Effects of mating on behavioral, neuroendocrine and neuronal responses to stressful stimuli in male and female Wistar rats: involvement of brain oxytocin

DISSERTATION

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Diesmal also nur für dich

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Sexual activity reduces acute stress-induced hypothalamic *c-fos* and CRH mRNA expression without affecting the hormonal stress response in male rats

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Martin Waldherr, Sandra Bäuml and Inga D. Neumann

Influence of hormone treatment and mating on anxiety-related behavior and central oxytocin release in female rats

(in preparation)

DATA! DATA! DATA! HE CRIED IMPATIENTLY.
I CAN'T MAKE BRICKS WITHOUT CLAY.
SHERLOCK HOLMES

Contents

I General Introduction

1 Introduction

Sexual activity and mating are the most essential interactions between females and males of sexually dimorphic species. The beneficial effects of sexual behavior, aside from reproduction, and its impact on the organism's response to environmental stress are just starting to be investigated in humans and animal models alike.

For a long time, the undisputed purpose of sexual behavior has been seen in the maintenance of the species. Sexual activity itself is a strong reinforcing stimulus, inducing a state of reward and thereby motivating the animal to seek out mating partners and engage in sexual activity (Agmo 1999). While these two facts are sufficient to explain the occurrence of sexual interactions from the evolutionary (reproduction) and the individual's (reward) point of view, the literature on various other positive individual consequences of sexual behavior is slowly emerging over the past two decades. Besides reproduction and acute pleasurable, rewarding effects, what are the benefits of engaging in sexual behavior for the individual, and how are these positive implications of mating mediated in the mammalian brain? Further investigation of this topic, in particular the effects of sexual activity on the response to stressful stimuli in male and female rats, is the main aim of this thesis.

In humans, sedation and calmness are common phenomena in the post-coital period. Recent studies delivered evidence, for example, for a decreased responsiveness to acute stressors and lower basal levels of stress-related hormones after sexual intercourse (Brody 2006) and intimacy between couples (Ditzen et al. 2008a). Similarly, in rats, the impact of a stressful situation has been reported to be less severe in previously mated animals (Fernandez-Guasti et al. 1989; Martinez-Mota et al. 2005). These positive effects of mating might not be the primary incentive for a

mammal to engage in sexual activity, but they are nevertheless an interesting and important consequence.

In the following, I shortly explain some of the key regulators of sexual behavior, with the main focus on the neuropeptide oxytocin (OT), as it is also implicated in conveying proximate or distal beneficial effects of mating. Secondly, a brief overview of the changes in hormone secretion and brain activation patterns induced by sexual behavior will be given, including a summary of mating-induced positive behavioral and neuroendocrine adaptations. Finally, the mating patterns of female and male rats and the terms used to describe and evaluate these behaviors are explained.

2 Regulation of sexual activity and mating in mammals

There are several hormones and neurotransmitters that are of major importance in the control of sexual activity. Detailed knowledge of their functions will in turn grant the basis for understanding the beneficial effects of sexual activity investigated in the course of this thesis.

2.1 The role of gonadal and adrenal hormones

The hypothalamo-pituitary-gonadal (HPG, Fig. 1) and -adrenal (HPA, Fig. 2) axes are important regulators of sexual behavior. However, their role in this context is not entirely clear, as they can exert facilitative or inhibitory actions on the expression of sexual behavior. It is undisputed that the HPG axis provides the physiological basis of sexual behavior, as its main agonists, estradiol (E) and progesterone (P) in females (Bale et al. 1995; Frye 2001), and testosterone (T) in males (Christensen

and Clemens 1974), control the development and continuous function of many primary and secondary sexual characteristics, e.g. ovulation and spermatogenesis. Generally, these steroids are involved in 'preparing' the organism for sexual behavior, for example by up-regulating OT receptor (OTR) expression in the ventromedial hypothalamus (VMH) (Bale et al. 1995), or by facilitation of dopamine (DA) release in the medial preoptic area (MPOA) (Putnam et al. 2005). Surprisingly, the role of the HPG axis for the display of sexual behavior is still unclear. In early studies undertaken in the 1970s (Moss and McCann 1973; Pfaff 1973), the starting point of this axis, gonadotropin-releasing hormone (GnRH), which is synthesized in the hypothalamus, has been found to be an important central agonistic factor inducing sexual behavior in females and males. However, soon thereafter, studies in rats and monkeys failed to find enhancing effects of GnRH on sexual behavior (Ryan and Frankel 1978; Phoenix and Chambers 1990) or even found inhibiting properties (Dorsa et al. 1981). Strong evidence suggesting that the HPG axis is vital for reproductive functions in general, but not for sexual behavior *per se*, could be gained from a natural knockout mouse model (Gibson et al. 1997). Bearing a specific deletion in the GnRH gene, which results in a lack of GnRH peptide in the brain, treatment with GnRH is necessary in these mice for the development of reproductive tissues, but once adult, male and female mice show sexual behavior in the absence of GnRH and a functional HPG-axis (Gibson et al. 1997). Additionally, there is also evidence from human and other primate studies, demonstrating that low levels of gonadal activity do not inhibit sexual behavior (Bancroft and Wu 1983; Kwan et al. 1983; Wallen 1990).

The HPA axis is activated when an individual is exposed to various kinds of stressors (Harbuz and Lightman 1992). Stressor exposure leads to an activation of parvocellular neurons within the hypothalamic paraventricular nucleus (PVN), where

corticotropin releasing hormone (CRH) and arginine vasopressin (VP) are synthesized. Both peptides are released from axon terminals into the hypophyseal portal circulation and stimulate corticotrophe cells in the anterior pituitary. As a result, adrenocorticotropic hormone (ACTH) is released into the bloodstream and stimulates the synthesis and release of glucocorticoids from the adrenal cortex.

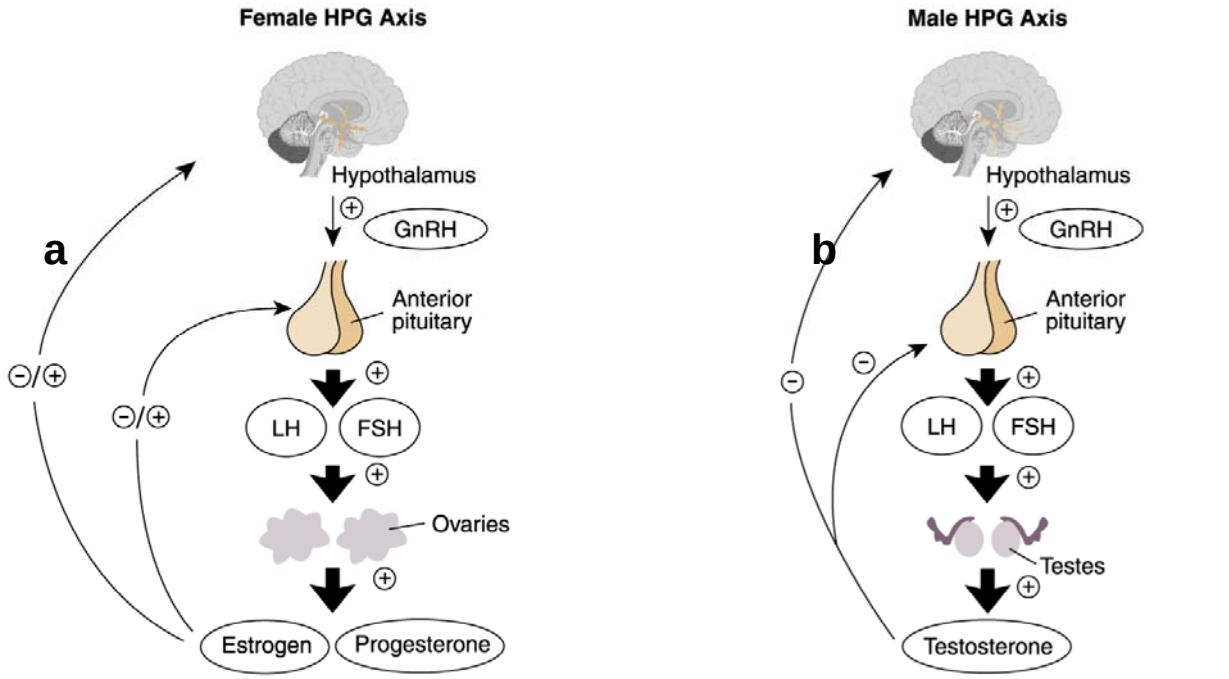

Fig. 1: Schematic drawing of the key components of the HPG axis. GnRH is synthesized in the parvocellular part of the PVN, released into the portal blood system in the median eminence and transported to the anterior pituitary. Here, luteinizing (LH) and follicle stimulating hormone (FSH) are released into the systemic blood circulation. In females, the ovaries respond primarily by releasing estrogens and progesterone (**a**) while in males the testes release testosterone (**b**). These steroid hormones can pass the blood-brain barrier and exert negative or positive feedback on the hypothalamus and pituitary (adapted from Hiller-Sturmhofel and Bartke 1998).

Stress has been shown to have a negative impact on sexual activity throughout several mammalian species, including mice, rats, monkeys and humans (Keverne 1978; Menendez-Patterson et al. 1980; Sapolsky 1982; Sugiura et al. 1997; Gorzalka et al. 1998; Amikishieva and Ovsyukova 2003; Retana-Marquez et al. 2003; Bodenmann et al. 2006; Ter Kuile et al. 2007). This is mainly due to the fact that increased release of glucocorticoids from the adrenals strongly inhibits the HPG axis (Collu et al. 1984).

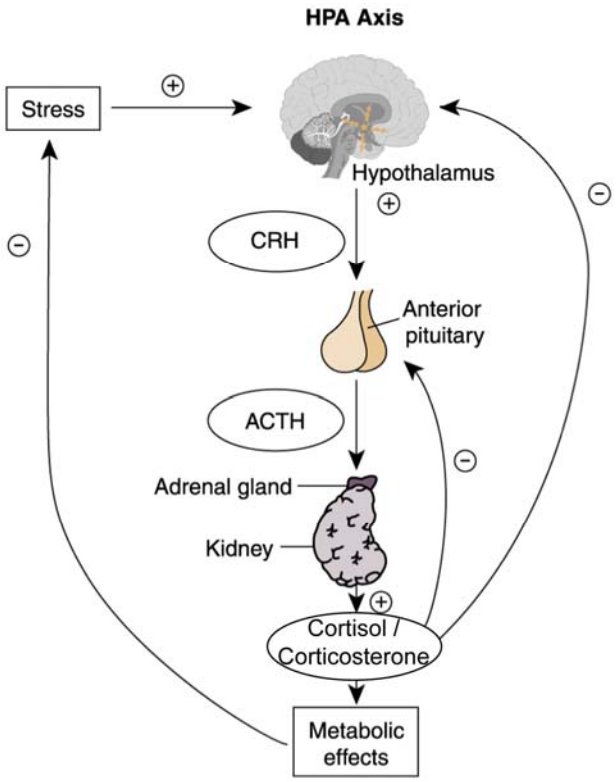

Fig. 2: Schematic drawing of the key components of the HPA axis. CRH is synthesized in parvocellular parts of the PVN, released into the portal blood system in the median eminence and transported to the anterior pituitary. Here, ACTH is released into the bloodstream and stimulates the release of cortisol (humans) or corticosterone (rats) from the adrenal cortex. These corticosteroids can pass the blood-brain barrier and, aside from their metabolic effects, exert negative feedback on the hypothalamus and pituitary (adapted from Hiller-Sturmhofel and Bartke 1998).

However, the HPA axis is highly activated during sexual activity (Szechtman et al. 1974; Craigen and Bronson 1982; Orchinik et al. 1988; Borg et al. 1991; Colborn et al. 1991; Retana-Marquez et al. 1998; Bonilla-Jaime et al. 2006) and does not inhibit the

release of gonadal hormones under these circumstances. In this context, glucocorticoid release is likely to facilitate the high level of arousal and physical activity during mating (Bonilla-Jaime et al. 2006).

In addition to the peripheral hormonal response, exposure to a given stressor elicits a behavioral and emotional response. This central stress reactivity is, at least partly, mediated by CRH. The neuropeptide CRH has not only been proven to trigger ACTH release from the pituitary, but also to play a major role in the promotion of anxiety- and depression-related behaviors in animal and human studies (Nemeroff et al. 1984; Bale and Vale 2004; Holsboer and Ising 2008) and to suppress sexual behavior on a central level in male and female rats (Sirinathsinghji 1986; 1987).

To summarize, the HPG axis facilitates sexual activity by providing the appropriate hormonal background and mediating the development of reproductive tissues, whereas an activated HPA axis acts mostly inhibitory on the expression of sexual behavior.

2.2 The role of centrally released neuropeptides and neurotransmitters

The number of neuroactive substances implicated in the central control of sexual activity is growing continuously (Table 1). Most of these transmitters have been intensively investigated and their role in sexual behavior is well documented (Bertolini and Gessa 1981; Pfaus and Gorzalka 1987; Carter 1992; Argiolas 1999). It has been shown that some of these transmitters influence physical components of sexual behavior like erections, but not emotional and motivational aspects that are of major interest for the work presented here. For example, ACTH, the pituitary hormone that stimulates corticosteroid release from the adrenals, and α-melanocyte stimulating

hormone (α - MSH), which is mainly responsible for skin pigmentation, are described as facilitating the physical requirements of sexual performance but not arousal (Bertolini and Gessa 1981). They act pro-erectile in male mammals (Bertolini et al. 1969; Spruijt et al. 1985) and increase lordosis (reflexive arching of the back to allow the male to copulate) in female rats (Thody et al. 1981), but fail to increase sexual behavior in male rats with low basal levels of sexual activity (Bertolini et al. 1975). The complexity of sexual behavior becomes clear from the number of different transmitters involved in its control, as listed in Table 1. However, here I will mainly focus on the role of OT.

2.3 Brain oxytocin: an important regulator of reproductive and sexual functions

The neuropeptide OT and its central actions in the control of sexual activity are of direct interest for the present work. OT is a well-acknowledged neuromodulator/ neurotransmitter of the brain regulating emotionality, stress coping, and prosocial behaviors (Insel and Young 2001; Landgraf and Neumann 2004; Choleris et al. 2007). In female rats, high activity of the brain OT system has been linked to the fine-tuned regulation of sexual behavior (Bale et al. 2001), parturition and milk letdown (Russell et al. 2003) and the performance of maternal behaviors (Pedersen et al. 1982; Pedersen et al. 1988; Insel and Young 2001). Furthermore, OT has been implicated in the low stress response characteristic for the peripartum period (Windle et al. 1997; Neumann et al. 2000b). Therefore, it has been viewed as a ‘female’ hormone and neuropeptide for a long time, as stated in 1987 by Murphy and colleagues: ”although men and women have similar concentrations of

immunoreactive and bioactive OT in the circulation, cerebrospinal fluid and hypothalamus, OT has *no known function* in men" (Murphy et al. 1987).

The role of OT in the control of mating behavior has been studied extensively in the last few years in both female and male rats (Flanagan et al. 1993; Witt and Insel 1994; Blaicher et al. 1999; Pedersen and Boccia 2002; Argiolas and Melis 2004; Pedersen and Boccia 2006; Succu et al. 2008). First evidence for the major importance of central actions of OT in the expression of female and male sexual behavior was delivered by intracerebroventricular (icv) injections of OT and OTR antagonists (OT-A), which were shown to facilitate and inhibit sexual behavior, respectively (Arletti et al. 1985; Arletti and Bertolini 1985; Caldwell et al. 1986; Argiolas et al. 1988).

Since then, the main sites of stimulatory OT action during sexual activity have been localized in the hypothalamus, specifically the VMH in female (Bale et al. 2001) and the PVN in male rats (Argiolas and Melis 2004) (Fig. 3). In female rats, OTR expression is increased 200-fold in the VMH during proestrus, the receptive phase of the cycle (Bale et al. 1995) and local injection of OT into the VMH facilitates sexual behavior (Bale et al. 2001). Interestingly, the closely related neuropeptide VP inhibits the facilitatory actions of OT when applied centrally in female rats (Pedersen and Boccia 2006). In male rats, OT-containing neurons are activated during sexual activity (Witt and Insel 1994), OT injection into the PVN induces penile erections (Melis et al. 1986), and lesions of the PVN strongly impair erections and sexual behavior (Ackerman et al. 1997; Liu et al. 1997).

Table 1: Neuropeptides and -transmitters implicated in the control of sexual behavior in rats.

Neuropeptide / Neurotransmitter	Effect on ♂ sexual behavior	Effect on ♀ sexual behavior	Main sites of action	References
ACTH / α - MSH	Facilitative	Facilitative	MPOA / VMH / CG	(Argiolas 1999; Drago et al. 1999)
CRH	Inhibitory	Inhibitory	PVN	(Argiolas 1999)
GNRH / LH	Facilitative / Inhibitory / No Effect	Facilitative / Inhibitory / No Effect	Not localized	(Stoleru et al. 1993; Argiolas 1999)
T	Facilitative	Facilitative	VMH	(Christensen and Clemens 1974; Tribollet et al. 1990; Putnam et al. 2005)
P	Facilitative	Facilitative	MPOA / VMH / LS / BNST / MeA	(Frye et al. 2006; Frye and Walf 2008)
E	Facilitative	Facilitative	MPOA / VMH / LS / BNST / MeA	(Christensen and Clemens 1974; De Kloet et al. 1985; Tribollet et al. 1990)
Excitatory amino acids (glutamic, aspartic)	Facilitative	---	PVN	(Melis et al. 2004)
GABA	Inhibitory	---	PVN	(Frye and Walf 2008)
NPY	Inhibitory	Inhibitory	MPOA	(Argiolas 1999)
Substance P	Facilitative	Facilitative	CG	(Argiolas 1999)
Opioids	Inhibitory	Inhibitory	PVN / MPOA / VMH	(Argiolas 1999)
OT	Facilitative	Facilitative	PVN / VTA / VMH	(Tribollet et al. 1990; Stoleru et al. 1993; Bale et al. 1995)
Serotonin	---	Inhibitory	MPOA / NAcc / AMY	(de Jong et al. 2006)
DA	Facilitative	Facilitative	PVN / NAcc	(Melis et al. 2003; Putnam et al. 2005)
NO	Facilitative	---	PVN	(Melis et al. 1998)

Abbreviations: ACTH / MSH / GNRH = adrenocorticotropic / melanocyte stimulating / gonadotropin releasing hormone, LH = Luteinizing hormone, T = testosterone, P = progesterone, E = estradiol, GABA = gamma-amminobutyric acid, NPY = neuropeptides Y, OT = oxytocin, DA = dopamine, NO = nitric oxide, MPOA = medial preoptic area, VMH = ventromedial hypothalamus, CG = central grey, PVN = nucleus paraventricularis, LS = lateral septum, BNST = bed nucleus of the stria terminalis, MeA = medial amygdala, AMY = amygdala;

OT transmission in the PVN and sexual activity are concomitantly activated by local DA release in the PVN (Melis et al. 2003) and in turn, OT infusion into the PVN stimulates the mesolimbic DA system (Melis et al. 2007), which mediates the reinforcing effects of natural rewards.

The complex regulatory functions of OT in the context of both emotionality as well as sexual activity implicate that OT is not only of major importance for the acute display of sexual behavior, but may also mediate subsequent effects of mating on the organism.

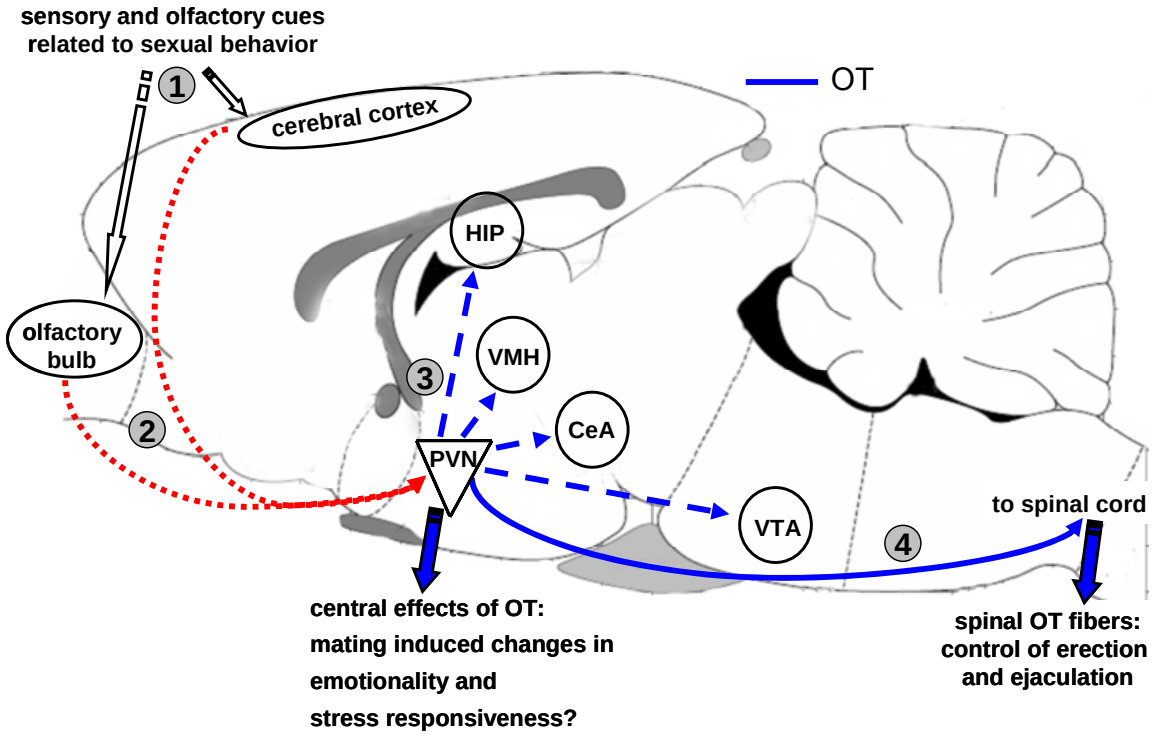

Fig. 3: OT pathways mediating central and peripheral responses to sexually related cues and sexual stimulation in male rats. ①and ②Environmental cues are gated in the cerebral cortex and olfactory bulb and, among other regions, lead to an activation of the PVN. ③In the PVN, OT-containing neurons that project to brain regions implicated in emotional responses show increased activity after mating. ④Activation of oxytocinergic neurons projecting to the spinal cord induces penile erections and facilitates ejaculation. (VTA = ventral tegmental area, CeA = central amygdala, VMH = ventromedial hypothalamus, HIP = hippocampus) (adopted from Argiolas and Melis 2004).

