR in the dLS, I decided to verify whether those two neural populations within the LS interact with each other in a single-cell level after OXTR activation using whole-cell *voltage-clamp*. OXTR activation increased GABAergic inhibition of dLS neurons whereas decreased GABAergic inhibition of vLS neurons. Next, I decided to evaluate whether those differences in activity were also reflected *in vivo* after an aggressive encounter, using pERK as a neural activity marker. Aggression differentially regulated pERK expression

in the LS, i.e. GABAergic neurons in the dLS showed decreased whereas in the vLS showed increased activation after the FIT. In line with that, pharmacological inhibition of the dLS and vLS enhanced and reduced female aggression, respectively. Taken together this part of my thesis shows that the balance between OXT and AVP release within the LS regulates female aggression in a receptor and region-specific manner via modulating GABAergic neurotransmission.

Overall, this thesis shows that females are able to develop escalated as well as abnormal aggression just like males. In addition, the OXT and the AVP system seem to be main players in regulating aggressive behavior in female Wistar rats, especially, regarding their role in controlling aggression by acting on the LS.

1. General Introduction

1.1. Aggression

Social aggression is typically defined as a social behavior displayed between conspecifics with the intention of physically harming one another (De Almeida *et al*, 2005; Koolhaas *et al*, 2013; Miczek *et al*, 2001; Nelson and Trainor, 2007). It is expressed by many, if not all animal species including humans, and usually the successful aggressor benefits by gaining access to limited resources, such as food, territory, nests, or mating partners (De Almeida *et al*, 2005; Nelson and Trainor, 2007). Further, aggressive behavior may also emerge, as a maladaptive response, whenever animals face challenges to their homeostasis, such as threats and/or stressors (Haller *et al*, 2014; Sandi and Haller, 2015).

When expressed out-of-context or in an exacerbated manner aggressive behavior becomes disruptive by harming both aggressor and victim. This is especially evident in the pathological levels of aggressive behavior displayed by humans suffering from aggression disorders, such as conduct (CD), anti-social personality (ASPD), and intermittent explosive disorder (De Almeida *et al*, 2005; Haller, 2016; Nelson and Trainor, 2007). Around 5% and 0.6-3% of the European population are affected by CD and ASPD, respectively. Those patients suffer from a broad spectrum of symptoms including excessive aggression towards others, damaging property, deceitfulness, lack of remorse or guilt, callousness or lack of empathy, shallow or deficient affect, manipulativeness, and egocentrism (DSM-V American Psychiatric Association, 2012; Freitag *et al*, 2018; Reynolds and Kamphaus, 2014; Wittchen *et al*, 2011). Exaggerated aggression also occurs as a symptom of other psychiatric and neurological disorders, such as autism, bipolar personality disorder, schizophrenia, post-traumatic stress disorder (PTSD), and dementia (Nelson and Trainor, 2007). Thus, aggression as

comorbidity or as a disorder constitutes a severe burden to society. In fact, estimations indicate that annually 1.3 million people worldwide die as a consequence of violence, and 126 billion US dollars are spent annually to either prevent or deal with the consequences of violence (WHO, 2014).

In humans, aggressive behavior is mainly classified into two types: *instrumental* or *controlled aggression* characterized by having a goal-oriented purpose, *and reactive* or *impulsive aggression* known to be a sudden, rather uncontrolled reaction to a stimulus, often related to anger. In general, reactive aggression is related to abrupt and inappropriate aggressive outbursts, as an example, when someone, by accident, punches a co-worker after a passionate argument, this type of aggression is typically linked with intermittent explosive disorder, PTSD and depression-related aggression. On the other hand, instrumental aggression can be associated with genocide, planned assassination and massive killings, in this case, people act less on impulse and plan their aggressive acts, as an example, we could mention planning for weeks on how to kill the co-worker who punched you after the argument. Patients suffering from CD and ASPD show high levels of instrumental aggression frequently accompanied by a lack of remorse (Viding *et al*, 2012). Both types of aggression also differ from a physiological point of view, reactive aggression is related to hyper-arousal, i.e. accompanied by high sympathetic activity and cortisol levels, whereas instrumental aggression has been linked to a hypo-arousal phenotype, i.e. low sympathetic activity and cortisol levels (Comai *et al*, 2012; Haller, 2013; Nelson and Trainor, 2007).

In order to reveal the brain regions and neurobiological mechanisms involved in aggression regulation, various strategies have been employed in animals. Seminal studies have used electrical and chemical stimulation of so-called aggression centers to evoke aggressive phenotypes similar to the ones seen in humans (De Almeida *et al*, 2005; Baxter, 1968; Haller, 2013; Potegal, M Blau, A and Glusman, 1981; Potegal

et al, 1981), current approaches preferred focusing on the animal's ethology in order to increase the biological significance of the findings. Therefore, in animals, aggression is usually classified accordingly with the subject's ethology (territorial, dominance, maternal) (De Almeida *et al*, 2005; Comai *et al*, 2012). Especially, territorial aggression has been studied in male rodents for years, using a reliable and consistent paradigm: the resident-intruder test (RI). This test relies on the fact that male residents will defend their territory against unfamiliar male intruders. Usually, either co-housing with a female over several days or weeks or social isolation are used to instigate the resident's territoriality and, consequently, aggressive behavior to defend its homecage. The test consists of releasing a slightly smaller same-sex intruder into the resident's homecage for 10-20 minutes. During this time aggressive behaviors, such as attack bites, threats, chases and tail rattles (mice) as well as dominant behaviors, such as keep down, offensive grooming, offensive up-rights are quantified. In addition, non-aggressive behaviors like non-aggressive social investigation (sniffing), investigation of the homecage, self-grooming and defensive behaviors might be also quantified in order to evaluate, whether the high aggressiveness impacts on other behavioral domains displayed by the residents (Koolhaas *et al*, 2013; Miczek *et al*, 2001). Surprisingly, although territorial aggression has been reported in wild female mice and hamsters (Harmon *et al*, 2002; Mcdonald *et al*, 2011; Miczek *et al*, 2001; Ross *et al*, 2019; Silva *et al*, 2010), this behavior has been mostly studied in males, whereas females have been only studied in the context of lactation associated with the prominent display of maternal aggression (see discussion on page 23).

Aiming to mimic pathological and out-of-context aggression seeing in humans, various rat and mouse models have been established, in order to understand the neural underpinnings of escalated aggressive behavior. In general, an animal becomes abnormally aggressive when shows: i) a mismatch between provocation and response,

i.e. attacking in inappropriate situations, as in a neutral arena or showing elevated aggression (excessive attack counts, short-latency to attack, causing severe tissue damage); ii) disregard of species-specific rules, such as attacking juveniles, anesthetized animals and females or attacking vulnerable body parts (head, paws, neck, and belly); iii) insensitivity towards the social signals of the intruder, i.e. sustained aggression despite submissiveness of the opponent or showing an inability in terminating aggression outbursts; iv) "offensive ambiguity", namely aggressive behavior lacking a normal structure, i.e., failure in signaling attacks from threats, attacking from defensive postures or attacking only smaller intruders (Haller, 2013; Miczek *et al*, 2013).

Among the approaches used to develop animal models of abnormal and excessive aggression five main strategies have been successfully described: i) using naturally aggressive animals such as hamsters (Ferris *et al*, 1997; Harmon *et al*, 2002; Potegal *et al*, 1981), Calfornia mice (Oyegbile and Marler, 2005; Silva *et al*, 2010) and feral rats (Koolhaas *et al*, 2013) ii) selecting mice (Caramaschi *et al*, 2008; Lagerspetz, 1968; Miczek *et al*, 2013; van Oortmerssen and Bakker, 1981) and rats (Beiderbeck *et al*, 2012; Koolhaas *et al*, 2013; Neumann *et al*, 2010; Walker *et al*, 2016) based on their levels of aggression in order to have animals showing feral aggression; ii) using ELS such as post-weaning social isolation (Toth *et al*, 2011), peripubertal stress (PPS) (Marquez *et al*, 2013) or maternal separation (MS) (Veenema *et al*, 2006); iii) manipulation of selected biological system to mimic features encountered in highly aggressive patients, such as adrenalectomy (Haller *et al*, 2004) to induce a state of hypo-CORT, and alcohol consumption (De Almeida *et al*, 2005; Miczek *et al*, 2013); and iv) aggression training, engaging and winning, repeatedly, conflicts have been implicated in decreasing attack latencies and increasing the number of attacks in hamsters (Been *et al*, 2016) and mice (Oyegbile and Marler, 2005; Silva *et al*, 2010).

Especially, ELS models have been repeatedly shown to induce consistent and reliable levels of high aggression in male rats (Haller *et al*, 2014; Masis-Calvo *et al*, 2018; Sandi and Haller, 2015). Those models are translationally relevant because they were built on the fact that patients with CD and ASPD as well as offenders, often come from troubled homes and face different types of abuse during their childhood and/or adolescence. In fact, several studies have described that stressful environmental conditions, especially during early life, correlate with aggressive as well as externalizing behavior in humans (Caspi *et al*, 2002; Dackis *et al*, 2017; Freitag *et al*, 2018; Glenn *et al*, 2013; Haller *et al*, 2014; Nelson and Trainor, 2007; Sandi and Haller, 2015). Most of the rat studies of ELS support the findings seen in humans (Marquez *et al*, 2013; Toth *et al*, 2011; Veenema *et al*, 2006), showing that an exaggerated stimulation of the hypothalamic-pituitary-adrenal axis (HPA) by stress during early life leads to abnormal aggressive behavior later in life underlined by a hyper-CORT phenotype (Biro *et al*, 2016; Marquez *et al*, 2013; Toth *et al*, 2011, 2012; Veenema *et al*, 2006; Veenit *et al*, 2013).

In the present thesis, PWSI has been used as a model of ELS for studying mechanisms of aggression in females in comparison to males. Therefore, I will describe the model in more detail in the next session.

1.2. POST-WEANING SOCIAL ISOLATION AND AGGRESSION

Among the ELS models of pathological aggression, PWSI seems to have the strongest effects, because it fulfills the 4 criteria needed to be classified as an abnormal aggression model as well as presents a robust translational component, psychosocial deprivation, including neglect and abuse, have been implicated in the development of ASPD and CD (Dackis et al, 2017; Glenn et al, 2013; Haller, 2016). The protocol consists of keeping pups (21 days old) either housed in groups (controls) or singly-housed for 7 weeks after weaning. It is based on the premise that lack of social contact

and the display of play behavior with conspecifics during puberty/adolescence impairs or prevents the development of the rat's social behavior repertoire, resulting in high and abnormal aggression (Toth *et al*, 2011).

PWSI induces abnormal aggression in male Wistar rats by increasing attack bites towards vulnerable targets and decreasing signaled attacks, i.e. PWSI rats attack from defensive postures. Conversely, isolated rats exhibit elevated plasma corticosterone (CORT) and autonomic responses during the RI (Toth *et al*, 2011), characterizing a hyper-arousal type of aggression, which has been linked rather to reactive aggression than to instrumental aggression expressed by ASPD and CD patients (Comai *et al*, 2012; Nelson and Trainor, 2007).

Concerning the neurobiological mechanisms underlying the high levels of aggression shown by isolated animals, it has been shown that PWSI enhances the neuronal activation of aggression-related regions, such as the hypothalamic-attack area (HAA), the bed nucleus of *stria terminalis* (BNST), the medial amygdala (MeA) and the orbital frontal cortex (OFC) (Toth *et al*, 2012). In addition, isolated rats exhibit a thinner right prefrontal cortex (PFC), which was a consequence of reduced glia and dendritic density as well as impaired vascularization of this area (Biro *et al*, 2016). Those changes have been related to the finding of decreased levels of the brain-derived neurotrophic factor (BDNF) in the PFC as well as in the MeA of PWSI rats (Mikics *et al*, 2018).

1.3. NEURAL CIRCUITS OF AGGRESSION

Throughout the years, neuroscientists have tried to fully characterize the brain networks involved in aggressive behavior display by using a variety of techniques, such as neural activity markers (c-fos, pERK, and zif268), chemical and mechanical lesioning/inhibition of target brain regions, electrophysiological recordings of neuronal populations during the display of aggression and, recently, calcium imaging in freely

moving animals (fiber photometry). All this effort culminated in the establishment of an aggressive behavior neuronal pathway, which completely overlaps with the social behavior network, in agreement with the theory that aggressiveness is an emergent property of social behaviors (Nelson and Trainor, 2007).

In rodents, social cues, including the ones eliciting aggression, are known to be mainly olfactory, therefore, they are initially processed in the main (MOB) and accessory (AOB) olfactory bulbs (Dulac and Torello, 2003; Nelson and Trainor, 2007; Stowers *et al*, 2013). In fact, the ablation of either the olfactory bulb by bulbectomy or of the olfactory nerves strongly impairs aggressive display in male mice (Mucignat-Caretta *et al*, 2004) and rats (Bergvall *et al*, 1991). Also, the existence of aggression evoking pheromones has been described in male mice (Chamero *et al*, 2007; Stowers *et al*, 2013). After this initial processing, the signals are forwarded to limbic and hypothalamic regions involved in aggression display such as the BNST, MeA, the lateral septum (LS), the medial pre-optic area (MPOA), the ventromedial (VMH) and anterior (AH) hypothalamus (mice), also known as part of the hypothalamic attack-area or mediobasal hypothalamus (MBH) (rats) (Beiderbeck *et al*, 2007, 2012; Toth *et al*, 2012; Trainor *et al*, 2010a). After, further processing all those signals seem to converge into the periaqueductal gray matter (PAG), in the midbrain, where the motor outputs are generated (Nelson and Trainor, 2007) (Figure 1.1).

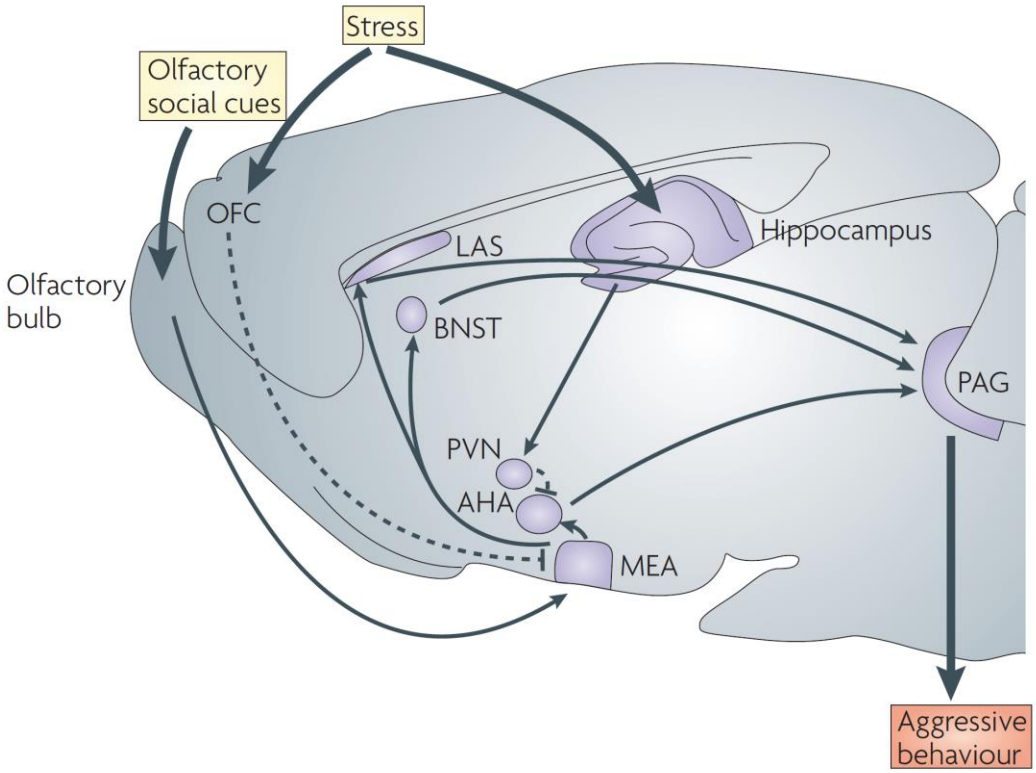


Figure 1.1: Neuroanatomical pathways of aggression in the rodent brain. Typically sensorial information arrives in the olfactory bulb and is further processed in the medial amygdala (MEA), the MEA projects to the lateral septum (LAS), bed nucleus of *stria terminalis* (BNST) and anterior hypothalamus (AHA). These brain areas are known to modulate the periaqueductal gray (PAG) activity, in order to originate the motor patterns need it during aggressive behavior display in rodents. Other regions such as the hippocampus, orbital frontal cortex (OFC) and the paraventricular nucleus of the hypothalamus (PVN) may also modulated aggressive behavior by acting on these regions, especially under stressful conditions. The OFC is thought to be one of the main inhibitors of aggressive behaviors in mammals (Adapted from Nelson and Trainor, 2007).

It is important to highlight the essential role of the HAA in eliciting attacks in male rodents. Early studies have shown that electrical stimulation of the HAA, which anatomically corresponds to several hypothalamic nuclei, leads to irritability and aggression in cats and rats (Brown *et al*, 1969; Haller, 2013; Kruk *et al*, 1983). Additionally, increased activation of the HAA after the RI has been reported, using neural activity markers, in rat models of abnormal aggression (Beiderbeck *et al*, 2012; Toth *et al*, 2012) and in *Peryomiscus californicus* (Trainor *et al*, 2010a). Recent experiments using immediate early genes, optogenetics, *in vivo* electrophysiology and calcium imaging, have shown that a specific population of neurons, expressing the estrogen receptor alpha (ERα) as well as progesterone receptors (PR) within the

ventrolateral portion of the ventromedial hypothalamus (VMHvl) work as a switch triggering aggression in solitary male mice independent of pheromone-sensing, gonadal hormones, opponents or social context (Lee *et al*, 2014; Lin *et al*, 2011; Yang *et al*, 2013, 2017). Further, the VMHvl ERαPR-neurons seem to have a pivotal role in aggressive behavior display, not only for controlling attack initiation and termination (Lee *et al*, 2014; Yang *et al*, 2013), but also for being responsible for controlling attack motivation/seeking in male mice (Falkner *et al*, 2016).

In addition to the VMHvl, there are other brain regions that should be drawn to attention for being constantly associated with male aggressive behavior in rodents such as the amygdaloid complex, the nucleus *accumbens* (NAcc) and the PFC. Particularly, hyperactivation of the central (CeA) and medial nuclei of the amygdala has been described in rat models of abnormal aggression (Marquez *et al*, 2013; Toth *et al*, 2012), also aromatase-positive neurons in the MeA have been shown to, specifically, induce aggressive behavior in male mice (Unger *et al*, 2015). Regarding the motivational aspects of aggression, the NAcc appears to play a main a role, addiction-like aggressive behavior has been described in male mice (Golden *et al*, 2017), this behavior is, at least, partially underlined by dopamine-sensitive neurons in the NAcc (Aleyasin *et al*, 2018; Golden *et al*, 2019). Accordingly, dopamine release within the NAcc as well as increased activity of this region have been shown in abnormally and highly aggressive male rats selectively bred for low anxiety-related behavior (LAB) during the RI (Beiderbeck *et al*, 2012). Last, although the PFC has been implicated in aggression inhibition in rodents (Nelson and Trainor, 2007), its role on it is still controversial, as both increased (Toth *et al*, 2012) as well as decreased (Marquez *et al*, 2013) neuronal activity has been seen in different subregions of the PFC in rat models of abnormal aggression. Moreover, direct stimulation of the medial part of PFC reduces aggression in male mice (Takahashi *et al*, 2014), whereas stimulation of

specific projections of the PFC to the hypothalamus seems to increase attack counts as well as abnormal attacks in PWSI rats (Biro *et al*, 2018).

1.3.1. THE LATERAL SEPTUM AS A GATE FOR AGGRESSIVE BEHAVIOR

The LS consists mainly of GABAergic neurons (~90%) (Alonso *et al*, 1990) located in different subnuclei, i.e. the dorsal (dLS), intermediate (iLS), ventral (vLS), rostral (rLS) and caudal (cLS) LS, placed in between the lateral ventricles in the rostrodorsal septal region. Although those subnuclei are mostly GABAegic, they are known to co-express different neuropeptides and steroid hormone receptors, such as substance P, neurotensin, encephalin, somatostatin, dynorphin, growth-hormone-releasing hormone, androgen receptors (AR), ERαs, mineralocorticoid receptors (MR), oxytocin receptors (OXTR) and vasopressin 1a receptors (V1aR) in a nuclei- and neuron-specific manner (Risold and Swanson, 1997a; Smith *et al*, 2017). The main source of inputs to the LS is the hippocampus, although it also receives projections from the brainstem (ventral tegmental area, *locus coeruleus* and *Raphé* nucleus) and presents bidirectional connections with the hypothalamus, BNST, preoptic area, and amygdala. Therefore, different neuropeptidergic, as well as monoaminergic terminals, can be found in the LS (DiBenedictis *et al*, 2017; Risold and Swanson, 1997b).

From a behavioral point of view, the LS has been implicated in several different types of social behaviors including social anxiety (Zoicas *et al*, 2014), stress-induced social avoidance (Guzmán *et al*, 2013), social memory (Camats Perna and Engelmann, 2017; Lukas *et al*, 2011a; Popik *et al*, 1992) and aggression (Wong *et al*, 2016). Early case studies have described the role of the septal area on inhibiting aggression in humans, patients, including women, with septal tumors exhibited aggression outbursts (septal rage) and increased irritability (Zeman, Wolfgang and King, 1958). Later studies performed in rodents have shown the same pattern: either lesion (Potegal, M Blau, A and Glusman, 1981) or pharmacological inhibition

(Muscimol-GABA agonist) (Borland *et al*, 2019) of the LS triggered septal rage and heightened aggression, in hamsters. Accordingly, electrical stimulation of the septum reduced aggression in highly aggressive male hamsters (Potegal *et al*, 1981). In male rats, a similar picture is seen, where lesions of the LS evoked septal rage (Albert and Chew, 1980). Moreover, reduced activation of the LS (c-fos) was found in highly aggressive LAB rats after an aggressive encounter (Beiderbeck *et al*, 2007). Recently, the mechanism by which the LS abolishes aggression has been elucidated. Optogenetic stimulation of LS-GABAergic projections to the VMHvl stopped attacks as well as reduced aggression in male mice (Wong *et al*, 2016). Intriguingly, new evidence has shown that this pathway is regulated by an intricate polysynaptic LS microcircuit, where GABA neurons in the dLS, under the influence of glutamatergic hippocampal V1bR-positive terminals, inhibit neurons in the vLS, leading to a disinhibition of the VMHvl and subsequently to aggression in male mice.

1.4. NEUROCHEMISTRY AND NEUROENDOCRINOLOGY OF AGGRESSION

Several neurotransmitters, neuropeptides, and hormones have been linked to aggressive behavior. Regarding the neurotransmitters, especially the monoamines serotonin (5-HT) and dopamine (DA), but also other neurotransmitters such as glutamate, GABA, noradrenaline (NA), acetylcholine (ACh) have been studied extensively (Comai *et al*, 2012). Drugs targeting both the DA and 5-HT systems such as haloperidol, risperidone or selective serotonin reuptake inhibitors (SSRIs) have been used for decades to treat aggressive patients in the clinic; nevertheless, the efficacy of those treatments is still arguable due to severe side effects (Carrillo and Ricci, 2009; Comai *et al*, 2012; Nelson and Trainor, 2007). Those treatments are based on the hypothesis that especially low levels of 5-HT in the brain might lead to disruptive aggressive behavior (Comai *et al*, 2012; Nelson and Trainor, 2007). In contrast to this hypothesis, low activity of the monoamine oxidase A (MAOA), the enzyme responsible

for degrading the monoamines in the brain, has been associated with high levels of emotional dysfunction and aggression in children with ADHD (Fowler *et al*, 2009). However, those results have to be interpreted with caution, because the effect of MAOA on aggression seems to be influenced by environmental conditions like maltreatment in childhood (Caspi *et al*, 2002; Fowler *et al*, 2009).

Although animal studies have shown that potentiating serotoninergic neurotransmission was able to reduce aggression in male hamsters (Ferris *et al*, 1997; Terranova *et al*, 2016), abnormally aggressive Wild-Type Groningen (WTG) rats (De Boer and Koolhaas, 2005), and mice (De Almeida and Miczek, 2002; Audero *et al*, 2013), other studies have shown rather opposite effects in similar models (Audero *et al*, 2013; Marquez *et al*, 2013; Mikics *et al*, 2018). This might be due to the fact that 5-HT receptors can also influence serotonin synthesis and release in the brain by acting on autoreceptors, which makes manipulating this system challenging (Carrillo and Ricci, 2009; Nelson and Trainor, 2007). Regarding the dopaminergic system, recent evidence shows that DA is released in the NAcc of LAB rats during the RI, and in this region, aggression was linked to activation of D2 receptors (Beiderbeck *et al*, 2012). Moreover, D1 but not D2 positive neurons in the NAcc shell seem to regulate aggression seeking as well as aggression self-administration in male mice (Golden *et al*, 2019).

Many studies have tried to link hormonal levels in blood with aggression in animals and humans. Especially, CORT concentration reflecting the activity of the HPA axis under basal or stimulated conditions have been associated with pathological aggression. Intriguingly, both hyper- as well as hypo-CORT levels have been strongly related to abnormal forms of aggression in different rat models (Masis-Calvo *et al*, 2018; Sandi and Haller, 2015; Walker *et al*, 2016). Specifically, heightened CORT responses to the RI or to an acute stressor have been seen in rat models of high and

abnormal aggression induced by ELS protocols such as MS (Veenema *et al*, 2006), PPS (Marquez *et al*, 2013), and PWSI (Toth *et al*, 2011). In agreement rats selected for high CORT responsiveness to restrain-stress exhibited increased levels of aggression in the RI (Walker *et al*, 2017), and acute inhibition of CORT release via metyrapone decreases attack counts in Wistar rats (Haller *et al*, 2004). However, abnormal aggression has been also described in Wistar rats after adrenalectomy (Haller *et al*, 2004). Altogether, this data fits the hypothesis that both low and high arousal states lead to increased aggression and that an aberrant function of the HPA axis underlines exaggerated aggressive behavior (Masis-Calvo *et al*, 2018; Nelson and Trainor, 2007; Sandi and Haller, 2015).

Sex hormones such as testosterone and estradiol have also been associated with aggressive behavior display in male rodents. For example, sexual investigation, i.e. exposition of a male to a receptive female, is known to increase testosterone levels as well as territoriality and aggressive display in rats (Koolhaas *et al*, 1980, 2013). In addition, castration is broadly known to abolish aggression in male rodents (Koolhaas *et al*, 1980; Miczek *et al*, 2001; Nelson and Trainor, 2007). In the brain, aromatized testosterone acts via estrogen receptors for masculinizing the undifferentiated brain of males, which contributes to the establishment of male-typical behaviors including components of mating and aggressive behavior (Lenz *et al*, 2012). Indeed, the deletion of ERα (Sano *et al*, 2016), estrogen receptor β (ERβ) (Nakata *et al*, 2016) and AR (Juntti *et al*, 2010) impairs aggressive behavior in male mice. It is important to highlight that the activational effects of those receptors are under the influence of environmental cues and may differ from the organizational effects seen after the deletion of the receptors (Trainor *et al*, 2007).

1.4.1. THE OXYTOCIN AND VASOPRESSIN SYSTEMS AND THEIR ROLE IN MODULATING AGGRESSIVE BEHAVIOR

The nonapeptides Oxytocin (OXT) and arginine vasopressin (AVP) are sister neuropeptides that emerged after the duplication of the single gene coding the peptide vasotocin in Vertebrata. Interestingly, more than a dozen homologous nonapeptides have been described among invertebrates and vertebrates, showing how relevant those peptides are from an evolutive point of view (Jurek and Neumann, 2018) (Figure 1.2). Although OXT and AVP are mainly found in magno- and parvocellular neurons of the paraventricular (PVN) and magnocellular neurons of the supraoptic (SON) nuclei of the hypothalamus (Grinevich *et al*, 2016; Jurek and Neumann, 2018; Koshimizu *et al*, 2012), AVP neurons can also be found in several other brain regions such as the MeA, BNST, MOB, AOB, piriform cortex, MPOA and suprachiasmatic nucleus of hypothalamus (SCN) (Tobin *et al*, 2010; De Vries and Panzica, 2006; Wacker and Ludwig, 2019). Both peptides are released into the capillaries of the neurohypophysis, reaching the periphery, where they act like hormones. In mammals, hormonal OXT is known to promote milk-ejection during lactation and the contraction of the myometrium during labor, whereas hormonal AVP supports water reabsorption in the kidney, and acts as a vasoconstrictor in the arterial capillaries. Moreover, parvocellular AVP originated in the PVN acts synergically with corticotrophin-releasing-hormone (CRH) to regulate corticotrophin (ACTH) secretion in the adenohypophysis (Grinevich *et al*, 2016; Jurek and Neumann, 2018; Koshimizu *et al*, 2012).

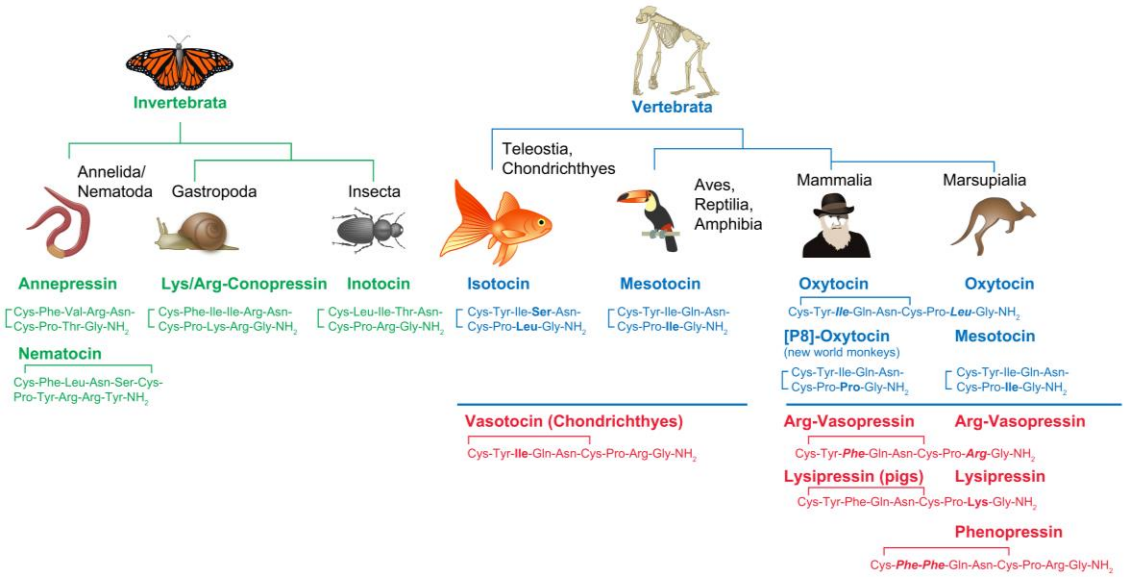


Figure 1.2: OXT and AVP sequences across different Taxa. Nonapeptide sequences of invertebrates are shown in green and vertebrates show in blue and red OXT and AVP, respectively, analogs across the animal kingdom. Each amino acid sequence is initiated by a 19 amino acid signal peptide, followed by the specific nonapeptide sequence depicted above, a processing signal consisting of glycine-lysine-arginine (GKR), and the neurophysin-glycopeptide-COOH-terminus. Italic/bold amino acids, difference between OXT and AVP; bold amino acids, difference between respective OXT or AVP. (Adapted from Jurek and Neumann, 2018).

Besides their hormonal role in the periphery, OXT and AVP also act as neuromodulators in the brain, where they are released from either axonal collaterals, terminals in various brain regions, or from soma and dendrites locally in the SON and PVN (Grinevich *et al*, 2016; Jurek and Neumann, 2018; Ludwig and Leng, 2006). Both peptides change neuronal excitability by binding to their respective receptors. OXT, typically binds to OXT receptors (OXTRs), whereas AVP binds to vasopressin 1a and 1b receptors (V1aRs and V1bRs) (Jurek and Neumann, 2018; Koshimizu *et al*, 2012), However, cross-activation of each others receptor has been described *in vitro* (Manning *et al*, 2012) as well as *in vivo* (Song *et al*, 2014; Tan *et al*, 2019). Moreover, OXT and AVP fibers, as well as OXTR and V1aR, are widespread throughout the rodent brain. Receptor binding is especially found in regions within the social behavior network, such as the amygdala nuclei, LS, BNST, NAcc, hypothalamic nuclei (VMH, AH, LH) and MPOA (DiBenedictis *et al*, 2017; Smith *et al*, 2017) (Figure 1.3).

Interestingly, in the rat brain, OXTRs and V1aRs seem to be uniquely distributed in different neuronal populations within the same region (Smith *et al*, 2017; Stoop *et al*, 2015). This is supposed to be the basis of the antagonistic effects of OXT and AVP on various behaviors. Indeed, in the lateral portion of the CeA, GABAergic neurons expressing OXTR inhibit V1aR-positive neurons in the medial part of the same region via collaterals, this process triggers opposing effects on fear, i.e. OXT decreases it whereas AVP increases it (Huber *et al*, 2005; Knobloch *et al*, 2012; Stoop *et al*, 2015).

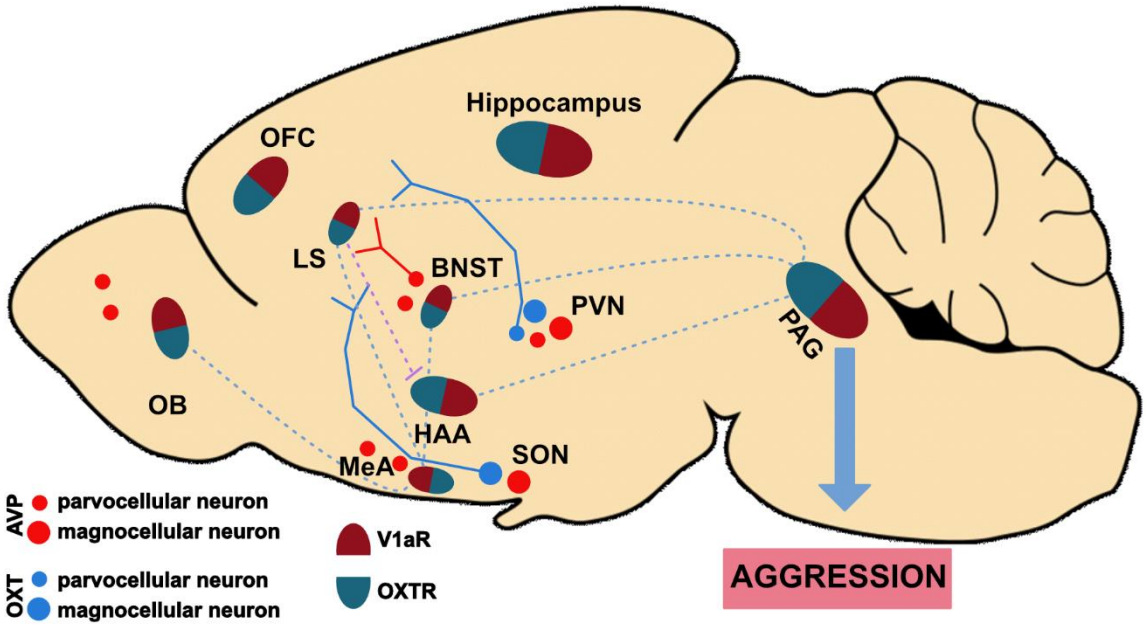


Figure 1.3:The oxytocin (OXT) and vasopressin (AVP) systems in the aggression network. Scheme shows localization of OXT neurons, in blue, in the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus, as well as AVP neurons, in red, in the PVN, SON, olfactory bulb (OB), medial amygdala (MeA), and bed nucleus of *stria terminalis* (BNST). OXT receptors (OXTR) and AVP 1a receptors (V1aR) are co-expressed in all regions involved in the aggression network, i.e. BNST, hypothalamic attack area (HAA), hippocampus, lateral septum (LS), MeA, OB and orbitalfrontal cortex (OFC), periaquedutal gray matter (PAG). Projections within the aggression network are shown in dotted blue lines. Blue (OXT) and red (AVP) plain lines show projections to the septum. Purple dotted line indicates inhibitory GABAergic projections from the LS to the HAA (Adapted from Jurek and Neumann, 2018; Nelson and Trainor, 2007; Stoop, 2015 and Swanson, 1997).

Another interesting fact about these systems is their sexual dimorphism in terms of receptor binding, neuronal number, and fiber densities. Male rats present more AVP-positive neurons in the BNST and MeA, and they also exhibit higher fiber density in the

LS, BNST, MeA and MPOA (DiBenedictis *et al*, 2017; De Vries and Panzica, 2006). Regarding the receptors, males showed increased OXTR binding in several regions of the social behavior network, such as posterior BNST (pBNST), MeA and VMH whereas females show increased OXTR binding only the iLS (Smith *et al*, 2017). On the other hand, V1aR binding is higher in female Wistar rats in the dLS, arcuate nucleus and ventromedial thalamus (Smith *et al*, 2017).

These sex differences might lead to functional differences. Indeed intracerebroventricular (i.c.v.) application of OXT triggered higher blood oxygen level-dependent (BOLD) activation in males than in female rats (Dumais *et al*, 2017). In addition, sexual dimorphic actions of the peptides have been reported in social behavior. For instance, endogenous OXT promotes social preference in male (Lukas *et al*, 2011c), but not in female Wistar rats (Lukas and Neumann, 2014). Also, the application of synthetic OXT into the pBNST was only able to prolong social memory persistence in males, but not females (Dumais *et al*, 2015). Regarding the vasopressinergic system, sex-dependent effects have been described in the context of aggression. Infusion of synthetic AVP into the AH exacerbates aggression male hamsters, whereas it reduces it in female hamsters (Terranova *et al*, 2016).

The role of OXT and AVP on modulating social behaviors has been extensively demonstrated in male rodents, the release of both neuropeptides has been associated to social behaviors, such as social investigation, social avoidance/ defeat, social memory, sexual behavior and aggression (Lukas and de Jong, 2017). Focusing specifically on aggression, there is not much known about how OXT may affect aggressive behavior in rodents and humans. In humans, conflicting data was found after intranasal OXT (de Jong and Neumann, 2017), those effects were also influenced by levels of anxiety (Pfundmair *et al*, 2018) and sociability, i.e. whether the participants were from in-groups or out-groups (de Dreu *et al*, 2012).

In rodents, the effects of OXT on aggression are also controversial, Excessively aggressive male WTG rats showed decreased OXT mRNA in the PVN when compared to low aggressive WTG rats, in those animals OXT mRNA was negatively correlated with offensive behavior in the RI, pointing towards the serenic role of OXT (Calcagnoli *et al*, 2014a). In addition, synthetic OXT administrated either intranasally (Calcagnoli *et al*, 2014b), i.c.v (Calcagnoli *et al*, 2013), or into the CeA (Calcagnoli *et al*, 2015) was able to reduce aggression in WTG rats, interestingly blockade of the endogenous system via an OXTR antagonist (OXTR-A) had no effect on those animals. Moreover, genetic approaches have also been used to target the OXT system in order to figure its role on intermale aggression, conventional OXTR knockout mice showed increased aggression, whereas conditional knockout mice, which had their OXTRs absent only in the forebrain postnatally, showed normal levels of aggression (Sala *et al*, 2013). In contrast with those results, the deletion of OXTR specifically in serotoninergic neurons in the *raphé* nucleus decreased aggression in male mice (Pagani *et al*, 2015). This shows a regional as well as a developmental-specific effect of the OXTR loss on aggression. The density of OXTRs also seems to be relevant for aggression display in male mice. In another study, OXTR knockouts, but not knockdowns (heterozygous subjects), showed increased aggression. Strikingly, OXT, as well as TGOT (OXTR specific agonist), rescued the social deficits of the knockouts probably by acting on other receptors, presumably V1aRs (Dhakar *et al*, 2012). In line with that, new evidence has shown anti-aggressive effects of OXT via binding to V1aRs in mice (Tan *et al*, 2019) and macaques (Jiang and Platt, 2018a).

Literature is also ambiguous about how AVP affects aggression in rodents. In male Wistar rats, a rise in AVP release was found in the LS of highly aggressive subjects, whereas low aggressive animals exhibited rising AVP levels in the BNST, during the RI. Accordingly, AVP levels in the LS are positively correlated with

aggression, and blockade of V1aR in the LS, as well as synthetic AVP administration into the BNST, decrease aggression in highly aggressive rats (Veenema *et al*, 2010). In male hamsters, pro-aggressive effects of synthetic AVP infusion into the AH have been found as well (Terranova *et al*, 2016), those effects were associated with increased V1aR binding in the AH of aggressive hamsters (Elliott Albers *et al*, 2006). In addition, V1bRKO mice displayed reduced aggression in the RI (Wersinger *et al*, 2002). Contrasting with those results, a blunted AVP release in the LS has been shown in abnormally aggressive rodents. For example, LAB rats exhibit a drop in AVP release in the LS during an aggressive encounter (Beiderbeck *et al*, 2007), also, short-attack latency (SAL) mice show decreased AVP fiber density in the same region (Compaam *et al*, 1993). In addition, a reduction of V1aR binding has been found in the LS of dominant male mice (Lee *et al*, 2019). In agreement with this data, activation of V1aR by synthetic AVP applied either i.c.v. in mice (Tan *et al*, 2019), intranasally or into the intra-cingulate cortex, in macaques reduced aggression (Jiang and Platt, 2018a). In addition, recent evidence has shown anti-aggressive and prosocial effects of AVP in humans (Brunnlieb *et al*, 2016; Parker *et al*, 2019). In summary, those results confirm that AVP, as well as OXT effects on aggression, are peptide-, region-, neuronal type- as well as sex-specific in rodents.

1.5. Sex differences in aggression: Female aggression

As mentioned before, aggressive behavior, as most of the other social behaviors, has been studied and validated predominantly in males, whereas females have been understudied or, alternatively, only studied in the unique physiological period of lactation (maternal aggression) (Denson *et al*, 2018; Freitag *et al*, 2018; Hashikawa *et al*, 2018). In fact, a few human studies led by neurobiologists, psychologists, and anthropologists, have compared aggressive behavior across the sexes and reported women as less aggressive than men in the laboratory, but also in

the real world (Campbell, 1999; Denson *et al*, 2018; Mancke *et al*, 2015). Consequently, two main hypotheses have been drawn to explain, why aggressive behavior is dimorphic in humans. From an evolutionary point of view, males and females are under different selective pressures, especially in terms of reproductive fitness. In general, males tend to adopt a polygynous strategy in order to guarantee their reproductive success. This may trigger high levels of competition and, consequently, aggression among males, in order to acquire sexual partners. In contrast, females are always "certain" of passing their genes to their offspring, thus aggression might be seeing as a potential risk to females due to the possibility of severe injury (Campbell, 1999). The exception is the state of lactation, when females have to defend the wellbeing of the offspring in order to guarantee their reproductive fitness. This might explain why mothers show high levels of aggression (even attacks to vulnerable targets) in order to protect their progeny. However, it is necessary to mention that females also have to compete for essential resources to achieve reproductive success such, as nutrition and space. Additionally, they should also be able to reject unsutable mating partners and to defend themselves in case of retaliation (Campbell, 1999; Hashikawa *et al*, 2018).

Another attempt to explain sex differences in aggression is based on cultural reasons. Despite evolution, culture is also seen as a powerful force shaping human behavior. Therefore, we have to acknowledge the fact that most of the human societies live under patriarchy ("a system of organization in which the overwhelming number of upper positions in hierarchies are occupied by males"), in this context sociobiological studies have pointed out how male-oriented culture has suppressed female aggressiveness and endorsed male aggression. Indeed, aggressive features tend to be seen as positive qualities in males, aggressive men are often described as brave, "war-heroes", heroical and assertive whereas the same aggressive features are seen

as detrimental in females being frequently associated with adjectives like pathological, male-like, emotional and illness-related. This might have led women to suppress their aggressive behaviors or alternatively use indirect forms of aggression (manipulation and relational aggression) in order to fit in the social construct imposed by men (Campbell, 1999; Denson *et al*, 2018).

Nevertheless, epidemiological evidence shows that non-lactating young girls and women may develop CD and ASPD just like males, although some sex differences have been found in terms of prevalence (1female:3males), affected females seem to show severer symptoms than affected males (Freitag *et al*, 2018). In addition, numbers of female offenders seem to arise in our society (Campbell, 1999; Denson *et al*, 2018; Freitag *et al*, 2018). From a neurobiological point of view sex differences in brain structure have been described in children with CD (Menks *et al*, 2017; Smaragdi *et al*, 2017). Furthermore, other neurobiological mechanisms underlying aggression in males and females seem also to differ (Denson *et al*, 2018; Hashikawa *et al*, 2018). Altogether, this shows how necessary is to develop new animal models in order to better understand the neural underpinnings of female aggressive behavior, especially in the context of intervention and treatment.

Similarly to humans, female aggression has been poorly studied in rodents, apart from maternal aggression. Curiously, territorial aggression has been observed in non-lactating female wild-mice (De Almeida *et al*, 2005; Miczek *et al*, 2001; Silva *et al*, 2010), hamsters (Harmon *et al*, 2002) and rats (Ho *et al*, 2001; De Jong *et al*, 2014). Especially, female hamsters (Been *et al*, 2016) and California mice (Silva *et al*, 2010) develop escalated aggression similarly to males. Recently, more studies where female aggressive behavior was assessed came to light. For example, female CD-1 mice form a hierarchy, among conspecifics, less despotic and linear than the ones established among males though (Williamson *et al*, 2019). In addition, co-housing with a mate

proved to be as efficient as in males in triggering enhanced aggression in non-lactating Swiss mice (Newman *et al*, 2019). Finally, our group established the female intruder test, based on the resident-intruder test of males, to assess female aggression in non-lactating Wistar rats. Although a direct comparison is inappropriate in this case because of different housing conditions, the results indicated that females did not differ from males in terms of quality or quantity of aggressive behavior displayed (De Jong *et al*, 2014).

In terms of neurobiological mechanisms, little is known regarding the regulation of female aggression. Lesions of the mediobasal hypothalamus seem to trigger exaggerated aggression in female rats (Haller *et al*, 1999). Accordingly, optogenetic stimulation of ERα-positive neurons in the posterior VMHvl (lateral part) leads to aggression in female mice (Hashikawa *et al*, 2017). Differently from males (Lee *et al*, 2014), the VMHvl of females developed distinct and specialized areas to control aggressive (lateral part) and sexual (medial part) behaviors in mice (Hashikawa *et al*, 2017). Sexually divergent mechanisms have also been described in hamsters concerning the involvement of 5-HT and AVP in aggression. In females, potentiating serotoninergic transmission, either by using a specific agonist (5-HT1a) into the AH or fluoxetine (SSRI) intraperitoneally (i.p.) was pro-aggressive, whereas AVP infusion in the AH was anti-aggressive; contrasting effects were seen in males after the infusion of the same treatments (Terranova *et al*, 2016).

The existence of evidence of aggression being displayed in virgin females as well as of dimorphic mechanisms regulating aggression reinforces the need for new rodent models to understand the neuronal mechanisms controlling female aggression. Such models of enhanced aggression in female rodents would also allow neuroscientists to understand the effect of social stress, such as acute or chronic social defeat on female behavior. This is indeed important, especially taking into account that

women suffer more from social phobia, depression, and PTSD caused by, among others, social stress (Laman-Maharg and Trainor, 2017; Wittchen *et al*, 2011).

1.6. AIMS OF THE PRESENT THESIS

Based on the evidence that i) females, including girls and women, show pathological and disruptive aggression, and ii) the mechanisms regulating aggression seem to be sexually dimorphic, the present thesis aimed to establish reliable and robust rat models of female aggression mimicking different etiological aspects of aggression in order to investigate the role of the OXT and AVP systems in regulating aggressive display in females. I specifically aimed to:

1. Evaluate whether PWSI is able to induce abnormal aggression in female Wistar rats similarly as it does in males. Additionally, I wondered whether aggressive behavior would also be sexually dimorphic in GH controls. Finally, I speculated whether the endogenous OXT and AVP system would also be affected in a sex-dependent manner by PWSI;

2. Establish a reliable rat model to enhance aggression displayed by female Wistar rats in order to evaluate the role of the neuropeptides OXT and AVP on female aggression, focusing especially on the LS.

1.6.1. COMPARING THE EFFECTS OF PWSI ON AGGRESSION ACROSS THE SEXES

PWSI is known as a consistent protocol to induce excessive and abnormal aggression in male Wistar rats (Toth *et al*, 2011, 2012). However, its effects on female behavior and neurobiology have not been yet assessed. In addition, although some papers have uncovered the neural underpinnings of PWSI induced aggression (Biro *et al*, 2016, 2018; Mikics *et al*, 2018; Toth *et al*, 2012), its effects on the OXT and AVP systems are still largely unknown. Since both neuropeptides are known to regulate aggressive display (Caldwell, 2017; de Jong and Neumann, 2017) it is very likely that

alterations on both systems are underlying the abnormal aggression seeing in PWSI rats.

Therefore, I first studied the effects of PWSI on aggressive, anxiety-like and social including aggressive behavior in female and male Wistar rats. Despite evaluating several components of aggressive behavior we also looked into social and anxiety-like behaviors due to the fact that CD as well as ASPD show comorbidity with anxiety disorders and socially deviant behavior (Freitag *et al*, 2018; Glenn *et al*, 2013). Moreover, we investigated whether PWSI would affect the endogenous OXT and AVP systems, at the peptide as well as at the receptor level.

1.6.2. Evaluating the role of the OXT and AVP systems on female aggression

In the second part of my thesis, I focused on establishing a model that relies on ethological features of aggression instead of the maladaptive ones induced by PWSI. This would be beneficial to better understand how the circuitry of aggression works in an ethologically relevant setting such as protecting a territory.

In order to do so, I used a combination of ethologically relevant approaches that have been used in male rodents as well as in female Syrian hamsters and California mice, i.e. a combination of short-term social isolation to induce territoriality (De Almeida *et al*, 2005; Elliott Albers *et al*, 2006; Koolhaas *et al*, 2013; Miczek *et al*, 2001; Ross *et al*, 2019) and aggression training to escalate aggressive behavior (winner effect) (Been *et al*, 2016; Oyegbile and Marler, 2005; Silva *et al*, 2010). Then, lowly aggressive, i.e. group-housed (GH), and highly aggressive, i.e. isolated and trained (IST), female Wistar rats were used in comparison to assess whether extreme phenotypes of aggression influence the endogenous OXT and AVP systems at the receptor (binding) and peptide level (release). Next, I used neuropharmacological and genetic approaches to manipulate OXT and AVP signaling within the brain in a central or local

manner. Because OXTR and V1aR are differently expressed in the LS, local experiments focusing on OXT targeted the vLS, predominantly OXTRs, whereas AVP experiment targeted the dLS, predominantly V1aRs. In addition, in order to understand how those neurons are wired in a circuit-level, we used two different approaches. First, whole-cell voltage-clamp was used to evaluate how the pharmacological activation of OXTRs would impact the spontaneous inhibitory activity of GABAergic neurons in those LS subregions. Second, immunohistochemistry (pERK) and neuropharmacology were used to evaluate and to manipulate neuronal activity in a LS region- and aggression level-specific manner.

CHAPTER 2: POST-WEANING SOCIAL ISOLATION EXACERBATES AGGRESSION IN BOTH SEXES AND AFFECTS THE VASOPRESSIN AND OXYTOCIN SYSTEM IN A SEX-SPECIFIC MANNER

This chapter was published in *Neuropharmacology* (Oliveira *et al*, 2019) as part of the special issue on Impulsivity and Aggression. The experiments were designed by Vinicius Oliveira, Trynke de Jong, and Inga Neumann. V.O. did all the behavioral assessments as well as analyzed the behavior. T.d.J. helped with brain collection. V.O. sliced the brains, performed, developed and evaluated *in situ* hybridization and receptor autoradiographs. V.O. prepared figures and tables, and wrote the first draft of the manuscript, which was revised by T.d.J. and I.N.

2.1. INTRODUCTION

Adverse and stressful early life experiences in humans, including parental neglect or abuse, often lead to impaired social behaviors such as exaggerated aggression in adulthood (Arseneault, 2017; Caspi *et al*, 2002; Dackis *et al*, 2017; Nelson and Trainor, 2007). This phenomenon is not limited to humans, as several rodent models of early life stress have found that they also affect social interactions in adulthood (Haller *et al*, 2014; Nelson and Trainor, 2007; Sandi and Haller, 2015). Adverse experiences occurring around puberty, in particular, appear to result in abnormal aggression in rodents (Marquez *et al*, 2013; Toth *et al*, 2011; Veenema *et al*, 2006, 2007).

Recently, post-weaning social isolation (PWSI) has emerged as a reliable rodent model of peri-pubertal stress leading to exacerbated aggressiveness in adulthood (Toth *et al*, 2011). The neurobiological underpinnings of the pro-aggressive effects of PWSI have been extensively studied in the last few years. Thus, isolated rats show enhanced corticosterone (CORT) levels after an aggressive encounter (Toth *et al*, 2011), and increased neural activity (c-Fos expression) in areas associated with threat

perception, social behaviors, and aggression such as the basolateral (BLA) and medial (MeA) amygdalar nuclei, the mediobasal hypothalamus, the bed nucleus of the *stria terminalis* (BNST) and the pre-frontal cortex (PFC) (Toth et al., 2012). In the medial PFC, PWSI caused reduced thickness accompanied by decreased spine density, number of astrocytes and vascularization (Biro *et al*, 2016).

As with most translational studies of aggression, the PWSI paradigm has been established and validated in males, whereas females have thus far not been tested. However, antisocial tendencies and abnormal aggression are prevalent in girls, and peri-pubertal stress may be a relevant factor in its onset (Denson *et al*, 2018; Freitag *et al*, 2018; Menks *et al*, 2017; Smaragdi *et al*, 2017). We, therefore, set out to establish whether PWSI can be implemented as an animal model of aggression in females by assessing whether PWSI has comparable effects on aggressive behavior in females and males using the female intruder test (FIT) (de Jong et al. 2013) alongside the male resident-intruder test (RIT) (Koolhaas *et al*, 2013), respectively.

A second goal of the study was to assess whether PWSI modifies two brain neuropeptide systems that both are essentially involved in the regulation of socio-emotional behavior, i.e. the oxytocin (OXT) and arginine vasopressin (AVP) systems. A role for central OXT and AVP in PWSI-induced aggression is likely, as the activity of both neuropeptides is affected by early life stress (Barrett *et al*, 2015; Beiderbeck *et al*, 2007; Lukas *et al*, 2010; Veenema *et al*, 2006) as well as social isolation (Albers et al., 2006; Tanaka et al., 2010). Furthermore, both nonapeptides play a strong role in the modulation of social interactions (Ebner et al., 2000; Lukas et al., 2011c;Lukas et al., 2011a; Zoicas et al., 2014) including aggression (Calcagnoli *et al*, 2013, 2014a; Harmon *et al*, 2002; De Jong *et al*, 2014; de Jong and Neumann, 2017; Lukas and de Jong, 2017; Masis-Calvo *et al*, 2018; Terranova *et al*, 2016; Veenema *et al*, 2010). Specifically, OXT was found to have anti-aggressive effects whereas AVP was

described to have sex and brain region-specific effects on aggression. Since profound sex differences have been found in the OXT and AVP systems in general (DiBenedictis *et al*, 2017; Smith *et al*, 2017), and in the role of OXT and AVP in social behaviors, including aggression (Bredewold et al., 2014; Dumais et al., 2015; Lukas et al., 2011c; Lukas and Neumann, 2014; Terranova et al., 2016), we hypothesized that PSWI affects OXT and AVP mRNA expression in the hypothalamic paraventricular (PVN) and supraoptic (SON) nucleus, as well as OXTR and V1aR receptor binding in aggression-related brain areas, in a sex-specific manner.

2.2. Material and Methods

Animals

All experiments were carried out in male and female Wistar rats that were bred in the animal facilities of the University of Regensburg, Germany. Intruders of both sexes were Wistar rats obtained from Charles Rivers Laboratories (Sulzfeld, Germany) that were kept in groups of 3 to 4 animals in a separate animal room. All rats were kept under controlled laboratory conditions (12:12 h light/dark cycle; lights off at 11:00, 21±1^{o}C, 60±5% humidity, standard rat nutrition (RM/H, Ssniff Spezialdia¨ten GmbH, Soest, Germany and water ad libitum).

PWSI procedure and housing conditions

The PWSI procedure was performed according to Toth et al., 2011. In brief, 6 litters were culled to 8 pups per litter (4 to 5 males and 3 to 4 females), 3-4 days after birth. On postnatal day (PND) 21, all pups were weaned and similarly distributed over four different experimental groups: isolated males and females (IS-m/IS-f, n = 13/11 per group) and group-housed males and females (GH-m/GH-f, n = 12 per group). IS animals were kept single-housed in a single rat cage (30.80 x 22.23 x 22.23 cm). GH rats were housed in non-related same-sex groups of 3-4 animals in standard group

cages (55x35x20 cm). All experimental animals were weighed once a week to monitor general health.