3 Neuroendocrine consequences of sexual behavior

3.1 Changes in HPG and HPA axis activity

The endocrine response to sexual activity and mating has been studied in many species, including rats (Kamel et al. 1977), rabbits (Agmo 1976), pigs (Borg et al. 1991), horses (Rabb et al. 1989) and, more recently, also humans (Kruger et al. 2003). Results are heterogenic between species and the importance of changes in hormonal release during and after sexual activity is poorly understood in most cases. In male rats, sexual activity has been shown to activate both the HPG (Kamel et al. 1975; Kamel et al. 1977) and the HPA axes (Szechtman et al. 1974; Retana-Marquez et al. 1998). Activation of the HPG axis leads to increased release of T from the gonads, which can be observed not only after mating, but also during the mere presence of a receptive female (Bonilla-Jaime et al. 2006) or the anticipation of sexual activity (Alexander et al. 1994). These findings indicate an important role of T not only in assuring the physiological basis for copulation, but also in mediating central aspects of sexual activity, for example sexual arousal and motivation. This is confirmed by studies in male rats showing that T stimulates DA release in the MPOA and thereby decreases ejaculation latency, which is indicative of a high level of arousal (Putnam et al. 2005).

The acute activation of the HPA axis during sexual activity leads to increased plasma levels of glucocorticoids and is likely due to physical activity and general arousal, as studied in amphibians, bulls, stallions, rats and mice (Szechtman et al. 1974; Craigen and Bronson 1982; Orchinik et al. 1988; Borg et al. 1991; Colborn et al. 1991; Retana-Marquez et al. 1998; Bonilla-Jaime et al. 2006). In this context, corticosterone facilitates the high level of arousal and physical activity during mating (Bonilla-Jaime et al. 2006).

However, it is not clear if sexual activity has long-term effects on HPA axis function and stress responsiveness. Mated male rats show an attenuated response to an emotional and physical stressful situations, i.e. less depressive-like behavior in the forced swim test (Martinez-Mota et al. 2005), but the reactivity of the HPA axis after sexual activity has not been studied so far.

In humans, data on both hormone systems is inconsistent. HPG and HPA axis activity has been shown to be either unaffected (Lee et al. 1974; Rowland et al. 1987; Carani et al. 1990; Kruger et al. 2003) or activated (Lincoln 1974; Purvis et al. 1976; Stoleru et al. 1993) by sexual activity. It is very likely that these discrepancies can be attributed to the type of stressor (Schedlowski et al. 1992), as well as general factors like circadian variation in hormonal release patterns (Richter et al. 1996).

3.2 Activation of distinct brain regions

In the early 1990s, the first studies describing activation of distinct brain regions by sexual activity in rats and Syrian hamsters were published (Robertson et al. 1991; Baum and Everitt 1992; Kollack and Newman 1992; Tetel et al. 1992). This was made possible by the developing technique of mapping the neuronal expression of the immediate early gene product c-Fos or its mRNA (*c-fos*). This allows the visualization of functional pathways in the brain, as *c-fos* transcription and c-Fos synthesis are elevated during neuronal activity (Hunt et al. 1987; Sagar et al. 1988; Morgan and Curran 1989; Robertson et al. 1991). These studies showed increased neuronal activity in the MPOA, medial amygdala (MeA), bed nucleus of the stria terminalis (BNST), VMH, VTA, NAcc and PVN (Robertson et al. 1991; Baum and Everitt 1992; Kollack and Newman 1992; Tetel et al. 1992). The MPOA and MeA are directly influenced by gonadal hormone release during mating (Greco et al. 1996; Greco et al. 1998) and control behavioral patterns of lordosis in female and mounting

in male rats (Meisel and Joppa 1994; Pfaus et al. 1996; Dominguez et al. 2007). The BNST is an important relay between sensory inputs and brain regions like the hypothalamus (Scalia and Winans 1975; Pfaff and Conrad 1978). The VMH is critical for the expression of pro- and receptive behaviors (Pfaus et al. 1996) and the main site for OT action in the context of female sexual behavior (Bale et al. 1995; Bale et al. 2001). Increased neuronal activity in the VTA and NAcc triggered by mating could recently be attributed to activated dopaminergic neurons in the VTA (Balfour et al. 2004) and release of DA in the NAcc (Melis et al. 2007) in the context of motivational and rewarding aspects of sexual behavior (Paredes and Agmo 2004). Finally, activation of the PVN during sexual behavior could be linked to OT-containing neurons in female (Flanagan et al. 1993) and male (Witt and Insel 1994) rats. These neurons were identified as parvocellular rather than magnocellular, pointing towards the importance of central oxytocinergic projections in the control of female and male sexual behavior (Flanagan et al. 1993; Witt and Insel 1994), as discussed in detail above. However, the central release of OT in the context of sexual activity could not be measured so far.

Human studies mapping brain activation patterns during sexual activity are still in the early stages of development. Functional magnetic resonance imaging (fMRI) requires fixation of the person being studied, which limits the participant's possibilities to engage in sexual activity. Studies using erotic video clips as a visual stimulus have found activation during sexual arousal in the amygdala and hypothalamus in men and women (Stoleru et al. 1999; Arnow et al. 2002; Karama et al. 2002). More recent studies on sexually stimulated participants (Holstege et al. 2003; Komisaruk et al. 2004) showed increased activity in the MeA and PVN in women (Komisaruk et al. 2004) and in the VTA in men (Holstege et al. 2003), partially confirming the results of animal studies (Fig. 4). However, these studies should best be seen as first glimpses

into the neuronal sexual response of humans, as is not clear how well these results are comparable to brain activation during sexual intercourse under naturalistic conditions.

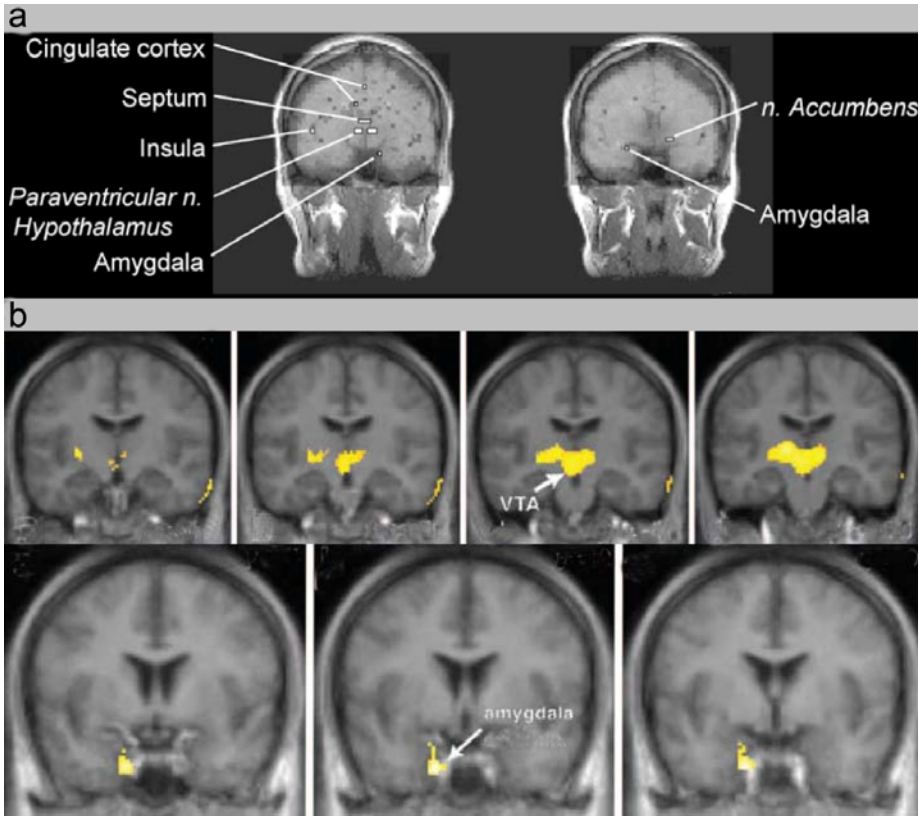

Fig. 4: Brain activation during sexual arousal and orgasm in humans. (**a**) Functional magnetic resonance images (fMRI) of activated forebrain regions at the time point of orgasm in a woman, showing increased activity in several regions, for example the amygdala and the hypothalamic paraventricular nucleus. (**b**) Positron emission tomography (PET) fMRI of a man's brain at the time point of orgasm. The upper panel shows a strong activation of the VTA, while the lower panel depicts a sharp drop of activity in the amygdala at the time point of ejaculation (adopted from Holstege et al. 2003; Komisaruk et al. 2004).

3.3 Beneficial consequences of sexual activity

In humans the postcoital period is associated with feelings of sedation and calmness. Interestingly, similar behavioral patterns of sedation and reduced activity can be observed in male rats after mating (Karen and Barfield 1975). While scientists have been studying the postcoital interval in rats to reveal the underlying mechanisms of

male refractoriness (for example Rodriguez-Manzo and Fernandez-Guasti 1994; Mas et al. 1995), studies dealing with the influence of sexual activity on subsequent behavioral parameters are scarce. In male rats, there is an early report on reduced defensive burying behavior after ejaculation, interpreted as an anxiolytic effect (Fernandez-Guasti et al. 1989). This finding was followed by the proof that the observed behavior cannot be explained by an analgesic effects of mating (Fernandez-Guasti and Saldivar 1991; Saldivar-Gonzalez and Fernandez-Guasti 1994). Furthermore, reduced depression-like behavior and a higher sensibility to antidepressant drugs was found in the forced-swimming paradigm after sexual activity in male rats (Fernandez-Guasti and Martinez-Mota 2005). Recent work by Bai and colleagues demonstrates that mating reduces the contextual fear memory of male rats (Bai et al. 2008). Here, rats exposed to a learned aversive stimulus show a reduced fear response, i.e. startle or freezing, if they are allowed to mate after the conditioning procedure (Bai et al. 2008). Recently, it has been reported in the African mole rat *Heterocephalus glaber* that sexual activity delays the process of aging and dramatically increases the lifespan of breeders in both sexes (Dammann and Burda 2006). In humans, there is only little neurobiological evidence for positive effects of sexual behavior. There are several studies stating, for example, that higher rates of sexual intercourse lower the risk of mortality (Davey Smith et al. 1997) or decrease weight gain (Brody and Kruger 2008), but there is few evidence for changes in brain activity or stress coping strategies as a result of sexual activity. One study demonstrates altered stress-reactivity in people with regular sexual intercourse, as they show reduced blood pressure reactivity to stressor exposure in comparison to control groups (Brody 2006). Heinrichs and colleagues could demonstrate that in women and men, social support by the partner reduces the hormonal stress response (Ditzen et al. 2007; Ditzen et al. 2008c). Based on these results, they

recently showed that intimacy between couples, which includes sexual intercourse, results in reduced salivary cortisol levels in both partners (Ditzen et al. 2008a), adding strong support to the assumption that sexual behavior positively affects stress reactivity in humans.

4 Sexual behavior in male and female rats

The expression of sexual behavior has diverse manifestations in different species. In rats, sexual activity can be described by a pattern of behavioral elements. These behavioral patterns and the terms used in describing them will be shortly explained in the following.

4.1 Male sexual behavior

At first contact, the male will quickly try to gain olfactory information about the hormonal state of the female by anogenital investigation, as the female will only mate during estrus, when pregnancy can be induced (Hull et al. 2002). If the female is receptive (and sometimes also if she is not) the male will display a fixed set of behaviors (Fig. 5).

- Mounting: While the female stays immobile, the male lifts his forebody over her hind quarters and begins a series of shallow thrusts with his pelvis. This somatosensory contact facilitates penile erections (Fig. 5 a).
- Intromitting: While every mount is an attempt to achieve intromissions (IM), its occurrence depends on how fast the male detects the vagina when thrusting and the intensity of the female's lordosis posture (Fig. 5 b). If IM is achieved, the male will display an intravaginal thrust, followed by a springing dismount and genital

licking and grooming. I preferably measured IM as indicator of sexual activity, as it is clearly discernible from mounting and includes ejaculations.

- Ejaculation: After several IM the male will show a longer thrust, lifting his forebody and releasing his grasp on the female (Fig. 5 c). Ideally, the female will move away from the male in this situation (Fig. 5 d), as opposed to IM. Ejaculations are followed by a refractory period of 3 to 5 min, with the male grooming himself and being unresponsive to further sexual stimulation.

It is interesting to note that all parts of sexual behavior in male rats are influenced by experience and learning. Sexually naive males can be ‘clumsy’ at times and even a receptive female may deny IM (McClintock and Adler 1978). The latencies until first mount, IM and ejaculation are decreased with experience in male rats (Sachs and Barfield 1970). Sexual satiety is reached after 7 or 8 ejaculations, at which point only an unknown female can elicit further sexual activity, known as the “Coolidge effect” (Hull et al. 2002). After approximately 4 h of mating the male will be exhausted and stay unresponsive for several days (Beach and Jordan 1956; Agmo 1997; Hull et al. 2002).

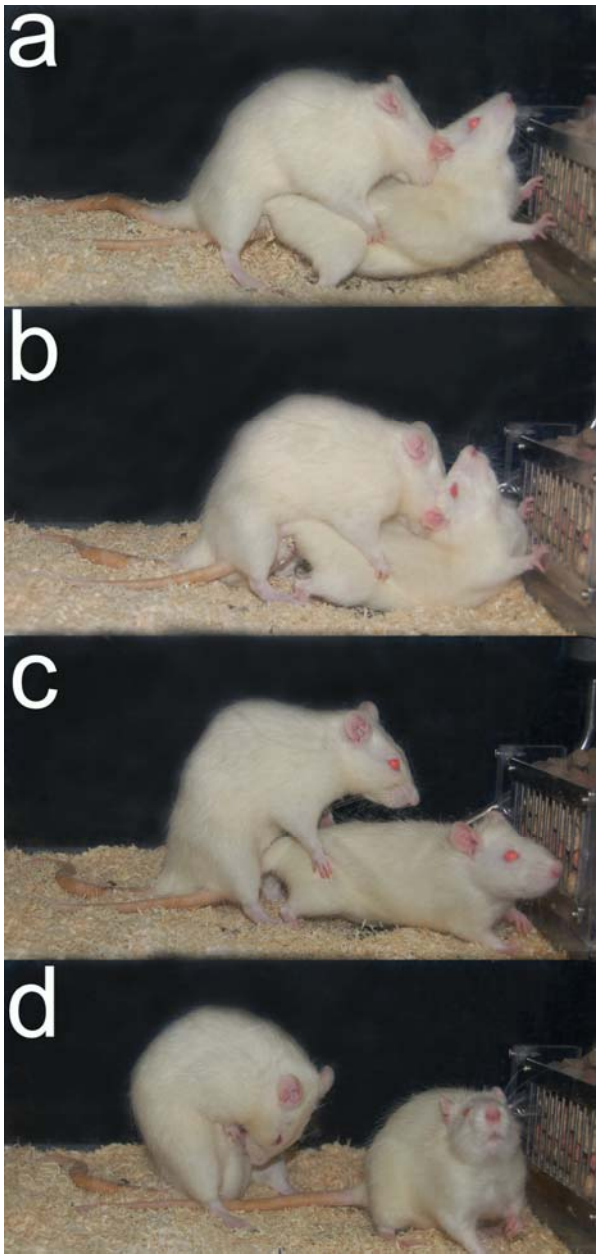

Fig. 5: Phases of a typical mating bout.

(**a**) Mounting: The male shows mounting behavior and grabs the female's flanks with his forepaws. The female begins to show lordosis.

(**b**) Intromitting: The female displays the typical lordosis posture (extension of the rear legs, dorsiflexion of the spine, elevation of the head and tail deviation) which allows the thrusting male to achieve IM.

(**c**) Ejaculation: The male has reached ejaculation, releases his grip of the female and stays in an upright position for several seconds.

(**d**) The female quickly moves away from the male. The male displays genital licking and grooming, a behavior that can be observed after successful IM.

4.2 Female sexual behavior and paced mating

Female sexual behavior includes a number of typical behaviors. **Proceptive** behaviors can be observed before and between mating activity and reflect the female's motivation to initiate mating (Blaustein and Erskine 2002).

These behaviors include:

- Hopping and darting: The female will perform quick jumps to present her hind quarters to the male, or run towards and away from him to motivate him to follow her.
- Ear wiggling: Fast lateral movement of the female's ears, probably as visual indication of receptivity.

The main **receptive** behavior of the female is called lordosis (Fig. 5 b). In this posture, the female becomes immobile, shows extension of the rear legs, dorsiflexion of the spine, elevation of the head and tail deviation (Pfaff et al. 1973). In contrast to proceptive behaviors, lordosis is triggered reflexively when tactile stimulation of the flanks and back occur during the female's receptive phase and is therefore used as the main indicator for receptivity in female rats (Pfaus et al. 1999). Under naturalistic or semi naturalistic conditions (which usually means: if given enough space), female rats will pace the sexual interactions with the male. By showing proceptive behaviors, the female will indicate her readiness for mating and quickly move away from the male after mounts and IM occur (Pfaus et al. 1999). This behavioral pattern allows the female to control the frequency of sexual interactions, as the male will not pursue her over long distances. In contrast, if mating takes place in a confined space, for example a standard laboratory experimental cage, the male will pace the occurrence of mating as he will continuously try to mount and intromit. This paradigm is stressful for the female and described as unpaced mating. The differentiation between female- and male-paced sexual activity is of major importance, as it has been shown that for both sexes mating is a rewarding experience only, if the respective paced mating condition is provided (Martinez and Paredes 2001).

5 Aim of this thesis

Several studies in humans and rodent animal models provide evidence for beneficial effects of sexual activity and mating on the emotional and hormonal stress responsiveness. However, the underlying central mechanisms responsible for these effects are mostly unclear. OT is a well-acknowledged neuromodulator/ neurotransmitter of the brain regulating sexual activity, emotionality, stress coping, and prosocial behaviors. This implicates that OT is not only of major importance for the physiological and behavioral regulation of mating, but may also mediate subsequent emotional changes.

Therefore, the present thesis aimed to study the effects of sexual activity and mating on the response to subsequent anxiogenic and stressful stimuli and the possible involvement of brain OT in male and female rats.

In a first set of experiments, male rats were mated, exposed to an emotionally challenging situation and their behavioral response was evaluated. I aimed to reveal the possible involvement of the brain OT system by measuring the central OT release during mating and manipulating the OTR system.

Furthermore, the hormonal and neuronal response to a physical stressor and the role of OT in this context was evaluated in mated male rats.

Additionally, I addressed the question whether paced and unpaced mating has differential effects on anxiety-related behavior and central OT release in female rats.

In chapter **II**, the effect of mating on the anxiety-related behavior of male rats and the involvement of brain OT are investigated. Therefore, male rats were mated and tested in two different established behavioral tests for anxiety at different time points after mating. In order to reveal the possible involvement of brain OT, we performed

microdialysis within the PVN of male rats during mating. Furthermore, I investigated if central actions of OT and mating-induced anxiolysis are causally linked, by testing the anxiety-related behavior after mating and subsequent administration of an OTR-A.

In chapter **III**, the HPA axis and neuronal reactivity of mated male rats to acute stressor exposure is described. Therefore, male rats were exposed to a short period of forced swimming (FS) at two different time points after mating and plasma concentrations of corticosterone and ACTH were measured via chronically implanted jugular vein catheters before and after mating as well as stressor exposure. Furthermore, the neuronal reactivity to FS was measured in mated males by evaluating the expression of *c-fos* and CRH mRNA after stressor exposure. Finally, the involvement of brain OT in the neuronal stress reactivity was addressed by central administration of an OT-A.

In chapter **IV**, the differential effects of paced and unpaced mating on anxiety and central OT release were assessed in female rats. Hence, female rats were mated under paced or unpaced conditions and tested in two different behavioral tests for anxiety with low or high light conditions after mating. Additionally, the release of OT within the PVN was measured by microdialysis during both mating conditions in order to reveal differences in the responsiveness of central OT transmission to paced and unpaced mating. Finally, the plasma concentrations of gonadal steroids were evaluated after ovariectomy (OVX) and hormone treatment to reveal their availability and, thereby, possible influence at the time of behavioral testing.

II Central oxytocin mediates anxiolysis in mated male rats

This chapter deals with the influence of sexual activity and mating on anxiety-related behavior in male rats. The main finding of the experiments described in this chapter is that sexual activity induces the release of the neuropeptide OT in the brain and is followed by reduces anxiety for up to 4 hours. Centrally released OT was found to be directly responsible for the anxiolytic effect of mating. These results have been presented in the following publication from the year 2007 and have been adopted for this thesis:

Centrally released oxytocin mediates mating-induced anxiolysis in male rats

Martin Waldherr and Inga D. Neumann

PNAS, October 16, 2007; 104 (42): 16681 – 16684.

Abstract

Sexual activity and mating are accompanied by a high level of arousal whereas anecdotal and experimental evidence demonstrate that sedation and calmness are common phenomena in the post-coital period in humans. These remarkable behavioral consequences of sexual activity contribute to a general feeling of well-being, but underlying neurobiological mechanisms are largely unknown. Here, we demonstrate that sexual activity and mating with a receptive female reduce the level of anxiety and increase risk-taking behavior in male rats for several hours.

The neuropeptide OT has been shown to exert multiple functions in male and female reproduction, and to play a key role in the regulation of emotionality following its peripheral and central release, respectively. In the present study we reveal that OT is released within the brain, specifically within the PVN, of male rats during mating with a receptive female. Furthermore, blockade of the activated brain OT system by central administration of an OT-A immediately after mating prevents the anxiolytic effect of mating, while having no effect in non-mated males. These findings provide the first direct evidence for an essential role of an activated brain OT system mediating the anxiolytic effect of mating in males.

Introduction

The neuropeptide OT is a well-acknowledged neuromodulator/ neurotransmitter of the brain regulating emotionality, stress coping, and prosocial behaviors (Insel and Young 2001; Landgraf and Neumann 2004; Choleris et al. 2007). In females, high activity of the brain OT system has been linked to the fine-tuned regulation of parturition and milk letdown (Russell et al. 2003), the performance of maternal behaviors (Insel and Young 2001), and to the low stress response characteristic for the peripartum period (Windle et al. 1997; Neumann et al. 2000b). Importantly, behavioral and physiological actions of intracerebral OT are not limited to females, as OT synthesis, local OT release and OT receptor binding have also been demonstrated in the male brain (Gimpl and Fahrenholz 2001; Ludwig and Pittman 2003; Landgraf and Neumann 2004). A major site of both OT synthesis and release within the brain is the PVN (Landgraf and Neumann 2004), a region integrating behavioral and neuroendocrine stress responses (Herman and Cullinan 1997). In male rats and mice, OT is an important regulator of sexual function (Argiolas and Melis 2004), of anxiety (Ring et al. 2006) and of stress-coping circuitries (Neumann et al. 2000c; Ebner et al. 2005; Huber et al. 2005). Moreover, in humans, intranasal OT was recently described to promote trust (Kosfeld et al. 2005), and to reduce the level of anxiety (Heinrichs et al. 2003), possibly at the level of the amygdala (Kirsch et al. 2005). With respect to sexual activity, pre-clinical (Stoneham et al. 1985) and human (Carmichael et al. 1987) research has shown elevated OT secretion into the blood during mating behavior and orgasm, respectively. Additionally, increased OT levels have been described in the cerebrospinal fluid after mating in rats (Hughes et al. 1987), which is indicative of stimulation of the OT system (Witt and Insel 1994).