OVERVIEW OF EXPERIMENTS (FIG 2.1)

At PND72, the first cohort of rats (IS males: n=5; GH males: n=5; IS females: n=5; GH females: n=5) were transferred to single-housed observation cages (40x24x35 cm, Plexiglas walls) 48 hours prior to the first test, the RIT (males) and the FIT (females), respectively, in order to induce reliable levels of aggression (Toth *et al*, 2011). On PND 74, these animals underwent the RIT or FIT one hour after lights went off. Immediately after the test they were deeply anesthetized by isoflurane, immediately after which animals were sacrificed, and brains were dissected and snap frozen for OXT and AVP mRNA quantification and OXTR and V1aR binding assays. Adrenals were dissected and weighed as an indirect marker of chronic stress (Haller *et al*, 2014; Sandi and Haller, 2015).

On PND 74, the second cohort (IS males: n=8; GH males: n=7; IS females: n=6; GH females: n=7) was singly housed in observation cages and tested in the RIT and FIT 48 hours later, similar to the first cohort. These animals were kept alive and underwent behavioral assessments in order to evaluate anxiety (PND78, elevated plus-maze) and social-related behaviors (PND 86 and PND88, social preference and social discrimination, respectively).

In both cohorts, vaginal smears were obtained from all females approximately 2 hours prior to the FIT. Only diestrous females underwent the FIT and, in cohort 1, brain and adrenal dissection. Vaginal smears were also taken after the other behavioral tests.

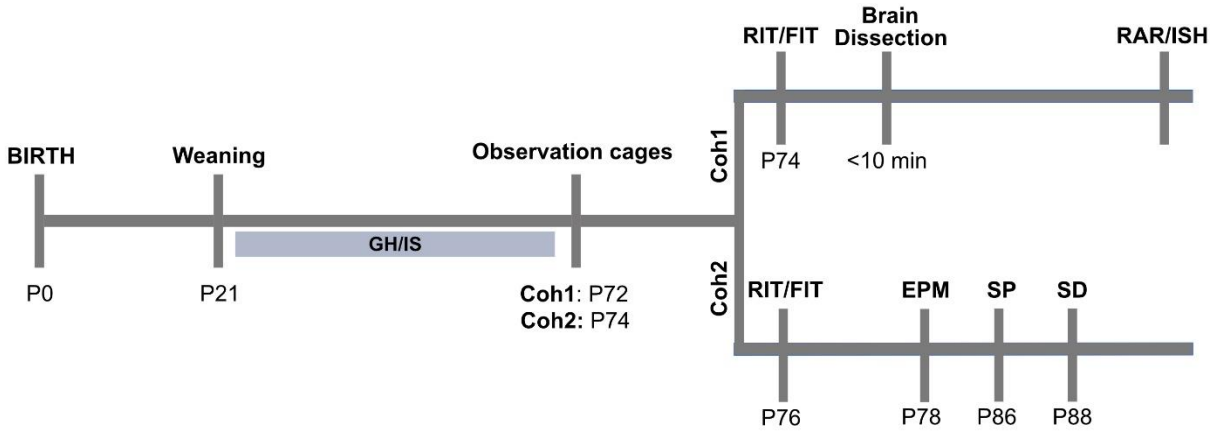


Figure 2.1: Schematic overview of the experimental design. Coh: Cohort; EPM: elevated plus-maze; FIT: female intruder-test; RIT resident intruder-test; SP: social preference test; SD: social discrimination task.

RESIDENT AND FEMALE INTRUDER TEST

The RIT and FIT took place in the early dark phase under dim red light conditions. An unfamiliar same-sex intruder was released into the observational cage of the resident for 10 minutes. Intruders weighed between 10-20% less than residents (De Jong *et al*, 2014; Koolhaas *et al*, 2013). The test was videotaped and ongoing behavior was continuously scored from video by a blind observer using JWatcher event recorder Program (Blumstein *et al*, 2000). The percentage of time of four major sets of behaviors were scored: i) aggressive behavior, consisting of attacks, keep down, threat, offensive grooming, offensive up-right; ii) neutral behaviors, consisting of exploring (investigating the home-cage), drinking and eating, autogrooming, immobility; iii) social behaviors (non-aggressive social interactions, sniffing); and iv) defensive behavior (submissive posture, kicking a pursuing intruder with hind limb). We also measured sexual behavior (lordosis, hopping, darting and mounting) when shown. In addition, we scored the frequency of attacks as well as the latency to the first attack. Furthermore, we re-scored the videos in slow frame speed in order to identify attacks towards vulnerable targets

(paws, head, belly, and throat) as a marker of abnormal aggression (Haller, 2013; Toth *et al*, 2011)

ELEVATED PLUS-MAZE

For analysis of anxiety-related behavior, the second cohort of animals was tested on the elevated plus-maze (EPM) on PND 78 in the late light phase (8:00–10.30 h). Animals were transferred to the EPM room two hours prior to the experiment in order to habituate to the novel environment. The EPM consisted of a plus-shaped platform elevated 80 cm above the floor, with two open (50x10 cm; 100 lux) and two closed arms (50x10x40 cm; 20 lux). Rats were placed in the center square facing a closed arm. The following parameters were recorded during the 5-min test using a video/computer system (Plus-maze version 2.0; ErnstFricke): time spent in open and closed arms, number of entries into open and closed arms, latency to enter an open arm. Here, statistical analysis is only presented for the percentage of time spent on the open arms: [time on open arms]/[time on open+closed arms] x100% as an indication of anxiety levels.

SOCIAL PREFERENCE TEST

One week later, on PND 86, the second cohort underwent a Social Preference Test. The test was performed according to standard procedures described in detail elsewhere (Lukas et al., 2011c; Lukas and Neumann, 2014). In brief, experiments were conducted in the early dark phase (13:00-16:00h). Rats were placed in a novel arena (40x80x40 cm) under dim red light conditions. Animals were allowed to habituate to the arena for 30 seconds, after which an empty wire-mesh cage (object stimulus: 2x9x9 cm) was placed at one side wall of the arena for 4 min. The empty cage was then exchanged by an identical cage containing an unknown same-sex conspecific (social stimulus) for an additional 4 min. Between each trial, the arena was cleaned with water

containing a low concentration of detergent. All phases were videotaped and videos were scored afterward by an observer who was blind to the experimental groups. The results were expressed using the Social Preference index: [time investigating the social stimulus]/[total time investigating both stimuli] x100%.

SOCIAL DISCRIMINATION TEST

On PND 88, the second cohort underwent a Social Discrimination Test. This test assesses the ability of an individual to discriminate a known conspecific from an unknown one, measured as the preference of the novel conspecific over the familiar one (Engelmann et al., 1998; Lukas et al., 2011a). The tests were performed in the dark phase (13:00-16:00) under the dim red light. Rats were individually housed in an observation cage with bedding from their home cage starting 2 h prior to the experiment. A same-sex juvenile rat (3 weeks old) was introduced into the cage of the experimental rat for 4 min (social memory acquisition period); 60 minutes later the now familiar juvenile rat was reintroduced along with an unfamiliar juvenile for 4 min (social discrimination period). All tests were videotaped and the time spent investigating the juveniles (sniffing the anogenital and head/neck regions) was measured by a researcher blinded to the housing conditions using JWatcher event recorder. The results were expressed using the discrimination index: [time investigating the unfamiliar conspecific]/[total time investigating both juveniles] x 100%. An index above 50% indicates a preference for the unfamiliar juvenile, in other words, social memory is present. We also measured latency to approach and total interaction time with the juveniles to exclude that possible effects were due to social avoidance. Furthermore, we measured aggressive behavior display towards the juveniles as a marker of abnormal aggression.

IN SITU HYBRIDIZATION

Brains were frozen and cut into 16-µm coronal cryostat sections, mounted on slides, and kept in -20°C. We adopted the hybridization protocol used by (Veenema *et al*, 2006). Briefly, slides were fixed in 4% paraformaldehyde, acetylated in 0.25% triethanolamine/acetic anhydride and dehydrated through a series of graded ethanols. Hybridization was performed using two specific 48-mer, 35S-labelled oligonucleotide probes: AVP: 5'-GCA-GAAGGC-CCC-GGC-CGG-CCC-GTC-CAG-CTG-CGT-GGC-GTT-GCTCCG-GTC-3' (Veenema *et al*, 2006) OXT: 5'-CTCGGAGAAGGCAG ACTCAGGGTCGCAGGCGGGGTCGGTCTCGGAGAAGGCAGACTCAGGGTCGCA GGCGGGGTCGGTGC-GGCAGCC-3' (Peters et al., 2014). Slides were incubated in hybridization solution [50% formamide, 10% dextran sulphate, 2 x standard sodium citrate (SSC), 2 mg/mL yeast tRNA, 10 mm dithiothreitol, 5 x Denhardt's]. The probe was applied to each slice at a concentration of 10^6 cpm/slide in 200 µL hybridization solution. The sections were hybridized overnight at 50°C in a humidified chamber. Then, slides were washed three times in 1 x SSC at 50°C, washed in 1 x SSC at room temperature, dehydrated in a graded series of ethanol and air dried. Hybridized sections were exposed to X-Omat film (Kodak, Rochester, NY, USA). Exposure time varied according to the peptide (OXT: 16h, AVP: 32h). All brain sections were hybridized at the same time and were exposed to the same film to avoid intrinsic variations between different in situ hybridizations and different films. Films were scanned using an EPSON Perfection V800 Scanner (Epson, Germany), the analysis was performed using the NIH Image program (ImageJ 1.31, National Institute of Health, http://rsb.info.nih.gov/ij/). Briefly, optical density was calculated in the PVN and SON for both peptides by taking the mean of bilateral measurements of 6 to 12 brain sections per region of interest. After subtraction of tissue background.

RECEPTOR AUTORADIOGRAPHY

Brains were cut into 16-µm coronal cryostat sections and mounted on slides kept in -20°C. The receptor autoradiography procedure was performed according to Lukas et al., 2010 using a linear V1A-R antagonist [125I]-d(CH2)5(Tyr[Me])-AVP (Perkin Elmer, USA) or a linear OXTR antagonist [125I]-d(CH2)5[Tyr(Me)2-Tyr-Nh2]9-OVT (Perkin Elmer, USA) as tracers. Briefly, the slides were thawed and dried at room temperature followed by a short fixation in paraformaldehyde (0.1%). Then slides were washed two times in 50 mM Tris (pH 7.4), exposed to tracer buffer (50 pM tracer, 50 mM Tris, 10 mM MgCl2, 0.01% BSA) for 60 min, and washed four times in Tris þ 10 mM MgCl2. The slides were then shortly dipped in pure water and dried at room temperature overnight. On the following day, the slides were exposed to Biomax MR films for 7-20 days depending on the receptor density and brain region (Kodak, Cedex, France). The films were scanned using an EPSON Perfection V800 Scanner (Epson, Germany). The optical density V1aR and OXTR were measured using ImageJ (V1.37i, National Institute of Health, http://rsb.info.nih.gov/ij/). Receptor density was calculated per rat by taking the mean of bilateral measurements of 6 to 12 brain sections per region of interest. After subtraction of tissue background.

STATISTICAL ANALYSES

Data were analyzed using GraphPad Prism 6.0. Normality was assessed using Kolmogorov-Smirnov analyses. Two-way ANOVA was carried out to analyze the main and interaction effects of sex and housing conditions on behaviors, adrenal weights, OXT, and AVP mRNA expression, and OXTR and $V_{1a}R$ binding. Body weight was evaluated in each sex with two-way ANOVA (main and interaction effects of housing condition and age. Social preference and social discrimination tests statistics were carried using the one-sample t-test comparing the groups with a hypothetical value of 50%. Data were presented as Means + SEM. Significance was accepted at $p \leq 0.05$.

2.3. RESULTS

PWSI DOES NOT AFFECT BODY AND ADRENAL WEIGHTS

During the PWSI period (PND21-74), the males' body weight increased from 58.96 ± 3.64 to 386.58 ± 20.39 g (GH-m) and from 60.69 ± 2.58 g to 416.83 ± 9.07 g (IS-m) (main effect of age: $F_{(7,167)}$= 1003.0; p<.001; main effect of housing: $F_{(1,167)}$= 0.24; n.s; interaction effect of age x housing: $F_{(7,167)}$= 0.84; n.s). In females, body weight increased from 57.96 ± 2.5 g to 265.50 ± 5.45 g (GH-f) and from 58.18 ± 2.07 to 254.41 ± 6.38 (IS-f) (main effect of age: $F_{(7,167)}$= 590; p<.001; main effect of housing: $F_{(7,167)}$= 1.68; n.s; interaction effect of age x housing: $F_{(7,167)}$= 0.84; n.s).

At PND 74, relative adrenal weights were higher in females (GH-f and IS-f: 0.31 ± 0.01 %) compared to males (GH-m: 0.17 ± 0.01 %, IS-m: 0.16 ± 0.01 %), but were not affected by housing condition (main effect of sex: $F_{(1,167)}$= 106.4; p<.001; main effect of housing: $F_{(1,167)}$= 0.12; n.s.; interaction effect of sex x housing: $F_{(1,167)}$= 0.11; n.s).

PWSI INCREASES AGGRESSION IN BOTH MALE AND FEMALE RATS

For this analysis, animals from cohort 1 and cohort 2 were pooled, since aggression levels did not differ between both cohorts. IS animals from both sexes showed increased aggression in the RIT or FIT (see table 2.1 for an overview of statistics). This increase was mainly reflected by an increase in threat behavior (Figure 2.2A). Both IS males and IS females engaged less time in neutral behaviors compared to GH animals during the RIT or FIT (Figure 2.2B). Females displayed more time in offensive grooming compared to males, independent of housing (Fig. 2.2A), whereas males displayed more autogrooming compared to females, independent of housing (Figure 2.2B). IS animals of both sexes showed a higher number of attacks (Figure 2.2C), and a higher percentage of attacks directed at vulnerable body parts compared with GH controls (Figure 2.2D). There was a trend toward a reduced attack latency in IS rats (Figure 2.2E).

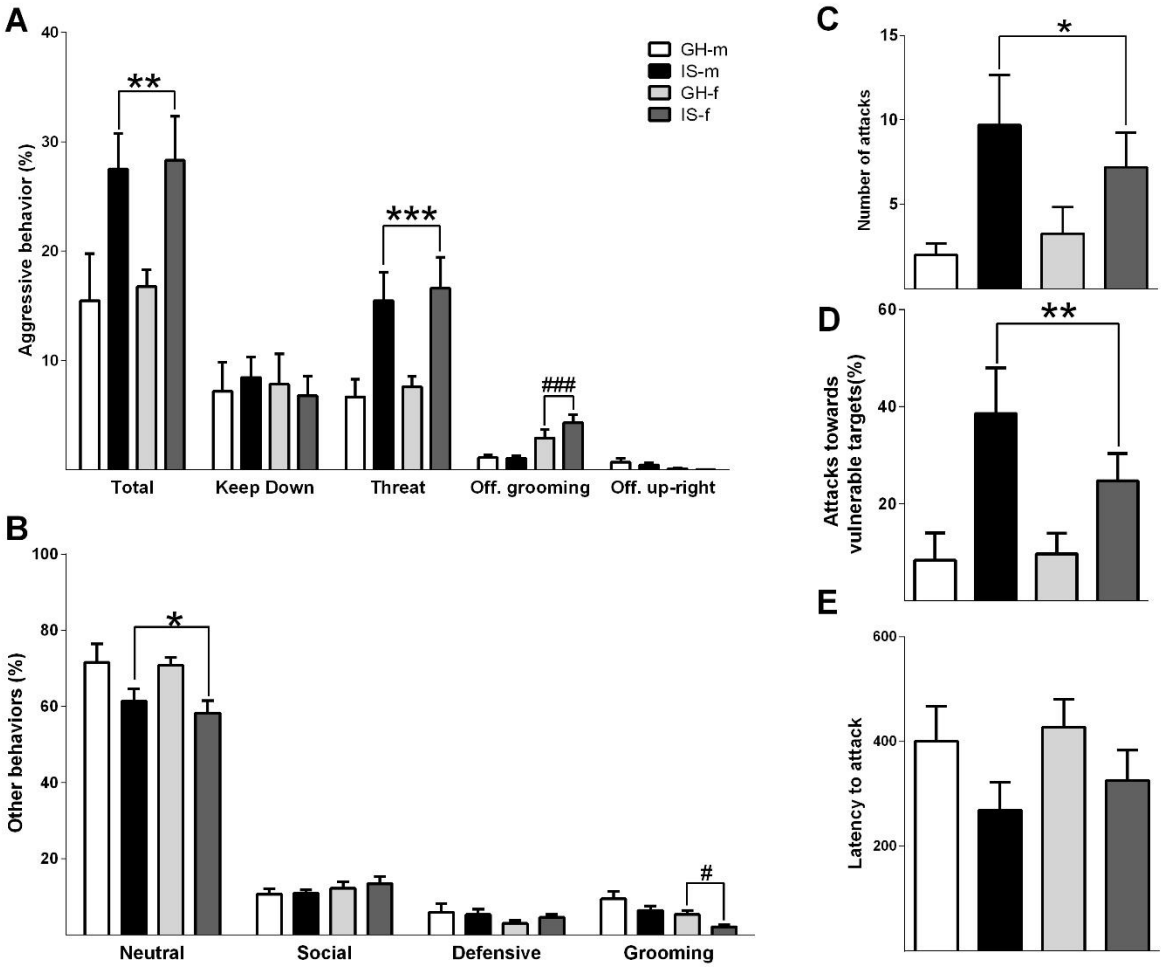


Figure 2.2: Effects of post-weaning social isolation (IS, black /dark grey) or group-housing (GH, white/light grey) on behavior of male (m, black/white bars) and female (f, grey bars) rats during a 10-min RIT/FIT, including (A) percentage of time behaving aggressively; (B) percentage of time behaving neutrally, socially and defensively; (C) number of attacks; (D) percentage of attacks towards vulnerable targets; (E) latency to attack. Effects of housing: * p≤.05; ** p≤.01; ***p≤.00; Effects of sex: #p≤.05; ###p≤.001.

Table 2.1: Main and interaction effects of housing (isolated (IS) vs. group-housed (GH)), and sex (male vs. female) on various behaviors in the RIT/FIT and social discrimination test. n.s. not significant.

Behavioral test	Behavior	Housing effect	Sex effect	HousingxSex
RIT/FIT	Aggression	$F_{(1,40)}$ =12.08; p=.001	n.s.	n.s.
	Threat	$F_{(1,40)}$=17.32; p<.001	n.s.	n.s.
	Offensive grooming	n.s.	$F_{(1,40)}$=20.99; p< .001	n.s.
	Attack	$F_{(1,40)}$=7.14; p=.011	n.s.	n.s.
	Vulnerable targets	$F_{(1,40)}$=9.15; p=.004	n.s.	n.s.
	Latency to atttack	$F_{(1,40)}$=4.08; p=.050	n.s.	n.s.
	Auto-grooming	n.s.	$F_{(1,40)}$= 11.59; p=.002	n.s.
	Neutral	$F_{(1,40)}$=11.09; p=.002	n.s.	n.s.
Social discrimination	Aggression	$F_{(1,22)}$=12.67; p=.002	$F_{(1,22)}$= 4.472; p=.04	n.s.

PWSI IMPAIRS SOCIAL DISCRIMINATION, BUT NOT ANXIETY-LIKE BEHAVIOR OR SOCIAL PREFERENCE

PWSI had no effect on anxiety-like behavior or social preference (see table 2.2 for statistical details). However, whereas GH animals were able to distinguish between a known and unknown juvenile (GH-m: $t_{(3)}$=3.337, p=0.0455; GH-f: $t_{(6)}$=2.612, p=0.0400), IS animals were not (IS-m: $t_{(6)}$=0.3042; p=0.7713; IS-f: $t_{(5)}$=1.304; p=0.2490) (Figure 2.3A). Furthermore, IS females presented increased aggression during the social discrimination test towards the juveniles compared to GH females and IS males (Figure 2.3B) (see table 1 for statistical details).

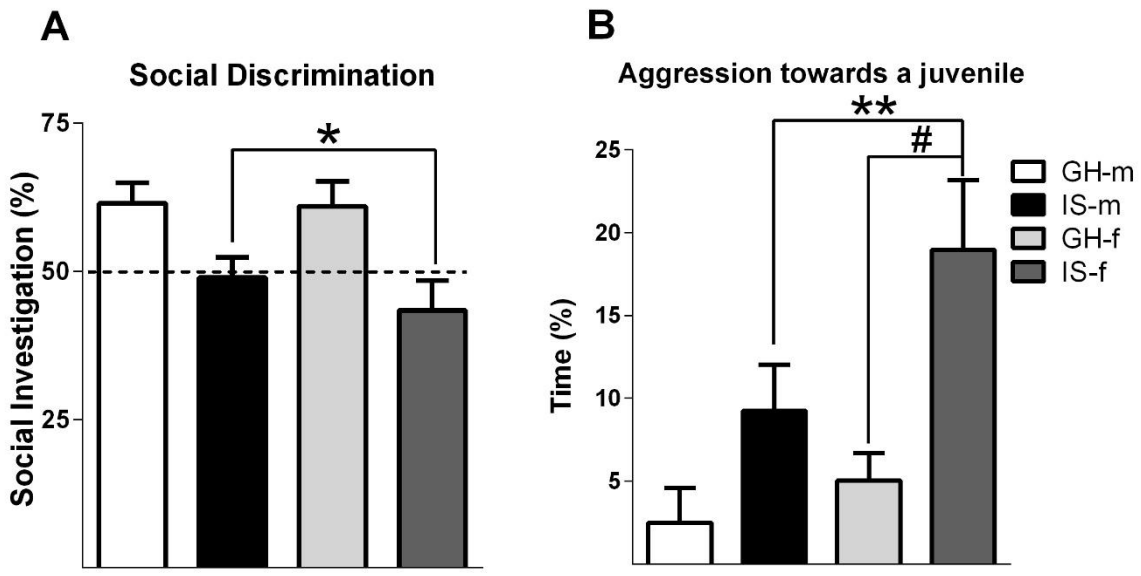


Figure 2.3: Effects of post-weaning social isolation (IS, black /dark grey) or group-housing (GH, white/light grey) on social memory (A) and aggression (B) of male (m, black/white bars) and female (f, grey bars) rats toward juveniles in a social discrimination task. Effects of housing: * p≤.05; ** p≤.01; Effects of sex: #p≤.05.

Table 2.2: Main and interaction effects of housing (isolated (IS) vs. group-housed (GH)), and sex (male vs. female) on the percentage of time spent in the open arm of the elevated plus-maze (EPM) and social preference index in the social preference test. Data are shown in mean±SEM. GH: group-housed; IS: isolated; m: male; f: female; n.s.: not significant

Behavior	GH-m	GH-f	IS-m	IS-f	p
EPM (% time)	11.5±2.8	33.3±7.4	34.8±9.2	34.5±9.1	n.s.
Social Preference Index	62.2±3.9	62.1±3.2	58.9±6.5	59.6±1.5	n.s.

PWSI INCREASES OXT, BUT NOT AVP MRNA EXPRESSION

PWSI increased OXT mRNA in the PVN but not in the SON in both sexes. In addition, males had a lower expression of OXT mRNA in the PVN compared to females (Figure 2.4A). Neither housing condition nor sex had any effect on AVP mRNA (see table 2.3 for statistical details).

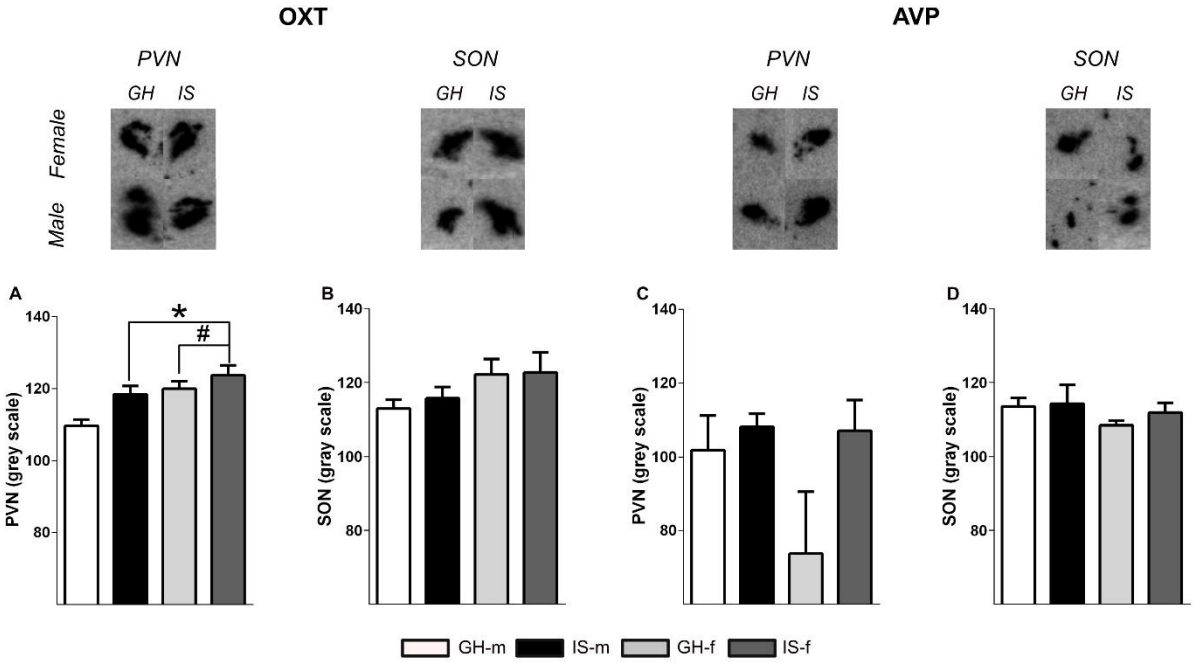


Figure 2.4: Effects of post-weaning social isolation (IS, black /dark grey) or group-housing (GH, white/light grey) on OXT and AVP mRNA expression in the PVN (A, C) and SON (B, D) of male (m, black/white bars) and female (f, grey bars) rats. Upper panel shows representative pictures of the brain regions evaluated. Effects of housing: * p≤.05; Effects of sex: #p≤.05.

Table 2.3: Main and interaction effects of housing (isolated (IS) vs. group-housed (GH)), and sex (male vs. female) on OXT and AVP mRNA expression and OXTR and V1aR binding. AVP: vasopressin; BNSTa: bed nucleus of *stria terminalis* anterior part; BNSTp: bed nucleus of *stria terminalis* posterior part; CeA: central amygdala; DG: dentate gyrus; IL: infralimbic cortex; LH: lateral hypothalamic area; LSd: lateral septum dorsal part; LSv: lateral septum ventral part; NAcca: nucleus *accumbens* anterior part; n.s.: not significant; OXT: oxytocin; OXTR: oxytocin receptor binding; PrL: Prelimbic cortex; VMH: ventromedial nucleus of the hypothalamus; V1aR: V1a receptor.

Peptide or Receptor	Region	Housing effect	Sex effect	HousingxSex
OXT	PVN	$F_{(1,12)}$=7.732; p=.02	$F_{(1,12)}$= 11.89; p=.005	n.s.
	SON	n.s.	*$F_{(1,13)}$= 4.117; p=.06*	n.s.
AVP	PVN	n.s.	n.s.	n.s.
	SON	n.s.	n.s.	n.s.
OXTR	BNSTa	n.s.	$F_{(1, 14)}$=8.05; p=.01	n.s.
	BNSTp	n.s.	$F_{(1, 12)}$=31.87; p< .001	n.s.
	CeA	n.s.	n.s.	n.s.
	IL	n.s.	n.s.	n.s.
	LSv	n.s.	n.s.	n.s.
	NAcca	$F_{(1, 13)}$=6.37; p= .02	$F_{(1, 13)}$=5.52; p= .03	n.s.
	PrL	n.s.	n.s.	n.s.
	VMH	n.s.	n.s.	n.s.
V1aR	BNSTa	n.s.	n.s.	$F_{(1, 15)}$= 22.96; p<.001
	CeA	n.s.	n.s.	n.s.
	DG	$F_{(1, 14)}$=9.98; p=.007	n.s.	n.s.
	LH	$F_{(1, 15)}$=6.69; p=.02	n.s.	n.s.
	LSd	n.s.	n.s.	n.s.
	NAcca	n.s.	n.s.	n.s.

PWSI ALTERS V1A AND OXT RECEPTOR BINDING

PWSI decreased OXTR binding in the NAcc independently of sex. Females presented higher binding than males in this brain region independent of the housing condition. Furthermore, males exhibited higher OXTR binding in the posterior and anterior part of the BNST than females, regardless of housing (Figure 2.5).

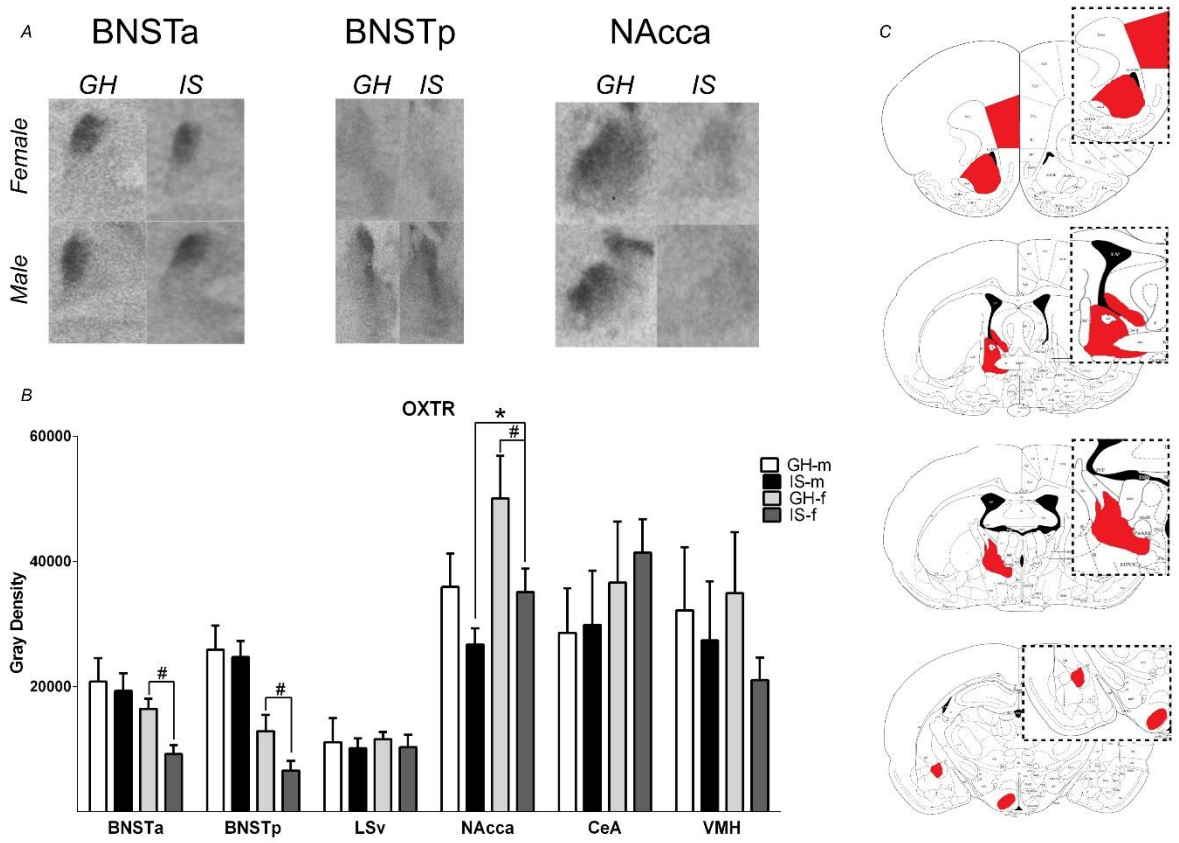


Figure 2.5: Effects of post-weaning social isolation (IS, black /dark grey) or group-housing (GH, white/light grey) on OXTR binding in male (m, black/white bars) and female (f, grey bars) rats. In (A) representative pictures showing main effects; (B), a graph depicting quantitative grey levels; (C) schematic diagrams showing the analyzed regions. BNSTa and BNSTp: bed nucleus of stria terminalis, anterior and posterior parts; LSv: lateral septum ventral part; NAcca: nucleus accumbens anterior part; CeA: central amygdala; VMH: ventromedial nucleus of the hypothalamus. Effects of housing: * p≤.05; Effects of sex: #p≤.05.

Regarding the V1a receptor, IS animals presented lower binding in the LH and DG regardless of sex. In the anterior portion of BNST, V1aR binding was differently regulated depending on sex: GH females presented higher binding than GH males, whereas IS females presented lower binding compared to IS males (Figure 2.6) (see table 2.3 for statistical details).

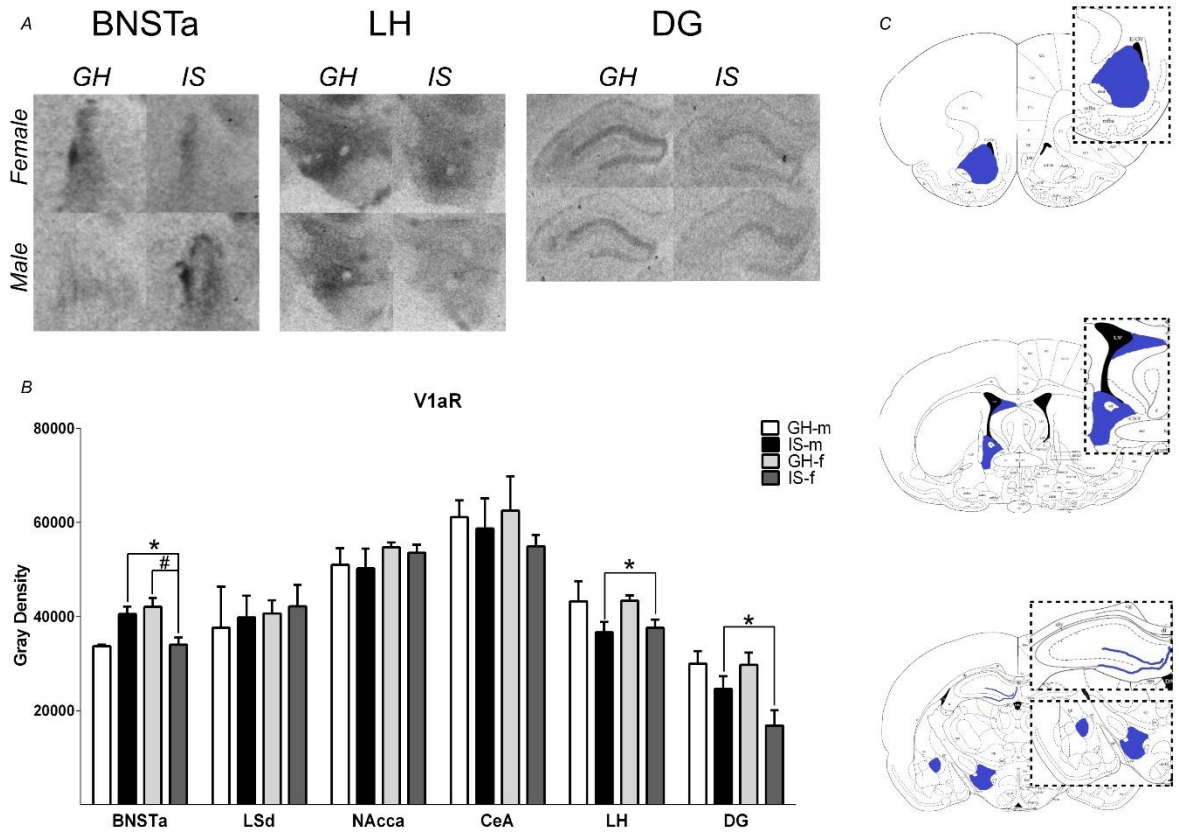


Figure 2.6: Effects of post-weaning social isolation (IS, black /dark grey) or group-housing (GH, white/light grey) on V1aR binding in male (m, black/white bars) and female (f, grey bars) rats. In (A) representative pictures showing main effects; (B), a graph depicting quantitative grey levels; (C) schematic diagrams showing the analyzed regions. BNSTa and BNSTp: Bed nucleus of stria terminalis, anterior and posterior parts; LSd: lateral septum dorsal portion; NAcca: nucleus accumbens anterior portion; CeA: Central amygdala; LH: lateral hypothalamic area; DG: dentate gyrus. Effects of housing: * p≤.05; Effects of sex: #p≤.05.

2.4. Discussion

The present study shows that PWSI has considerable long-lasting effects on two aspects of social behavior, i.e. aggression and social memory, and these effects are, overall, similar in males and females. Interestingly, males and females did not differ in the total level of aggression displayed during the RIT or FIT, independent of the housing condition. In other words, female Wistar rats were exactly as aggressive as male Wistar rats. This finding is somewhat surprising as female rodents are typically considered less aggressive than their male counterparts (Hashikawa *et al*, 2017, 2018; De Jong *et al*, 2014; Trainor *et al*, 2010b; Unger *et al*, 2015). However, only a few studies directly

compared aggressive behavior between males and females, showing that female house mice (*Mus musculus*) and California mice (*Peromyscus californicus*) indeed exhibited less aggression than their male conspecifics (Hashikawa *et al*, 2017; Trainor *et al*, 2010b) whereas female Syrian hamsters (*Mesocricetus auratus*) were as aggressive as males (Been *et al*, 2016; Terranova *et al*, 2016). To the best of our knowledge, this is the first study to directly compare aggression in virgin female and male rats under the same experimental conditions. Importantly, since aggression is a behavior that is particularly affected by breeding and housing conditions, as shown in this and other studies (Albers et al., 2006; Kohl et al., 2015; Oliva et al., 2010; Tulogdi et al., 2014), suboptimal conditions such as group versus single housing, limited bedding and nesting, and cage ventilation may have obscured aggressive behavior in female rats in previous studies. Overall, the similar levels of aggression demonstrated in the present study may be translated to humans. Recent studies have shown that men and women have the same potential to aggress, although men tend to engage more often in physical aggression, whereas women tend to engage more in verbal and psychological aggression (Denson *et al*, 2018; Freitag *et al*, 2018; Mancke *et al*, 2015). PWSI again proved to be a reliable model to enhance aggression in adulthood, similar to other peri-pubertal stress paradigms (Haller *et al*, 2014; Marquez *et al*, 2013; Sandi and Haller, 2015; Toth *et al*, 2011; Veenema *et al*, 2006). Aside from the quantitative increase, IS rats also presented a higher percentage of attacks towards vulnerable targets during the RIT or FIT (especially males), as well as aggression towards a juvenile during the social discrimination test (especially females). A recent study has reported that isolated female Swiss-Webster mice attacked juvenile intruders in response to optogenetic stimulation of estrogen receptor α-positive neurons in the ventrolateral part of the ventromedial hypothalamus, suggesting that attacking a "weaker" intruder is hard-wired in the female brain (Hashikawa *et al*, 2017). Further

studies are needed to dissect the neural pathways involved in the specific features of abnormal aggression exhibited by males and females after PWSI.

PWSI changed neither explorative behavior in the elevated plus-maze nor social preference behavior in adult male and female rats, indicating normal levels of anxiety-related behavior and social motivation in these animals (Lukas et al., 2011c; Lukas and Neumann, 2014). However, IS subjects showed impaired social memory as they failed to discriminate a known juvenile from an unknown one in the social discrimination test. Previous studies have also reported that early life stress affects social memory and social discrimination in rats. Both male and female Long Evans rats exposed to 2 weeks of PWSI were not able to discriminate between a familiar and an unfamiliar juvenile within a 30 minutes interval (Tanaka *et al*, 2010). In addition, exposure to maternal separation or limited nesting also impaired social discrimination in adult male rats and mice, respectively (Kohl et al., 2015; Lukas et al., 2011a). Our study confirmed these previous findings and showed that the effects of PWSI on social discrimination last until adulthood in both sexes.

Our final aim was to evaluate whether exposure to PWSI leads to an alteration of central OXT and AVP systems as a possible mechanism underlying the behavioral effects. Our results show that OXT mRNA was increased (5%) in the PVN of IS rats in both sexes, whereas AVP mRNA was not altered by sex or housing condition in our subjects. These findings appear to be somewhat in contrast with (Tanaka *et al*, 2010), who found a reduced number of OXT-immunoreactive neurons in the medial parvocellular dorsal zone of the PVN after 2 weeks of PWSI in female, but not male rats, and a decreased number of AVP-ir neurons in the medial parvocellular ventral zone of the PVN in males but not in female rats. This difference may be a result of the quantification of mRNA expression versus protein levels, which do not always correlate (Steinman et al., 2015). Alternatively, OXT and AVP mRNA expression and protein

synthesis may shift depending on the duration of the isolation period (two weeks versus seven weeks in our study). Furthermore, both IS and GH females presented higher levels of OXT mRNA than males in this brain region. Several studies have shown a lack of sexual dimorphism in the OXT system in rats (both basal mRNA expression and basal peptide immunoreactivity) (DiBenedictis *et al*, 2017; Dumais *et al*, 2013). A possible explanation for this discrepancy is that our experiments differed in terms of experimental settings such as the light-dark cycle and context (animals were transferred to a new environment, single-housed observation cages, 48 hours before the RIT and FIT), which may have induced the sex difference. Rats in our study also underwent a 10-min aggressive encounter immediately before the killing, and it is possible that this induced some rapid sex-dependent changes in OXT mRNA expression.

Concerning the receptors, we found housing and sex-related changes in both OXTR and V1aR binding. Males presented a higher OXTR binding in the anterior and posterior part of the BNST compared to females. This sex difference has been reported before and has been linked to sexual dimorphisms in persistence of social memory (Dumais *et al*, 2013, 2015; Smith *et al*, 2017). Furthermore, PWSI decreased OXTR binding in the anterior part of the NAcc of both sexes. The NAcc has been reported to regulate appetitive (seeking) and consummatory (execution) aspects of aggression in mice (Aleyasin *et al*, 2018). In addition, NAcc is involved in abnormal aggression in male rats bred for low anxiety behavior (LAB rats) (Beiderbeck *et al*, 2012). Interestingly, OXT signaling in the NAcc has also been shown to be related to the positive aspects of social interactions and stress resilience in voles (Barrett *et al*, 2015; Bosch *et al*, 2016; Yu *et al*, 2016). Therefore, it is possible that altered OXT signaling in this region plays a role in PWSI-induced abnormal aggression. Our lab currently

explores the effects of OXTR manipulation in the amygdala and septum on female aggression. The present results suggest that the NAcc should also be studied.

PWSI affected V1aR binding in both males and females in several brain areas. Receptor binding was decreased in the LH and DG. The LH has been associated with predatory aggression in rats (Haller, 2013). This brain region was highly activated in both GH and IS male rats who fought a conspecific in the RIT (Toth et al., 2012). In addition, AVP-ir was increased in this region under basal conditions and 2 hours after exposition to the RIT in MS male rats (Veenema *et al*, 2006). Therefore, it can be hypothesized that PWSI affects aggression in part via changes in AVP neurotransmission in the LH. The DG, on the other hand, has been implicated in social memory acquisition (Fernandes *et al*, 2016) and persistence in mice (Pereira-caixeta *et al*, 2017). This region also presented a blunted neural activity (c-Fos expression), in response to a pubertal con-specific, in rats isolated for four weeks, independently of the sex (Ahern *et al*, 2017). A reduction of AVP signaling in the DG through low V1aR binding may disrupt rats social discrimination ability.

Finally, we found an interaction effect of housing and sex on V1aR binding in the anterior portion of the BNST. GH females presented high levels of V1aR binding when compared to GH males. Surprisingly, this relation was reversed after PWSI, as males had higher binding than females. The BNST is known to be involved in the effects of PWSI on aggression in male rats, as IS males exhibited enhanced neural activity in this brain area after the RIT compared to GH males (Toth et al., 2012). The BNST also contains a sexually dimorphic AVP system, with males presenting a higher number of AVP-ir cell bodies (DiBenedictis *et al*, 2017). Furthermore, activity of AVP neurons located in the caudal portion of the BNST was correlated positively with aggression in male, but negatively in female California mice (Steinman et al., 2015). Taking into account that AVP may have sex-specific effects on aggression (for example, AVP

injection into the anterior hypothalamus decreased aggressiveness in females whereas it potentiated aggression in males (Terranova *et al*, 2016)), it is possible that PWSI resulted in opposite effects on AVP neurotransmission in the BNST in males compared to females, which in turn lead to pro-aggressive effects in both sexes. Further studies should focus on the sex-specific effects of AVP neurotransmission in the anterior BNST on aggressive behavior. Ongoing experiments in our lab are focusing on the effects of manipulation of V1aR in the amygdala and septum on female aggression, the present results suggest that the anterior BNST should be targeted as well.

2.5. CONCLUSION

Our study is the first study to directly compare aggressive behavior between virgin female and male rats, both under normal (GH) conditions and after PWSI. Importantly, both females and males of the IS group showed increased aggressive behavior as adults, mainly reflected by exacerbated threat behavior. Besides its overall effect on general aggression, PWSI induced abnormal aggression, i.e. increased attacks towards vulnerable targets and increased aggression towards a juvenile. Finally, whereas OXT and AVP mRNA expression remained largely unchanged, PWSI altered OXTR binding in the NAcc and V1aR binding in the LH, DG and BNST, regions likely to contribute to the effects of prolonged PWSI on social interactions.

In summary, the present study supports PWSI as a reliable animal model to induce aggression in both male and female rats. We also show evidence of the contribution of the OXT and AVP system in the aggressive behavior induced by PWSI.

CHAPTER 3: THE OXYTOCIN-VASOPRESSIN BALANCE WITHIN THE LATERAL SEPTUM DETERMINES AGGRESSION LEVELS IN VIRGIN FEMALE RATS

This chapter was submitted for publication in *Nature Communications* (Oliveira et. al.). The behavioral experiments were designed by Vinicius Oliveira with the help of Trynke de Jong and Inga D. Neumann. The electrophysiology experiments were designed by V.O. and Michael Lukas with the help of Veronica Egger. V.O. did all the behavioral assessments as well as analyzed the behavior with the help of Hanna Wolff, Elisa Durante, Alexandra Lorenz, and Anna-Lena Mayer. V.O. sliced the brains, performed, developed and evaluated the receptor autoradiographs with the help of Elisa Durante. V.O. performed and analyzed all the immunohistochemistry data. Patch-clamp was performed by V.O. and posterior analyses were done by VO with the help of M.L. Surgeries were done by V.O. with the help of Oliver Bosch, Rodrigue Maloumby and Thomas Grund. All the figures and tables were prepared by V.O. who also wrote the manuscript with the input of I.N, all authors proofread the manuscript.

3.1. INTRODUCTION

Aggression is defined as a social behavior that has the intention of inflicting physical damage to a conspecific. Aggressive behavior is expressed by most, if not all, mammalian species, including humans, and it typically benefits the aggressor by gaining access to resources such as food, territory or mating partners (Nelson and Trainor, 2007). When expressed out-of-context or in an exacerbated manner, aggressive behavior becomes disruptive and harms both aggressor and victim. In humans, excessive or pathological aggression as seen, for example, in individuals suffering from conduct or antisocial personality disorder constitutes a severe burden to society (Freitag *et al*, 2018; Glenn *et al*, 2013; Nelson and Trainor, 2007; Wittchen *et al*, 2011). To better understand the neurobiology of social aggression and to develop potential treatment options, laboratory animal models of aggression have been

successfully used for decades (Koolhaas *et al*, 2013; Nelson and Trainor, 2007). However, as with most animal models available, aggressive behavior has been studied and validated predominantly in male rodents, whereas females have been studied rarely and if so, only in the physiologically unique period of lactation(Hashikawa *et al*, 2018). Given the facts that i) girls and women also demonstrate disruptive social aggression and may develop conduct as well as anti-social personality disorder just like males (Colins *et al*, 2016; Denson *et al*, 2018; Freitag *et al*, 2018), and ii) the neurobiology of aggression appears to be sexually dimorphic (Borland *et al*, 2019; Denson *et al*, 2018; Hashikawa *et al*, 2017, 2018; Newman *et al*, 2019; Oliveira *et al*, 2019; Terranova *et al*, 2016), the use of non-lactating female animal models is required to fully understand female aggression and to identify potential targets for treatment of extreme aggression in both sexes.

First, in order to study the neurobiological mechanisms underlying female aggression, we established an animal model to robustly enhance the mild levels of spontaneous aggression displayed by female Wistar rats (De Jong *et al*, 2014). We hypothesized that a combination of social isolation and repeated aggression training by exposure to the female intruder test (FIT) (De Jong *et al*, 2014), i.e. to an unknown same-sex intruder, enhances female aggressiveness. Indeed, it has been shown that both social isolation (Oliveira *et al*, 2019; Ross *et al*, 2019), as well as repeated engagement in conflict with conspecifics (winner effect) (Been et al, 2016; Oyegbile and Marler, 2005; Silva et al, 2010), exacerbate aggression in solitary and aggressive rodent species, independently of the sex. Next, we sought to investigate the role of the neuropeptides oxytocin (OXT) and vasopressin (AVP) in female aggression. Both OXT and AVP have been strongly associated with aggressive behavior in males and lactating females (Beiderbeck *et al*, 2007; Bosch *et al*, 2005; Bosch and Neumann,

2010; Calcagnoli *et al*, 2013; Lukas and de Jong, 2017; Nephew *et al*, 2010; Oliveira *et al*, 2019; Ross *et al*, 2019; Veenema *et al*, 2010) and are known to be affected by social isolation in a sex-dependent manner (Oliveira *et al*, 2019; Ross *et al*, 2019).

We also hypothesized that the effects of OXT and AVP on female aggression are predominantly mediated in the lateral septum (LS), which is known to inhibit aggressive behavior in both sexes (Borland *et al*, 2019; Zeman, Wolfgang and King, 1958). Early studies have shown that electrical stimulation of the LS reduced aggression (Albert and Chew, 1980; Potegal *et al*, 1981), whereas either pharmacological inhibition or lesioning of the LS triggered exaggerated aggression ('septal rage') in male and female hamsters (Borland *et al*, 2019; Mcdonald *et al*, 2011; Potegal, M Blau, A and Glusman, 1981). Also, patients (including women) with septal tumors show increased irritability and aggressive outbursts (Zeman, Wolfgang and King, 1958). Recently, it has been shown that optogenetic stimulation of LS GABAergic neurons to the VMHvl abolishes attacks in male mice(Wong *et al*, 2016).

Although several pieces of evidence confirm the gatting role of the LS in aggression, the neuromodulatory mechanisms involved in the regulation of septal activity during aggressive encounters, especially in virgin females, are still largely unknown. Interestingly, in the rat LS, OXT receptors (OXTR) and AVP V1a receptors (V1aR) are differentially expressed in two distinct subregions of the LS, i.e. OXTRs are located exclusively in the ventral LS (vLS) whereas V1aRs are predominantly found in the dorsal LS (dLS) (Smith *et al*, 2017). In addition, both OXT and AVP release in the LS have been tied to various social behaviors, including aggression, although the results are sometimes conflicting (Lukas and de Jong, 2017). For example, Wistar rats bred for low anxiety-related behavior (LAB), which display high and abnormal aggression (Beiderbeck *et al*, 2012), showed an attenuated LS AVP release

(Beiderbeck *et al*, 2007), whereas the high aggression of male Wistar rats (Veenema *et al*, 2010) during the resident-intruder test (RI) was found to be associated with increased AVP release in the same region. Regarding OXT even less is known about its role in the LS in the context of aggression. At the receptor level, increased OXTR binding has been reported in both dominant male mice (Lee *et al*, 2019) and lactating females (Caughey *et al*, 2011).

Here, using a novel, reliable rat model of female aggression, we first investigated differences in central OXT or AVP release and receptor binding in response to an aggressive encounter in group-housed (GH), i.e. lowly aggressive, females, versus isolated and trained (IST), i.e. highly aggressive, females. Next, neuropharmacological, chemogenetic and optogenetic approaches were used to selectively manipulate OXT or AVP signaling within the brain, either centrally or targeting specifically the vLS or dLS to assess its behavioral consequences in the FIT. Finally, in order to dissect the neuronal mechanisms within the LS controlling aggressive behavior, we (i) specifically modulated OXTR activation while recording spontaneous GABAergic inputs to vLS and dLS GABAergic neurons *in-vitro* , and (ii) monitored and manipulated neuronal activity in those subregions using pERK immunohistochemistry and neuropharmacology, respectively, in rats that exhibited opposite levels of aggression.

3.2. METHODS

RATLINES AND ANIMAL CARE

The rats used for behavioral experiments were female Wistar rats (10-14 weeks old) that were bred in the animal facilities of the University of Regensburg, Germany. Female intruders were Wistar rats obtained from Charles Rivers Laboratories (Sulzfeld, Germany) that were kept in groups of 3 to 5 animals in a separate animal room. All rats were kept under controlled laboratory conditions (12:12 h light/dark cycle; lights off at

11:00, 21±1ºC, 60±5% humidity), with access to standard rat nutrition (RM/H, Ssniff Spezialdiäten GmbH, Soest, Germany) and water *ad libitum*. For patch-clamp and pERK immunohistochemistry analyses, Venus-VGAT rats (VGAT: vesicular GABA transporter; lineVenus-B, W-Tg(Slc32a1-YFP*)1Yyan)(Uematsu *et al*, 2008) were used that were bred in the animal facilities of the University of Regensburg. All procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Local Government of Oberpfalz and Unterfranken.

FEMALE INTRUDER TEST (FIT)

The FIT took place in the early dark phase under dim red light conditions. An unfamiliar same-sex intruder was released into the observational cage of the resident for 10 minutes. Intruders weighed between 10-15% less than residents (De Jong *et al*, 2014). The test was videotaped for later analysis by an experienced observer blind to any treatments using JWatcher event recorder Program (Blumstein *et al*, 2000). The percentage of time of four major groups of behaviors were scored: i) aggressive behavior, consisting of keep down, threat behavior, offensive grooming, and attacks; ii) neutral behaviors, consisting of exploring (investigating the home-cage), drinking and eating, self-grooming; iii) social behaviors (non-aggressive social interactions, sniffing, following); and iv) defensive behavior (submissive posture, kicking a pursuing intruder with hind limb). We also measured sexual behavior (lordosis, hopping, darting and mounting) and immobility when displayed. In addition, we scored the frequency of attacks as well as the latency to the first attack. Vaginal smears were taken after the FIT in order to verify the estrus cycle; all phases of the estrus cycle were included in the study.

Chapter 3: The oxytocin-vasopressin balance within the lateral septum determines aggression levels in virgin female rats

OVERVIEW OF THE *IN-VIVO* EXPERIMENTS

Animal groups for endogenous OXT/AVP measurements: Female Wistar rats were split into three different conditions: Group-housed (GH) females were kept in groups of 3 to 5 animals per cage; Isolated (IS) females were singly housed for 8 days in observational cages and Isolated and trained (IST) females were also kept singly for 8 days, but from day 5 on they underwent 3 consecutive FITs (training). On day 9, GH females were transferred to observational cages 4 hours prior to the behavioral experiments. One hour after lights went off, all residents were assigned into two different conditions FIT, rats which got an intruder, and control, rats that were left undisturbed in their home-cages. Immediately after the FIT rats were deeply anesthetized using intraperitoneal urethane (25%, 1,2 ml/kg) to allow cerebrospinal fluid (CSF) collection via *cisterna magna* puncture. Next, these animals were decapitated and brains and trunk blood were collected for receptor binding and hormonal measurements, respectively (Figure 1a).

Neuropharmacology design: In the following experiments, subjects were split only into GH and IST conditions. IST females underwent surgery for intracerebroventricular (i.c.v.) or local cannula implantation and were respectively left undisturbed for recovery for 3 or 5 days. The training protocol was performed as described above, except for the fact that residents received a sham-injection before the FIT in order to get used to the infusion procedure. GH animals were kept single-housed overnight for recovery and brought back to their original groups in the next day until the experiments took place. As described previously, before pharmacology experiments GH residents were transferred into observational cages. All animals were handled daily in order to get used to the infusion procedure. Typically, a cross-over, within-subjects design was

used for all the intracerebroventricular (i.c.v.) agonist experiments whereas a between-subjects design was used for the local infusions and i.c.v. antagonists experiments.

Microdialysis: After 4 days of training, IST rats had their microdialysis probes implanted into the LS. After one day of recovery, IST subjects received the fifth FIT in order to confirm their previous aggression levels. On the following day, both GH and IST animals underwent the microdialysis procedure. Briefly, rats were connected to a syringe mounted onto a microinfusion pump via polyethylene tubing and were perfused with sterile Ringer's solution (3.3 µl/min, pH 7.4) for 2 hours, prior to the experiment, in order to establish an equilibrium between inside and outside of the microdialysis membrane. One hour after lights went off, three consecutive dialysates were collected every 30 min: 1 and 2 were collected before the FIT (baseline represented as an average of both time-points) and dialysate 3 included the 10-min FIT. Dialysates were collected into Eppendorf tubes containing 10 µl of 0.1 M HCl and were immediately frozen on dry ice, and subsequently stored at −20 °C until AVP and OXT radioimmunoassay measurements took place.

Opto- and Chemogenetics design: Chemo- and optogenetics experiments were only performed using GH rats. After virus infusion into the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus, animals were kept single-housed for one week in order to recover. After recovery, rats were brought back to their original groups for two weeks until either the experiments took place (chemogenomics) or they had their optical fiber implanted (optogenetics). Similarly to the pharmacology experiments, chemogenetic rats were kept in groups and isolated shortly before the dark phase (see above). Subjects received an i.p. infusion of clozapine-N-oxide dihydrochloride (CNO, 2mg/kg) 45 min before the FIT. They were brought back to their original groups directly after the FIT. In the optogenetic experiments, after optical fiber

implantation rats were single-housed for three days for recovery as well as to avoid damaging the fiber. Similarly to the microdialysis experiments, animals were connected to the optogenetics cables two hours before the experiment to get used to the cables. Especially, in this experiment, the FIT lasted 12 minutes. Blue-light (30ms pulses of 30Hz delivered for 2min) was delivered twice: at 2 and 8 minute after the beginning of the FIT. For both, chemo- and optogenetic experiments, animals were tested twice according to their estrus-cycle phase. Once when they were receptive (proestrus and estrus) and the second time when they were non-receptive (metestrus-diestrus). All the animals were transcardiacally perfused after the last test and handled daily during the experiment.