In combination, these findings lead us to hypothesize that mating results in reduced anxiety-related behavior, possibly mediated by an elevation in local OT release within

the brain, specifically within the PVN. Therefore, after providing evidence for the anxiolytic effect of mating in males, intracerebral microdialysis was performed within the PVN of conscious male rats in the presence of either a non-receptive (non-primed; non-mated group) or a receptive (E & P-primed; mated group) female. Moreover, the causal involvement of brain OT in the reduced anxiety-related behavior found after copulation was examined using a selective OT-A administered immediately after mating.

Materials and methods

Animals. Sexually naïve adult male (350 – 400g body weight) and female (200 – 250g) Wistar rats (Charles River, Bad Sulzfeld, Germany) were used. They were kept under standard laboratory conditions (12:12 light/dark cycle, lights off at noon, 22°C, 60% humidity, food and water ad libitum). Female rats were OVX under isofluran anesthesia at least three weeks before the experiments, and were subcutaneously (s.c.) treated with E (200 µg /0.2 ml oil) and P (500 µg / 0.2 ml oil, Fluka Chemie GmbH, Buchs, Switzerland) 48 h and 6 h before start of the mating experiment, respectively, to increase sexual receptivity. Non-primed female rats received 0.2 ml oil. All experiments were approved by the local Bavarian government and performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

Experimental design

Effects of mating on anxiety in males. Single-housed male rats were divided into the following three groups: single-housed males, males with a non-receptive female, and males with an E/P-primed, receptive female. During the 30-min presentation of the respective female in the male's home cage, the number and frequency of mounts with IM were recorded.

Males were tested on the elevated plus-maze (EPM) or in the black-white box (BWB) 30, 120 or 240 min after removal of the female. Mating experiments were performed in the dark between 02.00 and 05.00 p.m., i.e. 2 to 5 hours after lights off. In a 2nd set of male rats, mating effects on anxiety were confirmed during the light phase, i.e. 6 to 8 hours after lights on.

In an additional experiment, an E/P-primed, receptive female was placed into the male's cage behind a perforated polycarbon wall allowing auditory, visual and olfactory communication, but no physical contact for 30 min; males were tested on the plus-maze after 30 min and compared to single-housed controls.

Monitoring of OT release in the PVN of male rats during mating. Two days after stereotaxic surgery (Ebner et al. 2005), eight consecutive 15-min dialysates were collected: samples 1 and 2 were taken under basal, single-housed conditions; samples 3 and 4 were collected in the presence of a receptive or non-receptive female behind a perforated wall allowing sensory communication, but no physical contact. Afterwards, the wall was removed allowing sexual behavior (mated group) or physical contact (non-mated group) during collection of samples 5 and 6, before the respective female was removed and collection of samples 7 and 8. OT content in lyophilised microdialysates was quantified by radioimmunoassay (Landgraf et al. 1995).

Administration of an OT or a VP receptor antagonist. To test the involvement of endogenous OT in the anxiolytic effect of mating, a selective OT-A (desGly-$NH_{2(9)}$,d$(CH_2)_5$[Tyr$(Me)_2$,Thr_4]OVT; 0.75 µg/ 5 µl) (Manning and Sawyer 1989) or vehicle (sterile isotonic saline) were slowly infused into the lateral ventricle of the male rat . Infusions were performed after termination of the 30-min mating period and removal of the receptive female to avoid interference with sexual functions (Melis et al. 1999). Thirty min after icv treatment, the anxiety-related behavior of mated male rats was tested on the plus-maze. In a second experiment performed under otherwise identical conditions, a VP V1a receptor antagonist (d$(CH_2)_5$Tyr(Me)vasopressin; 0.75 µg/5 µl) (Manning and Sawyer 1989) or vehicle were infused.

Elevated plus-maze. Anxiety-related behavior was tested on the EPM (Pellow et al. 1985; Neumann et al. 2000b). Increased open-arm exploration (percentage of time spent on and number of entries performed into open arms; 140 lux) indicates reduced anxiety. The number of entries into closed arms (20 lux) monitored during the 5-min observation period indicates locomotor activity (Korte et al. 1999).

Black-white box. To confirm the anxiolytic effect of mating in another established test for anxiety-related behavior, rats were monitored in the BWB (Henniger et al. 2000) (lit compartment: 40 x 50 cm, 350 lux; dark compartment: 40 x 30 cm, 70 lux) 30 min after mating. The percentage of time spent in the lit compartment indicates anxiety-related behavior; the number of rearings is recorded as exploratory behavior (Henniger et al. 2000).

Surgical procedures. Stereotaxic implantation of the microdialysis probe and the guide cannula for icv infusion were performed in male rats under isoflurane anesthesia as described (Neumann et al. 1993; Neumann et al. 2000c). For monitoring OT release within the PVN, a U-shaped microdialysis probe (dialysis membrane: molecular cutoff of 18 kDa; Haemophan; Gambro Dialysatoren, Hechingen, Germany) was implanted into the right PVN (1.5 mm caudal to bregma, 1.8 mm lateral to midline, 9.1 mm beneath the surface of the skull, angle of 10° to avoid sagittal sinus damage) (Paxinos and Watson 1998). For icv infusion of the receptor antagonists, a guide cannula (21 G, 12 mm long) was implanted with its tip resting 2 mm above the right lateral ventricle (1.0 mm caudal to bregma, 1.6 mm lateral to midline, 1.8 mm beneath the surface of the skull). The microdialysis probe and the icv guide cannula (closed with a stylet) were secured in place with dental cement to two stainless steel screws inserted into the skull. After surgery, the rats received 30 µl antibiotics s.c. (Baytril; Bayer Vital GmbH, Leverkusen, Germany). Following surgery, animals were kept individually in transparent experimental cages (24 x 40 x 36 cm polycarbon walls) allowing detailed behavioral observation. They were handled daily to reduce nonspecific stress responses during the experiment. The correct placement of the microdialysis probe within the PVN and of the icv infusion was verified after termination of experiment on 40-µm cryocut slices by Nissl-staining and by icv infusion of Evans Blue dye (5 µl), respectively.

Intracerebral microdialysis. Microdialysates were sampled at 4.5 µl / min (sterile Ringer's solution, pH 7.4) into 1.5-ml Eppendorf tubes containing 10 µl of 0.1N HCl and immediately stored at -80°C (Neumann et al. 1993).

Icv infusion. The receptor antagonist was slowly infused into the lateral ventricle of the well handled, freely moving animal in the home cage using a 27-gauge icv

cannula attached to a microliter-syringe via 70-cm PE-50 tubing and inserted into the previously implanted guide cannula. After infusion, the icv cannula was kept in place for 30 s to allow substance diffusion. Both, the OTR and VP V1a receptor antagonists were the generous gift of Dr. M. Manning, Toledo, OH, U.S.A.

Statistics. Data are presented as mean + S.E.M. Either a one-way ANOVA (factor mating: single housed, contact without or with mating; factor treatment) or a two-way ANOVA (factors treatment X time, or factors mating X treatment) was performed. All interactions were followed up by Bonferroni *post hoc* tests for pair-wise comparisons. Significance was accepted at $P < 0.05$. All statistics were performed using SPSS 13.0 (SPSS Inc., Chicago, USA).

Results

Effects of mating on anxiety in males. Male Wistar rats, which were successfully mated with a receptive female for 30 min during the dark phase, showed significantly reduced anxiety-related behavior, both on the EPM (Pellow et al. 1985) (Fig. 6 a) and in the BWB (Henniger et al. 2000) (Fig. 6 b), 30 min after removal of the female rat. Males which were mated spent a higher percentage of time in the unprotected and exposed open arms of the plus-maze (factor mating: $F_{2,25}$ 3.62, P = 0.042, analysis of variance; ANOVA) and entered the open arms more often ($F_{2,25}$ 6.72, P = 0.005) compared with single-housed and non-mated males (P < 0.05, Bonferroni test). In confirmation, in the BWB, mated males spent a higher percentage of time ($F_{2,25}$ 6.70, P = 0.005) in the aversive lit compartment compared with the single-housed group (P < 0.05; Fig. 1b). The anxiolytic effect of mating was found to be a long-lasting and robust phenomenon, as increased risk-taking behavior on the plus-maze could be confirmed both after 120 min (P < 0.05 versus single-housed, Fig. 6 c) and 240 min (P < 0.001 versus single-housed and non-mated controls, Fig. 6 d) after mating.

Moreover, an anxiolytic effect of mating was also seen, when the rats were mated in the light phase (i.e. 6 to 8 hrs after lights on; Fig. 7 a) However, the level of anxiety found after mating did not correlate with the number of IM (Fig. 7 b). Sexual activity did not significantly alter the locomotor activity and exploratory behavior after 30, 120 and 240 min, as the number of entries into the closed arms of the maze (Korte et al. 1999) and the frequency of rearings displayed in the white or black box (Henniger et al. 2000), respectively, were similar among groups (Fig. 6 a-d).

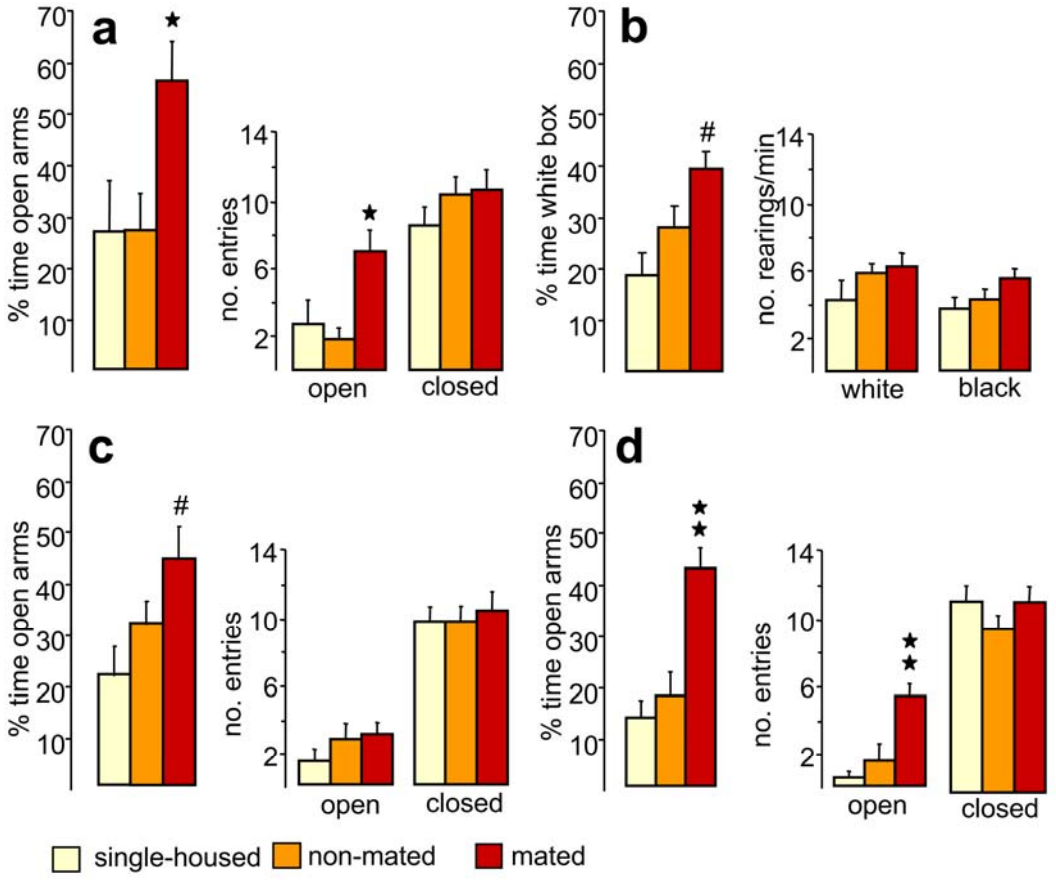

Fig. 6: Anxiolytic effect of mating in males in the dark phase. Male Wistar rats were tested on the EPM (**a, c, d**) and in the BWB (**b**) 30 min (**a, b**), 120 min (**c**) and 240 min (**d**) after termination of a 30-min mating period. Male rats were either single-housed (yellow) or exposed to a non-receptive (non-mated group; orange) or an E/P-primed (mated group; red) female rat in their home cage in the dark phase. Reduced anxiety levels were found in mated males at all 3 time points as indicated by increased exploration of the open arms of the plus-maze (**a, c, d**) and of the lit compartment of the BWB (**b**), respectively. The locomotor activity (number of entries into the closed arms of the plus-maze (**a, c, d**) was not altered by mating. Data represent means + S.E.M. Group size: single-housed: a, n = 8; b, n = 8; c, n = 12; d, n = 9; non-mated : a, n = 3; b, n =9; c, n = 11; d, n = 10; mated: a, n = 7; b, n = 11; c, n = 10; d, n = 11; ⁑ $P < 0.001$, ⋆ $P < 0.05$ versus single-housed and non-mated males, # $P < 0.05$ versus single-housed males after ANOVA.

In a subsequent experiment, which has been performed because OT release already started to rise during the presence of the primed female (Fig. 8, dialysates 3, 4), the anxiety-related behavior on the EPM was compared between males, which were either single-housed (n = 9) or exposed to a primed female behind a perforated polycarbon wall (n = 9).

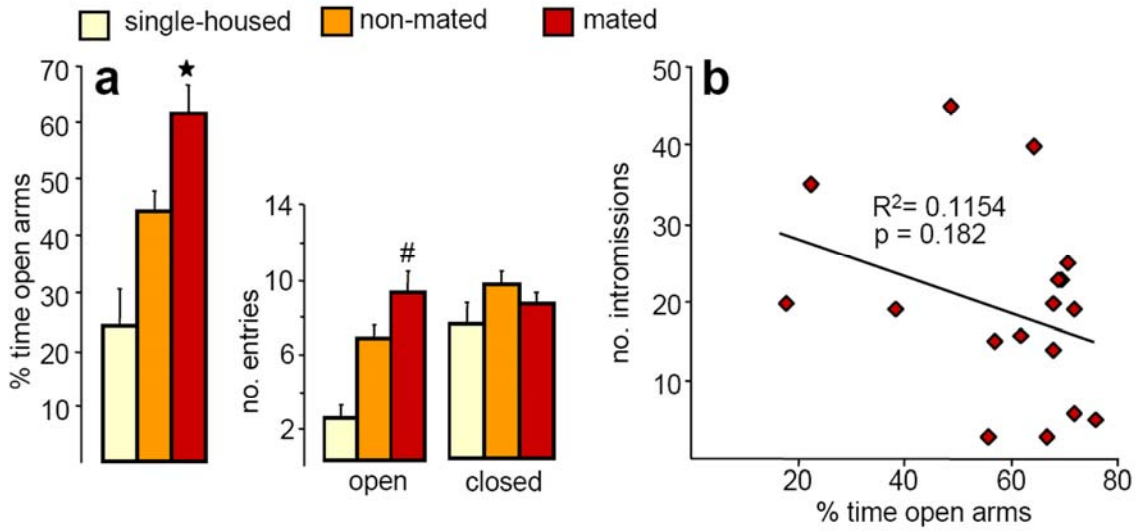

Fig. 7: **Anxiolytic effect of mating in males in the light phase.** (**a**) Male Wistar rats were tested on the EPM 30 min after termination of a 30-min mating period performed in the light phase, i.e. 6 to 8 h after lights on. Adult male rats were either single-housed (yellow) or exposed to a non-receptive (non-mated group; orange) or an E/P-primed (mated group; red) female rat in their home cage. Reduced anxiety levels in mated males were confirmed by increased exploration of the open arms of the plus-maze. The locomotor activity as reflected by the number of entries into the closed arms was not significantly altered by mating in the light phase. Group size: single-housed: n = 12; non-mated: n = 26; mated: n = 10; data represent means + S.E.M. ⋆P < 0.05 versus single-housed and non-mated males, # P < 0.05 versus single-housed males after ANOVA. (**b**) The anxiolytic effect of mating did not correlate with the numbers of intromissions recorded during the 30-min mating period (mated animals from 6 a and Fig. 5 a).

Statistical analysis revealed no differences in anxiety-related behavior between these two groups 30 min after removal of the female (percentage of entries into open arms: single-housed: 34.0 ± 2.8%; presence of primed female: 36.9 ± 4.0%, $F_{1,16}$ 0.36, P = 0.56; percentage of time on open arms: 13.5 ± % 2.6 versus 14.2± 3.6 %; $F_{1,16}$ 2.64, P = 0.86) or in the locomotor activity (number of entries into closed arms: 10.9 ± 1.5 versus 10.8 ± 1.5).

Monitoring of OT release in the PVN of male rats during mating. In the next stage, we wanted to demonstrate that activation of brain OT due to sexual activity is likely to enforce these behavioral adaptations. Thus, in order to link the brain OT system with mating-induced anxiolysis, the dynamics of neuronal OT release was monitored within the PVN via intracerebral microdialysis. Fifteen-min microdialysates were collected from male rats in their home cage during single-housing, in the presence of the receptive (mated group) or non-receptive (non-mated group) female behind a perforated wall, as well as during and after a 30-min mating period. A significant change in OT release within the PVN across the 8 samples was found (factor time: $F_{7,91}$ 3.0, P = 0.007), which was dependent upon the presence of either the receptive or the non-receptive female rat (factor mating: $F_{1,13}$ 6.88, P = 0.021; Fig. 8 a). It is important to note that even the presence of a receptive female behind the perforated wall tended to elevate the intra-PVN release of OT in the male rat (dialysates 3, 4; Fig. 8 a). Therefore, auditory, visual and/or olfactory stimuli originating from a receptive female are likely to contribute to the activation of the brain OT system in preparation for the anticipated sexual activity. However, significant activation of local OT release within the PVN was only witnessed during successful mating (P < 0.05 versus single-housing and versus non-primed group; dialysate 5). In contrast, OT release was unchanged in males, which were in contact with a non-receptive female (non-mated group, Fig. 8 a). The microdialysis procedure *per se* did not affect mating behavior, as the number of IM (14.0 ± 3.68) was not different to non-operated rats (13.2 ± 1.52). The mating-induced release of OT was locally restricted to the PVN as revealed by unchanged concentration in samples collected from outside the PVN (Fig. 8 a + 9).

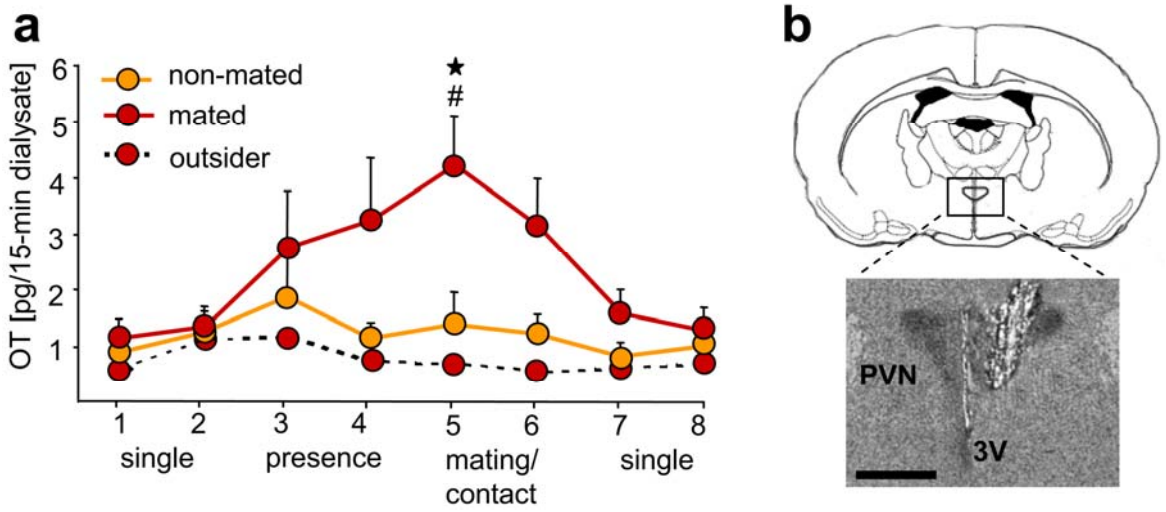

Fig. 8: OT release within the brain during sexual activity in male rats. Mating triggers OT release into the extracellular fluid of the PVN of male Wistar rats as indicated by elevated OT content in microdialysates sampled during exposure to a receptive female (**a**). Two 15-min microdialysates were sampled during single-housing (dialysates 1, 2), during the presence of a non-receptive (non-mated group, orange; n = 5) or receptive (mated group, red; n = 8) female behind a perforated wall (dialysates 3, 4), during physical contact (non-mated) or mating with the respective female (dialysates 5, 6) and after removal of the female from the male's home cage (dialysates 7, 8). The dotted line (red symbols) indicates OT content in microdialysates sampled from outside the PVN of a mated male (see also Fig. 9). Note the tendency of elevated OT release in males in the presence of the receptive female (dialysates 3, 4, mated group). Data represent means + S.E.M. ★$P < 0.05$ versus single-housing (dialysates 1, 2), # $P < 0.05$ versus non-mating group**.** (**c**) Schematic drawing of the rat brain at the level of the hypothalamus and the PVN (upper panel, at Bregma –1.5 mm) (Paxinos and Watson 1998), and microphotograph of a Nissl-stained coronal brain section after removal of the microdialysis probe located inside the right PVN (lower panel); 3V = third ventricle; scale bar = 1.0 mm.