Neural activation after aggression The LS consists mostly of GABAergic neurons (Risold and Swanson, 1997a). Therefore, in order to detect neural activity differences induced by an aggressive encounter, we used female Venus-VGAT rats (10-14 weeks old). As described previously, those animals have been divided into GH and IST groups. Immediately after the last FIT animals were deeply anesthetized, in the room, with isoflurane, followed by $CO_{2,}$ before being transcardiacally perfused.

STEREOTAXIC SURGERY

Briefly, rats were anesthetized with isoflurane (ForeneH, Abbott GmbH & Co. KG, Wiesbaden, Germany) and fixed in a stereotaxic frame. Further, rats were intraperitoneally (i.p.) injected with the analgesic Buprenovet (0.05 mg/kg Buprenorphine, Bayer) and the antibiotic Baytril (10 mg/kg Enrofloxacin, Baytril, Bayer, Germany). i.c.v. cannulas (21 G, 12 mm; Injecta GmbH, Germany) and microdialysis probes (2mm, self-made, molecular cut-off 18 kDa) (Neumann I., Russel J.A. and Group, 1993) were implanted unilaterally, whereas local cannulas were implanted bilaterally (26 G, 12 mm; Injecta GmbH, Germany). Cannulas were implanted 2 mm

above the target region in order to avoid lesion before the experiment, fixed to the skull with two jeweler's screws and dental cement (Kallocryl, Speiko-Dr. Speier GmbH, Muenster, Germany), and closed by a stainless steel stylet (i.c.v. 25 G, local 27 G). For virus surgeries, a solution of ketamine and xylazine (100 and 10 mg/kg, respectively, i.p.) was applied as anesthesia. After virus delivery skin was sutured. Optogenetic animals underwent another surgical procedure similar to the one used for cannula implantation to implant the optic fiber (PlexBright optogenetic stimulation system fiber stub implant; 6 mm length). Here, fibers were fixed with light-hardening dental cement. For specific coordinates please see Table 1.

Table 3.1: Stereotaxic surgery coordinates. * Second optic fiber was implanted in a 15° angle.

Procedure	Region	Coordinates		
		AP	ML	DV
i.c.v.	Lateral ventricle	-1.0	+1.6	+2.0
Local cannula	dLS	-0.4	±0.7	+2.4
	vLS	-0.4	±0.7	+3.5
Microdialyses	dLS	-0.4	+0.7	+5.0
	vLS	-0.4	+0.7	+5.2
Virus delivery	PVN	-1.7	±0.3	+8.1
	SON	-1.25	±1.9	+9.3
Optic Fiber*	vLS 0°	-0.4	-2.25	+5.8
	vLS 15°		+0.7	+5.1

RECEPTOR AUTORADIOGRAPHY

Brains were cryo-cut into 16-µm coronal sections, slide-mounted, and stored at -20°C. The receptor autoradiography procedure was performed using a linear V1A-R antagonist [125I]-d(CH2)5(Tyr[Me])-AVP (Perkin Elmer, USA) or a linear OXTR antagonist [125I]-d(CH2)5[Tyr(Me)2-Tyr-Nh2]9-OVT (Perkin Elmer, USA) as tracers. Briefly, the slides were thawed and dried at room temperature followed by a short fixation in paraformaldehyde (0.1%). Then slides were washed two times in 50 mM Tris

(pH 7.4), exposed to tracer buffer (50 pM tracer, 50 mM Tris, 10 mM MgCl2, 0.01% BSA) for 60 min, and washed four times in Tris buffer 10 mM MgCl2. The slides were then shortly dipped in pure water and dried at room temperature overnight. On the following day, the slides were exposed to Biomax MR films for 7-25 days depending on the receptor density and brain region (Kodak, Cedex, France). The films were scanned using an EPSON Perfection V800 Scanner (Epson, Germany). The optical density of V1aR and OXTR were measured using ImageJ (V1.37i, National Institute of Health, http://rsb.info.nih.gov/ij/). Receptor density was calculated per rat by subtracting the background and calculating the mean of bilateral measurements of 6 to 12 brain sections per region of interest.

ELISA FOR PLASMA CORTICOSTERONE

Quantification of plasma corticosterone was performed using ELISA. As described before, trunk blood of FIT animals was collected after decapitation. Approximately 1 ml blood was collected in EDTA-coated tubes on ice (Sarstedt, Numbrecht, Germany), centrifuged at 4°C (2000g, 10 min), aliquoted and stored at -20°C until the assay was performed using a commercially available ELISA kit for corticosterone (IBL International, Hamburg, Germany).

RADIOIMMUNOASSAY FOR OXT AND AVP

OXT and AVP content in blood, CSF and lysates were measured via radioimmunoassay (RIAgnosis, Germany). All samples were measured within the same assay to avoid inter-assay variability.

DRUGS AND VIRUSES

Animals were treated either with endogenous ligands, agonists or antagonists in order to modulate the OXT and AVP system. Usually, drugs were infused 10 min before the FIT in i.c.v cannula experiments (OXT: 50ng/5µl and AVP: 0.1ng or 1ng/5µl, Tocris).

Antagonists were infused 10 min prior to the infusion of the respective agonist with a final volume (agonist+antagonist) of 5µl (OXTR-A, des-Gly-NH2,d(CH2)5[Tyr(Me)2,Thr 4]OVT, or V1aR-A, d(CH2)5Tyr(Me)2AVP: 750ng)(Manning *et al*, 2012). For cannulas placed in the LS agonists (OXT, AVP, TGOT, [Thr4,Gly7]OT: all at: 0.1ng, /0.5µl per side) were infused 5 min before the FIT whereas antagonists (OXTR-A and V1aR-A: 100ng/0.5µl, per side) and muscimol (10ng/0.5µl, per side, Tocris) were infused 10 min before the FIT. In order to modulate the activity of the OXT neurons, the PVN and SON of GH females were infused with 280nl of rAAV1/2 OTprhM3Dq:mCherry (4×10^{11} genomic copies per ml) and rAAV1/2 OXTpr-ChR2:mCherry (4×10^{11} genomic copies per ml) for chemo- and optogenetic experiments, respectively. Viruses were slowly infused by pressure infusion at 70nl/min. After infusion, optogenetic animals had their water replaced by salt loaded water for 1 week (2% NaCl) in order to enhance virus expression. Chemogenetic animals received i.p. infusions either with CNO (HB6149, HelloBio, United Kingdom) or saline (1ml/kg).

PATCH-CLAMP

Slice preparation: Juvenile VGAT-Rats (postnatal day 15-21) were deeply anesthetized with isoflurane and decapitated. Coronal brain slices containing the lateral septum (300 µm) were cut in ice-cold carbogenized (O_2 [95 %], CO_2 [5 %]) artificial extracellular fluid (ACSF; [mM]: 125 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 20 Glucose, 2.5 KCl, 1 $MgCl_2$, and 2 $CaCl_2$) using a vibratome (Vibracut, Leica Biosystems, Germany) followed by incubation in carbogenized ACSF for 30 min at 36°C and then kept at room temperature (~21° C) until experimentation. *Electrophysiology:* Neurons in the dLS and vLS were visualized by infrared gradient-contrast illumination via an IR filter (Hoya, Tokyo, Japan) and patched with pipettes sized 8–10 MΩ. Somatic whole-cell patch-clamp

recordings were performed with an EPC-10 (HEKA, Lambrecht, Germany). Series resistances measured 10-30 MΩ. The intracellular solution contained [mM]: 110 CsCl, 10 HEPES, 4 $MgCl_2$, 10 TEA, 10 QX-314, 2.5 Na_2ATP, 0.4 NaGTP, 10 NaPhosphocreatine, 2 ascorbate, at pH 7.2. The ACSF was gassed with carbogen and contained [mM]: 125 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 20 Glucose, 2.5 KCl, 1 $MgCl_2$ and 2 $CaCl_2$. Additionally, biocytin (5 mg/ml) was added to the intracellular solution for post-hoc fluorescent labeling of the patched neurons. The average resting potential of lateral septal neurons was -60 mV (Allaman-Exertier *et al*, 2007). Leaky cells with a holding current above ~ -30 pA were rejected. Spontaneous activity (i.e. IPSCs) was recorded in voltage-clamp mode at resting membrane potential (-60 mV). The frequency, amplitude, and coefficient of variance (CV) of spontaneous IPSCs were analyzed with Origin 2019 (OriginLab Corporation, Northampton, MA, USA). *Histology:* After the experiment *in-vitro* slices were post-fixed in 4% paraformaldehyde in PBS (room temperature, overnight) and prepared for fluorescent labeling.

PERFUSION

After deep anesthesia with isoflurane followed by CO_2 rats were transcardiacally perfuse first with 0.1 PBS followed by 4% paraformaldehyde. Brains were post-fixated in PFA 4% overnight.

IMMUNOHISTOCHEMISTRY

Brains were cut into 40µm slices, which were collected in cryoprotectant solution and stored at -20°C until usage. Typically, a series of 6-8 slices comprehending the whole anteroposterior axis of the region of interest was used for immunostaining. First, slices were washed in 0.1 PBS and then raised in Glycine buffer (0.1M in PBS) for 20 min. After slices were washed with PBST (0.1 PBS with 0.3% triton-x 100) and blocked for 1 hour in blocking solution. Directly after blocking, slices were incubated in primary

antibodies for 1-2h at room temperature and then at 4°C overnight. On the next day, slices were again left in room temperature for 1-2h then washed in PBST and incubated in the secondary antibody. Next, slices were raised in 0.1 PBS and mounted on adhesive microscope slides (Superfrost Plus, Thermo Fisher Scientific Inc, Waltham, MA, USA). Slides were kept in the dark at 4°C until imaging. Especially, for the pERK immunostaining slices were pre-incubate in ice-cold methanol at -20°C for 10 min before the Glycine buffer step. Also for this staining primary antibody incubation lasted 64h. For details of tissue, mounting medium, blocking solution, and antibodies please see Table 2. Imaging from the neural activity, patch-clamp and molecular identification of the LS neurons was done using an inverted confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems, Wetzlar, Germany). Chemo- and optogenetic imaging was performed using an epi-fluorescence microscope (Thunder Imaging Systems, Leica). Digital images were processed (Merging and Z-projections) using the Leica Application Suite X (Leica) and Fiji (Schindelin *et al*, 2019). Cell counting was done by an experienced observer blind to the treatments. For patch-clamp, the detailed morphology of the neurites was reconstructed and analyzed with the Fiji plugin Simple Neurite Tracer(Longair *et al*, 2011) from the z-stack. From this analysis, number of branch points, junctions, and total branch length of the neurites were extracted and compared between dorsal and ventral neurons of the lateral septum. We also analyzed the area and diameter of the soma comparing both subregions.

Table 3.2: Immunohistochemistry specifics: NGS (normal-goat serum), BSA (bovine serum albumin).

Experiment	Tissue	Blocking solution	Primary antibody	Secondary antibody	Mounting medium
Neuronal activity	Venus-VGAT	5% NGS	rabbit-anti-pERK antibody (1:250 CellSignalling #9101 or #4370, Danvers, MA, USA)	Alexa-fluor 594 goat-anti-rabbit antibody (1:200)	Bectastain har-set (H-1400, Vector Laboratories, Inc., Burlingame, CA, USA
Patch-clamp	Venus-VGAT	5% NGS	Streptavidin conjugated with CF633 (1:400; Biotium, Fremont, CA, USA)		DAPI Fluoromount-G (SouthernBiotech, Birmingham, AL, USA)
Molecular identification of LS neurons	Venus-VGAT	5% NGS	rabbit-anti-ERα (1:500, C1355, Milipoer, USA); chicken-anti-Somatostain (1:500, 366-006, Synaptic Systems, Gottingen, DEU)	Alexa-fluor 594 goat-anti-rabbit antibody (1:250): Alexa-fluor 647 goat-anti-chicken (1:250)	Bectastain har-set (H-1400, Vector Laboratories, Inc., Burlingame, CA, USA
Chemo- and optogenetics	Wistar	1%BSA 5% NGS	mouse-anti-neurophysin1/oxytocin (1:500, Harold Gainer, p38); rabbit-anti-mCherry (1:1.000, ab167453, Abcam)	Alexa-fluor 488 goat-anti-mouse antibody (1:1000): Alexa-fluor 594 goat-anti-rabbit (1:1000)	DAPI Fluoromount-G (SouthernBiotech, Birmingham, AL, USA)

STATISTICS

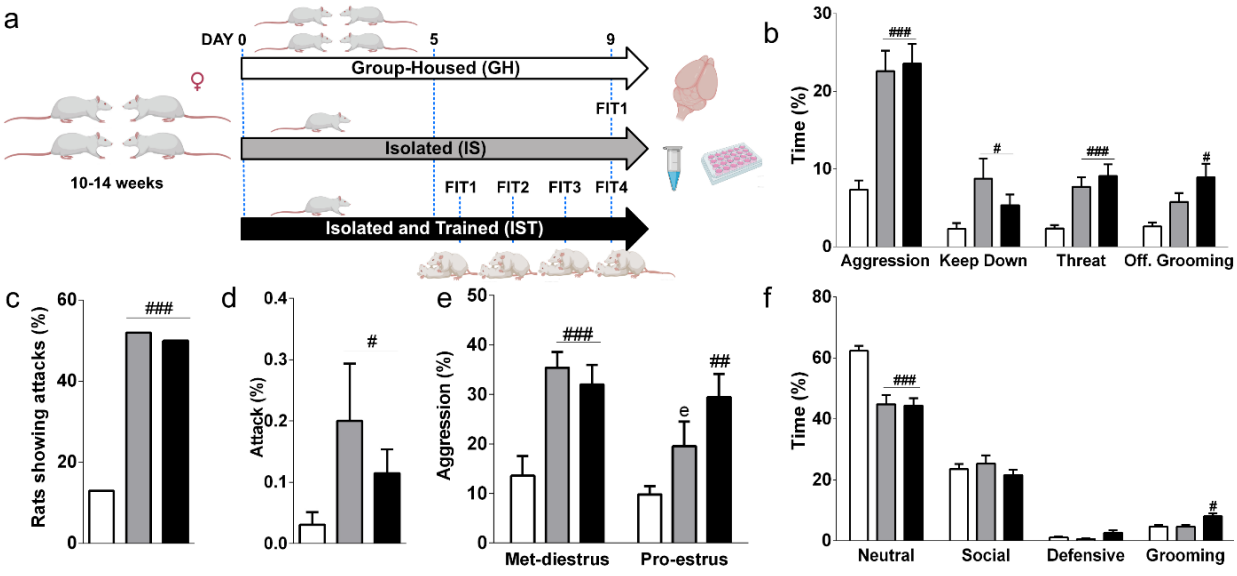
Data normality was checked using the Kolmogorov-Smirnov test. If normality was reached, data were analyzed using either Student t-tests (paired or unpaired), chi-

square test, or analyses of variance (one or two way ANOVA) followed by a posthoc comparison corrected with Bonferroni, when appropriate. In case data were not normally distributed, either Mann-Whitney U-test or Dumms multiple comparison tests were performed. For detailed statistics information see Supplementary Table 1.

3.3. RESULTS

SOCIAL ISOLATION AND TRAINING RELIABLY ENHANCED FEMALE AGGRESSION

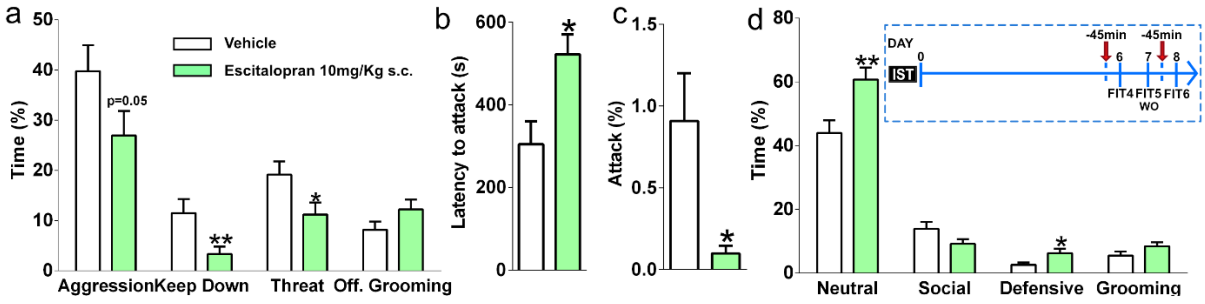
Training consisted of daily 10-min exposure to a FIT (De Jong *et al*, 2014) on three consecutive days. GH and isolated non-trained rats (IS) were used as control groups to assess the effects of isolated housing and of aggression training (Figure 3.1a). Both IST and IS females displayed increased aggression, i.e. increased time spent on keep down, threat, offensive grooming (Figure 3.1b), and attacking, and engaged more frequently in attacks (Figure 3.1c,d), when compared to GH controls (Supplementary Movie1). Further, we found a major effect of the estrus-cycle on aggression, i.e. IS females were less aggressive in the proestrus or estrus phase than in the metestrus or diestrus phase (Figure 3.1e). Probably as compensation for increased aggression, IS and IST rats displayed less neutral behaviors. Only IST females showed increased self-grooming behavior when compared to GH controls.



Chapter 3: The oxytocin-vasopressin balance within the lateral septum determines aggression levels in virgin female rats

Figure 3.1. Social isolation and training reliably enhance female aggression, independently of the estrous cycle. a Scheme illustrating the animal groups and behavioral timeline. Female Wistar rats were kept either housed in groups (GH) or socially-isolated (IS and IST). IST females underwent three consecutive female intruder tests (FIT), those consisted of aggressive encounters with an unknown same-sex intruder, partially drawn using https://biorender.com/). **b** Both IS and IST rats increased total aggression (one-way ANOVA followed by Bonferroni $F(2,63)=16.26$, $p< 0.0001$), keep down (Kruskal-Wallis test followed by Dunn's $H3=6.691$, $p=0.035$), threat ($H3=18,98$, $p<0.0001$) and offensive grooming ($H3=10.78$, $p=0.0046$). IS and IST females engaged more in attacks (Chi-squared test, $X2=40.81$, $p<0.0001$) **c**, and spend more time attacking ($H3=8.8$, $p=0.0123$) **d** than GH females. **e** IS females in the proestrus-estrus (Pro-estrus) phase of the estrous-cycle displayed less aggressive behavior than metestrus-diestrus (Met-diestrus) females (two-way ANOVA followed by Bonferroni; factor housing: $F(2, 57)=14.24$, $p<0.0001$; estrous cycle: $F(1, 57)=5.470$, $p=0.0229$; housingxestrous cycle: $F(2, 57) =1.780$, $p=0.1779$) . **f** IST and IS females compensated their increased aggression with decreased neutral behaviors ($F(2, 63)=18.76$, $p<0.0001$), only IST females spent more time auto-grooming ($F(2, 63)=5.787$, $p=0.0049$). All data are shown as mean+SEM. $^{\#}p<0.05$,$^{\#\#}p<0.001$, ,$^{\#\#\#}p<0.001$ vs GH; e$p<0.05$ vs met-diestrus.

In order to further validate our model of female aggression, we infused aggressive IST females with the selective serotonin reuptake inhibitor (SSRI) escitalopram (ESC) (s.c.10mg/kg), since SSRIs typically decrease aggression in rodents (Carrillo and Ricci, 2009). Indeed, ESC significantly reduced the time spent on aggression, i.e. on keeping down, threat, and attacking, and increased the latency to attack, compared to vehicle. Also, ESC-treated females displayed more defensive and neutral behaviors (Supplementary Figure 1).



Supplementary Figure 1. Escitalopram (ESC) decreases aggression in isolated and trained rats (IST). Insert illustrates experimental design (FIT=female intruder test; WO=wash-out). **a** Intraperitoneal infusion of ESC 45 min before the FIT decreased total aggression (Paired two-tailed t-Student's test $t_{(10)}=2.157$, $p=0.05$) keep down (Mann-Whitney test $U=26.0$, $p=0.0233$), threat ($t_{(10)}=2.506$, $p=0.0311$) and **c** number of attacks ($U=24.0$, $p=0.011$) in IST rats. **c** Latency to attack was increased in ESC rats ($U=24.0$, $p=0.011$) . **d** ESC treatment increased the time spent in neutral ($t_{(10)}=2.821$, $p=0.0181$) as well as defensive behaviors ($t_{(10)}=2.201$, $p=0.05$) in IST rats. All data are shown as mean+SEM. $^{*}p<0.05$,$^{**}p<0.001$, vs vehicle.

Chapter 3: The oxytocin-vasopressin balance within the lateral septum determines aggression levels in virgin female rats

THE HIGH LEVELS OF AGGRESSION DISPLAYED BY ISOLATED AND TRAINED RATS ARE UNDERLINED BY HIGH LEVELS OF OXT AND LOW LEVELS OF V1A RECEPTOR BINDING

Next, rats were either exposed to the FIT or not (control), after being housed in their respective housing-conditions in order to evaluate the role of the endogenous OXT and AVP systems on aggression (Figure 3.1a). IST and IS rats exposed to the FIT exhibited higher levels of OXT in the CSF compared with their respective control groups. Additionally, IST females had elevated OXT levels when compared with GH females that underwent the FIT (Figure 3.2a). Consequently, aggression levels were found to be positively correlated with OXT content in the CSF (Figure 3.2b). However, none of these effects of aggression on intracerebral OXT release were reflected in plasma OXT concentrations (Supplementary Figure 2a-b). We also analyzed OXTR binding in selected brain regions involved in the social/aggressive behavior network (Supplementary Figure 2c). Among 9 regions analyzed, only within the LS OXTR were affected by the housing and training conditions, as IST females exhibited less OXTR binding in the vLS, when compared to GH controls (Figure 3.2c and 3.2e). However, local OXTR binding did not correlate with aggression (Figure 3.2d).

In contrast to OXT, AVP concentrations in the CSF were reduced in IST females after the FIT without any effect of housing/training conditions (Figure 3.2f). Consequently, AVP content in the CSF did not correlate with aggression (Figure 3.2e). Interestingly, FIT exposure tended to heighten plasma AVP regardless of housing condition, although the magnitude of this effect seemed to be reduced in IST females (Supplementary Figure 2d). Plasma AVP did not correlate with aggression (Supplementary Figure 2e). IST females exhibited lower V1aR binding in the dLS, when compared with GH controls (Figure 3.2h and 3.2j, for the other regions, see

Supplementary Figure 2f), and V1aR binding negatively correlated with aggression, i.e. female rats with reduced binding were found to be more aggressive (Figure 3.2i).

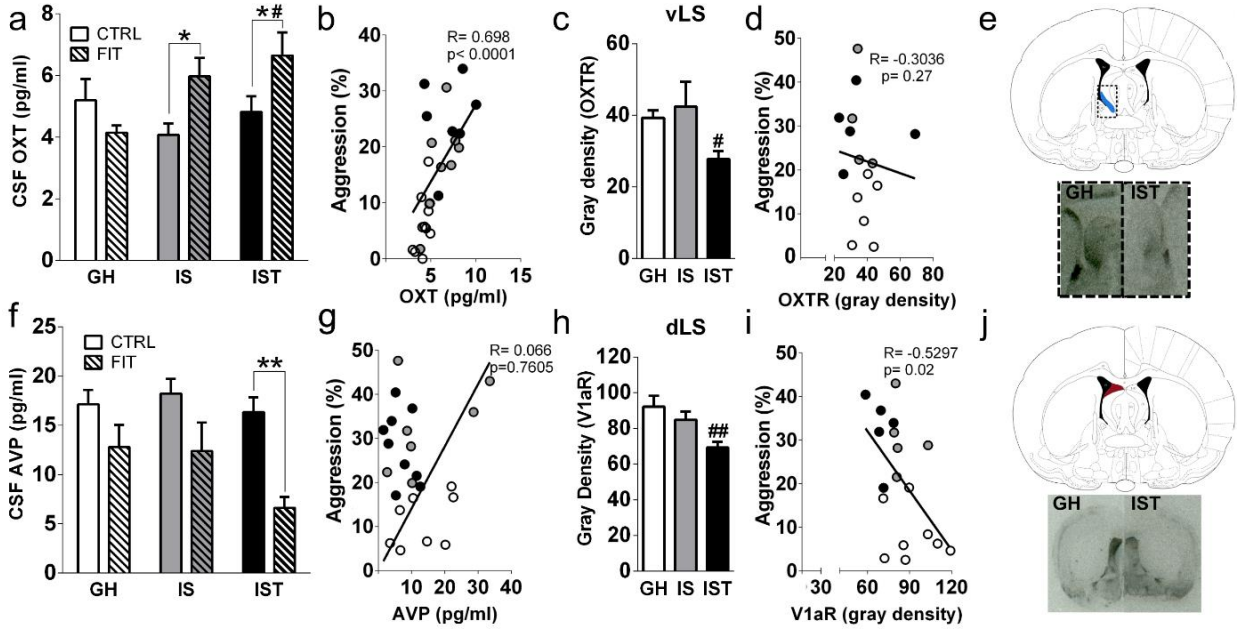
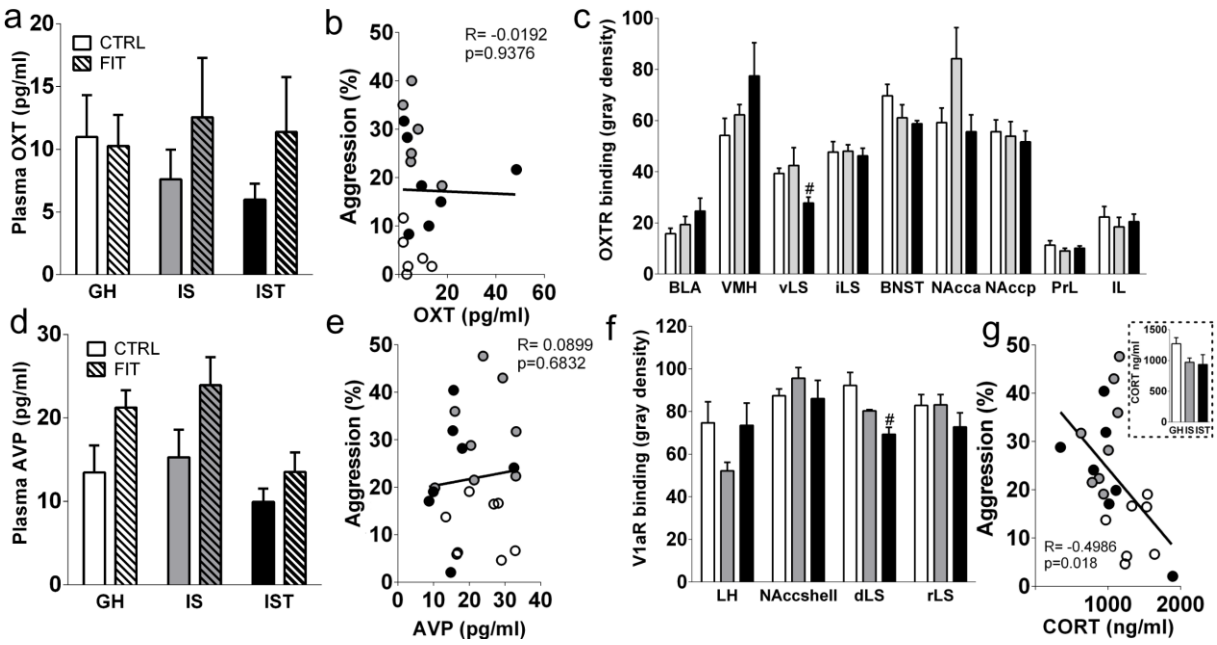


Figure 3.2. The high levels of aggression displayed by isolated and trained rats are underlined by high levels of OXT and low levels of V1a receptor binding. a IS and IST females showed higher levels of OXT in the cerebrospinal fluid (CSF) immediately after the FIT compared to respective control rats, also IST females which underwent the FIT exhibited higher OXT levels than GH females (GH=group-housed; IS= isolated; IST= isolated and trained; CTRL= control; FIT= female intruder test) (two-way ANOVA followed by Bonferroni FIT effect: $F_{(1,44)}$=3.913, p=0.0542; housing effect: $F_{(2,44)}$=1.965, p=0.1523; FITxhousing effect: $F_{(2,44)}$=4.683, p=0.0143). **b** Aggression correlated with CSF OXT levels of FIT rats (Pearson's correlation r=0.698, p<0.0001). **c** IST females presented decreased OXT receptor (OXTR) binding in the ventral portion of the lateral septum (vLS) (Kruskal-Wallis test followed by Dunn's: $H_{(3)}$=7.124, p=0.0284). **d** Aggression levels did not correlate with OXTR binding (Spearman's correlation r=-0.3036, p=0.2708). **e** Scheme illustrating localization of OXTR in the vLS and magnification of the autoradiograph showing example subjects (left: GH; right: IST). **f** FIT exposure decreased CSF AVP levels only in IST rats (two-way ANOVA FIT effect: $F_{(1,50)}$=15.98, p=0.0002; housing effect: $F_{(2,50)}$=2.134, p=0.1290; FITxhousing effect: $F_{(2,50)}$=0.9042, p=0.4114). **g** Aggressive behavior did not correlate with CSF AVP content (r=0.066, p=0.7605). **h** IST females exhibited decreased V1a receptor (V1aR) binding in the dorsal part of the LS (dLS) ($H_{(3)}$=8.724, p=0.0062). **i** Aggression negatively correlated with V1aR binding in the dLS (r=-0.5297, p=0.02). **j** Scheme illustrating localization of the V1aR in the dLS and representative autoradiograph (left: GH, right: IST). All data are presented as mean + SEM. $^{\#}$p<0.05,$^{\#\#}$p<0.01 vs GH; *p<0.05,**p<0.01 vs control.