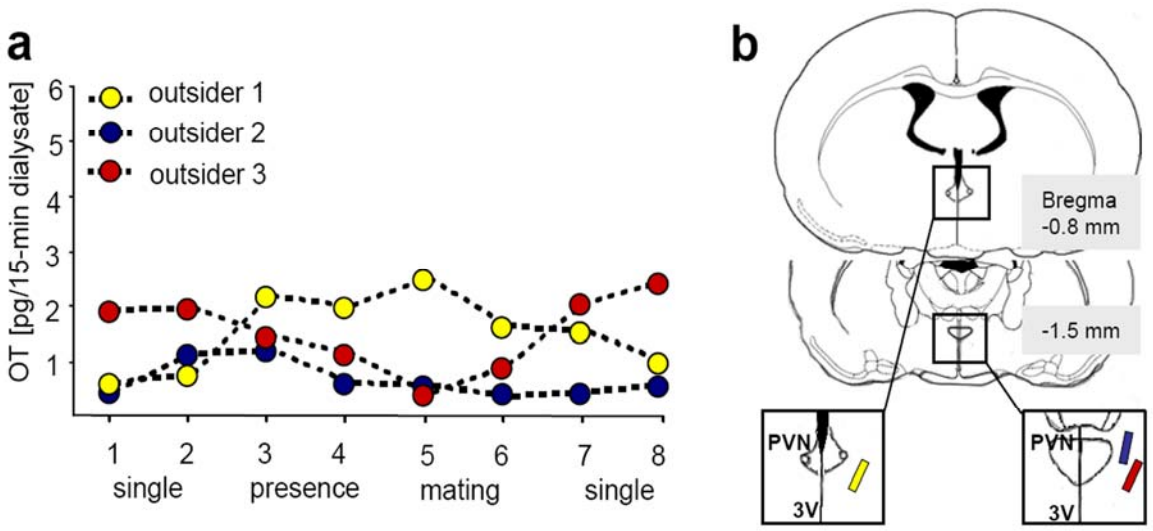

Fig. 9: OT concentrations in individual microdialysates sampled from outside the PVN. (**a)** Two 15-min microdialysates were sampled during single-housing (dialysates 1, 2), during the presence of a receptive female behind a perforated wall (dialysates 3, 4), during mating with the female (dialysates 5, 6) and after removal of the female from the male's home cage (dialysates 7, 8). Histological verification revealed placement of microdialysis probes outside the PVN. The colours in **9 a** correspond to the respective colour of microdialysis probe location in **9 b**. (**b**) Schematic drawings of the rat brain at the level of the hypothalamus and the PVN at two anterior-posterior planes (at Bregma -0.8 mm and –1.5 mm) (Paxinos and Watson 1998) where outsiders were located. The coloured bars indicate the placement of microdialysis probes. 3V = third ventricle;

Administration of an OT or VP receptor antagonist. The demonstration of a significant release of OT within the male brain in response to mating consequently led us to determine whether there is a causal link between mating-induced activation of the brain OT system and the subsequent anxiolysis. Therefore, the widely distributed brain OTRs (Gimpl and Fahrenholz 2001) of mated male rats were blocked by administration of a selective OT-A into the lateral ventricle via a previously implanted guide cannula. The antagonist treatment was performed immediately after removal of the receptive female from the male's cage to prevent adverse effects on penile erection and thereby sexual activity (Argiolas and Melis 2004). Confirming our hypothesis, the reduced level of anxiety-related behavior seen in vehicle-treated males on the plus-maze 30 min after mating (2-way ANOVA, factor

mating: $F_{1,27}$ 12.0, P = 0.022, compared with the respective single-housed group) was prevented by the OT-A (factor treatment: $F_{1,27}$ 9.13, P = 0.036; Fig. 10 a, compared with the vehicle-treated mated males). This effect was neuropeptide specific, as in an additional group of male rats administration of the receptor antagonist for the related neuropeptide VP had no effect on mating-induced anxiolysis (Fig. 10 b). Furthermore, OT-A administration had no effect on anxiety-related behavior in non-mated rats, which further proves that the up-regulation of the OT-system is responsible for the anxiolytic effect of mating.

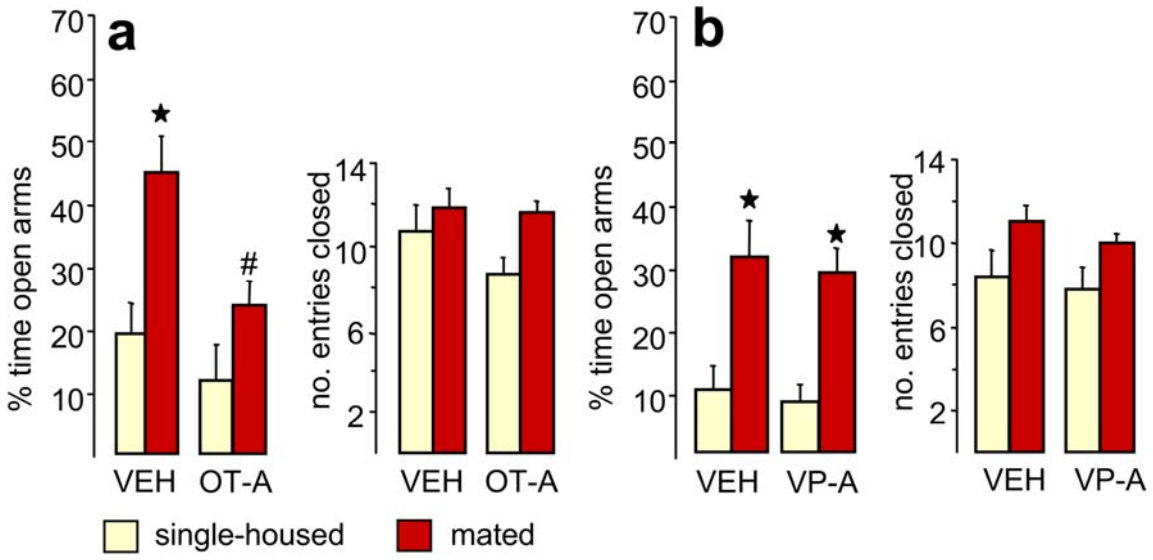

Fig. 10: Brain OT mediates the anxiolytic effect of sexual activity. Male rats were infused with a selective OT-A **(**0.75 µg/5 µl; **a**), or a VP receptor antagonist (VP-A; 0.75 µg/ 5 µl; **b**), into the right cerebral ventricle immediately after a 30-min mating period with a receptive female rat. Treatment with the OT-A, but not the VP-A, prevented the reduced anxiety-related behavior seen in vehicle (VEH) -treated mated males compared with VEH-treated single-housed male rats on the plus-maze. The percentage of time spent in the open arms of the maze indicates anxiety-related behavior, the number of entries performed into the closed arms indicates the locomotor activity. Data represent means + S.E.M. Group size: single-housed (yellow): vehicle, n=7 (a), n=11 (b); OT-A, n=8, VP-A, n=5; mated (red): VEH, n=8 (a), n=11 (b), OT-A, n=8, VP-A, n=6; ★P < 0.01 versus respective single-housed group, # P < 0.05 versus VEH-treated mated group.

Discussion

These results provide the first evidence that an elevated activity of the brain OT system, as a consequence of sexual activity, mediates the anxiolytic effect of successful mating in males. Thus, increased release of OT, as demonstrated within the PVN, is not only important for the regulation of sexual functions (Argiolas and Melis 2004), but also for supporting future beneficial behavioral strategies. Multiple behavioral consequences of reduced anxiety are possible, which are likely to be species-dependent. In rats, the most beneficial behavioral strategy for a male is to embrace the risky search for novel mating partners to ensure optimal distribution of his genes, especially in sparsely populated environments (Robitaille and Bovet 1976). Also, OT has been shown to exert reinforcing and rewarding actions (Liberzon et al. 1997). Therefore, the combination of these behavioral traits of increased OT after successful mating in the polygamous male rodent may facilitate the potentially risky search for receptive females (Robitaille and Bovet 1976), increasing the chances for distribution of his genes. In addition, the activated brain OT system has also been linked to a general attenuation of stress responsiveness (Windle et al. 1997; Neumann et al. 2000b; Heinrichs et al. 2003; Windle et al. 2004; Kirsch et al. 2005), and brain OT was shown to promote non-REM sleep (Lancel et al. 2003). Therefore, the phenomenon of increased relaxation, calmness and sedation found in humans after sexual activity and orgasm (Kruger et al. 2002; Brody 2006), which are clearly related to anxiolysis, may also be mediated by an activated OT system. Moreover, although highly speculative, the possibility exists that enforced / reinforced trust to the sexual partner involves brain OT (Liberzon et al. 1997; Kosfeld et al. 2005). However, the dominant behavioral trait from the wide range of possible OT effects in the post-coital period may depend upon natural environmental conditions and the species considered (Pfaus et al. 2003).

The question arises via which brain circuitries OT exerts the anxiolytic effects after mating. The demonstration of mating-induced release of OT within the PVN points towards the possibility of direct OT effects within the PVN (Neumann et al. 2000c; Windle et al. 2004), a brain region integrating inputs from other stress-related brain sites and regulating anxiety (Li et al. 1996; Herman and Cullinan 1997; Windle et al. 2004). Also, we may consider OT diffusion to remote regions (Landgraf and Neumann 2004) after local release in the PVN, as well as mating-induced OT release from axonal terminals originating within the PVN and projecting to other brain sites, such as the amygdala. Here, OT was shown to exert anxiolytic effects, to inhibit autonomic fear responses (Huber et al. 2005) and to promote sedation (Ebner et al. 2005). Moreover, the activation of the amygdala was reduced after intranasal OT-treatment (Kirsch et al. 2005), and after ejaculation in men (Holstege et al. 2003), further implicating similar anxiolytic mechanisms of OT actions across species. Thus, multiple brain regions relevant for OT actions might be involved in the anxiolytic effects seen after mating in male rats. This may also partially explain the fact that a rather transient increase in local OT release results in a behavioral effect which lasts over several hours. Additionally, the role of other neurotransmitter systems, including serotonin, DA and opioids all activated during sexual activity (Pfaus 1999; Argiolas and Melis 2004; Devidze et al. 2006), and their regulation by OT needs to be considered.

The maintenance of emotional homeostasis including the regulation of a well-balanced level of anxiety is a key function of limbic brain regions, which developed early in evolutionary terms. Brain OT, which is conserved throughout numerous species (Acher et al. 1995), has been demonstrated to be a vital component of this

circuitry. Our results demonstrate that sexual activity and mating behavior activate OT release within the male brain. In turn, the activated OT system contributes to reduced emotional responses to anxiogenic stimuli, which could be an important neurobiological mechanism supporting the positive effects of sexual activity on mental and physical health. In parallel, the increased risk-taking behavior found after copulation is likely to have evolved as an important behavioral strategy in male polygamous mammals to secure the successful distribution of their genes. Thus, our findings may further our neurobiological understanding of behavioral and emotional consequences of sexual activity, and the important involvement of brain OT.

III Reduced neuronal stress reactivity in mated male rats

This chapter deals with the influence of sexual activity and mating on the stress-responsiveness of male rats. The main finding of the experiments described in this chapter is that sexual activity reduces the central neuronal reactivity to an acute stressor, but does not change the hormonal stress response of the HPA axis. The adaptations of the neuronal stress response where found to be independent of the brain OT system. These results have been presented in the following publication from the year 2009 and have been adopted for this thesis:

Sexual activity reduces acute stress-induced hypothalamic *c-fos* and CRH mRNA expression without affecting the hormonal stress response in male rats

Martin Waldherr, Kewir Nyuyki, Rodrigue Maloumby and Inga D. Neumann

(submitted)

Abstract

Beneficial effects of sexual activity and mating on the responsiveness to environmental stress can be seen in humans and other mammal species alike, but the underlying neurobiological mechanisms are largely unknown. Because sexual activity and mating with a receptive female has been shown to activate the brain OT system and thereby reduce the emotional stress response, we investigated the hormonal and neuronal response to FS in mated male rats. Mating itself was found to trigger a hormonal stress response of the HPA axis which was absent in males which had contact with an unreceptive female. There were no significant differences in the hormonal stress response between mated and non-mated males when they were exposed to FS 45 min or 4 h after mating or female contact. However, the neuronal reactivity to FS was attenuated 4 h after mating within the PVN, measured as a lack of stress-induced increase in *c-fos* and CRH mRNA expression. Furthermore, blockade of the activated brain OT system by central administration of an OT-A after mating did not prevent the reduced expression of *c-fos* mRNA in response to stressor exposure. These findings provide evidence for a stress-protective effect of sexual activity and mating in male rats.

Introduction

Stress has been shown to have a negative impact on sexual behavior throughout several mammal species including mice (Sugiura et al. 1997; Amikishieva and Ovsyukova 2003), rats (Menendez-Patterson et al. 1980; Gorzalka et al. 1998; Retana-Marquez et al. 2003), monkeys (Keverne 1978; Sapolsky 1982) and humans (Bodenmann et al. 2006; Ter Kuile et al. 2007), likely via an inactivation of the HPG axis and, consequently, reduced T release. On the other hand, little is known about the possible impact of sexual activity on the acute or long-term stress responsiveness. Sexual activity has been shown to acutely activate the HPA axis, possibly due to physical activity and general arousal as studied in amphibians, bulls, stallions, rats and mice (Szechtman et al. 1974; Craigen and Bronson 1982; Orchinik et al. 1988; Borg et al. 1991; Colborn et al. 1991; Retana-Marquez et al. 1998; Bonilla-Jaime et al. 2006). Regarding the long-term effects of sexual activity on HPA axis function and the emotional stress responsiveness, reduced depressive-like behavior in the forced swim test (Martinez-Mota et al. 2005), reduced defensive burying (Fernandez-Guasti et al. 1989), increased risk-taking behavior (Kavaliers et al. 2008) and a reduced fear response after contextual fear conditioning (Bai et al. 2008) has been shown in male rats. Moreover, we could recently demonstrate a reduced level of anxiety-related behavior 30 min and up to 4 h after mating (Waldherr and Neumann 2007). In humans, sexual intercourse or physical contact with the partner reduces the intensity of the peripheral stress response (Brody 2006; Ditzen et al. 2007). These findings suggest that mating exerts inhibitory effects on the neuronal activity within stress- and anxiety-relevant brain regions.

The neuropeptide OT is a possible mediator between mating and the subsequent inhibition of physiological and behavioral stress responses. OT is released within the brain, specifically within the PVN during mating (Hughes et al. 1987; Waldherr and

Neumann 2007), where it is of major importance for the regulation of male sexual behavior (Stoneham et al. 1985; Argiolas and Melis 2004). Moreover, such centrally released OT was shown to mediate the anxiolytic effect of mating in male rats (Waldherr and Neumann 2007). Generally, in both males and females, brain OT exerts anxiolytic effects (Neumann et al. 2000b; Bale et al. 2001; Ring et al. 2006; Blume et al. 2008) and has been shown to attenuate the stress responsiveness of the HPA axis (Windle et al. 1997; Neumann et al. 2000c; Windle et al. 2004). Therefore, it is a promising candidate for conveying possible stress-protective effects of mating.

Our goal was, therefore, to elucidate the acute response of the HPA axis after a 30-min mating period, as well as the effects of sexual activity on the hormonal and neuronal responses to an acute, short stressor either 45 min or 4 h after termination of mating and the possible involvement of brain OT. In the first experiment, male rats were implanted with a chronic jugular vein catheter and were housed with either a primed or non-primed female for 30 min. Subsequently, they were exposed to FS (1 min) as an acute physical and psychological challenge either 45 min or 4 h after termination of the mating period with ongoing blood sampling for determination of plasma concentrations of ACTH and corticosterone. In a second experiment, mated and non-mated males were forced to swim 4 h after mating (10 min) and stress-induced neuronal activity within the PVN was quantified by *in situ* hybridisation for mRNA expression of the immediate early gene *c-fos* and CRH. Lastly, based on the inhibitory effect of mating on stress-induced *c-fos* mRNA expression, additional groups of mated and non-mated males received an icv infusion of an OT-A directly after mating in order to reveal a possible involvement of brain OT in the mating-induced inhibition of the *c-fos* mRNA response.

Materials and Methods

Animals. Sexually naïve adult male (350 – 400 g body weight) and female (200 – 250 g) Wistar rats (Charles River, Bad Sulzfeld, Germany) were kept under standard laboratory conditions (12:12 light/dark cycle, lights off at 11.00 a.m., 22°C, 50% humidity, food and water ad libitum). Female rats were OVX under isofluran anesthesia at least three weeks before the experiments, and were s.c. treated with E (200 µg /0.2 ml oil) and P (500 µg / 0.2 ml oil, Fluka Chemie GmbH, Buchs, Switzerland) 48 h and 6 h before start of the mating experiment, respectively, to increase sexual receptivity as described before (Waldherr and Neumann 2007). Non-primed female rats received 0.2 ml oil. Males were mated once for 30 min at least one week before behavioral experiments to ensure sexual activity. All experiments were approved by the local Bavarian government and performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

Experimental design

Experiment 1: Plasma ACTH and corticosterone responses to mating and subsequent stressor exposure. We evaluated the ACTH and corticosterone plasma responses to mating as well as to an acute stressor applied thereafter. Males were fitted with a chronic jugular vein catheter 5 days prior to mating and blood sampling. On the experimental day, males were allowed contact with a primed (mated group = M) or non-primed (non-mated group = NM) female for 30 min, or were single-housed (SH). Either 45 min or 4 h after removal of the female, they were exposed to FS (1 min, 20 °C). Before and after female contact as well as before and after FS, 0.2-ml blood samples were collected for quantification of ACTH and corticosterone concentrations (Fig.11).

Experiment 2: *C-fos* and CRH mRNA expression in the PVN in response to acute swim stress 4h after mating. In order to elucidate the effect of mating on neuronal stress responses, i.e. *c-fos* and CRH mRNA expression in the PVN, mated, non-mated or single-housed rats were exposed to FS (10 min, 20 °C) or remained non-stressed 4 h after female contact and brains were removed 30 min (*c-fos* mRNA) or 2 h (CRH mRNA) after stressor exposure. Brain slides were processed for analysis of *c-fos* mRNA or CRH mRNA expression within the PVN by *in situ* hybridisation.

Experiment 3: *C-fos* mRNA expression in the PVN in response to swim stress 4 h after mating and icv infusion of an OT-A. To investigate the possible role of OT in the reduced neuronal stress-response in mated males, single-housed and mated rats received an icv infusion of either a selective OT-A (desGly-$NH_2(_9)$,d$(CH_2)_5$[Tyr$(Me)_2$,Thr$_4$]OVT; 0.75 µg/ 5 µl; generous gift of Dr M. Manning, Toledo, OH, USA) (Manning and Sawyer 1989) or vehicle (sterile isotonic saline, 5 µl) immediately after mating. Animals were then treated as in experiment 2 for evaluation of *c-fos* mRNA expression within the PVN.

Surgery. Surgery was performed under isofluran anesthesia 5 days prior to the beginning of the experiment using semiaseptic conditions. Following surgery, animals were kept individually in transparent polycarbon cages (24 x 40 x 36 cm) and handled carefully each day to familiarize them with the blood sampling or icv infusion procedures and reduce non-specific stress responses during the experiments.

Jugular vein surgery and blood sampling. As described previously (Neumann et al. 1998; Bosch et al. 2007) the jugular vein was exposed by blunt dissection, a catheter consisting of silicone tubing (Dow Corning Corp., Midland MI) and PE-50

polyethylene tubing was inserted approximately 3 cm into the vessel through an incision in a cardiac direction and exteriorized at the neck of the animal. The catheter was filled with sterile saline containing gentamicin (30 000 IU/ml, Centravet, Bad Bentheim, Germany). Five days after surgery, at 10.00 h, the catheter of each rat was attached to an extension tube connected to a 1-ml plastic syringe filled with sterilized heparinized 0.9% saline (30 IU／mL, Heparin-Natrium, Ratiopharm, Ulm, Germany). Each rat was then left undisturbed for 2 h (see Fig. 11). Following two basal blood samplings (shown as basal 1), taken 30 min and directly before female exposure, either a receptive (M group), non-receptive (NM group) or no female (SH group) was introduced into the male's home-cage for 30 min. A third sample was collected directly after female exposure (contact peak). Either 45 min or 4 h later another basal sample was taken (basal 2) before rats were forced to swim and returned to their home-cage. Further blood samples were taken 5 (ACTH peak), 15 (corticosterone peak) and 60 min after FS (basal 3). All blood samples were immediately replaced by sterile 0.9% saline.

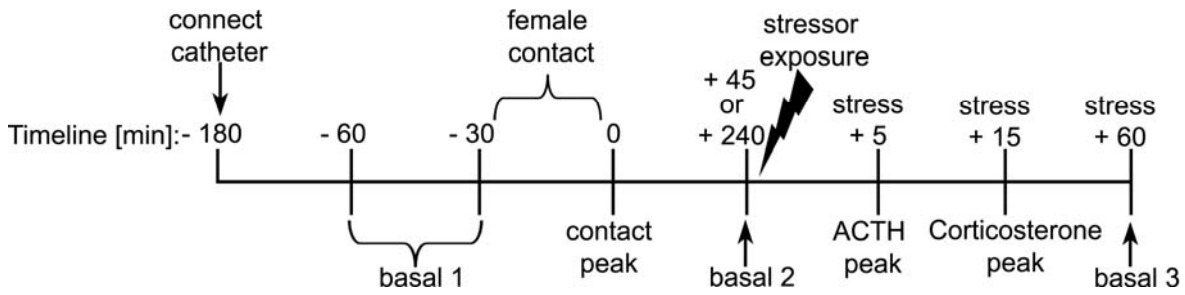

Fig.11: Schematic drawing of the blood sampling protocol. The indwelling jugular vein catheters were connected to polyethylene tubing and 1-ml syringes 2 h before the start of the experiment. Thirty min and directly before the receptive or unreceptive female was introduced into the male's homecage (female contact) two basal samples were taken. At the end of the 30-min mating period another sample was taken after removal of the female. Either 45 or 240 min later, another basal sample was collected before rats were exposed to 1 min of FS and additional samples were collected 5, 15 and 60 min after termination of FS.

Icv guide cannula implantation and infusion of OT-A. An icv guide cannula was implanted as previously described (Neumann et al. 2000c). Briefly, the guide cannula (21 gauge) was inserted stereotaxically with its tip resting 2 mm above the right lateral ventricle (coordinates: 1.0 mm caudal to Bregma, 1.6 mm lateral to midline, 1.8 mm beneath the surface of the skull) (Paxinos and Watson 1998), fixed to the skull and two jeweller screws with dental acrylic, and closed with a stylet. During the experiment, either vehicle or the OT-A were slowly infused into the lateral ventricle of the well handled, freely moving animals in the home cage using a 27-gauge icv cannula attached to a microliter-syringe via 70-cm PE-50 tubing and inserted into the previously implanted guide cannula. After infusion, the icv cannula was kept in place for 30 s to allow substance diffusion.

Treatment of blood samples and radioimmunoassays for ACTH and corticosterone. All blood samples were collected on ice in EDTA-coated tubes containing 10 µl aprotinin (Trasylol, Bayer AG, Leverkusen, Germany) and centrifuged at 4 °C (4000 r.p.m., 5 min). Plasma samples for evaluation of ACTH (70 µl) and corticosterone (20 µl) were stored at -20 °C until assay. Plasma ACTH and corticosterone were measured using commercially available kits (ICN Biomedicals, Costa Mesa, U.S.A.) according to the respective protocols. Detection limits for ACTH and corticosterone were < 4 pg/ml and < 10 ng/ml, respectively.

***In situ* hybridisation for *c-fos* and CRH mRNA.** Brains were sectioned coronally at 16 µm on a cryostat and thaw mounted onto poly-l-lysine-coated slides and stored at -80 °C. Brain sections were matched for level among the groups. The same number of brain slices was used for each animal. The hybridization protocol was adopted from a previous established one (De Vries et al. 1994). 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 for CRH mRNA using a highly specific, 48-mer 35S-labelled oligonucleotide probe (5´-GGC-CCG-CGG-CGC-TCC-AGA-GACGGA-TCC-CCT-GCT-CAG-CAG-GGC-CCT-GCA-3´) (Bosch et al. 2006) complementary to bases 64–111. For *c-fos* mRNA detection, a 45-mer 35S-labelled oligonucleotide (5´-CAGCG-GGA-GGA-TGA-CGC-CTC-GTA-GTC-CGC-GTT-GAAACC-CGA-GAA-CATC-3´) (Erdtmann-Vourliotis et al. 1999) complementary to bases 141–185, was used. One set of slides with six brain sections per slide and per animal, targeting the PVN, was used in each setup. Sections 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 section 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, sections 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) along with ^{14}C autoradiographic standards for 21 days (*c-fos* mRNA) or for 17 days (CRH mRNA). All brain sections from one experiment 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. Using the NIH Image program (ImageJ 1.31, National Institute of Health, http://rsb.info.nih.gov/ij/), the optical density of mRNA expression in the PVN was determined bilaterally in the brain slices with the highest density of mRNA expression. This resulted in bilateral measurements in two to four brain sections for each rat, which were pooled to provide an average per rat. For tissue background, the optical density of a non-hybridized region outside the PVN was measured.

Statistics. Data are presented as mean + S.E.M and were analyzed using a two-way ANOVA for repeated measures (factor mating X factor time), a two-way ANOVA (factor mating X factor stress condition) or a three-way ANOVA (factor mating X factor stress condition X factor icv treatment) followed up by Bonferroni *post hoc* tests for pair-wise comparisons unless stated otherwise. Significance was accepted at $P < 0.05$. All statistics were performed using SPSS 16.0 (SPSS Inc., Chicago, USA).

Results

Experiment 1: Plasma ACTH and corticosterone responses to mating and subsequent stressor exposure. In male rats, mating changed plasma ACTH (factor mating X factor time: $F_{2,34}$ 8.69, P = 0.006) and corticosterone ($F_{2,34}$ 7.67, P = 0.002) concentrations. In detail, immediately after 30 min of mating, plasma ACTH and corticosterone were found to be increased (both P < 0.01 versus basal and versus SH and NM groups; Fig. 12 a, b). Plasma ACTH and corticosterone levels remained unchanged in SH and NM rats.

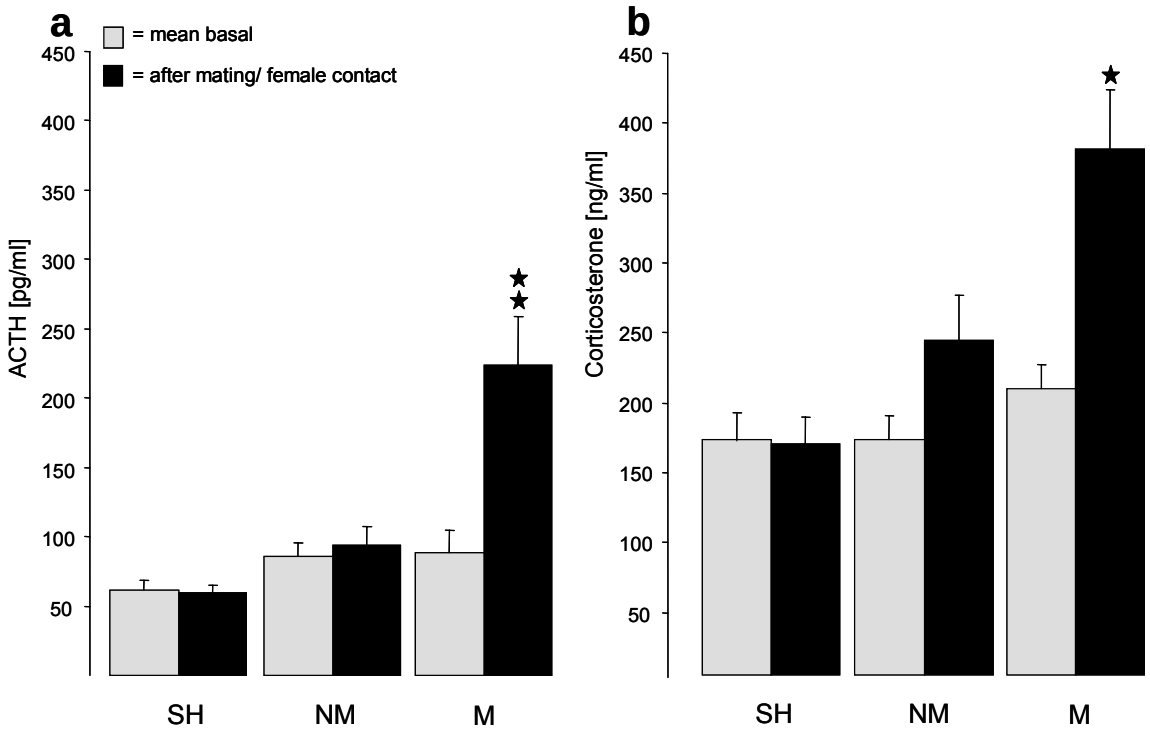

Fig. 12: Changes in plasma ACTH and corticosterone concentrations in response to mating/ female contact. M males (n = 16) show significantly increased plasma ACTH (**a**) and corticosterone (**b**) concentrations directly after a 30-min mating period compared to basal values as well as SH (n = 11) and NM (n = 10) animals. ⁑ P < 0.01; ★P < 0.05 versus mean basal and SH and NM groups.

After exposure to FS 45 min after mating/ female contact, peak ACTH and corticosterone plasma concentrations were found at 5 min and 15 min, respectively, in all rats. FS changed plasma ACTH (factor stress: $F_{1,30}$ 68.7, $P < 0.001$) and corticosterone ($F_{1,30}$ 106, $P < 0.001$) concentrations in all experimental groups with elevated hormone levels in SH, NM and M rats compared with pre-stress levels ($P < 0.01$; Fig. 13 a, b). No differences in ACTH and corticosterone stress responses were detectable among groups. However, ACTH pre-stress levels were still elevated in mated males (factor mating: $F_{2,30}$ 3.75, $P < 0.035$; $P < 0.01$ versus SH group; Fig. 13 a). A second set of animals was exposed to FS 4 h after mating/ female contact. Again, stressor exposure altered plasma ACTH (factor stress: $F_{1,35}$ 86.2, $P < 0.001$) and corticosterone ($F_{1,35}$ 125, $P < 0.001$), which was found to be independent of mating condition (factor mating: ACTH: $F_{2,35}$ 0.54, $P = 0.58$; corticosterone: $F_{2,35}$ 0.36, $P = 0.70$). Plasma concentrations of both hormones were increased after stress in all three experimental groups to a similar extent ($P < 0.01$ for both hormones; Fig. 13 c and d). Basal plasma levels of ACTH and corticosterone 4 h after mating, immediately before and 60 min after FS were similar in all groups (Fig. 13 c and d).

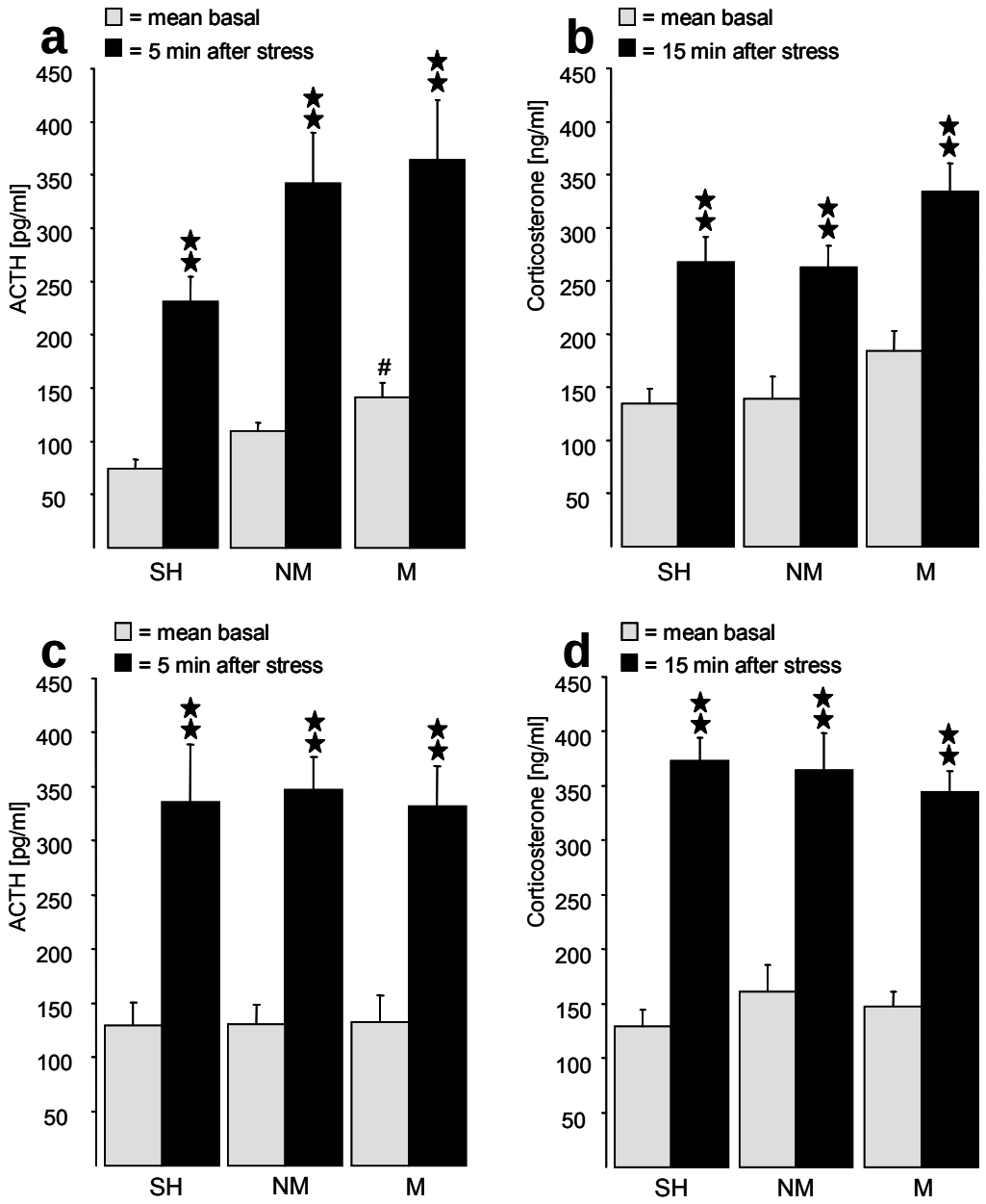

Fig. 13: Changes in plasma ACTH and corticosterone concentrations in response to 1 min of FS 45 min or 4 h after female contact. Plasma ACTH (**a**) and corticosterone (**b**) concentrations were significantly increased in response to 1 min of FS applied 45 min after mating/ female contact in all experimental groups when compared to respective basal values. Basal ACTH was significantly higher in previously mated animals when compared to SH animals. Four hours after mating/ female contact FS significantly increased plasma concentrations of ACTH (**c**) and corticosterone (**d**) in all groups compared to pre-stress values. SH (n = 10/10), NM (n = 10/12), M (n = 13/16). ⁑ $P < 0.01$ versus mean basal; # $P < 0.01$ versus SH mean basal.

Experiment 2: *C-fos* and CRH mRNA expression in the PVN in response to stressor exposure 4h after mating. Four hours after mating/ female contact, exposure to FS changed the expression of *c-fos* mRNA within the PVN (factor stress: $F_{1,29}$ 12.6, P = 0.001) which was independent of mating condition (factor mating: $F_{2,29}$ 2.66, P = 0.087). Specifically, in both SH (P < 0.01) and NM (P < 0.05) animals *c-fos* mRNA expression was significantly increased 30 min after FS (Fig. 14 a). In contrast, in mated animals *c-fos* mRNA synthesis remained unchanged (P = 0.655).

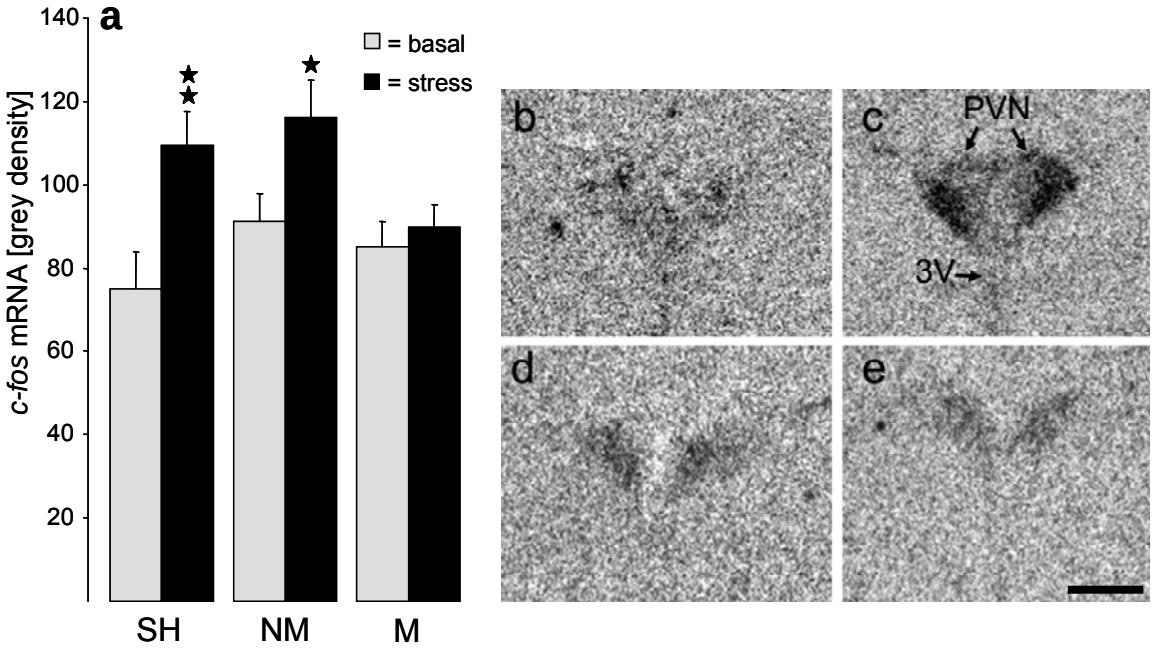

Fig. 14: Changes in *c-fos* mRNA expression in the PVN in response to 10 min of FS 4 h after female contact. (**a**) *c-fos* mRNA expression was significantly increased 30 min after stress in SH (n = 5/6) and NM (n = 6/6) animals but was unchanged in M animals (n = 6/6) when compared to respective basal values. ⁑ P < 0.01; ⋆P < 0.05 vs. respective basal *c-fos* mRNA expression. (**b** - **e**) Microphotographs of coronal brain sections demonstrating the distribution of hybridized *c-fos* mRNA in the PVN of SH and M males under basal conditions (**b**, **d**) or following 10 min of FS (**c**, **e**); 3V = third ventricle. (Scale bar, 1.0 mm)

In another set of animals, CRH mRNA expression measured in the PVN 2 hours after FS was found to depend on stressor exposure (factor stress: $F_{1,34}$ 4.43, P = 0.043) and mating condition (factor mating: $F_{2,34}$ 4.45, P = 0.019), but Bonferroni *post-hoc* testing did not reveal any differences between groups. Separate Mann-Whitney-U test comparison of non-stressed versus stressed animals revealed a significant increase in CRH mRNA expression in SH (P = 0.01 versus unstressed; Fig. 15 a) and NM (P = 0.016 versus unstressed) rats, but not in mated animals (P = 0.914). These results indicate that mating attenuates the subsequent activation of CRH mRNA expression in the PVN in response to an acute stressor.

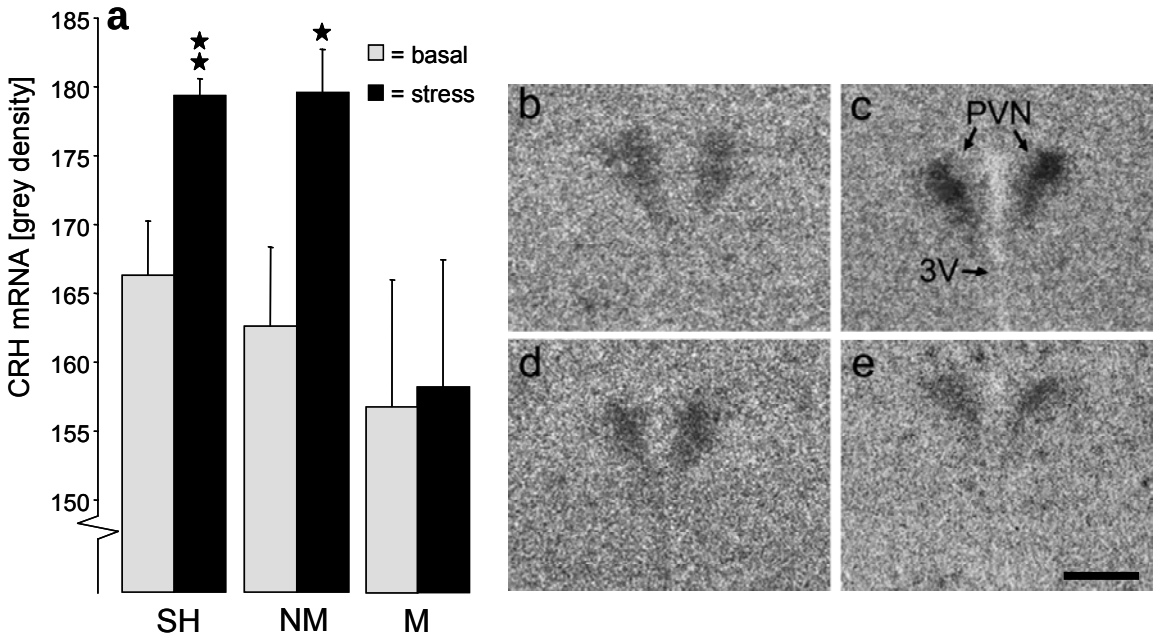

Fig. 15: Changes in CRH mRNA expression in the PVN in response to 10 min of FS 4 h after female contact. (**a**) CRH mRNA expression was significantly increased 30 min after stress in SH (n = 6/6) and NM (n = 6/7) animals but was unchanged in M animals (n = 7/8) when compared to respective basal values. ⁑ P < 0.01; ⋆ P < 0.05 vs. respective basal *c-fos* mRNA expression. (**b** - **e**) Microphotographs of coronal brain sections demonstrating the distribution of hybridized CRH mRNA in the PVN of SH and M males under basal conditions (**b**, **d**) or following 10 min of FS (**c**, **e**); 3V = third ventricle. (Scale bar, 1.0 mm)

Experiment 3: *C-fos* mRNA expression in the PVN in response to stressor exposure 4 h after mating and icv infusion of an OT-A. *C-fos* mRNA expression in the PVN was dependent on mating condition and stressor exposure (factors mating X stress: $F_{1,30}$ 4.72, P = 0.038) but independent of icv treatment ($F_{1,30}$ 0.437, P = 0.954). In SH males, FS resulted in increased *c-fos* mRNA expression (P < 0.01; Fig. 16 a) in both treatment groups. In mated animals, there was no significant increase in *c-fos* mRNA expression after FS in VEH- and OT-A treated rats, confirming the findings from experiment 2 and indicating an inhibition of neuronal responsiveness to an acute challenge 4 h after mating. The lack of stress-induced *c-fos* mRNA expression in mated animals was not mediated by central OT.

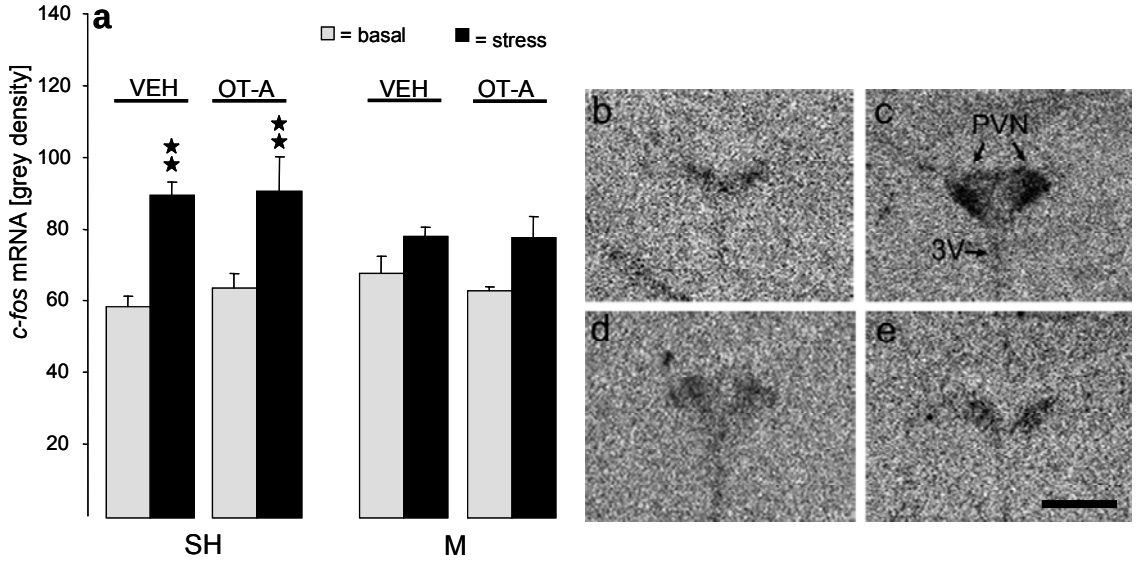

Fig. 16: Changes in *c-fos* mRNA expression in the PVN in response to 10 min of FS 4 h after female contact and icv infusion of either VEH or OT-A. (**a**) *c-fos* mRNA expression was significantly increased 30 min after FS in SH VEH and OT-A treated animals. M animals showed a tendency but no significantly increased *c-fos* mRNA expression after FS in both treatment groups. Group size was as follows: SH (VEH: n = 4/6; OT-A: n = 4/5), M (VEH: n = 4/5; OT-A: n = 4/5).
⁑ P < 0.01 versus respective basal *c-fos* mRNA expression. (**b** - **e**) Microphotographs of coronal brain sections demonstrating the distribution of hybridized *c-fos* mRNA in the PVN of SH and M OT-A treated males under basal conditions (**b**, **d**) or following 10 min of FS (**c**, **e**); 3V = third ventricle. (Scale bar, 1.0 mm)

Discussion

Based on our recent finding that mating reduces anxiety in male rats (Waldherr and Neumann 2007), we investigated the effects of mating on the central neuronal and peripheral response to a subsequent stressor. While sexual behavior itself elicited a strong hormonal stress response, it did not alter HPA axis reactivity after subsequent acute stressor exposure when compared to single-housed and non-mated animals. However, neuronal activity in the PVN in response to acute stressor exposure was reduced in previously mated animals, shown here as a lack of stress-induced increase in *c-fos* and CRH mRNA synthesis. The blunted *c-fos* and CRH responses after mating did not depend on central OT activation, as *c-fos* mRNA expression in response to an acute stressor was still inhibited after icv treatment with an OT-A.