Since hypo-corticosterone has been implicated in the development of male aggression in humans and animals (Denson *et al*, 2018; Nelson and Trainor, 2007), we decided to assess plasma corticosterone concentrations after exposure to the FIT.

Although there was no effect of housing/training conditions, plasma corticosterone indeed negatively correlated with female aggression levels (Supplementary Figure 2g).



Supplementary Figure 2. Peripheral OXT, AVP and CORT are not changed after exposure to the FIT, but aggression negatively correlates with plasmatic CORT in female Wistar rats. a Neither IS or IST females exihibited any changes in plasmatic OXT after the FIT compared to respective control rats. **b** Consequently aggression did not correlate with plasmatic OXT levels. **c** Among all the regions analyzed only ventral lateral septum (vLS) OXT receptor (OXTR) binding was decreased in IST females (Kruskal-Wallis test followed by Dunn's: $H_{(3)}$=7.124, p=0.0284).**d** FIT exposure tended to increase plasmatic AVP levels but this effect seems to be reduced in IST rats (two-way ANOVA FIT effect: $F_{(1,55)}$=8.45, p=0.0053; housing effect: $F_{(2,55)}$=4.387, p=0.0171; FITxhousing effect: $F_{(2,55)}$=0.4883, p=0.6163). **e** Aggressive behavior did not correlate with plasmatic AVP content. **f** V1a receptor (V1aR) binding was decreased only in the dorsal LS (dLS) ($H_{(3)}$=8.724, p=0.0062). **g** Neither IS or IST females showed differences in plasmatic levels of CORT after FIT exposure (insert). However Aggression negatively correlated with plasmatic CORT levels (Pearson's correlation r=-0.4986, p=0.018) All data are presented as mean + SEM. #p<0.05 GH.Abbreviations: basolateral amygdala (BLA), ventromedial hypothalamus (VMH), intermediate portion of the lateral septum (iLS), bed nucleus of Stria terminalis (BNST), anterior Nucleus accumbens (Nacca), posterior Nucleus accumberns (Naccb), Nucleus accumbens shell (Nacc shell), Lateral hypothalamus (LH), rostral portion of the lateral septum (rLS).

OXT PROMOTED WHEREAS AVP REDUCED AGGRESSION IN A CENTRAL APPROACH

Since female aggression was positively correlated with levels of OXT in the CSF, we decided to combine neuropharmacological and chemogenetic methods to increase OXT availability in the brain of low aggressive GH females, on the other hand,

we centrally infused highly aggressive IST rats with an OXTR antagonist (OXTR-A) in order to block endogenous release (Figure 3.3a).

Intracerebroventricular (i.c.v.) infusion of synthetic OXT (50ng/5µl) enhanced aggression in GH females, whereas, surprisingly, the same treatment decreased aggression in IST females (Figure 3.3b), and this anti-aggressive effect of OXT was reflected in all behaviors analyzed. Although these results might seem puzzling at first, the high similarity between OXT and AVP which co-evolved from a single peptide(Jurek and Neumann, 2018) results in cross-reactivity with each other's receptors *in vitro* (Manning *et al*, 2012) and *in vivo* including in the context of aggression (Jiang and Platt, 2018a; Tan *et al*, 2019). In order to test for a possible cross-reactivity of OXT on V1aRs, we blocked either OXTR or V1aR (V1aR-A, both at 750ng/5µl, i.c.v.) with respective specific antagonists 10 min prior to i.c.v. OXT infusion. The OXTR-A could not abolish the anti-aggressive effects of OXT, but V1aR-A could (Figure 3.3c). This clearly indicates that the anti-aggressive effect of synthetic OXT is mediated via V1aR.

In order to reveal the involvement of the *endogenous* OXT system in female aggression, we first blocked OXTRs in IST females and found that i.c.v. infusion of the OXTR-A reduced the total aggressive behavior (Fig 3.3d). Next, we infected the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus of GH female rats with an rAAV to selectively express GqDREADD under the control of an OXT promoter fragment (Figure 3.3g) and thus enable chemogenetical stimulation of endogenous OXT release as shown before (Grund *et al*, 2019). The virus showed a penetrance (percentage of OXT neurons that co-expressed mCherry) of 64.4% (PVN) and 75.1% (SON), respectively; however, some mCherry-labelled cell bodies were found outside the target infusion site indicating leakage of the virus (transfection specificity PVN: 73.9%; SON: 77.9%). Intraperitoneal application of the DREADD

ligand clozapine-N-oxide dihydrochloride (CNO, 2mg/kg) increased aggression in diestrus and metestrus GH females, thereby confirming our pharmacological results. Intriguingly, estrus and proestrus GH females showed no increase in aggression (Figure 3.3f).

Finally, since synthetic OXT decreased aggression via activation of V1aRs, and because IST rats exhibited low levels of AVP in their CSF, we infused aggressive IST females with synthetic AVP (0.1 and 1ng/5µl, i.c.v.). Elevation of brain AVP availability prior to the FIT resulted in decreased total aggression, keeping down, threat behavior as well as time spent on attacks in IST rats rats (Figure 3.3e). Although the i.c.v. experiment showed strong serenic effects we decided to not manipulate the AVP system in a central manner using the GqDREADD approach due to two reasons i) AVP is one of the main players in brain physiology and stimulation of several cell bodies could disturb homeostasis, in fact, high doses of i.c.v. AVP are known to elicit barrel rotations in rats(Wurpel *et al*, 1986), also ii) the AVP neurons are widespread in several nuclei in the rat brain such as the medial amygdala, the bed nucleus of *stria terminalis* (BNST), PVN and SON (DiBenedictis *et al*, 2017) what makes the infusion of all targeted neurons challenging in terms of animal welfare.

Taken together, our results demonstrate a pro-aggressive effect of brain OXT acting via OXTRs, and anti-aggressive effects of OXT and, particularly, AVP acting via V1aRs, in female Wistar rats.

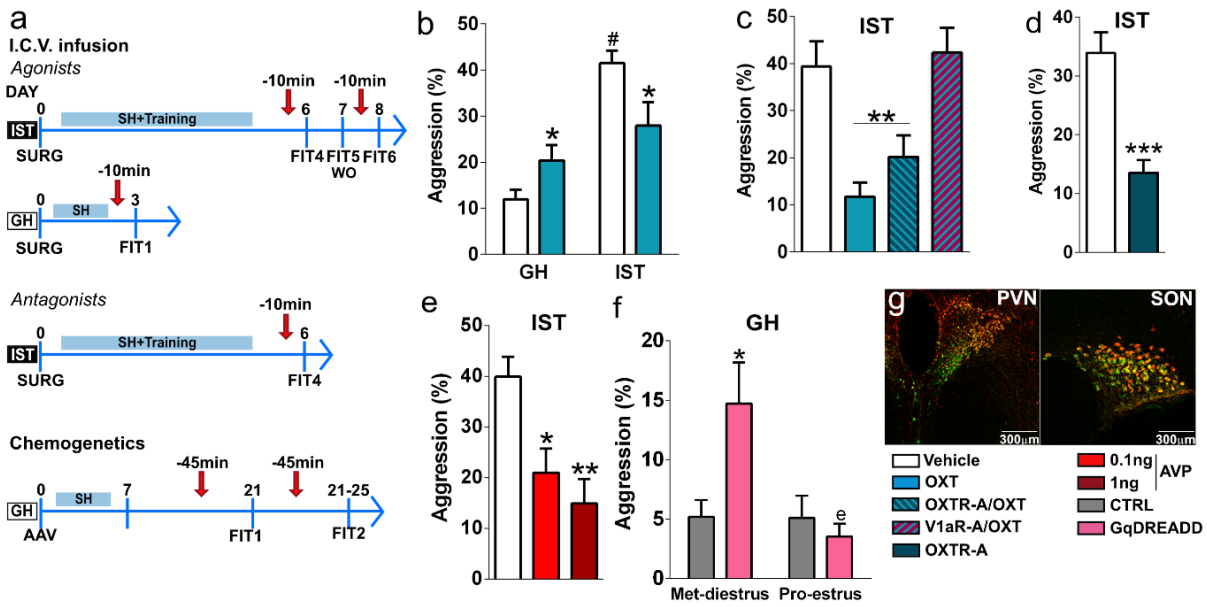


Figure 3.3. OXT promotes aggression, whereas AVP reduces aggression in central approach. **a** Experimental design for pharmacological and chemogenetic experiments targeting the OXT and AVP systems in isolated and trained (IST) and group-housed (GH), rats (AAV= adeno-associated DREADD virus infusion into the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei; AVP=vasopressin; arrow= drug infusions; FIT= female intruder test; IS= social isolation after surgery; OXT= oxytocin; OXTR-A= OXT receptor antagonist; SH= single-housing; SURG= surgery; V1aR-A= V1a receptor antagonist; WO= Wash-out). **b** I.c.v. infusion of OXT (50ng/5ul) increased aggression in GH (two-tailed t-Student's test $t_{(19)}$=2.46, p=0.0237), but decreased aggression in IST females ($t_{(8)}$=2.33, p=0.0482). **c** I.c.v. infusion of V1aR-A (750ng/5µl), but not OXTR-A (750ng/5µl) blocked the anti-aggressive effects of OXT in IST females (one-way ANOVA followed by Bonferroni $F_{(3,28)}$=10.08, p=0.001). Both, **d** i.c.v infusion of OXTR-A ($t_{(28)}$=4.964, p<0.0001) and **e** i.c.v. AVP (0.1 or 1ng/5µl) reduced total aggressive behavior ($F_{(3,54)}$=7.483, p=0.0003) in IST rats. **f** Chemogenetic activation of OXT neurons in the PVN and SON increased aggression only in met-diestrus GH rats (two-way ANOVA, factor treatment: $F_{(1,19)}$=3.342, p=0.0833; estrous cycle: $F_{(1,19)}$=6.677, p=0.0182; teatmentxestrous cycle: $F_{(1,19)}$= 6.449, p=0.02). **g** Confirmation of the virus infection in the PVN (right) and SON (left). OXT-neurophysin I staining: green; mCherry (virus): red. Scale bars 300µm. Data are shown as mean + SEM. $^{\#}$p<0.05 vs GH; *p<0.05**p<0.01,***p<0.0001 vs either vehicle or control; ^{e}p<0.05 vs met-diestrus.

THE PRO-AGGRESSIVE EFFECT OF OXT WAS MEDIATED IN THE VLS

Based on the fact that OXTR binding was exclusively reduced in the vLS of IST females (Figure 3.2c), and that the LS is a target region for aggression regulation (Potegal, M Blau, A and Glusman, 1981; Potegal *et al*, 1981; Wong *et al*, 2016), we hypothesized that the pro-aggressive effect of OXT is mediated in the vLS. Thus, we combined local *in vivo* microdialysis, neuropharmacological and optogenetic

approaches to specifically reveal the role of the OXT system in the vLS on female aggression (Figure 3.4a).

In vivo monitoring of OXT release within the vLS under basal conditions and during ongoing behavioral testing revealed an increased release in IST rats during the FIT, which was found to correlate with the total aggression displayed (Figure 3.4b-c). Consequently, local OXT release occurring during the FIT was higher in IST females compared with GH controls (Figure 3.4b). Next, to test for the involvement of such locally released OXT and subsequent OXTR-mediated signaling in the vLS in female aggression, we bilaterally infused an OXTR-A (100ng/0.5µl) into the vLS of IST rats, 10 min prior to the FIT. Blockade of OXTR resulted in decreased total aggression (Figure 3.4d).

With the aim to specifically prove the involvement of septal OXT neurotransmission in triggering female aggression, we used optogenetics to selectively stimulate oxytocinergic terminals and thereby local release of OXT in the vLS of GH rats. To this end, GH rats were infected with a channelrhodopsin (ChR2) rAAV in the PVN and SON, which is expressed under the control of an OXT promoter fragment (Figure 3.4e). Similarly to the GqDREADD rAAV, the ChR2 rAAV showed a penetrance of 64.4% for the PVN and 75.5% to the SON, respectively. Although the specificity of the virus in targeting the OXT system has been proven by another group (Knobloch *et al*, 2012), we also had leakage of the virus outside of the target regions (transfection specificity PVN:74.4% and SON: 76.9%). Blue-light stimulation of vLS OXT fibers during the FIT increased the level of aggression in a time-dependent manner, particularly in metestrus/diestrus females, thereby confirming our results from the i.c.v and CSF experiments (see Figure3 4f-h). Cumulative analyses showed a main effect of the virus transfection, i.e. metestrus/diestrus animals that expressed the ChR2 and

received blue-light stimulation exhibited higher aggression compared to controls, whereas aggression was not affected in proestrus/estrus females (Figure 3.4h, Supplementary movie 2 and 3).

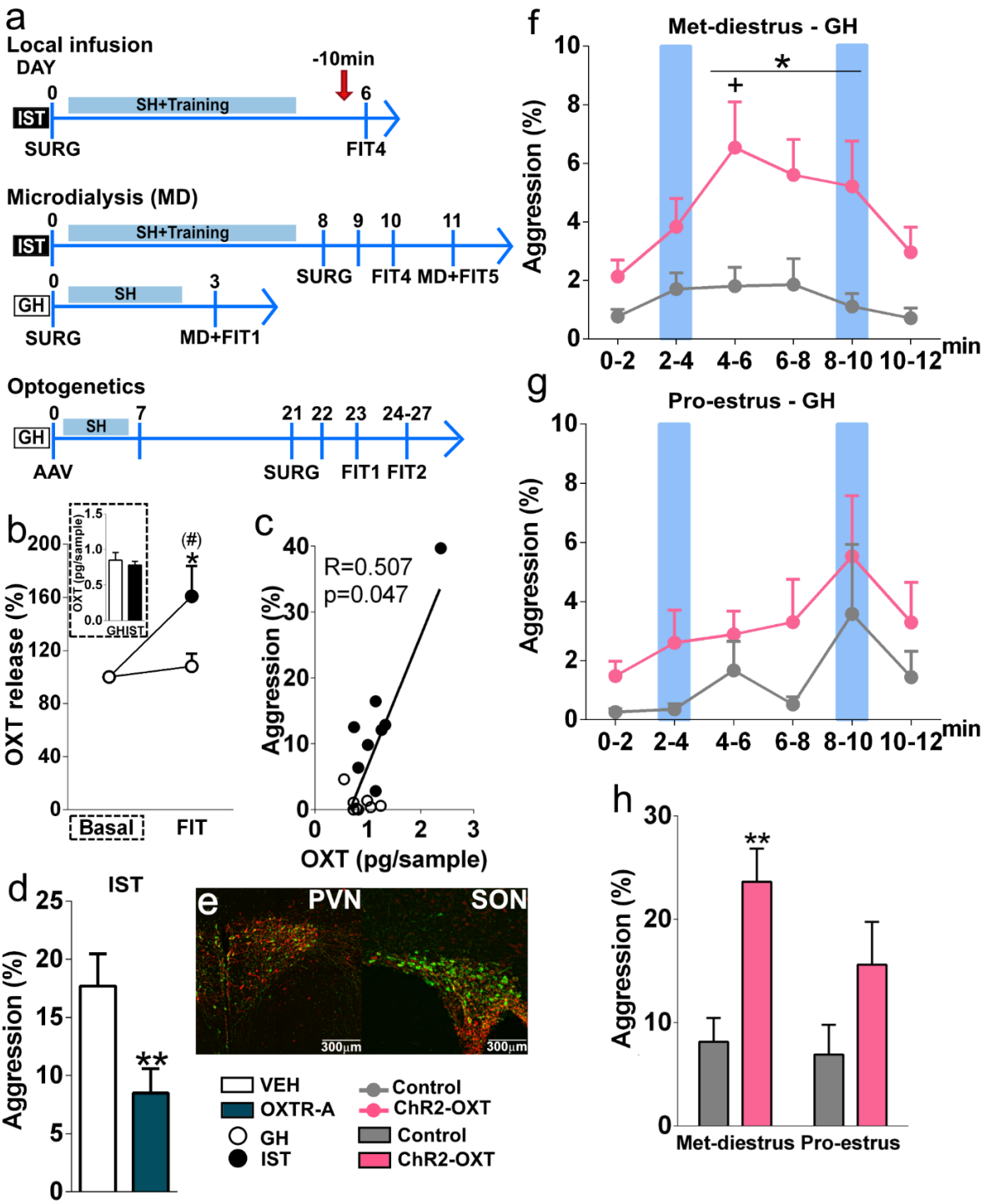


Figure 3.4. The pro-aggressive effect of OXT is mediated in the vLS. a Scheme illustrating the experimental design for pharmacological, microdialysis and optogenetic experiments (AAV= adeno-associated virus microinfusion into the paraventricular (PVN) and supraoptic (SON) nucleus of hypothalamus; arrow= drug infusions; FIT= female intruder test; GH= group-housed; IS= social isolation after surgery; IST= isolated and trainded; MD= microdialysis; SH= single-housing; SURG= surgery; OXT= oxytocin; OXTR-A= OXT receptor antagonist; vLS: ventral part of the lateral septum). **b** OXT release in the vLS is indicated by the percentage of rise in OXT content. IST but not GH females exhibited a rise in OXT release during the FIT (One sample t-Student's test IST: $t_{(7)}$=2.649, p=0.033; GH: $t_{(7)}$=0.8274, p=0.435), thus IST rats strongly tended to have a higher OXT release than GH rats ($t_{(14)}$=2.12, p=0.0527), insert depics basal OXT levels in GH and IST females ($t_{(14)}$= 0.5397, p=0.597). **c** Aggression displayed during the FIT correlated with OXT content in the vLS (Spearman's correlation r=0.507, p=0.047).**d** OXTR-A (100ng/0.5µl) infusion into the vLS reduced total aggression (two-tailed t-Student's test $t_{(26)}$=2.583, p=0.0158) in IST females. **e-h** Optogenetic stimulation (indicated by blue columns) of OXT termainals in the vLS of GH females during the FIT. **e** Confirmation of the virus infection in the PVN (right) and SON (left). OXT-neurophysin I staining: green; mCherry (virus): red. Scale bars 300µm. Blue-light stimulation of ChR2-OXT fibers enhanced aggressive behavior in met-diestrus **f** (two-way ANOVA followed by Bonferroni factor: time: F(5,75)=8.826, p=0.0256; virus: F(5,75)=20.03, p=0.0004; timexvirus: F(5,75)=1.056, p=0.3917), but not in pro-estrus females **g** (factor: virus: F(1,15)=13.06, p=0.0026; estrous cycle: F(1,15)=2.07, p=0.1708; virusx estrous cycle: F(1,15)=1.114, p=0.308). **h** Cumulative analyses show an effect of light stimulation in enhancing aggression in ChR2-OXT females only when they were in the met-diestrus phase of the cycle (factor virus: $F_{(1,15)}$=13.06, p=0.0026; estrous cycle: $F_{(1,15)}$= 2.07, p=0.1708; virusx estrous cycle: $F_{(1,15)}$= 1.114, p=0.308). Data are shown as mean+SEM. $^{(\#)}$p=0.05 vs GH; *p<0.05, **p<0.01 vs either vehicle, baseline or ChR2 control; $^{+}$p<0.05 vs 0-2 time-point**.**

AVP EXERTED ANTI-AGGRESSIVE EFFECTS WITHIN THE DLS

In order to localize the anti-aggressive effects of AVP identified above, we used intracerebral microdialysis and neuropharmacology within the dLS (Figure3 5a), as local V1aR binding was decreased in highly aggressive IST females and found to be negatively correlated with aggression levels (Figure 3.2h-i). Optogenetic stimulation of AVP septal terminals was not possible due to the low specificity and penetrance of the virus in parvocellular neurons of the BNST, source of AVP innervation to the LS (DiBenedictis *et al*, 2017; De Vries and Panzica, 2006) (data not shown).

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AVP release was monitored within the dLS of GH and IST rats under basal conditions and during exposure to the FIT. Differently from GH controls, in which AVP release was found to significantly increase to 130% during FIT, such a rise was not found in IST females resulting in lower AVP levels compared with GH controls (Figure 3.5b). However, no obvious negative correlation between AVP release and aggressive behavior could be identified (Figure 3 5c). Next, in order to prove that AVP acts on V1aRs specifically in the dLS to inhibit female aggression, we infused AVP, TGOT (a specific OXTR agonist), or OXT (all at 0.1ng/0.5µl) either into the dLS, where we identified predominantly V1aRs or into the vLS, where predominantly OXTRs were found (Figure 3.2e and j). Bilateral infusions of AVP, but not TGOT or OXT, into the dLS of IST rats resulted in decreased total aggression (Figure 3.5d). In contrast, none of the treatments in the vLS affected aggression (Figure 3.5e). Surprisingly, V1aR-A (100ng/0.5µl) administration into the dLS alone had a moderate effect on enhancing aggression in IST rats (Figure 3.5f).

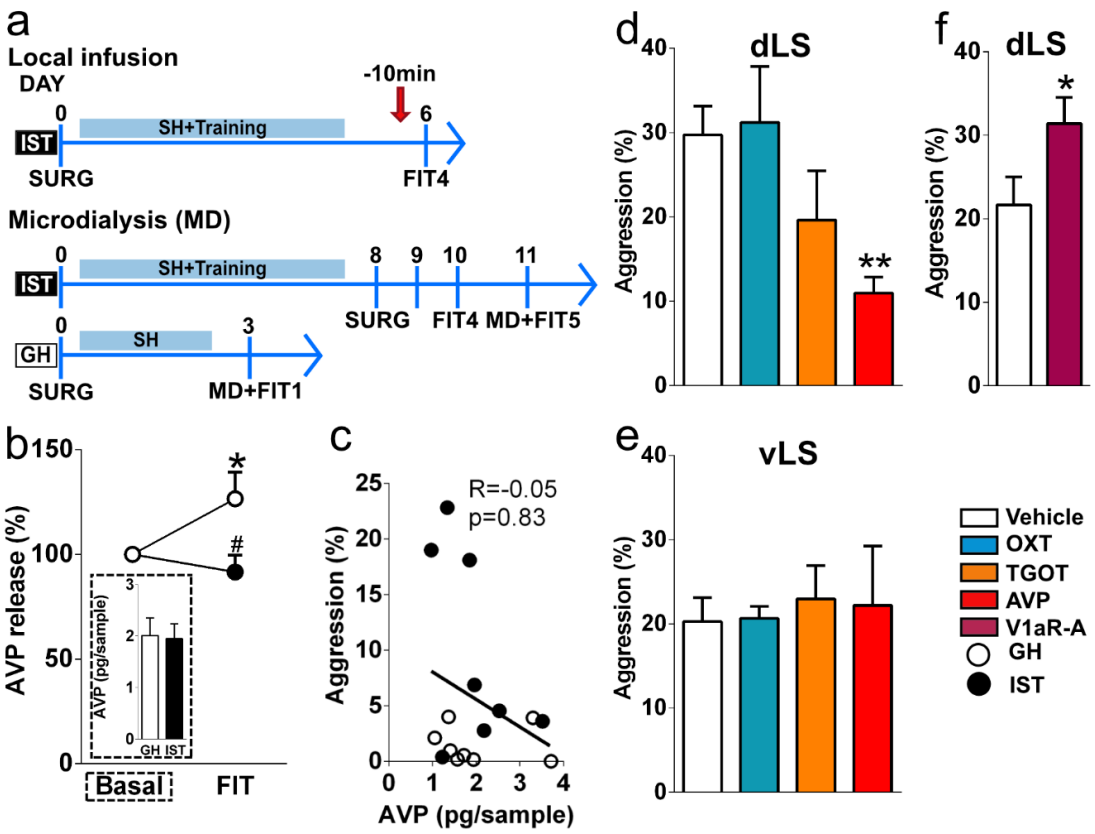

Figure 3.5. AVP exerts anti-aggressive effects within the dLS. a Scheme illustrating the experimental design for pharmacology and microdialysis experiments targeting the AVP system (AVP= vasopressin; arrow= drug infusions; FIT= female intruder test; GH= group-housed; i.c.v.= intracerebroventricular; IS= isolation after surgery; IST= isolated and trained; MD= microdialysis; SH= single-housing; SURG= surgery; dLS: dorsal part of the lateral septum; OXT= oxytocin; TGOT= [Thr4,Gly]OXT, OXT receptor agonist; vLS: ventral part of the lateral septum; V1aR-A= V1aR receptor antagonist). **b** AVP release in the dLS is indicated by the percentage of rising in AVP content. GH but not IST females showed increased rise in AVP release in the dLS during the FIT (Wilcoxon Signed Rank test GH: $W_{(9)}$=45, p=0.0039; IST: $W_{(7)}$=-4.00, p=0.8125), thus AVP release during the FIT was higher in GH than in IST rats (Mann-Whitney test U=6.00, p=0.0052). Insert shows that basal AVP levels did not differ between the groups. **c** Female aggression did not correlate with AVP content in the dLS during the FIT (Pearson's correlation r=-0.1971, p=0.4632).**d** Infusion of AVP, but not OXT or TGOT (all at: 0.1 ng/0.5 µl), into the dLS decrease total aggression in IST females ($F_{(3,30)}$= 7.292, p=0.0008). **e** AVP infusion into the ventral lateral septum (vLS) did not change aggression in IST females ($F_{(3,25)}$=0.1097, p=0.9536). **f** Local blockade of V1aR (100ng/0.5µl) prior to the FIT increased female aggression in IST rats (two-tailed t-Student's test $t_{(20)}$=2.137, p=0.0451). Data are shown as mean+SEM. $^{\#}$p<0.05 vs GH; *p<0.05, **p<0.01 vs either vehicle or baseline.

TWO DISTINCT SUBREGIONS OF THE LS ARE DIFFERENTLY REGULATED BY THE ACTIVATION OF OXTRS

The LS consists mostly of GABAergic neurons grouped into different subnuclei according to the expression of different receptors and neuropeptides (Risold and Swanson, 1997a); most importantly, specific expression of V1aRs and OXTRs

(Dumais and Veenema, 2016). It was reported that spontaneous IPSCs in these neurons are modulated by AVP in various ways (Allaman-Exertier *et al*, 2007). In our behavioral paradigm, activation of OXTRs in the vLS enhanced aggression, whereas activation of V1aRs in the dLS rather reduced aggression in female Wistar rats. In order to tie these region- and neuropeptide-specific effects to neuronal processing at the single-cell level, we recorded spontaneous activity from dLS and vLS neurons *in vitro* (whole-cell voltage-clamp, -60mV) and investigated OXTR-mediated effects by applying TGOT (1mM) to the bath. We also characterized these two neuronal populations with respect to morphological (biocytin filling) and molecular parameters (Figure 6a).

Neurons in the dLS exhibited a higher morphological complexity compared to vLS neurons (Figure 3.6b-f), as indicated by higher numbers of neurite branches and junction points (Figure 3.6i). Apart from the specific expression of V1aRs and OXTRs, dorsal and ventral LS neurons also differed regarding the expression of other markers: somatostatin-positive cell bodies were only found in the dLS (Figure 3.6g), whereas estrogen receptor α (ERα) expressing cells were exclusively located in the vLS (Figure 3.6h), suggesting that those are indeed distinct neuronal populations.

Selective activation of OXTRs differentially affected the spontaneous inhibitory activity in dLS versus vLS (Figure 3.6j and l). In the dLS, cells responded to TGOT with an increased IPSC frequency. IPSCs were entirely blocked by further addition of bicuculline (50µM, GABA-A antagonist) in the bath (Figure 3.6j-k). Conversely, in the vLS, neurons responded to TGOT with a decreased IPSC frequency (Figure 3.6l-m), indicating an excitatory effect of OXTR activation. Again, activity was entirely blocked by bicuculline. Altogether, our data demonstrate that activation of OXTR in the vLS triggers concomitant excitation of vLS neurons and inhibition of dLS neurons. In

addition, dLS neurons exhibited higher morphological complexity and different individual markers when compared to vLS neurons.