Mating is accompanied by a significant activation of several brain and body systems, including the autonomic nervous system (Dail et al. 1989; Keast 1999), the HPA and HPG axes (Bonilla-Jaime et al. 2006), the mesolimbic DA system (Melis et al. 2003; Balfour et al. 2004), and release of OT into the blood (Stoneham et al. 1985) as well as in the brain (Waldherr and Neumann 2007). Our results confirm the findings the mating elicits an activation of the HPA axis from several other species (Rabb et al. 1989; Borg et al. 1991), as reflected by a significant rise in plasma ACTH and corticosterone at the end of a 30-min mating period. Several explanations for mating-induced activation of the HPA-axis have been proposed, including physical activity (Colborn et al. 1991) and sexual motivation and arousal (Bronson and Desjardins 1982; Bonilla-Jaime et al. 2006). The fact that plasma ACTH and corticosterone remained unchanged during exposure to an unprimed female, which is, however, extensively investigated, indicates that HPA axis activation is part of the sexual arousal response triggered only by contact with a primed female. As increased levels of corticosterone have been shown to stimulate motivation (De Wied 1980) and, thus,

exploratory behavior (Takahashi et al. 1989), activation of the HPA axis in mated males might contribute to the state of arousal and increased behavioral activity needed for sexual behavior.

In male rats several behavioral consequences of mating were described, including anxiolysis (Fernandez-Guasti et al. 1989; Waldherr and Neumann 2007), reduced avoidance of, and analgesic responses to, predator threat (Kavaliers et al. 2008), reduced floating in the forced swim test and increased sensitivity to antidepressant drugs (Martinez-Mota et al. 2005).Therefore, we hypothesized that these positive behavioral effects of mating could be, at least partially, due to changes in the central processing of stressful stimuli. This is supported by our finding that the acute stress-induced rise in *c-fos* mRNA expression in the PVN seen in single-housed and non-mated males is abolished by previous mating. Expression of the immediate early gene *c-fos* is an established indicator of neuronal activation and elevated levels of *c-fos* mRNA expression in the PVN have been found in response to emotional and physical stressful stimuli (Arnold et al. 1992; Smith et al. 1992; Chan et al. 1993; Chen and Herbert 1995; Cullinan et al. 1995). Thus, we can conclude that sexual activity and mating attenuate the subsequent neuronal responsiveness to an acute stressful challenge. This is further supported by our finding of a reduced expression of CRH mRNA within the PVN in response to an acute stressor 4 hours after mating. CRH has not only been proven to trigger ACTH release from the pituitary, but also to play a major role in the promotion of anxiety- and depression-related behaviors in animal and human studies (Nemeroff et al. 1984; Bale and Vale 2004; Holsboer and Ising 2008) and to suppress sexual behavior in male and female rats (Sirinathsinghji 1986; 1987). Thus, our finding may explain positive behavioral effects of mating in the context of emotional and physical challenging situations, as seen for example

during EPM or FS exposure (Martinez-Mota et al. 2005; Waldherr and Neumann 2007).

The discrepancy between increased HPA axis activity and blunted CRH mRNA expression is more difficult to interpret. The undiminished reactivity of the HPA axis to stressor exposure we see in mated animals despite reduced neuronal activity and CRH mRNA synthesis could be due to the fact that the corticotrope cells of the anterior pituitary are either hypersensitive to CRH or to other transmitters, e.g. VP. VP is known to co-stimulate ACTH release together with CRH under certain physiological conditions (Gillies et al. 1982) and may sustain the peripheral functionality of the HPA axis even though CRH mRNA synthesis is reduced. Additionally, we have to consider the possibility that a readily releasable pool of CRH exists in axon terminals of CRH neurons located in the median eminence that is similar to the one described for neurohypophysial VP (Sachs et al. 1967; Burford et al. 1973; Neumann et al. 1994b). These stores will be sufficient to trigger ACTH release from the pituitary in response to an acute stressor (de Goeij et al. 1991) and could explain the undiminished reactivity of the HPA axis observed in mated males. A dissociation between increased CRH mRNA expression within the PVN and concomitant unchanged HPA axis activity has also been recently described in macaques (Centeno et al. 2007; Herod et al. 2008). This supports our finding of a discrepancy between central and peripheral CRH functions in the context of stressor exposure.

Mating-induced changes in the central processing of a stressful stimulus are likely to cause the reduced CRH mRNA synthesis in the PVN. Limbic forebrain regions like the medial amygdala and lateral septum have been implicated in regulating the emotional and systemic consequences of stress in rats and mice (Cullinan et al.

1995; Dayas et al. 2001; Calfa et al. 2006; Singewald et al. 2008) and show differential changes in neuronal activity in dependence of the type and duration of the stressor (Martinez et al. 1998; Girotti et al. 2006; Singewald et al. 2009). These limbic regions stimulate CRH release via noradrenergic projections to the PVN in response to various types of stressors (Alonso et al. 1986; da Costa et al. 1996; Koob 1999; Lu et al. 2008).

Brain OT has been shown to inhibit the basal and stimulated activity of the HPA axis in male and female rats (Windle et al. 1997; Neumann et al. 2000c; Windle et al. 2004; Slattery and Neumann 2008). In this context, it has been shown that injection of OT into the PVN results in a lack of increased *c-fos* mRNA and CRH mRNA expression in response to restraint stress (Windle et al. 2004). This inhibitory effect of OT is either direct, by acting on the CRH producing parvocellular neurons in the PVN, or indirect, via an activation of brain regions that modulate stress reactivity (Windle et al. 1997; 2004). As mating induces OT release in the PVN has recently been shown to mediate the anxiolytic effect of sexual activity (Waldherr and Neumann 2007) we expected local OT effects on the neuronal stress-responsiveness. However, the blunted *c-fos* mRNA expression patterns in the PVN found after mating and subsequent stressor exposure were not disinhibited after icv treatment with an OT-A. We conclude that brain OT is not causally involved in the reduced neuronal stress response within the PVN of mated animals.

In conclusion, the results presented here demonstrate that sexual activity modulates HPA axis activity in a complex manner. Mating itself induces a strong hormonal response, probably as part of the complex mechanisms of arousal and activity in the context of sexual behavior. Although mating did not alter the hormonal HPA axis

response to an acute stressor, the neuronal reactivity within the PVN was strongly attenuated 4 h after mating. From this we conclude that sexual activity influences the central reactivity to a subsequent environmental stressor and therefore, may have stress-protective properties.

IV Paced and unpaced mating: influence on anxiety and central oxytocin release in female rats

This chapter deals with the influence of mating and hormone treatment on anxiety-related behavior and central OT release of female rats. The main finding of the experiments described in this chapter is that female-paced mating does not influence anxiety-related behavior, but increases central OT release. Unpaced mating, on the other hand, is shown to be anxiogenic. Hormone treatment increases plasma levels of E and P, with highest levels of P coinciding with low levels of anxiety. These results have been presented in the following publication:

Influence of hormone treatment and mating on anxiety-related behavior and central oxytocin release in female rats

Martin Waldherr, Sandra Bäuml, and Inga D. Neumann

(in preparation)

Abstract

Sexual activity and partner intimacy can have various positive consequences for women in the context of emotional stress. This is highly dependent on the fact that the engagement in sexual activity is voluntary. Similarly, in female rats, mating is a rewarding experience only, if they are able to control the rate of sexual interaction. Here, we demonstrate that mating under paced conditions does not affect the low level of anxiety found in E and P treated females, while unpaced mating results in increased anxiety. The neuropeptide OT has been shown to play a key role in the regulation of female emotionality, following its central release. In this study, we reveal that OT is released within the brain, specifically within the PVN, during paced mating only. This central oxytocinergic activity is not affected by peripheral hormone treatment. Furthermore, plasma concentrations of E and P are elevated for more than 27 h after respective subcutaneous injection and P reaches peak levels at the time of mating and behavioral testing. These findings provide evidence for an anxiolytic state in hormone-treated females and a differential effect of paced and unpaced mating on anxiety and the brain OT system.

Introduction

Sexual activity has been shown to have various positive effects in humans and other mammal species. Interestingly, there are several studies from the group of Brody et al., demonstrating positive correlations between sexual intercourse and various psychological and physiological parameters, for example relationship quality (Costa and Brody 2007), weight gain (Brody and Kruger 2008) and stress-reactivity (Brody 2006) in women. These results are supported by the finding that regular couple intimacy reduces basal cortisol levels (Ditzen et al. 2008a). Further proof of a positive effect of sexual activity in female mammals has been found in several animal studies. Mating increases the life expectancy in mole rats (Dammann and Burda 2006) and induces a reward state in female rats (Paredes and Vazquez 1999; Martinez and Paredes 2001). However, the central mechanisms behind these findings are largely unknown. One possible mediator of the positive effects of sexual activity is the neuropeptide OT. In female rats, high activity of the brain OT system has been linked to the regulation of luteinizing hormone release (Johnston et al. 1992), parturition and milk letdown (Neumann et al. 1993; Russell et al. 2003), the performance of maternal behaviors (Insel and Young 2001), and to the low stress response characteristic for the peripartum period (Windle et al. 1997; Neumann et al. 2000b). Additionally, OT is an important regulator of sexual function (Caldwell et al. 1986; Pfaus 1999) and anxiety (Ring et al. 2006). In a previous study in our lab, we demonstrated that OT release in the PVN, a region integrating behavioral and neuroendocrine stress responses (Herman and Cullinan 1997), is directly involved in the anxiolytic effect of mating in male rats (Waldherr and Neumann 2007). Sexual activity is known to activate oxytocinergic neurons in the PVN of female rats (Flanagan et al. 1993; Polston et al. 1998) and this can only be partially explained by OT release from the pituitary, as it has been shown that OT increases in the cerebrospinal fluid as a result

of vaginocervical stimulation even after hypophysectomy (Sansone et al. 2002). Therefore, increased activity of OT-containing neurons in the PVN during sexual activity can be linked to central OT release at the level of the spinal cord (Sansone et al. 2002) and distinct brain regions. Here, OT release during mating has been proposed for the VMH in females (Flanagan et al. 1993) and has recently been shown in the PVN of males in the context of anxiolysis (Waldherr and Neumann 2007). This led us to hypothesize that mating may reduce the emotional stress response in females, with a possible involvement of brain OT.

In female rats, it is important to differentiate between paced and unpaced mating. Under semi-naturalistic conditions 90 % of the occurring IM performed by a male rat are preceded by female approach behavior (McClintock and Adler 1978), clearly demonstrating that the female controls the rate of sexual interactions. This approach behavior consists of several proceptive (e.g. solicitation, hopping and darting) and receptive (lordosis) behaviors, indicating the readiness of the female to engage in sexual activity (Beach 1976). If the prerequisites for these behaviors are met in the laboratory, mostly achieved by enabling the female to escape the male (Pfaus et al. 1999), so called paced mating will occur. On the other hand, unpaced mating occurs when rats engage in sexual behavior in a confined space, where the male will dictate the rate of IM and the female can only use defensive and antagonistic behaviors to try to enforce the rate of copulation (Everitt 1990). While an increased activity of OT-containing neurons within the PVN has been demonstrated under paced (Flanagan et al. 1993) and unpaced mating conditions (Polston et al. 1998), the induction of a reward state, as evaluated by conditioned place preference, can only be seen after paced mating (Martinez and Paredes 2001; Gonzalez-Flores et al. 2004).

In cycling female rats, the fluctuations of ovarian hormones across the estrous cycle are considered to be responsible for behavioral changes that reflect emotionality and

anxiety (Mora et al. 1996). Plasma concentrations of E and P peak during proestrus, when lowest levels of anxiety are found on the EPM (Mora et al. 1996; Marcondes et al. 2001) and in the defensive burying paradigm (Fernandez-Guasti and Picazo 1990). The anxiolytic state during proestrus is dependent on the high plasma levels of P, as OVX rats show higher anxiety-related behavior than cycling rats, and s.c. injections of P reduces anxiety in OVX females (Mora et al. 1996). Data about a potential modulation of anxiety-related behavior by E, which peaks in late di- and early proestrus, is less clear, as both anxiolytic (Nomikos and Spyraki 1988; Frye and Walf 2004; Hiroi and Neumaier 2006) and anxiogenic effects were described in rats (Mora et al. 1996) and mice (Morgan and Pfaff 2001). Thus, modulatory effects of ovarian hormones on anxiety-related behavior must be taken into account when studying mating-induced effects on anxiety in females.

Here, we investigated the influence of hormone treatment and paced and unpaced mating on anxiety-related behavior in OVX and primed female rats and the possible involvement of brain OT. OT release within the PVN was monitored using intracerebral microdialysis during paced and unpaced mating. Also, plasma concentrations of both E and P during priming were measured via chronically implanted jugular vein catheters.

Materials and Methods

Animals. Sexually naïve adult female (200 – 250 g) and male (350 – 400 g body weight) Wistar rats (Charles River, Bad Sulzfeld, Germany) were kept under standard laboratory conditions (12:12 light/dark cycle, lights off at 10.00 a.m., 22 °C, 50 % humidity, food and water ad libitum). Female rats were OVX under isofluran anesthesia at least three weeks before the experiments, single-housed for one week after surgery and kept in groups of 4 animals afterwards. They were treated s.c. with E (200 µg / 0.2 ml oil) and P (500 µg / 0.2 ml oil, Fluka Chemie GmbH, Buchs, Switzerland) 48 h and 4 - 6 h before start of the mating experiments, respectively, to increase sexual receptivity (priming). Non-primed female rats received 0.2 ml oil. Females were carefully handled each day after OVX and submitted once to the priming regimen in the week before the experiments to reduce nonspecific stress. Mating experiments were performed in the dark between 12.00 and 03.00 p.m., i.e. 2 to 5 hours after lights off. All experiments were approved by the local Bavarian government and performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.

Experimental design

Experiment 1: Effects of priming and paced or unpaced mating on anxiety-related behavior in females. OVX females were divided into the following three groups: single-housed (SH) non-primed females, SH primed females (SH primed) and primed females mated with a sexually experienced male (mated). Preliminary experiments including primed females with contact to an unknown female consistently showed no differences between these animals and primed SH females. Therefore, the social contact group was not included in experiment 1. For the paced mating experiment, all animals were accustomed to the mating arena (see below and

Fig.17) for 30 min on two consecutive days before the experiment. On the experimental day, each female was placed in the mating arena for 5 min before a sexually experienced male was introduced into the arena on the opposite side of the dividing wall (mated group), or females stayed alone in the arena for an additional 30 min (SH). The animals were returned to their home cage and 30 min later, tested on the EPM (light intensity on the open arms of the EPM: 120 lux). Unpaced mating was performed in the female's home cage for 30 min, where the limited space did not allow the female to escape the male and, thus, pace mating behavior (Paredes and Alonso 1997). After removal of the male, females were tested in the BWB 30 min later. In order to test a possible effect of paced mating on anxiety levels under more anxiogenic testing conditions, a 2nd set of paced mated females was tested on the EPM 30 min after mating (light intensity on the open arms of the EPM: 240 lux). One week later, the same females were paced mated at the end of the dark phase (08.00 p.m.) and tested in the BWB 3 h later during the light phase, i. e. when anxiety levels are elevated and a reduced influence of ovarian hormones on anxiety has been shown (Mora et al. 1996). During each 30-min mating interval, the number and frequency of mounts with IM were recorded.

Experiment 2: OT release within the brain during sexual activity in females. In order to elucidate if paced and unpaced mating differentially activate the brain OT system, we measured OT release within the PVN during paced and unpaced mating. Two days after stereotaxic implantation of a microdialysis probe into the PVN, six consecutive 15-min dialysates were collected. Samples 1 and 2 were taken under basal, single-housed conditions; samples 3 and 4 were collected in the presence of either an unknown OVX female (non-mated group) or a sexually experienced male (mated group). After removal of the respective animal, samples 5 and 6 were

collected. Two additional groups of primed and unprimed females stayed single-housed during collection of 6 consecutive microdialysates samples to evaluate the influence of hormone treatment on central OT release. Two experiments were performed, in which the microdialysis sampling was performed either in the mating arena (paced mating) or the female's home cages (unpaced mating). However, as control groups showed similar levels of OT in both setups, release data was combined into one figure. OT content in lyophilised microdialysates was quantified by radioimmunoassay (Landgraf et al. 1995).

Experiment 3: E and P plasma concentrations during the priming and mating regimen. As a significant influence of priming on anxiety-related behavior was observed and P has been shown to exert anxiolytic effects (Mora et al. 1996), we measured the plasma concentrations of E and P during the priming and mating regimen (Fig. 18). OVX females were fitted with a chronic jugular vein catheter 5 days prior to priming and blood samples were collected as described in the sampling protocol (Fig. 18) and analyzed for E (day 1, 2) or P (day 3, 4) concentrations using ELISA.

Paced mating chamber. Various designs of paced mating conditions have been used before to observe female and male mating behavior (e.g.,Erskine 1985; Mendelson and Gorzalka 1987; Pfaus et al. 1990). In order to perform microdialysis experiments during paced mating, we constructed an open-topped paced mating arena (34 x 31 x 53 cm) bisected into two equally sized chambers by a barrier (Fig. 17). Between the barrier and the outer walls of the arena an interspace of 3.5 cm was left, allowing only the female animal to pass and to run behind the barrier, thus being out of the male's reach.

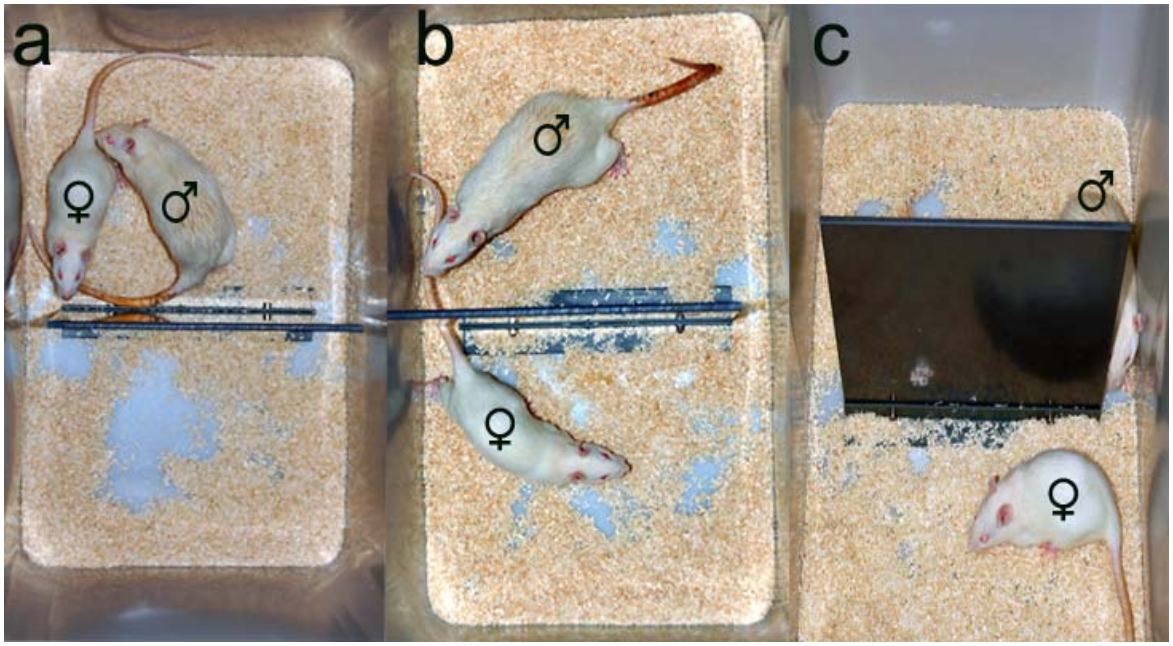

Fig. 17: Paced mating arena. For behavioral analysis of mating behavior a paced mating arena was used (34 x 31 x 53 cm), divided in the middle by a vertical barrier with an interspace of 3.5 cm between the barrier and the cage's walls. The arena was required to be open-topped for the performance of microdialysis during paced mating. In this setup, the female can freely move from side to side, while the larger male can't and will, therefore, remain on one side of the barrier. In (**a**), the male shows anogenital investigation of the female. (**b**) The female escapes to the other side of the barrier. (**c**) The male stays behind the dividing wall because of his size.

This setup effectively simulates a semi-naturalistic condition (Robitaille and Bovet 1976; Erskine 1985), allowing the female to pace sexual behavior without spatially confining the male, which can disrupt the occurrence of mounting and intromitting (Pfaus et al. 1999). Additionally, the setup allows detailed behavioral observation.

Elevated plus-maze. The EPM test is based on creating a conflict between the rat's exploratory drive and its innate fear of open and exposed areas. This test has been validated for the detection of emotional responses to anxiogenic and anxiolytic stimuli and substances (Pellow et al. 1985; Neumann et al. 2000b). Shortly, the plus-maze consisted of a plus-shaped platform elevated 70 cm above the floor, with two closed (50 × 10 × 40 cm) and two open (50 × 10 cm) arms and a central platform (10 x 10

cm). At the beginning of each test, the rat was placed on the central platform, facing a closed arm. During the 5-min test, the percentage of time spent on the open arms (120 and 240 lux respectively, as described in experiment 1) was scored using a video/computer system. The number of entries into closed arms (20 lux) was taken as a parameter of locomotor activity.

Black-white box. The BWB is another established test for the measurement of anxiety-related behavior (Crawley and Goodwin 1980; Henniger et al. 2000) and consisted of a brightly lit compartment with white floor (40 x 50 cm, 350 lux) and a dark compartment with black floor (40 x 30 cm, 70 lux). The compartments are connected by a small opening (7.5 x 7.5 cm) and the floors are divided by lines into 20 and 12 squares (10 x 10 cm). At the beginning of each test, each rat was placed in the black box facing the opening. During the 5-min test, the following parameters were scored using a video/computer system: the percentage of time spent in the white box as a measure of anxiety and the number of rearings in the black box per minute as a parameter of exploratory behavior.

Surgery. Surgery was performed under isofluran anesthesia and semiaseptic conditions. Following surgery, the rats received 30 µl antibiotics s.c. (Baytril; Bayer Vital GmbH, Leverkusen, Germany), were kept individually in transparent polycarbon cages (24 x 40 x 36 cm) and handled carefully each day to familiarize them with the microdialysis or blood sampling procedures and to reduce non-specific stress responses during the experiments.

Jugular vein surgery and blood sampling. The jugular vein was exposed by blunt dissection. A catheter consisting of silicone tubing (Dow Corning Corp., Midland MI) and PE-50 polyethylene tubing was inserted approximately 3 cm into the vessel

through an incision in a cardiac direction and exteriorized at the neck of the animal. The catheter was filled with sterile saline containing gentamicin (30 000 IU/ml, Centravet, Bad Bentheim, Germany).

Five days after surgery, at 10.00 a.m., the catheter was attached to an extension tube connected to a 1-ml plastic syringe filled with sterilized heparinized 0.9% saline (30 IU／mL, Heparin-Natrium, Ratiopharm, Ulm, Germany). The rats were then left undisturbed for 2 h (see Fig. 18). Following two basal blood samplings (30 min and directly before s.c. injection), the animals were injected s.c. with 200 µg E or vehicle (0.2 ml corn oil, VEH). Additional blood samples were taken at 1 h, 2 h, 4 h, 6 h, 8 h and 27 h after injection. Two days later, further basal samples were taken at 09.30 and 10.00 a.m., animals were injected s.c. with either 500 µg P or VEH and additional samples were taken at 1 h, 2 h, 4 h, 6 h, 8 h and 27 h after injection. The 0.2 ml blood samples were immediately replaced by sterile 0.9% saline. Blood plasma concentrations of E and P were analyzed using standard ELISA kits (DRG Diagnostics GmbH, Marburg, Germany).

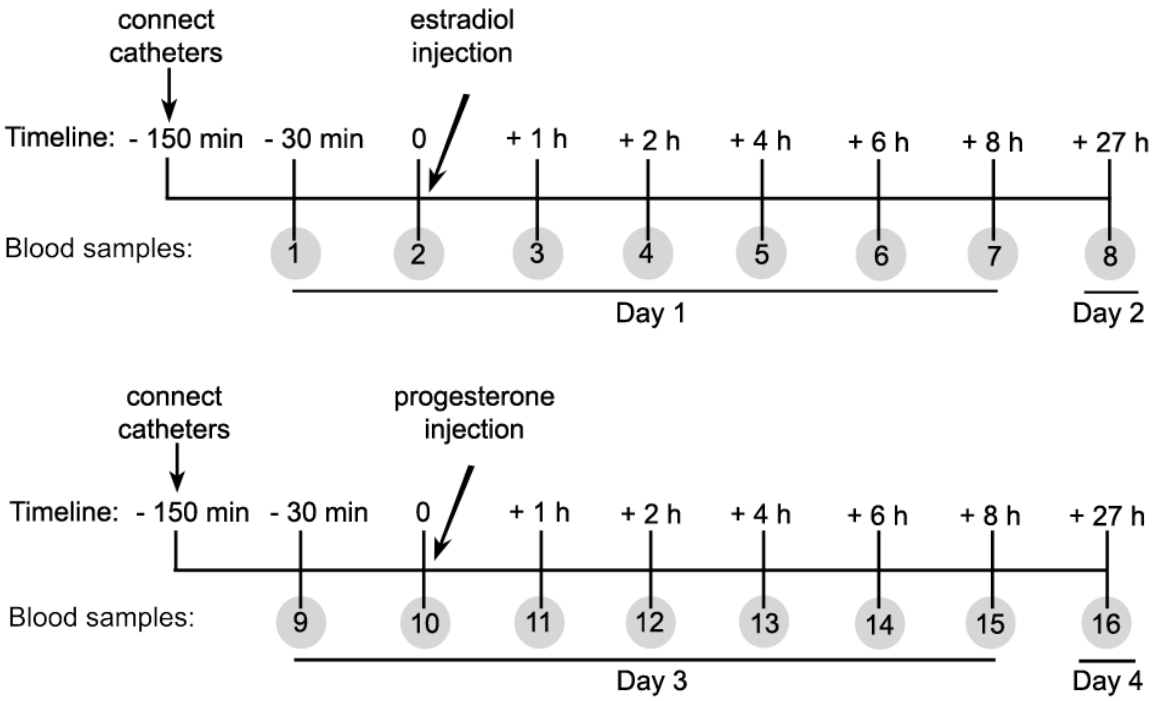

Fig. 18: Blood sampling protocol. Blood samples from OVX female rats were collected during the priming regimen via chronically implanted jugular vein catheters. On day 1, i. e. 5 days after surgery, 2 basal samples were collected before injection of E (200 µg; s.c.) or VEH (sample 1, 2) and 1 h, 2 h, 4 h, 6 h, 8 h and 27 h thereafter (samples 3 to 8). On day 3, samples 9 and 10 were collected before females were injected with P (500 µg; s.c.) or VEH (sample 9, 10). Six additional samples were collected beginning 1 h after injection (samples 11 to 16).

Intracerebral microdialysis. For monitoring OT release within the PVN, a U-shaped microdialysis probe (dialysis membrane: molecular cutoff of 18 kDa; Haemophan; Gambro Dialysatoren, Hechingen, Germany) was implanted into the right PVN (1.5 mm caudal to bregma, 1.8 mm lateral to midline, 8.6 mm beneath the surface of the skull, angle of 10° to avoid sagittal sinus damage) (Paxinos and Watson 1998) as described before (Neumann et al. 1993).The microdialysis probe was secured in place with dental cement to two stainless steel screws inserted into the skull. Microdialysates were sampled at 4.5 µl / min (sterile Ringer's solution, pH 7.4) into 1.5-ml Eppendorf tubes containing 10 µl of 0.1 N HCl and immediately stored at -80 °C. The correct placement of the microdialysis probe within the PVN was verified after termination of the experiment on 40-µm cryocut slices by Nissl-staining.

Statistics. Data are presented as mean + S.E.M. EPM and BWB data were analyzed using a two-way ANOVA (factor treatment X factor mating), for microdialysis and blood sampling data, a two-way ANOVA for repeated measures was performed (microdialysis: factor mating X factor time; blood sampling: factor treatment X factor time). All interactions were followed up by Bonferroni *post hoc* tests for pair-wise comparisons. Significance was accepted at $P < 0.05$. All statistics were performed using SPSS 13.0 (SPSS Inc., Chicago, USA).

Results

Experiment 1: Effects of priming and paced or unpaced mating on anxiety-related behavior in females. Anxiety on the EPM and in the BWB was significantly different between experimental groups 30 min and 3 h after mating, depending on the hormonal state of the females under low light (factor treatment: EPM: $F_{1,34}$ 13.1, P = 0.001; BWB: $F_{1,31}$ 5.78, P = 0.022) and high light conditions (EPM: $F_{1,42}$ 4.77, P = 0.035; BWB: $F_{1,42}$ 17.3, P < 0.001). In detail, SH primed females showed significantly reduced anxiety-related behavior when compared with non-primed females (P < 0.01, Fig. 19 a, d; P < 0.05, Fig. 19 b, c) in both behavioral tests under low and high light conditions. Unpaced mating resulted in increased anxiety (factor mating: $F_{1,31}$ 4.13, P = 0.05; P = 0.05 versus SH primed, Fig. 19 b), whereas anxiety-related behavior was similar between paced mated and SH primed females (Fig. 19 a, c, d).

Experiment 2: OT release within the brain during sexual activity in females. In order to reveal differences in OT release in the PVN between unpaced and paced mating, intracerebral microdialysis was performed during ongoing mating experiments. Significant differences in OT release within the PVN were found between experimental groups (factor mating X time: $F_{10,110}$ 2.76, P = 0.004; Fig. 20 a). Only during paced mating (sample 3), an increased OT release was found compared with basal values (samples 1, 2) and with OT levels during unpaced mating or social contact with another female (P < 0.05).

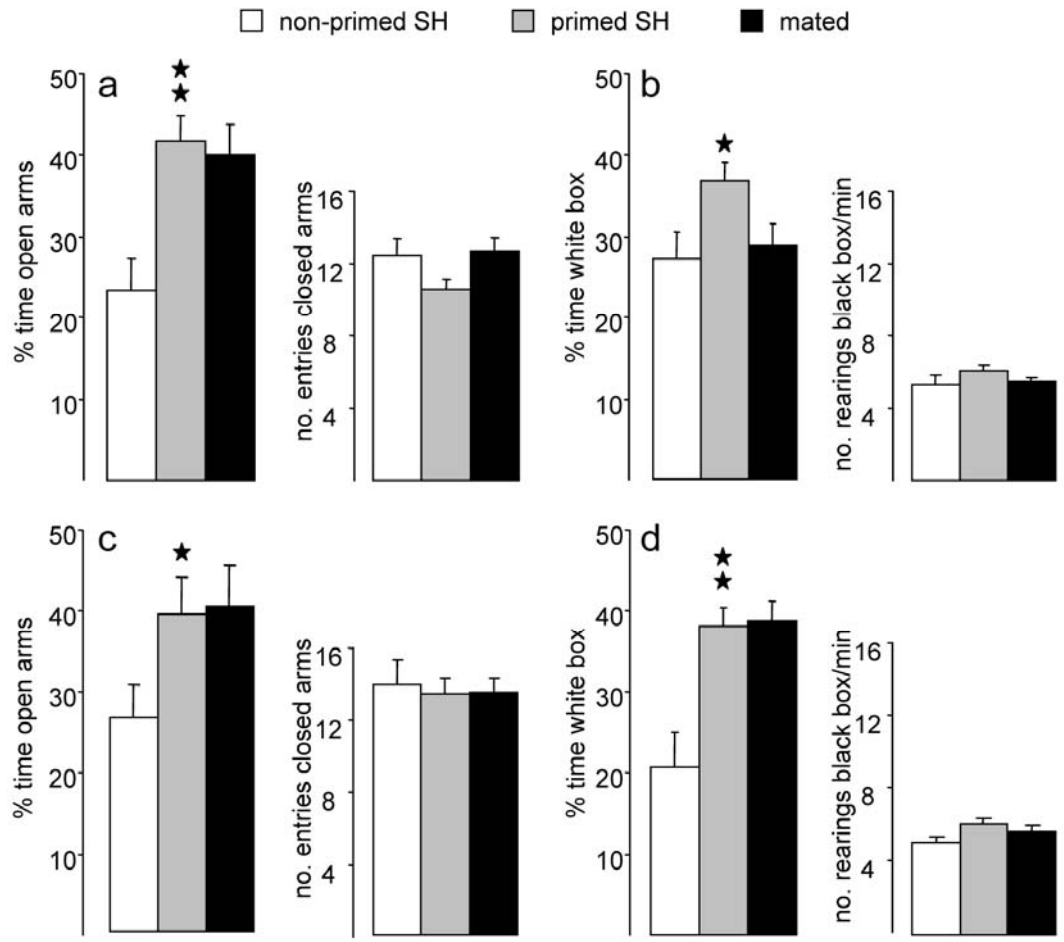

Fig. 19: Effects of priming and paced or unpaced mating on anxiety-related behavior in females. Female Wistar rats were tested on the EPM (**a, c**) and in the BWB (**b, d**) 30 min (**a, b, c**) or 3 h (**d**) after termination of a 30-min paced (**a, c, d**) or unpaced (**b**) mating period. Female rats were either single-housed (SH), non-primed or estradiol- and progesterone-primed or primed and exposed to an experienced male (mated). Reduced anxiety levels were found in primed SH females as indicated by increased exploration of the open arms of the plus-maze with standard (120 lux, **a**) and increased light intensity (240 lux, **c**) and of the lit compartment of the black-white box in the dark (**b**) or light phase (**d**). Unpaced mating increased anxiety in the BWB (**b**). The locomotor activity (number of entries into the closed arms of the plus-maze (**a, c, d**) was not altered by priming or mating. Data represent means + S.E.M. Group size between 11 and 17; ★★$P < 0.01$,★$P < 0.05$ versus non-primed SH, # $P < 0.05$ versus mated group.

OT release stayed elevated in paced mated females until the end of the experiment ($P < 0.05$ versus social contact). Priming *per se* did not influence basal OT release within the PVN, as unprimed (3.7 ± 0.53 pg / 15-min dialysate) and primed (3.0 ± 0.6 pg / 15- dialysate) SH females showed similar basal levels of measurable OT. Mating elicited an increase in OT release when compared with baseline OT concentrations (mean from samples 1, 2; factor mating: $F_{1,13}$ 7.6, $P = 0.016$). However, only paced mating increased local OT release significantly in female rats ($P < 0.01$ versus baseline, Fig. 20 b).

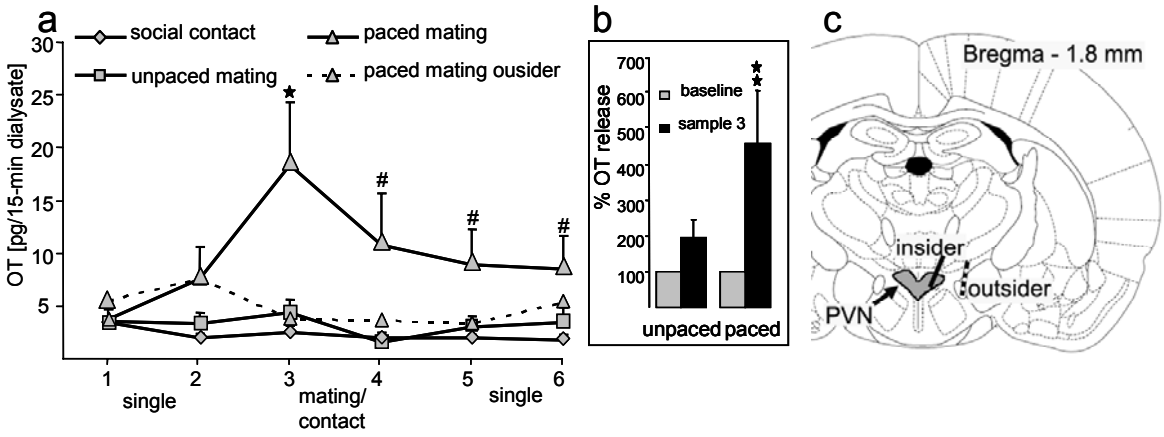

Fig. 20: OT release within the brain during sexual activity in females. (**a**) Paced mating triggers OT release into the extracellular fluid of the PVN of female Wistar rats, as indicated by elevated OT content in microdialysates sampled during and after exposure to an experienced male. Two 15-min microdialysates were sampled during single-housing (dialysate 1, 2), during physical contact with an unknown female (social contact) or paced / unpaced mating with an experienced male (dialysate 3, 4) and after removal of the female or male from the female's home cage (dialysate 5, 6). The dotted line indicates OT content in microdialysates sampled from outside the PVN of a paced mated female. (**b**) Paced mating increases OT release within the PVN when compared to mean basal values. Data represent means + S.E.M. ⁑ $P < 0.01$ versus basal OT release, ⋆ $P < 0.05$ versus single-housing (dialysates 1, 2) and other groups (dialysate 3), # $P < 0.05$ versus social contact group. (**c**) Schematic drawing of the rat brain at the level of the PVN (Paxinos and Watson 1998) and representative placement of microdialysis probes inside (insider) and outside (outsider) the PVN.

Experiment 3: E and P plasma concentrations during the priming and mating regimen. As priming reduced anxiety in experiment 1, we monitored the time course of plasma E and P during the priming regimen. As expected, injection of E (200 µg, s.c.) altered plasma levels of E (factor treatment X time: $F_{7,42}$ 17.1, P < 0.001, Fig. 21 a), with increased plasma levels found at all time points up to 27 h after injection (P < 0.05 versus pre injection and VEH group). Females injected with P (500 µg, s.c.) showed a long lasting increase in plasma concentrations of P (factor treatment: $F_{1,5}$ 63.1, P = 0.004, Fig. 21 b) 4h, 8 h and 27 h after injection (P < 0.05 versus VEH).

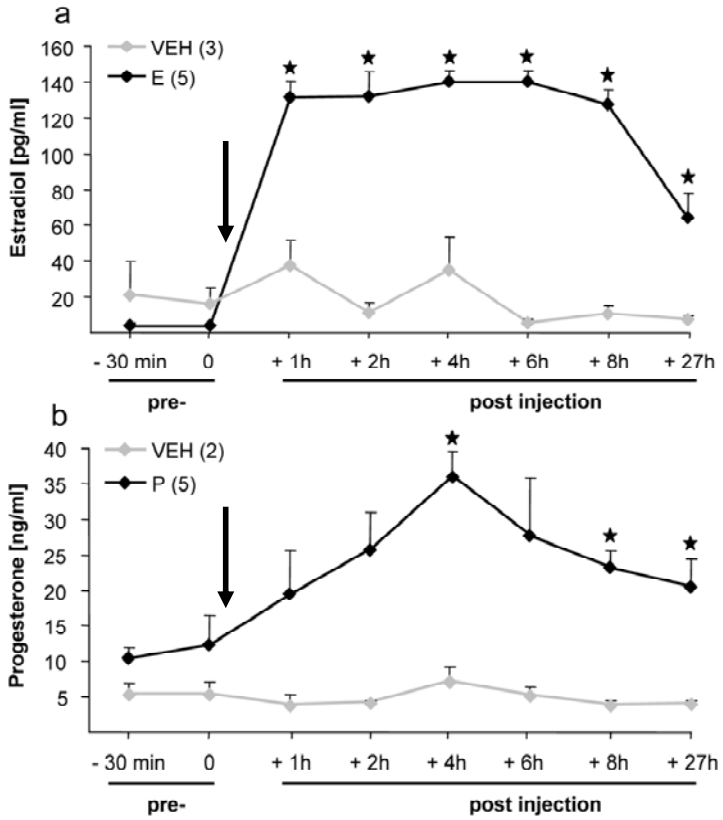

Fig. 21: E and P plasma concentrations during the priming and mating regimen. Female Wistar rats received s.c. injections of 200 µg E or oil (VEH) 48 h (**a**), and 500 µg P or VEH 4 - 6 h (**b**) prior to mating. Plasma concentrations of E and P were increased for at least 27 h after the respective s.c. injection. Note the peak concentration of P at the time point when mating and behavioral testing occurs (+4 to +6 h). Data represent means + S.E.M. ★P < 0.05 versus pre-injection (- 30 min and 0) and respective SH samples.

Discussion

We have shown here that anxiety-related behavior is reduced by high levels of gonadal steroids in female rats and that mating activates the brain OT system only when females were allowed to pace the sexual contact. Primed females were found to be less anxious on the EPM and in the BWB, and their anxiety-related behavior was unchanged by paced mating under low and high light conditions. However, if primed females were mated under unpaced conditions, their level of anxiety was found to be increased in the BWB. Microdialysis during paced and unpaced mating revealed an increase in local OT release within the PVN in paced mated females only. Local OT release in the PVN was not changed by priming and the resulting high concentrations of E and P in the plasma. Measurement of plasma concentrations of both E and P during the priming regimen showed a long lasting increase after s.c. administration of the respective steroid hormone.

Based on our recent findings of reduced anxiety after mating in male rats (Waldherr and Neumann 2007) we tested here, if females show similar alterations of the emotional stress response after sexual behavior. However, we found a strong anxiolytic effect of priming, which was not altered by paced mating and reversed by unpaced mating. The role of gonadal hormones and their fluctuation across the menstrual cycle in the context of anxiety-related behavior has been described before in rats (Mora et al. 1996; Marcondes et al. 2001) and mice (Morgan and Pfaff 2001). The role of E, or estrogens in general, is not clear, as both anxiolytic (Nomikos and Spyraki 1988; Frye and Walf 2004; Hiroi and Neumaier 2006) and anxiogenic (Mora et al. 1996; Morgan and Pfaff 2001) effects have been described. In contrast, P has been almost exclusively described as acting anxiolytic, both endogenously and after peripheral or central administration (Fernandez-Guasti and Picazo 1990; Mora et al.

1996; Frye et al. 2000; Frye and Walf 2004). There is evidence that P influences central dopaminergic and GABAergic transmitter systems, which have been implicated in the regulation of anxiety (Costa et al. 1975; Gray 1979; Iversen 1984). P has been shown to modulate GABA receptors in the rat brain (Maggi and Perez 1984) and central effects of P have been shown to be, at least partly, mediated via DA (D1) and GABA (A) receptors (Frye and Walf 2008), suggesting that apart from inducing lordosis via progestin receptors (Whalen and Luttge 1971), P may also affect anxiety-related behavior via GABAergic or dopaminergic pathways.

In female rats, the oxytocinergic system is involved in both sexual (Arletti and Bertolini 1985) and anxiety-related behaviors (Neumann et al. 2000b; Bale et al. 2001). Central OT receptors are highly up regulated during proestrus, a finding that has been suggested to be P dependent (Bale et al. 1995). Therefore, administration of P may result in an activated brain OT system, which in turn modulates anxiety-related behavior.

Our finding of increased OT release within the PVN during paced mating is difficult to interpret, as it does not result in reduced anxiety-related behavior, as has recently been shown in mated males (Waldherr and Neumann 2007). Primed females show decreased levels of anxiety and therefore, the lack of behavioral changes after paced mating may be due to a ceiling effect. However, there are other possible explanations for the increased OT release within the PVN during paced mating. It has been shown that only paced mating induces a reward state in females (Paredes and Alonso 1997; Martinez and Paredes 2001). Reward processing depends on the mesolimbic DA system (Wise and Bozarth 1985; Kelley and Berridge 2002), which in turn receives oxytocinergic innervations from PVN (Buijs 1978; Melis et al. 2007). It has recently been shown in male rats that brain OT directly activates the mesolimbic DA system

and thereby facilitates sexual behavior and induces a state of motivation and reward (Succu et al. 2007; Succu et al. 2008). Based on these findings, we hypothesize that OT release in the PVN during paced mating may activate the mesolimbic system and, therefore, play a role in the reward state observed after paced mating in female rats (Martinez and Paredes 2001).

In conclusion, we have shown here that priming leads to reduced anxiety in OVX females which is not altered by paced mating reversed by unpaced mating. Additionally, we were able to perform intracerebral microdialysis during mating and found increased local OT release in the PVN only during paced mating. Finally, as we showed that plasma levels of P are highest 4 – 6 h after s.c. injection, which coincides with the time point of mating and behavioral testing, we suggest a possible role of P in the mediation of reduced anxiety-related behavior observed in primed females.

V General Discussion

It is a capital mistake to theorize before one has data.
Insensibly one begins to twist facts to suit theories,
instead of theories to suit facts.
Sherlock Holmes

1 Summary and discussion of results

The experiments presented in this thesis were designed to investigate the effects of sexual activity and mating on the response of male and female rats to anxiogenic or stressful stimuli. In addition, underlying mechanisms were studied, with the main focus on the involvement of brain OT as a possible mediator of mating-induced anxiolysis and reduced stress-reactivity. The knowledge gained by these experiments might help to further our understanding of the emotional impact of sexual behavior on the organism, and provide important implications for the study of the psychological consequences of sexual activity in humans.