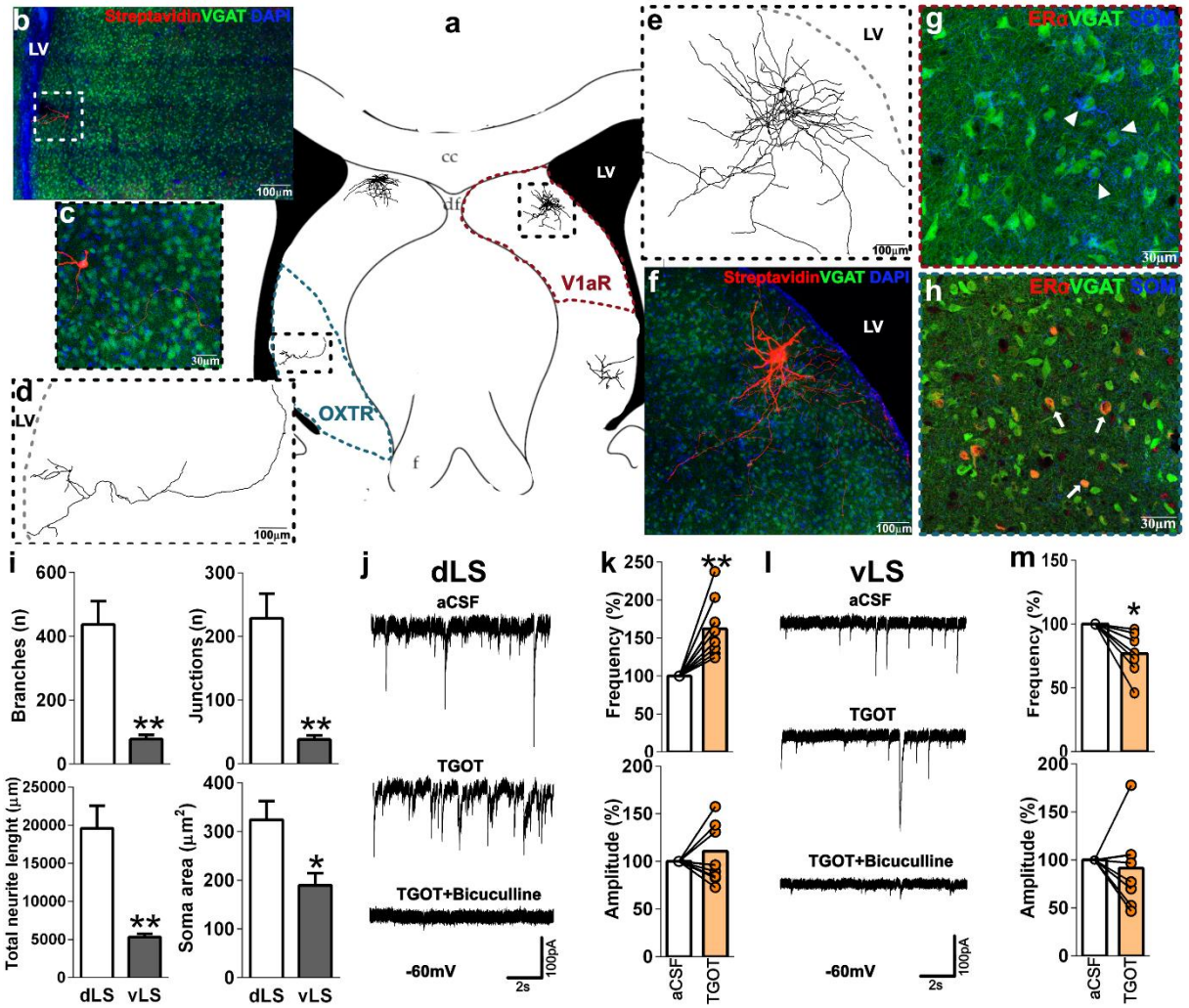

Figure 3.6. Two distinct subregions of the LS are differently regulated by the activation of OXTRs. a Scheme indicating the receptor binding-specific delimitation of the vLS (OXTR) and dLS (V1aR) used during voltage-clamp experiments including two representative dLS and vLS cells located in the respective subregions **b** Maximal z-projection of biocytin-filled vLS cell (streptavidin-CF633) overlaid with a single z-plane of VGAT and DAPI. **c** Magnification of cell body from the cell shown in b. **d** Neurite reconstruction of cell (b), the gray-dotted line indicates the border of the lateral ventricle (LV). **e** Neurite reconstruction of the cell shown in f, gray-dotted line indicates the border of the LV. **f** Maximal z-projection of a biocytin-filled dLS (Streptavidin-CF633) cell overlaid with a single z-plane of VGAT and DAPI. **g** Magnification of a single z-plane indicating the presence of somatostatin (SOM) cell bodies (arrowheads) only in the dLS. **h** Magnification of a single z-plane indicating the presence of ERα cell bodies (arrows) only in the vLS. **i** Morphological characterization of LS neurons. dLS cells exhibited more neurite branches (two-tailed Student's t-test $t_{(4)}$= 4.841, p=0.0072) and junctions ($t_{(4)}$= 4.849, p=0.0072) longer neurite length ($t_{(4)}$= 4.798, p=0.0078)**,** and wider soma areas (Mann-Whitney test U= 1.00, p=0.0156) than vLS cells. Representative spontaneous current traces during TGOT (1µM) and bicuculline (50µM) bath application in dLS **j** and vLS **l** cells . **k** TGOT increased IPSC frequencies in dLS cells (Wilcoxon Signed Rank test $W_{(9)}$=55, p=0.002) and **m** decreased IPSC frequencies in vLS cells ($W_{(7)}$=-36, p=0.0078). Ventral cells also presented increased FHWM ($W_{(7)}$=30, p=0.0391) TGOT had no effect whatsoever on the amplitude of the IPCS independent on the subregion. Data are shown as mean+SEM. *p<0.05, **p<0.01, vs either vLS or aCSF.

AN INTRINSIC GABAERGIC CIRCUIT WITHIN THE LS REGULATES FEMALE AGGRESSION

After we have shown that i) activation of vLS OXTR increases and dLS V1aR decreases aggression and ii) spontaneous inhibition is increased in dorsal and decreased in ventral cells by OXTR activation, we hypothesized that those subregions would also be differentially activated after an aggressive encounter. Therefore, we assessed neuronal activity in Venus-VGAT GH and IST rats exposed to the FIT using pERK as a neuronal activity marker(De Jong *et al*, 2014; Oyegbile and Marler, 2005; Silva *et al*, 2010).

We found striking regional differences in neuronal activity in the LS of highly aggressive IST females (Figure 3.7a-d). In detail, FIT exposure and the display of high aggression resulted in a decreased total number of both pERK-positive and VGAT/pERK-positive cells in the dLS (Figure 3.7a-b and e), with a negative correlation found between the amount of double-labeled cells in the dLS and total aggression (Figure 3.7f). In contrast, in the vLS of IST females, we found a trend towards more

pERK-positive cells and an increased number of VGAT/pERK positive cells (Figure 3.7c-d and g), which did not correlate with aggression (Figure 3.7h).

To further confirm the dLS as an anti-aggressive center and the vLS as a pro-aggressive center, and consequently to create a causal link between neuronal activity and behavior, we infused rats with the GABA-A agonist muscimol (10ng/0.5µl) 10min prior to the FIT. As predicted, inactivation of the dLS enhanced aggression, threat behavior and the percentage of GH rats showing attacks (Figure 3.7i-j). In contrast, opposing effects were seen in the vLS of IST rats. Muscimol decreased aggression and the percentage of rats showing attacks (Figure 3.7k-l).

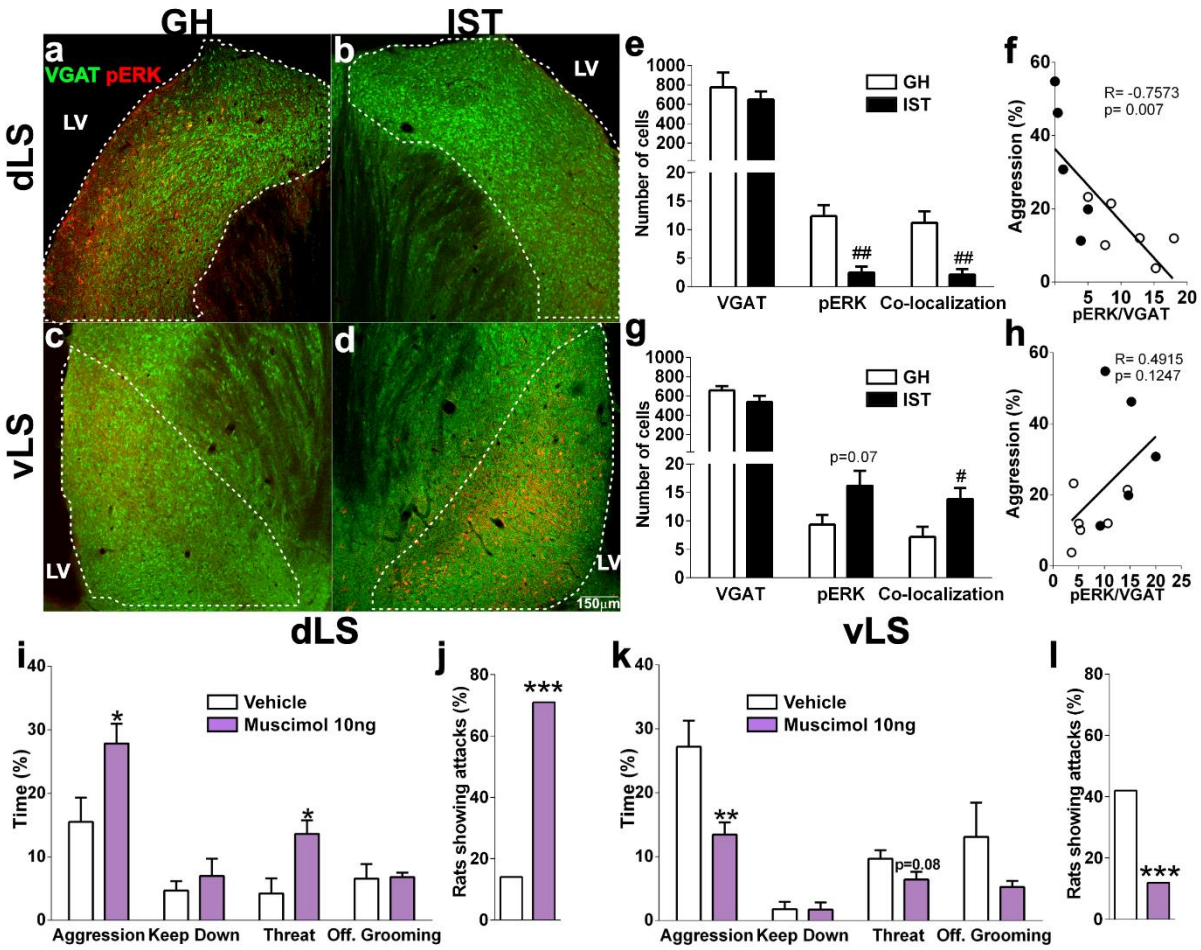

Figure 3.7. An intrinsic GABAergic circuit within the LS regulates female aggression. a-d Example average z-projects showing pERK (Alexa594) immunostaining in Venus-VGAT females after FIT exposure. **a** dLS of a GH female, **b** dLS of an IST female, **c** vLS of a GH female, **d** vLS of an IST female (dLS= dorsal part of the lateral septum; GH=group-housed; IST= isolated and trained; LV=lateal ventricle; VGAT= vesicular GABA transporter; vLS= ventral part of the lateral septum). **e-h** Bar chart depicting quantitative analyses of activation patterns in the LS after FIT. **e** In the dLS of IST females less pERK-positive cells (two-tailed t-Student's test $t_{(9)}$=4.205, p=0.0023) and fewer pERK/VGAT co-localized cells were found ($t_{(9)}$=3.753, p=0.0045). **f** Aggression negatively correlates with the number of pERK/VGAT-positive cells in the dLS (Pearson's correlation r=-0.7459, p=0.0084). **g** In the vLS of IST females a tendency of more pERK-positive cells ($t_{(9)}$=2.069, p=0.0685) and a higher number of pERK/VGAT-positive cells was found ($T_{(9)}$=2.28, p=0.0486). **h** Aggressin does not correlate with the number of pERK/VGAT-positive cells in the vLS (r=0.4915, p=0.1247). **i** Infusion of muscimol (10ng/0.5µl) into the dLS increased total aggression ($t_{(12)}$= 2.515, p=0.0272) and threat (Mann-Whitney test U=5.00, p=0.0111) in GH rats. **j** Inhibition of the dLS also enhanced the percentage of rats showing attacks (Fisher exact test, p<0.0001). **k** Infusion of muscimol into the vLS decreased total aggression ($t_{(13)}$=3.191, p=0.0071) and tended to decrease threat behavior ($t_{(13)}$= 1.832, p=0.0899). **l** Inhibition of the vLS also reduced the percentage of rats showing attacks (p<0.0001). Data are shown as mean+SEM. $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs GH; *p<0.05, **p<0.01, ***p<0.0001 vs vehicle.

3.4. DISCUSSION

Our results demonstrate that a combination of social isolation and aggression training is able to exacerbate the mild levels of aggression displayed by GH female Wistar rats. In addition, female aggression seems to be controlled by a sophisticated mechanism involving differences in GABA neurotransmission as well as a fine-tuning between OXT and AVP neurotransmission in two distinct neuronal populations within the LS.

In highly aggressive IST females, OXT release within the vLS was found to be elevated during the FIT, which was also reflected by higher OXT levels in their CSF. We could also find that aggression levels positively correlated with both OXT release in the vLS and into CSF. Importantly, the involvement of endogenous OXT in female aggression was directly demonstrated by complementary pharmacological, chemogenetic and optogenetic approaches: (i) Pharmacological blockade of OXTR either i.c.v. or locally in the vLS resulted in reduced aggression in IST rats, whereas (ii) either chemogenetically intracerebral or optogenetically intra-vLS induced release of OXT heightened aggression in low aggressive and non-receptive GH females.

The role of OXT in multiple aspects of social behaviors has been established in male rodents (Jurek and Neumann, 2018; Lukas and de Jong, 2017), where OXT is known to exert prosocial (Lukas *et al*, 2011c) and anti-aggressive (Calcagnoli *et al*, 2013) effects. In contrast, OXT actions on female social behavior are less known and have mainly been studied in the context of maternal behavior (Jurek and Neumann, 2018). Differently from males, OXT does not seem to be involved in social motivation and naturally occurring social preference in non-lactating female rats, and OXT was not able to reverse social defeat-induced social avoidance (Lukas and Neumann, 2014). Further, OXTR binding in the central amygdala (CeA) has been negatively

correlated with social interest in female Wistar rats (Dumais *et al*, 2013). In line with our findings, pro-aggressive and/or antisocial effects of OXT have been reported in lactating female rats (Bosch *et al*, 2005), female rhesus monkeys (Jiang and Platt, 2018b), and non-lactating women (Denson *et al*, 2018; Pfundmair *et al*, 2018). It should be pointed out that the motivating factors behind the high aggression found in lactating females may differ from the ones found in non-lactating females as well as the severity of the aggression (Caughey *et al*, 2011; Hashikawa *et al*, 2017, 2018; Newman *et al*, 2019). However, from an evolutive point of view, co-opting the same neuropeptidergic system for promoting aggression in lactating females (to protect the offspring) and in non-lactating females (to fight for resources such as territory and food) makes sense especially knowing that high activity of the brain OXT system with elevated synthesis and release of OXT, as well as OXTR binding (Bosch *et al*, 2005; Caughey *et al*, 2011; Jurek and Neumann, 2018; Lukas and de Jong, 2017) has been shown during pregnancy/lactation. Interestingly, increased OXTR binding in the LS, our region of interest, has been found in highly aggressive lactating females (Caughey *et al*, 2011).

In addition, aggression was influenced by the estrus-cycle especially in IS females and in the chemo and optogenetics induced OXT release experiments. Low aggression has been described in receptive (estrus) Wistar rats (Ho *et al*, 2001) also ovariectomy seems to decrease aggression in aggressive rats and mice (Ho *et al*, 2001; Newman *et al*, 2019). In this context, it is of note that OXTR binding in regions involved in the regulation of aggression differs during the estrus-cycle and after ovariectomy, typically OXTR binding is decreased by low estrogen (Dumais *et al*, 2013). Furthermore, OXT release is affected by sex steroids as shown *in vitro* (Widmer *et al*, 2003) and *in vivo* (Nyuyki *et al*, 2011). Since ERα in the vLS shows similar expression patterns to the one shown by OXTRs, we could hypothesize that estradiol would either act in

interneurons or directly on OXTR expressing neurons to counteract the effects of OXT. Further experiments should focus on unraveling the relation among the estrus-cycle, aggression and the OXT and AVP systems.

Concerning the involvement of the AVP system in female aggression, we could reveal a completely different picture. In comparison to GH, IST females showed blunted AVP release within the dLS during an aggressive encounter, which was also reflected by lower AVP content in the CSF. Also, IST rats exhibited reduced V1aR binding in the dLS, and aggression negatively correlated with V1aR binding. As further proof of AVP's inhibitory effect on female aggression, infusion of AVP either i.c.v. or directly into the dLS was efficient to decrease the high aggression seen in IST rats, whereas blockade of V1aRs in the dLS exacerbated their aggression.

Although several pieces of evidence have reported AVP to exert pro-aggressive effects in males (Ferris *et al*, 1997; Leroy *et al*, 2018; Terranova *et al*, 2016; Veenema *et al*, 2010), conflicting data have been shown in animal models of abnormal aggression, where a blunted AVP system in the LS has been described. For example, LAB rats show decreased AVP release in the LS during the RI (Beiderbeck *et al*, 2007) and short-attack latency mice exhibited decreased AVP innervation of the same region (Compaam *et al*, 1993). Additionally, high aggression displayed by dominant animals seems to be also linked to reduced AVP signaling, i.e. alpha male mice showed decreased V1aR binding in the LS when compared to subordinate males (Lee *et al*, 2019) and synthetic AVP is able to flatten dominance in rhesus macaques (Jiang and Platt, 2018a). In lactating females, the link between AVP and aggression seems to be complex as well, under basal conditions either synthetic or endogenous AVP has been shown to decrease maternal aggression in Sprague-Dawley rats (Nephew *et al*, 2010; Nephew and Bridges, 2008), however, anxiety dependent effects have been also

described in the CeA, where AVP release was associated with maternal aggression only in rats bred for high anxiety-related behavior (Bosch *et al*, 2010). Nevertheless, in accordance with our data, serenic effects of AVP have been shown in non-lactating hamsters (Gutzler *et al*, 2010; Terranova *et al*, 2016) and rhesus macaques (Jiang and Platt, 2018b). In humans, recent studies have also highlighted the prosocial role of AVP as a potential therapeutical drug for social disorders, since intranasal AVP was able to increase risky cooperative behavior in men (Brunnlieb *et al*, 2016) and to enhance social skills in autistic children (Parker *et al*, 2019).

After having identified the specific pro-aggressive effect of OXT in the vLS and anti-aggressive effect of AVP in the dLS, in the final set of experiments we used pharmacology (muscimol) and neural activity (pERK), to show how those two distinct regions in the LS are differently activated by aggression. Dorsal neurons inhibit aggressive behavior whereas ventral neurons seem to promote aggression. Those neurons also responded differently to OXTR activation *in vitro*. TGOT increased inhibitory spontaneous activity dorsally whereas it decreased it ventrally. As the inhibitory currents that were modulated by TGOT were shown to be strictly GABAergic, this indicates that activation of OXTRs located exclusively in the vLS leads to GABAergic inhibition of neurons in the dLS.

The role of the LS in suppressing aggression has been described for decades. However, only one recent paper has shown the existence of local microcircuits within the LS regulating aggression in male mice (Leroy *et al*, 2018). Supporting the pro-aggressive role of AVP in males (Elliott Albers *et al*, 2006; Ferris *et al*, 1997; Terranova *et al*, 2016; Veenema *et al*, 2010), V1b receptor activation on presynaptic terminals of hippocampal fibers to the LS increased aggression via stimulation of inhibitory interneurons in the dLS projecting to the vLS. Although this data contrast with ours we

have to keep in mind that i) we did not access the role of V1bRs in the present study, thereby their effects could possibly differ from the V1aR's effect, ii) V1bR's effect was not shown to be modulated by endogenous AVP release, iii) the OXT and AVP systems as well as their effects on social and aggressive behaviors are known to be sexually dimorphic (DiBenedictis *et al*, 2017; Dumais *et al*, 2013, 2015; Lukas *et al*, 2011c; Lukas and Neumann, 2014; Oliveira *et al*, 2019; Smith *et al*, 2017; Terranova *et al*, 2016), and iv) the distribution of V1aRs and OXTRs in the LS seems to differ in mice(Lee *et al*, 2019) and rats (Smith *et al*, 2017), i.e. in mice V1aR and OXTR distribution appears to overlap what does not occur in rats. Additionally, in our model, activation of V1aRs in the dLS seems to overrule whatever possible pro-aggressive effect of V1bRs activation in any brain region, since AVP administration in the dLS was able to mimic the anti-aggressive effects of i.c.v. AVP in IST females.

Supporting our findings, the antagonistic effects of OXTR and V1aR activation have been described in the context of fear in the CeA. Similar to our data, activation of OXTR-positive cells led to inhibition of V1aR-positive cells via GABAergic transmission, which abolished the behavioral effects of V1aR activation (Huber *et al*, 2005; Knobloch *et al*, 2012). From the best of our knowledge, such a mechanism has never been described before in the context of aggression. Supporting our results showing that high levels of OXT in the vLS leads to inhibition of the dLS via GABAergic projections, the blockade of GABA-A receptors in the LS (dorsally) of lactating mice has been shown to reduce maternal aggression (Lee, Grace and Gammie, 2010). This indicates that aggression in animals with a higher activity of the OXT system (Jurek and Neumann, 2018) is at least partially modulated by increased GABA release in the LS.

Chapter 3: The oxytocin-vasopressin balance within the lateral septum determines aggression levels in virgin female rats

Taken together, our results demonstrate that female aggression is regulated by the fine balance between OXT and AVP within the LS. Thus disturbances in this balance elicit major behavioral consequences, i.e. whenever the balance shifts in the OXT direction, high OXT binding to OXTRs in the vLS, exacerbates aggression whereas a shift in the AVP direction, high AVP binding to V1aRs in the dLS, reduces aggression in female Wistar rats. Accordingly, dorsal neurons in the V1aR expressing area seem to be the main inhibitors of aggression centers such as the HAA, whereas ventral neurons in the OXTR expressing area are the main generators of aggression. We also have shown that activation of OXTRs in the vLS elicits GABA release onto the dorsal cells implying that they are postsynaptic to vLS neurons (Figure 3.8).

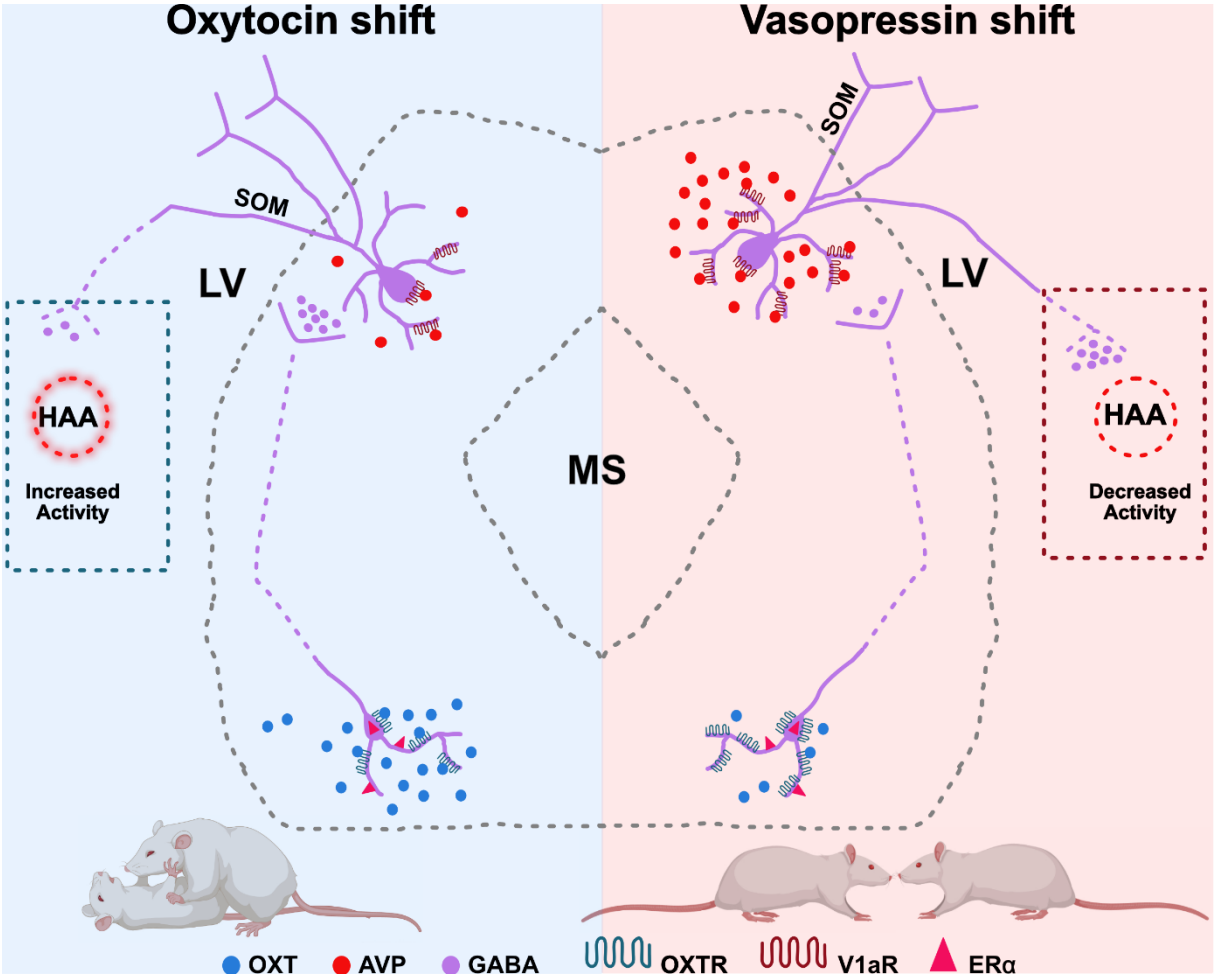

Figure 3.8. The balance between OXT and AVP regulates the inhibitory tonus within the LS in order to control female aggression in Wistar rats. The scheme depicts the main findings of this study (partially drawn using https://biorender.com/). Further, the scheme hypothesizes how alterations in OXT and AVP release may impact a microcircuit in the LS (gray-dotted outline) in order to generate aggressive display in female Wistar rats. Dorsal and ventral cells are shown in proportion to their real size (AVP= vasopressin; ERα= estrogen receptor α; HAA= hypothalamic attack area; LS= lateral septum; LV= lateral ventricle; MS= medial septum; OXT= oxytocin; OXTR= oxytocin receptor; SOM= Somatostatin; V1aR= V1a receptor). On the left, a shift into the OXT direction underlined by high OXT release ventrally and low AVP release dorsally culminate in increased inhibition of dLS (dotted line indicates hypothetical pathway). Inhibition of the dLS probably triggers the disinhibition of aggression centers such as the HAA what leads to female aggression. On the right side, the AVP shift is shown where a combination of low OXT release ventrally and high AVP release dorsally evokes increased activation of the dLS neurons what might trigger an inhibition of the HAA thereby abolishing female aggression.

4.1 General Discussion

Since the neurobiological meaning of our findings has been extensively discussed in the respective chapters, here I am going to focus on the implications, relevance, and perspectives related to those findings. In the present thesis, I introduced two rat models of female aggression. The first one, PWSI was adapted from Toth and Haller (Toth *et al*, 2011) whereas the second one was established based on previous work showing that female aggression in hamsters and Calfornia mice is induced/triggered by social isolation (Borland *et al*, 2019; Ross *et al*, 2019; Silva *et al*, 2010) as well as by winning conflicts (Been *et al*, 2016; Silva *et al*, 2010). Thus, I achieved to mimic different etiologic factors of aggression. First, with PWSI, I was able to show that adverse early life experiences, which are known to trigger maladaptive aggression in humans (Dackis *et al*, 2017; Freitag *et al*, 2018; Glenn *et al*, 2013; Sandi and Haller, 2015) and male Wistar rats (Marquez *et al*, 2013; Toth *et al*, 2011; Veenema *et al*, 2006), are also able to induce exaggerated and abnormal aggression in female Wistar rats. Second, using an ethological approach, I proved that the combination of social isolation and training, used to stimulate territoriality and aggression in males (Koolhaas *et al*, 2013; Miczek *et al*, 2001; Oyegbile and Marler, 2005; Ross *et al*, 2019), elicits offensive behavior towards an unknown same-sex intruder in female Wistar rats, in a RI setting.

Those differences in the etiology of aggression were also reflected in neurobiological, physiological and behavioral contexts. PWSI affects V1aR and OXTR in several brain regions (BNST, LH, DG, and NAcc, respectively) whereas IST affected only those receptors in the LS. In addition, PWSI females showed a hyper-CORT phenotype (Figure 4.1., preliminary data) whereas IST females seem to show a hypo-CORT (Supplementary figure 2) response to the FIT. From a behavioral point of view, those animals also diverged, PWSI induced severer aggressive behavior than IST; i.e.

PWSI females spent more time on threat behavior as well as displayed more attacks than IST females (Figure 4.2. for illustrative porpuses). However, those comparisons should be taken carefully because in the PWSI experiments only diestrus females underwent the FIT, whereas in the IST group we have mixed estrus-cycles. As the estrus cycle has been shown to affect female aggression by this own study and others (Ho *et al*, 2001), we could expect that PWSI rats would also display less aggressive behavior when they are in proestrus or estrus.

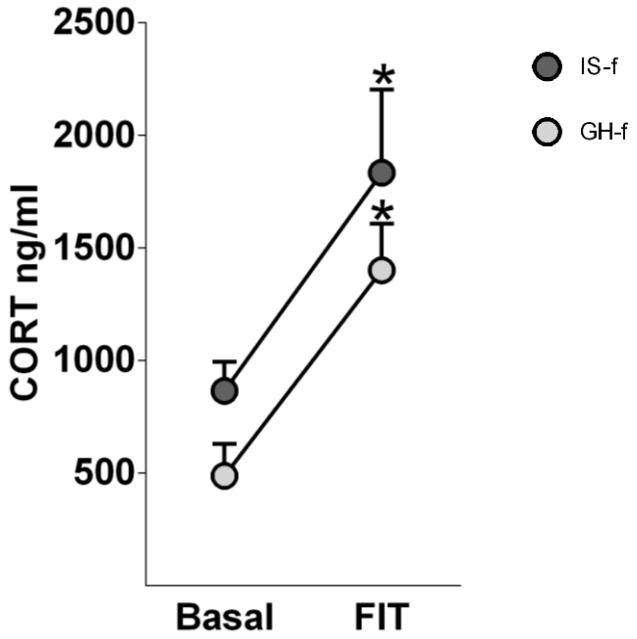

Figure 4.1: Effects of post-weaning social isolation (IS dark grey) or group-housing (GH, light grey) on corticosterone levels in female (f, circles) rats after an exposure to either the female (FIT) (two-way ANOVA followed by Bonferoni's: **Females:** FIT: $F_{(2,17)}$= 16.41, p=0.0009. Data show in Mean+SEM. Effects of the test: * p≤.05.

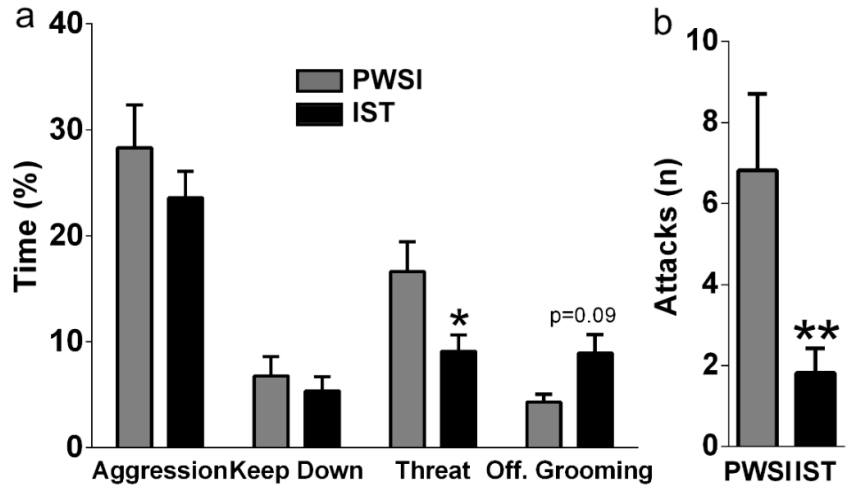

Figure 4.2: Comparison of aggression levels displayed by PWSI and IST female rats. Bar chart showing PWSI females in dark gray and IST females in black. **a** PWSI rats spend more time in threat (Mann-Whitney test U=47, p=0.013) behavior as well as displayed more attacks when compared to IST rats **b** (U=54, p=0.007). Data shown Mean+SEM. * p≤.05; ** p≤.01 vs IST.

Since both models mimic different features of aggressive behavior and show robust effects, scientists may wonder which one of them better emulates female aggression. Although addressing this question is a challenging task I will try to make a comparison between both models. As shown in chapter 2, PWSI is a suitable model to investigate: a) how early life stress events affect aggressive behavior; b) abnormal aggression in females, especially considering the sex-specific effects shown towards juveniles, c) hyper-arousal (hyper-CORT) induced aggression and d) the pharmacology of violent aggression. As a disadvantage I have to mention that those animals may also show impairments in other behavioral domains such as social memory, as shown in chapter 2, sexual behavior (Kercmar et al, 2014) and depressive-like behavior (Shetty and Sadananda, 2017). Although this might seem a synergistic feature due to the fact that aggressive patients also show comorbidities, we should keep in mind that creating a direct causal link in between the alterations induced by PWSI and aggression might be difficult in this context

The isolation and training model is beneficial to a) unravel the neural circuitry involved in territorial aggression of female Wistar rats, by comparing the neurobiology of IST, i.e. highly aggressive, versus GH, i.e, low aggressive rats; b) investigate how different aggression levels are linked to hormone as well as neurotransmission in the brain; c) address whether rats selected for high aggressiveness show impairments in other behavioral domains such as anxiety, depressive-like, sexual and social behaviors; d) manipulate different neurotransmitter, hormones and neural pathways to uncover their role on territorial aggression; and e) test the efficacy of potential serenic drugs.

4.2 OUTLOOK FOR PWSI

In the second chapter, I was able to show that males and females did not differ in their levels of total aggression either in control or PWSI conditions. Nevertheless, sex differences in aggressive behavior display were found, i. e. females exhibited more offensive grooming and aggression towards a juvenile whereas males tended to display more attacks towards vulnerable body parts. Indeed, child maltreatment leading to externalizing and offending behavior in both boys and girls has been reported (Denson *et al*, 2018; Glenn *et al*, 2013; van der Put *et al*, 2015). However, the manner of how aggressive behavior is displayed might change across sex, as in chapter 2. Indeed, women (Colins *et al*, 2016) and girls (Ackermann *et al*, 2019) with psychopathic traits and CD, respectively, exhibited unchanged physical but increased relational aggression, i.e. aggression aiming at harming someone's relationships and social status, when compared to their male counterparts.

Although the reasons behind those qualitative differences in aggressive behavior are still obscure, it is possible to draw a hypothesis in order to explain those findings. As mentioned before, Campbell (1999) has already attempted to explain those differences making use of an evolutive approach, she hypothesized that females will not engage in conflicts to avoid serious harming, because their reproductive fitness does not depend directly on fighting for getting a larger number of sexual partners. We can extend this hypothesis by stating that females would only engage in a conflict when they are certain of winning by facing a "weaker" opponent such as a juvenile, in order to reduce risks of getting injured. Applying Campbell's logic again, a physical injury would mean more energetic costs in terms of reproduction for females due to the fact that they have to invest more energy on pregnancy, lactation and maternal care. However, this changes completely once birth takes place, in that case, going into a new pregnancy would mean higher energetic costs. Therefore females probably

escalate their aggression in order to protect their offspring (Campbell, 1999; Hashikawa *et al*, 2017; Unger *et al*, 2015). Accordingly to this hypothesis, attacking juveniles (weaker opponent) seems to be hard-wired in the virgin female brain since spontaneous aggression towards a juvenile was shown by virgin Swiss-webster females whereas lactating mice showed aggression towards juveniles and adults (Hashikawa *et al*, 2017). Another, point raised by Campbell is that patriarchal culture might have suppressed aggression in women. In laboratory rodents, empirical observations based on breeding guidelines might have endorsed the study of male aggression whereas eclipsed the study of female aggression in virgins, during breeding male's territorial and aggressive behavior, is facilitated by co-housing with a female and subsequent social isolation, this probably lead scientists to use those factors to induce territorial aggression and to focus their research on male rodents (De Almeida *et al*, 2005; Koolhaas *et al*, 1980, 2013; Miczek *et al*, 2001). In females, aggression display has been typically described only during lactation what might have lead scientists to ignore the study of virgin's aggression (Bosch *et al*, 2005; Hashikawa *et al*, 2018; Lukas *et al*, 2011a; Nephew and Bridges, 2008; Newman *et al*, 2019). Interestingly, recent evidence shows that sexual experience also induces aggression in laboratory female mice as it does in males (Newman *et al*, 2019), in addition, wild female rodents such as mice (Miczek *et al*, 2001; Silva *et al*, 2010) and hamsters (Ross *et al*, 2019) seem to show aggressive and dominant behavior just like males.

Future studies should focus on testing these hypotheses by measuring PWSI induced female aggression towards different innocuous stimuli such as anesthetized or much smaller intruders in order to see whether this aggressive behavior is only directed to infants or any harmless stimuli. In addition, it would be interesting to investigate, in a sex-specific context: a) which are the neural correlates of "infant aggression" and b) if those correlates overlap with neural pathways known to regulate

territorial and/or abnormal aggression. Among the regions evaluated in this study, the BNSTa seems like a promising target region as V1aR binding was affected in a sex-dependent manner there, i.e. IS-f exhibited reduced whereas IS-m had enhanced V1aR binding. Since the effects of AVP on aggression have been reported to be sex-specific in hamsters (Terranova *et al*, 2016) and AVP is released in the BNST of low-aggressive male Wistar rats (Veenema *et al*, 2010), we could hypothesize that differential release of AVP within the BNSTa of PWSI males and females would regulate “infant aggression”. In that context, it would be interesting to monitor as well as manipulated AVP release into the BNSTa before and/or during an aggressive encounter in male and female PWSI rats.

Furthermore, several pieces of evidence point towards the NAcc as a promising target region for this type of aggression. Thus, NAcc activity has been associated with: i) abnormal and escalated aggression in rodents (Been *et al*, 2016; Beiderbeck *et al*, 2012), ii) the appetitive (seeking) and consummatory (attack on-set) aspects of aggression (Aleyasin *et al*, 2018; Golden *et al*, 2019) and iii) the establishment of social dominance in rodents (Van Der Kooij *et al*, 2018; Larrieu *et al*, 2017). In addition, OXT release in the NAcc seems to be linked with the rewarding aspects of social interactions (Barrett *et al*, 2015; Bosch *et al*, 2016; Yu *et al*, 2016). PVN OXT neurons are especially active during social interactions with a juvenile in adult male mice (Hung *et al*, 2017). In that context, we could hypothesize that the effects of PWSI on increasing OXT mRNA and decreasing OXTR binding in the NAcc, independently of the sex, would impair the reward component of interacting with a juvenile and turn the social investigation into aggression. Since females under control conditions had increased OXT mRNA in the PVN and OXTR binding in the NAcc when compared to males they could be particularly affected by the reduction in OXTR binding thus showing more aggression towards a juvenile. In line with that scalable control of aggression has been

shown in male mice, where different levels of neural activation of the ERα-positive neurons in the VMHvl triggered contrasting social behaviors, i.e. low activation lead to social investigation whereas high activation culminated in aggression (Lee *et al*, 2014). Further experiments should focus on: a) assessing the activity of OXT neurons in the PVN projecting to the NAcc as well as of the NAcc neurons during infant aggression, b) manipulating OXT neurotransmission before and during infant aggression in the NAcc and c) monitoring OXT release within the NAcc.

4.3 Outlook for the IST protocol

In this section, I will discuss the main open questions regarding Chapter 3 as well as introduce some suggestions for future experiments and new potential targets. In chapter 3, I was able to show that social isolation and aggression training were able to heighten the levels of aggression displayed by female Wistar rats (GH). This effect was accompanied by striking changes in OXT and AVP release which turned out to act on a GABAergic microcircuit within the LS. Specifically, I could show that exacerbated aggression was underlined by increased OXT release within the vLS and decreased AVP release within the dLS.

Concerning this data set, there are still some open questions. For example, how social isolation and training differentially act in OXT and AVP neurons to trigger enhanced and decreased neuropeptide release, respectively. OXT release seems to be mainly affected by isolation since both, IS and IST females show increase OXT levels in their CSF after the FIT. In contrast, AVP release seems to be mainly affected by experience as only IST females showed reduced AVP levels in their CSF and dLS during the FIT. Further experiments should focus on identifying which are the specific plasticity mechanisms (neural activity, synaptic proteins, vesicle trafficking or docking, innervation of target regions) that lead to those differences in neuropeptidergic release. From an AVP perspective it would be especially interesting to know how aggressive

experience (training) changes the presynapse to induce a drop in the release. This type of mechanism associating plasticity with escalated aggression has been described before in the literature, but most of the studies focus on the postsynapse, for example, successive aggressive encounters elicit increased number of spines in the NAcc of female hamsters (Been *et al*, 2016). Thus, understanding which are the presynaptic components of those changes in OXT and, especially, AVP release would add new knowledge to the aggression field. Apart from answering how the protocol induces changes in peptide release, another interesting question to be answered is how IST females react to different social stimuli such as juveniles, young males, anesthetized intruders and ovariectomized females at the behavioral and neurobiological level.

I also showed that the effects of OXT and AVP on aggression are mediated within specific subregions of the LS, namely OXT acted via OXTRs in the vLS whereas AVP acted via V1aR in the dLS. The neurons in these subregions are differently regulated by aggression and OXTR activation. The FIT decreased the activity of GABAergic neurons in the dLS and increased the activity of GABAergic neurons in the vLS. Accordingly, inhibition of those regions differently regulated aggression, i.e. inhibition of the vLS decreased whereas inhibition of the dLS enhanced female aggression. Furthermore, OXTR activation also had opposing effects on GABAergic spontaneous inhibition in the LS, i.e. decreased inhibition in ventral neurons but increased inhibition in dorsal neurons of the LS.

Although we already described in detail how the LS is regulating female aggression there are still some unanswered questions that are worth to be investigated, for example, a) how vLS and dLS cells interact in a physiological and morphological context, this could be evaluated using a combination of tracing, electrophysiological and optogenetic techniques to target the specific neuronal populations; b) how this circuit is wired in males since AVP is known to have pro-aggressive effects in the LS

of male Wistar rats (Veenema *et al*, 2010) and not much is known about how septal OXT affects intermale aggression; c) how this circuit is engaged in other social behaviors known to be LS- as well as OXT- and AVP-dependent such as social discrimination (Lukas *et al*, 2011a; Popik *et al*, 1992).

I also have shown that being receptive, i.e. in the proestrus or estrus phases of the estrus cycle, not only decreases aggression but also abolishes the pro-aggressive effects of endogenous OXT release. Ho and co-workers (2001) have shown that being in estrus decreases aggression display in rats. Also, the surgical removal of the ovaries (ovariectomy) abolishes aggression in mice (Newman *et al*, 2019) and rats (Ho *et al*, 2001). As estrogen and progesterone receptors are known to affect aggression in male (Lee *et al*, 2014; Nakata *et al*, 2016; Sano *et al*, 2016; Yang *et al*, 2013) and female rodents (Hashikawa *et al*, 2017; Spiteri *et al*, 2010) and sex hormones and the estrous cycle are known to modulate OXT release (Nyuyki *et al*, 2011; Widmer *et al*, 2003) and OXTR binding (Dumais *et al*, 2013; Dumais and Veenema, 2016), respectively, we could hypothesize that the effects of the estrous cycle are either mediated directly by affecting vLS neurons, since ERαs are expressed only in this region, or indirectly by regulating OXTR signaling and/or binding. Further experiments should depict this hypothesis and evaluate how sex hormones affect female aggression.

Furthermore, the IST paradigm could also be used to study how other brain regions may engage in the aggressive behavior network during female aggression. Especially, it would make sense to asses how regions that are known to be involved in excessive and pathological aggression in humans such as the amygdala and the PFC (Rosell and Siever, 2015) are affected by the IST protocol. Preliminary data on the CeA acquired during my Ph.D. shows that an aggressive encounter (FIT) enhanced the colocalization of pERK/VGAT cells in IST females, indicating that the CeA is overactivated in aggressive females (Figure 4.3d). In addition, IST females exhibited

increased OXTR but unchanged V1aR binding in the CeA when compared to GH controls (Figure 4.3a-c). In contrast to that synthetic AVP but not TGOT (all at: 0.1ng/0.5µl) increased female aggression in IST rats (Figure 4.3e). Further experiments should focus on a) monitoring OXT and AVP release in the CeA during the FIT, b) manipulating OXT and AVP neurotransmission within the CeA and c) assessing whether those effects are related to the state of anxiety of the animals since the amygdala is known to be strongly involved in anxiety-like behaviors/traits (Calhoon and Tye, 2015; Janak and Tye, 2015) that are known to influence aggressive behavior in rats (Neumann *et al*, 2010).

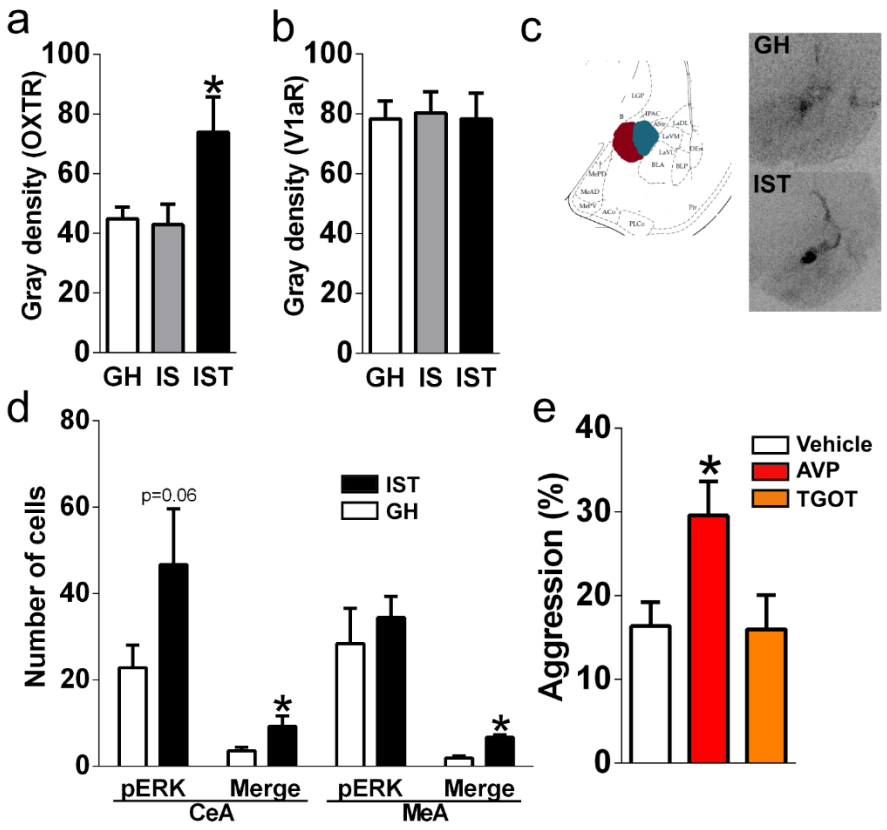

Figure 4.3 Preliminary data on the neuropeptidergic regulation of female aggression within the central amygdala (CeA): a Isolated and trained (IST) but not isolated (IS) females exhibited higher oxytocin receptor (OXTR) binding in the CeA than group-housed (GH) controls (one-way ANOVA followed by Bonferoni's: $F_{(2,19)}$=4.933, p=0.02). **b** no changes were seen in V1a receptor (V1aR) binding independent of the group (Kruskal-Wallis test followed by Dunn's: $H_{(3)}$=4.551, p=0.101). **c** Scheme illustrating localization of OXTR (blue) as well as V1aR (dark red) in the CeA and magnification of the autoradiograph showing example subjects for OXTR binding (upper-right: GH; lower-right: IST). **d** Bar chart depicting quantitative analyses of pERK and VGAT co-localization. IST females tended to have an increased number of pERK-positive cells and showed an increased number of pERK/VGAT cells in the CeA (two-tailed t-Student's test $t_{(9)}$=2.319, p=0.0455). Interestingly, VGAT/pERK cells were also found to be increased in the medial amygdala (MeA) although no differences were seen in total positive-pERK cells ($t_{(10)}$=6.088, p=0.0001). **e** Local infusion of AVP but not TGOT (all at: 0.1ng/0.5µl) enhanced female aggression in IST females ($F_{(2,24)}$=4.534, p=0.02). Data shown in Mean+SEM. * p≤.05 vs either GH or vehicle.

Finally, despite focusing on the resident's perspective the IST protocol allows scientists to study the effects of acute as well as chronic social defeat on females by studying the intruders. Several studies have attempted to assess the effects of social defeat on female psychopathology, however, most of the protocols show practical complications due to the use of either lactating females (Lukas and Neumann, 2014; Newman *et al*, 2019) or optogenetic stimulation of the residents (Takahashi *et al*, 2017). Thus, an ethological and simple protocol such as the IST protocol would be beneficial to understand how being a victim of aggression may affect neurobiology as well as physiology and behavior in a sex-specific manner. From a translational point of view, this is extremely relevant since women are more affected by depression, anxiety and PTSD than men (Laman-Maharg and Trainor, 2017; Wittchen *et al*, 2011), which are

all known to be caused, among other reasons, by social stress (Haller *et al*, 2014; Laman-Maharg and Trainor, 2017; Masis-Calvo *et al*, 2018; Sandi and Haller, 2015)

4.4 CONCLUDING REMARKS

Overall, in the present thesis, I have shown that although aggression may differ qualitatively in male and female Wistar rats, they seem to display similar levels of aggression from a quantitative perspective. Accordingly, the same factors known to stimulate male aggression appear to stimulate female aggression, since PWSI evoked exacerbated aggression in male and female Wistar rats and IST evoked female aggression at comparable levels to intermale aggression. Particularly, PWSI induced abnormal aggression towards juveniles. Regarding the neurobiology, the OXT and AVP systems emerged as important players in female aggression in both models. Especially, using the IST protocol we have unraveled an elaborated mechanism where differential OXT and AVP release within specific regions of the LS triggered changes in GABAergic activation and subsequent neurotransmission to elicit aggression in females.

ABBREVIATION LIST

GH-m
Group-housed males 37, 43, 44, 46
5-HT
Serotonin 20, 21, 32
ACTH
Corticotrophin 23
ADHD
Attention deficit hyperactivity disorder 20
AH
Anterior hypothalamus 15, 25, 27, 29, 32
AOB
Acessory olfactory bulb 15, 23
AR
Androgen receptors 18, 22
ASPD
Antisocial personality disorder 5
AVP
Vasopressin 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, 28, 29, 32, 33, 34, 35, 36, 37, 41, 42, 43, 48, 49, 50, 56, 57, 58, 59, 60, 57, 58, 59, 60, 61, 62, 65, 66, 72, 73, 74, 75, 76, 77, 80, 82, 83, 90, 91, 92, 93, 94, 95, 98, 100, 101, 102, 103, 105
BDNF
Brain derived neurotrophic factor 14
BLA
Basolateral amygdala 35
BNST
Bed nucleus of stria terminalis 7
BNSTa
Bed nucleus of stria terminalis anterior portion 7, 49, 50, 51, 98
CD
Conduct disorder 5
CeA
Central amygdala 17, 25, 27, 49, 51, 91, 93, 94, 103
ChR2
Channelrhodopsin 66, 79, 80
cLS
Lateral septum cauldal nucleus 18
CORT
Corticosterone 10, 12, 13, 14, 21, 34, 93, 95
CRH
Corticotrophin releasing hormone 23
CSF
Cerebrospinal fluid 8, 59, 61, 66, 72, 73, 75, 76, 90, 92, 100, 101
D1
Dompamine receptor 1 21
D2
Dopamine receptor 2 21
DA
Dompamine 20, 21
DG
Dentate gyrus 7
dLS
Dorsal nucleus of the lateral septum 8
DREADD
Designed receptor exclusively actiavated by designed drugs 76
EPM
Elevated plus-maze 39, 47, 48
ERα
Estrogen receptor alpha 17, 22, 32, 69, 85, 99
ERβ
Estrogen receptor beta 22
ESC
Escitalopram 72
ELS
Early Life Stress..6
FIT
Female intruder test 8
GABA
Gamma-aminobutyric acid 8
GH
Group-housed 7, 8, 37, 38, 43, 44, 46, 47, 49, 53, 57, 58, 59, 60, 59, 61, 62, 63, 66, 70, 71, 72, 73, 75, 76, 79, 82, 83, 87, 88, 90, 96, 100, 103
GH-f
Group-housed females 37, 43, 47
HAA
Hypothalamic-attack-area 14, 16, 95
HPA
Hypothalamic-pituitary-axis 13, 21, 22
IL
Infralimbic cortex 49, 51
iLS
Lataral septum intermediate nucleus 18, 26
IS
Isolated 37, 38, 43, 44, 46, 47, 49, 53, 54, 55, 56, 57, 58, 59, 60, 61, 70, 71, 72, 98, 100
IS-f
PWSI females 37, 43, 47, 98
IS-m
PWSI males 37, 43, 44, 47, 98
IST
Isolated and trained 7, 8, 59, 61, 62, 63, 70, 71, 72, 73, 75, 76, 77, 78, 79, 82, 83, 87, 88, 90, 91, 92, 93, 94, 96, 99, 100, 101, 103, 105
LAB
Rats selectively bred for low anxiety-related behavior 18, 19, 21, 29, 58, 92
LH
Lateral hypothalamus 7
LS
Lateral septum 8
MAOA
Monoamine oxidase A 20
MBH
Mediobasal hypothalamus 15
MD
Microdialysis 79

MeA
Medial amygdala 14, 15, 17, 23, 26, 35
MOB
Main olfactory bulb 15, 23
MPOA
Medial pre-optic area 15, 23, 25, 26
MS
Maternal separation 12, 21, 58
NAcc
Nucleus Accumbens 6
NAcca
Anterior nucleus accumbens 49, 51, 52
OFC
Orbital frontal cortex 14
OXT
Oxytocin 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, 28, 29, 33, 34, 35, 36, 37, 41, 42, 43, 48, 49, 50, 53, 56, 57, 58, 60, 57, 58, 59, 60, 61, 62, 66, 72, 73, 74, 75, 76, 77, 78, 79, 80, 83, 90, 91, 93, 94, 95, 99, 100, 101, 102, 103, 105
OXTR
Oxytocin receptor 7
OXTR-A
OXTR antagonist 28, 66, 75, 76, 79, 100
Oxytocin receptor antagonist 8
PAG
Periaqueductal gray matter 15
pBNST
Bed nucleus of stria terminalis posterior part 26, 27
pERK
Phosporilated extracellular signal-regulated kinase 8
PFC
Prefrontal cortex 14, 17, 18, 35, 103
PPS
Peripubertal stress 12, 21
PR
Progesterone receptor 17
PrL
Prelimbic cortex 49, 51
PTSD
Post-traumatic stress disorder 9, 10, 33, 105
PVN
paraventricular nucleus of the hypothalamus 6
Paraventricular nucleus of the hypothalamus 6, 23, 24, 27, 36, 42, 48, 50, 56, 57, 62, 64, 66, 76, 79, 80, 99
PWSI
Post-weaning social isolation 6
rAAV
adenoassociated virus 76, 79
RI
Resident-intruder test 11, 14, 16, 18, 21, 27, 28, 29, 59, 92, 93
RIT
Resident intruder test 35, 37, 38, 44, 46, 54, 55, 57, 58, 59
rLS
Lateral septum rostral nucleus 18
SCN
Suprachiasmatic nucleus of the hypothalamus 23
SON
Supraoptic nucleus of the hypothalamus 23, 24, 36, 42, 48, 50, 63, 65, 66, 76, 79, 80
SSRI
Serotonin selective re-uptake inhibitor 32, 72
TGOT
[Thr4,Gly7]OXT 28, 66, 83, 85, 86, 93, 103
V1aR
V1a receptor 7
V1aR-A
V1aR antagonist 66, 75, 83
vLS
Vental nucleus of the lateral septum 8
VMH
Ventromedial hypothalamus 15, 25
VMHvl
Ventromedial hypothalamus ventrolateral portion 17, 19, 32, 99
WTG
Wild-Type Groningen rats 20, 27, 28

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