We investigated in male rats, if sexual activity and mating influence the behavioral response to anxiogenic stimuli (chapter II) and the neuronal and HPA axis response to an acute stressful situation (chapter III). Additionally, the effect of paced and unpaced mating on anxiety-related behavior and central OT release was investigated in female rats (chapter IV).

Anxiety-related behavior was reduced in mated male rats, an effect that could be observed up to 4 h after mating. Sexual activity induced an increased release of the neuropeptide OT within the PVN and the mating-induced anxiolysis was found to depend on central oxytocinergic actions.

The stress-responsiveness of male rats was differentially affected by mating. While the hormonal reactivity of the HPA axis was unchanged at various time points after mating, mated males showed a lack of increased neuronal reactivity and synthesis of *c-fos* and CRH mRNA in the PVN after stressor exposure 4 h after mating. This effect of sexual activity, however, was found to be independent of central actions of OT.

In female rats, unpaced mating increased anxiety-related behavior, while paced mating did not affect anxiety. However, only paced sexual activity induced an increased release of the neuropeptide OT within the PVN of females. Finally, elevated levels of circulating gonadal steroid hormones were found to act highly anxiolytic in female rats.

In summary, these findings give a clear indication of positive effects of mating, as the reactivity to fear- and stressful situations is attenuated by sexual activity in males. Additionally, sexual activity was shown for the first time to increase central OT release in both male and female rats, and brain OT mediates the positive consequences of mating, at least in males.

In the study presented in chapter II we were able to provide the first direct evidence for an essential role of an activated brain OT system in mediating the anxiolytic effect of mating in males. Increased release of OT was found during mating within the PVN and proven to be causally linked to the anxiolytic effect of sexual activity, as icv infusions of an OT-A directly after the mating period abolished the anxiety-reducing effect. Therefore, central OT is not only important for the regulation of sexual functions (Buijs 1978; Tang et al. 1998; Argiolas and Melis 2004), but also for supporting future beneficial behavioral strategies after sexual activity. It is likely that these effects of sexual activity are species specific, as in male rats, reduced anxiety after mating might help to facilitate the competition or potentially risky search for another receptive female (Robitaille and Bovet 1976), while, in humans, an activated brain OT system and reduced anxiety may rather facilitate relaxation and the emotional bonding to the partner (Liberzon et al. 1997; Kruger et al. 2002; Kosfeld et al. 2005; Brody 2006). With regard to our findings in chapter IV, which show that paced mating elicits a pronounced increase of OT release within the PVN without

affecting anxiety-related behavior in female rats, the beneficial effects of mating also seem to be sex specific. This does not necessarily mean that mating and central OT release have no positive emotional effects in females, as paced mating has been shown to induce a state of reward (Pfaus et al. 1999; Martinez and Paredes 2001). OT has been shown to exert reinforcing and rewarding actions (Liberzon et al. 1997) and to activate the mesolimbic DA system (Melis et al. 2007). Additionally, an activated brain OT system has also been linked to a general attenuation of stress responsiveness (Windle et al. 1997; Neumann et al. 2000b; Heinrichs et al. 2003; Windle et al. 2004; Kirsch et al. 2005), implying that the increased OT release we found after paced mating might modulate the females' stress-reactivity during the encounter with the larger male conspecific. This is supported by a study of Witt and Insel that found reduced rejection of male advances and increased physical contact with male partners after central OT application (Witt and Insel 1991). These facilitatory actions of brain OT might also explain the increased anxiety after unpaced mating, as this mating condition does not change OT release in the PVN and therefore, the female has a low tolerance and higher stress-sensitivity towards the tactile stimulation by the male (Carter 1992).

The finding that icv infusion of an OT-A blocked the anxiolytic effect of mating in male rats points towards two possible mechanisms of OT involvement in this context. One possibility is a direct effect of OT within the PVN (Neumann et al. 2000c; Windle et al. 2004), as this brain region integrates inputs from other stress-related brain sites and regulates anxiety (Li et al. 1996; Herman and Cullinan 1997; Windle et al. 2004). Here, OT has recently been shown to act anxiolytic in a direct and dose-dependent manner via an activation of the extracellular signal-regulated kinase 1/2 (Blume et al. 2008).

Secondly, OT may diffuse to remote regions after local release in the PVN (Landgraf and Neumann 2004), or thirdly, mating might induce OT release from axonal terminals originating within the PVN and projecting to other brain sites, such as the amygdala or VTA (Gimpl and Fahrenholz 2001; Melis et al. 2007). Additional results about possible sites of actions of brain OT will be presented and discussed in a following part of this discussion (3). In conclusion, the activated brain OT system seen during mating in male and female rats contributes to reduced emotional responses to anxiogenic stimuli, at least in males, which could be an important neurobiological mechanism in mammal species supporting the beneficial effects of sexual activity on mental and physical health.

In chapter III of this thesis we investigated if sexual behavior and mating might have a positive influence on the hormonal and neuronal response to subsequent stressor exposure in male rats. By measuring plasma concentrations of ACTH and corticosterone we were able to show that mating itself elicits a strong peripheral stress response, but does not influence the secretory response of the HPA axis to stressor exposure up to 4 h after mating. The finding that mating is, to some extent, stressful is confirmed by studies in other mammal species and can likely be explained by physical hyperactivity as well as sexual arousal and motivation (Szechtman et al. 1974; Craigen and Bronson 1982; Borg et al. 1991; Colborn et al. 1991; Retana-Marquez et al. 1998; Bonilla-Jaime et al. 2006). Increased release of corticosterone has been shown to stimulate exploratory behavior (Takahashi et al. 1989), sniffing (Morley and Levine 1982), motivation (De Wied 1980) and alertness (Korte et al. 1992; Vazquez-Palacios et al. 2001). Therefore, increased corticosterone release during mating may prepare the animal for the physical demands of copulatory behavior (Bonilla-Jaime et al. 2006).

In contrast to the unchanged peripheral stress responsiveness, the neuronal stress reactivity is severely blunted in mated rats 45 min and 2 h after stressor-exposure, as *c-fos* and CRH mRNA expression was not elevated in response to FS in mated males. The immediate early gene *c-fos* is an established indicator of neuronal activation and elevated levels of *c-fos* mRNA expression in the PVN have been found in response to emotional and physical stressful stimuli (Arnold et al. 1992; Smith et al. 1992; Chan et al. 1993; Chen and Herbert 1995; Cullinan et al. 1995). From our finding of an attenuated stress-induced increase in *c-fos* mRNA synthesis, which was in fact measured more than 4.5 h after mating, we conclude that sexual activity leads to a long-lasting attenuation of the subsequent neuronal responsiveness to an acute stressful challenge. This finding is supported by a reduced expression of CRH mRNA within the PVN in response to an acute stressor 4 hours after mating. CRH plays a major role in the promotion of anxiety- and depression-related behaviors in animals and human alike (Nemeroff et al. 1984; Bale and Vale 2004; Holsboer and Ising 2008) and can suppress sexual behavior in male and female rats (Sirinathsinghji 1986; 1987). Our finding of reduced CRH mRNA synthesis in mated males may help explain positive behavioral effects of sexual activity in the context of emotional and physical challenging situations, as seen for example during EPM testing or FS exposure (Martinez-Mota et al. 2005; Waldherr and Neumann 2007).

The discrepancy between attenuated neuronal, but unchanged hormonal responsiveness of the HPA axis is difficult to interpret at the moment. The undiminished reactivity of the HPA axis despite reduced neuronal activity and *c-fos* and CRH mRNA synthesis could be due to the fact that the corticotrope cells of the anterior pituitary are either hypersensitive to CRH or to other transmitters, e.g. VP. Additionally, we have to consider the possibility that a readily releasable pool of CRH exists in axon terminals of CRH-containing neurons located in the median eminence,

which is similar to the one described for neurohypophysial VP (Sachs et al. 1967; Burford et al. 1973; Neumann et al. 1994b). These stores will be sufficient to trigger ACTH release from the pituitary in response to an acute stressor (de Goeij et al. 1991) and could explain the undiminished hormonal reactivity of the HPA axis observed in mated males. Additionally, it is also possible that mating influences the activity of limbic and brainstem regions that have been implicated in regulating the emotional consequences of stressor exposure and regulate the synthesis and release of CRH via noradrenergic projections to the PVN (Cullinan et al. 1995; Dayas et al. 2001; Calfa et al. 2006; Singewald et al. 2008).

Brain OT has been shown to inhibit the basal and stimulated activity of the HPA axis in male and female rats (Windle et al. 1997; Neumann et al. 2000c; Windle et al. 2004; Slattery and Neumann 2008). Studies by Windle and colleagues have shown that injection of OT into the PVN results in a lack of increase in *c-fos* mRNA and CRH mRNA expression in response to restraint stress (Windle et al. 2004). This inhibitory effect of OT could be either direct, by acting on the CRH producing parvocellular neurons in the PVN, or indirect, activating remote brain regions that modulate stress reactivity (Windle et al. 1997; Windle et al. 2004). Based on these findings, together with our results from chapter II, we expected local OT effects on the neuronal stress-response. However, the blunted *c-fos* mRNA expression found within the PVN after mating and subsequent stressor exposure was not disinhibited after icv treatment with an OT-A. From this we conclude that brain OT is not causally involved in the reduced neuronal stress response within the PVN of mated male rats.

It is interesting to note that in this experiment, mated males showed a strong tendency towards increased *c-fos* expression in response to FS, regardless of OT-A or VEH infusion (Fig. 16 a). If the experimental groups of SH and M animals are pooled regardless of their icv treatment, we find a significant stress-induced increase

in *c-fos* mRNA expression also in mated males, which was, however, still lower compared with the one seen in SH males, supporting our earlier results from chapter III (Fig. 22). This finding indicates that mating reduces rather than inhibits the neuronal stress response in male rats.

In summary, it can be speculated that mating changes the emotional reaction to a subsequent stressor without affecting the peripheral hormonal response that might be necessary for the animal to regain physical homeostasis.

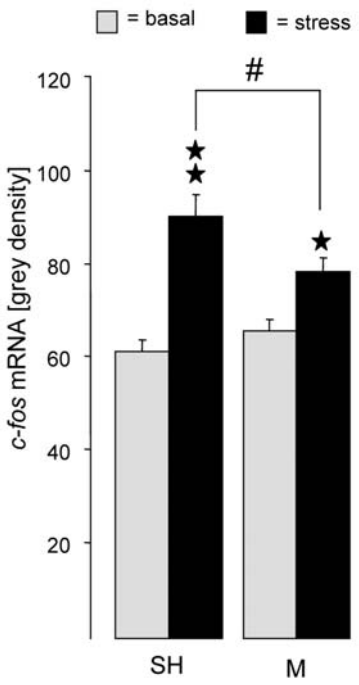

Fig. 22: Stress-induced *c-fos* mRNA expression in the PVN of male rats. Merged groups of SH and M males treated icv with VEH and OT-A (chapter III, experiment 3) show a significant effect of mating and stressor exposure on *c-fos* mRNA expression in the PVN (factor mating X stress: $F_{1,33}$ 5.03, P = 0.032) with increased expression found 30 min after 10 min of FS in SH (P < 0.01; n = 8/11) and M (P < 0.05; n = 8/10) animals. Mating significantly reduced the stress-induced *c-fos* mRNA expression compared to SH stressed animals (P < 0.05). ‡ P < 0.01;★ P < 0.05 versus respective basal mRNA expression; # P < 0.05 versus SH stressed males.

Based on the results in males presented in chapter II, we conducted similar studies in female rats (chapter IV). However, the experimental demands proved to be more complicated and the results gained are less clear. This is due to the fact that in female rats, we have to differentiate between paced and unpaced mating (see chapter I) and they will only mate in the receptive phase of their cycle. Therefore, it is necessary to OVX the experimental animals and treat them with steroid hormones to elicit receptivity. OVX surgery, s.c. injection procedures and of course, the resulting fluctuation in steroid hormone concentrations are factors that may possibly influence behavioral testing in female rats as opposed to the experiments performed in males.

Paced mating did not directly influence the anxiety-related behavior of females and the stressful conditions of unpaced mating were found to increase anxiety. On the other hand, we found that the brain OT system is activated by paced mating, and therefore, other positive effects of mating besides anxiolysis are possible. As only the respective paced mating condition is rewarding in both males and females (Paredes and Vazquez 1999; Martinez and Paredes 2001) and a direct interaction between OT and the mesolimbic DA system has been recently demonstrated (Melis et al. 2003; Melis et al. 2007; Succu et al. 2007), an activated OT system may mediate the rewarding and reinforcing properties of paced mating in both male and female rats.

Additionally, OT has repeatedly been shown to increase social approach behavior and pair bonding in nonhuman mammals (Arletti and Bertolini 1985; Carter et al. 1995; Young and Wang 2004), to increase trust and social memory (Kosfeld et al. 2005; Guastella et al. 2008) and positively affect couple interactions in humans (Ditzen et al. 2008b). Therefore, OT release during paced mating could possible facilitate the pro- and receptive behaviors displayed by the female to solicitate sexual interactions, and reduce the acute fear and stress responses to allow the approach of the larger male conspecifics.

As mentioned above, the fluctuations of gonadal steroids across the ovarian cycle strongly influence various behavioral parameters in female rats, including anxiety (Mora et al. 1996; Marcondes et al. 2001). As we found elevated plasma P at the time point of behavioral testing and P has been described as being highly anxiolytic (Fernandez-Guasti and Picazo 1990; Mora et al. 1996; Frye et al. 2000; Frye and Walf 2004), we conclude that this is a possible explanation for the inability of paced mating to further decrease anxiety in female rats. It is interesting to note that in an attempt to circumvent the anxiolytic effect of P we also managed to induce receptivity and, therefore, were able to mate females by E treatment only, as has been described previously (Brandling-Bennett et al. 1999). However, a high level of P is necessary for the expression of proceptive behaviors (Gonzalez-Flores et al. 2004) and the development of a place preference as a sign of rewarding properties of paced mating can only be observed after P treatment (Paredes and Alonso 1997; Paredes and Martinez 2001). In our experiment, E primed females failed to show any proceptive behaviors and, although they did mate, we observed a high amount of antagonistic behavior towards the male conspecific (unpublished data). Based on these observations during the mating period we decided to forego further behavioral testing of these females.

To summarize, there was no anxiolytic effect of mating in primed females, but an activated brain OT system during paced mating has been shown here for the first time, and provides important knowledge about the central effects of paced and unpaced mating.

2 Differential effects of sexual activity on anxiety in males of three breeding lines

The results presented in chapter II demonstrated that sexual activity and mating affect the emotional and behavioral reactivity of mated male rats to subsequent stressors for up to 4 h. In continuation of these results, we assessed if the anxiolytic effect of mating can still be observed at a later time point in unselected male rats, and if there are similar anxiolytic effects of sexual activity in males of an animal model for extreme anxiety-related behavior, i.e. the high (HAB) and low (LAB) anxiety-related behavior breeding lines.

Based on our finding of reduced anxiety for up to 4 h after mating and a study performed by the group of Fernández-Guasti, which showed long-lasting central adaptations, namely increased enkephalin contents in various brain regions, after sexual activity for at least 48 h (Rodriguez-Manzo et al. 2002), we tested if changes in anxiety-related behavior can still be observed 24 h after a 30-min mating period. Therefore, male rats were mated for 30 min as described in chapter II and tested on the EPM 24 h later. However, no anxiolytic effect of mating could be observed (Fig. 23). This result is supported by findings from Fernández-Guasti and co-workers, showing that one or several ejaculations lead to an instant anxiolytic effect in the defensive burying paradigm that can not be observed 24 h after mating (Rodriguez-Manzo et al. 1999).

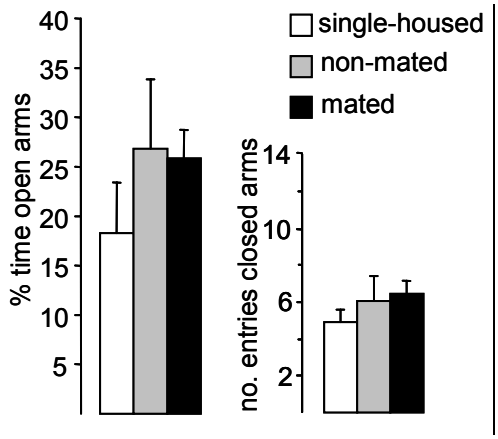

Fig. 23: Anxiety-related behavior of male rats 24 h after mating. Male rats were tested on the EPM 24 h after termination of a 30-min mating period. No differences in anxiety were found between single- housed (n = 8), non-mated (n = 10) and mated (n = 13) males.

From this we conclude that although sexual activity leads to long-lasting central changes, e.g. an activated opioid system (Rodriguez-Manzo et al. 2002) and reduces anxiety for up to 4 h, a 30-min mating period does not lead to long-term adaptations in anxiety-related behavior in male rats.

The HAB and LAB rat lines were selectively bred over several years for differences in anxiety-related behavior on the EPM (Liebsch et al. 1998a; Liebsch et al. 1998b; Landgraf et al. 1999). HAB rats consistently show a high level of inborn anxiety, which is reflected by their behavior not only on the EPM, but also in other behavioral tests, e.g. the BWB or open field (Henniger et al. 2000). Additionally, they show reduced exploratory and aggressive, as well as increased depression-like behavior (Liebsch et al. 1998a; Veenema et al. 2007). LAB rats show a lower reactivity of the HPA axis to non-social stressors (Landgraf et al. 1999; Salome et al. 2004) but an increased HPA axis activation during social stress and intermale aggression (Veenema et al. 2007). These behavioral traits have been proposed to, at least partially, depend on a genetic variation that leads to VP overexpression in HAB rats (Murgatroyd et al. 2004). Taken together, HAB and LAB rats provide an excellent animal model for studying the neurobiology of inborn anxiety and the actions of anxiolytic and antidepressant compounds (Liebsch et al. 1998a; Keck et al. 2003). In

the context of this thesis, I wanted to elucidate if mating is able to change the inborn levels of trait anxiety in HAB and LAB males, or if anxiolytic effects of mating are limited to the acute expression of anxiety in unselected rats. Therefore, HAB and LAB males were mated with unselected OVX females for 30 min, and 30 min or 4 h later tested for anxiety-related behavior on the EPM or BWB, respectively. During the mating period, we scored the number of IM to characterize possible differences in the mating behavior between the two breeding lines.

During the interaction with a receptive female, LAB males were more active than HAB animals and showed a higher number of IM during the 30-min mating period (LAB: 33.8 ± 2.5; HAB: 20.7 ± 2.8). However, although LAB animals engage in sexual activity very quickly and intensively when presented with a receptive female, the effect of mating on anxiety-related behavior was more pronounced in HAB males 30 min and 4h after sexual activity (Fig. 24 a, c, 25 a, c). HAB males showed decreased anxiety-related behavior on the EPM and in the BWB (Fig. 24 a, 25 a) and their level of anxiety positively correlated with the number of IM they achieved during the respective mating period (Fig. 24 b, 25 b).

Mated LAB males did not show any behavioral changes when tested on the EPM 30 min after mating (Fig. 24 c), but showed reduced anxiety in the BWB 4 h after mating (Fig. 25 c) and the number of IM achieved did not correlate with anxiety-related behavior (Fig. 24 d, 25 d). It is notable that basal anxiety in SH LAB males was very low on the EPM (Fig. 24 c), possibly creating a ceiling effect and, therefore, allowing no additional anxiolytic effect of mating in this test.

These results confirm our findings in unselected male Wistar rats (chapter II) and demonstrate that sexual activity is able to reduce the state anxiety of male HAB and LAB rats.

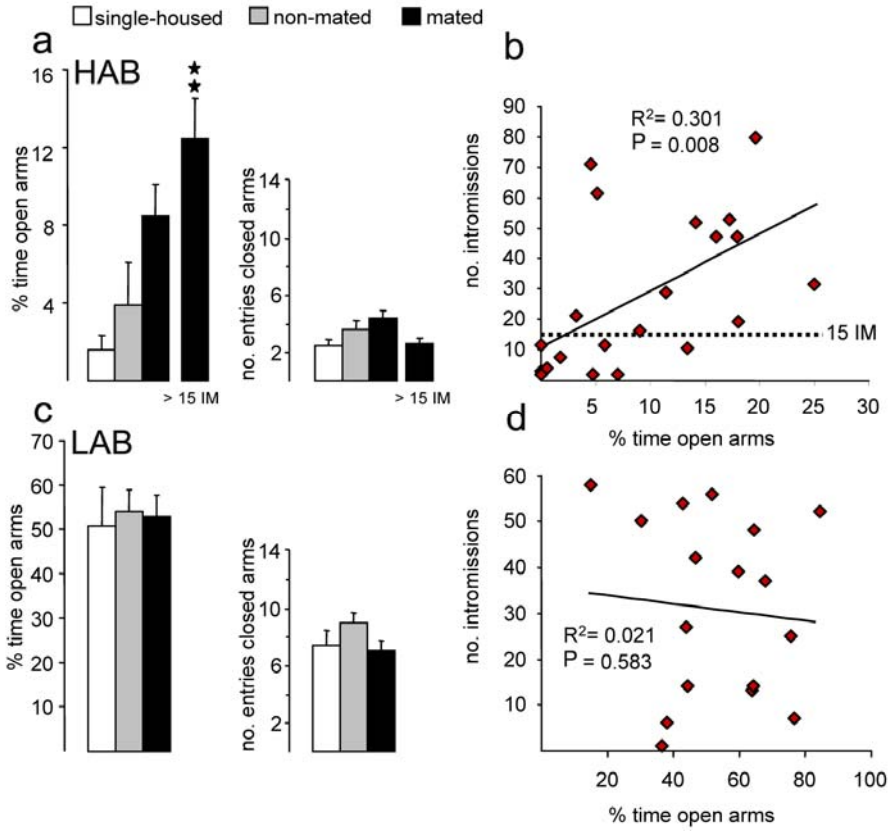

Fig. 24: Anxiety-related behavior of HAB and LAB males 30 min after mating. Male HAB (**a**) and LAB (**c**) rats were tested on the EPM 30 min after termination of a 30-min mating period. The level of mating-induced anxiolysis in HAB males was found to positively correlate with the number of IM achieved (linear regression: $F_{1,20}$ 8.61, P = 0.008, **b**). HAB males with more than 15 IM showed reduced anxiety (factor mating: $F_{2,33}$ 5.46, P = 0.009; P < 0.01 versus single-housed males, **a**). No differences in anxiety-related behavior were found between mated and non-mated/ single-housed LAB males (**c**) and their number of IM did not correlate with their time spent on the open arms of the EPM (**d**). The locomotor activity as reflected by the number of entries into the closed arms was not significantly altered by mating. Group size HAB/ LAB: single-housed: n = 10/10; non-mated: n = 13/9; mated: n = 21/17; data represent means + S.E.M. ⁑ P < 0.01 versus single-housed males.

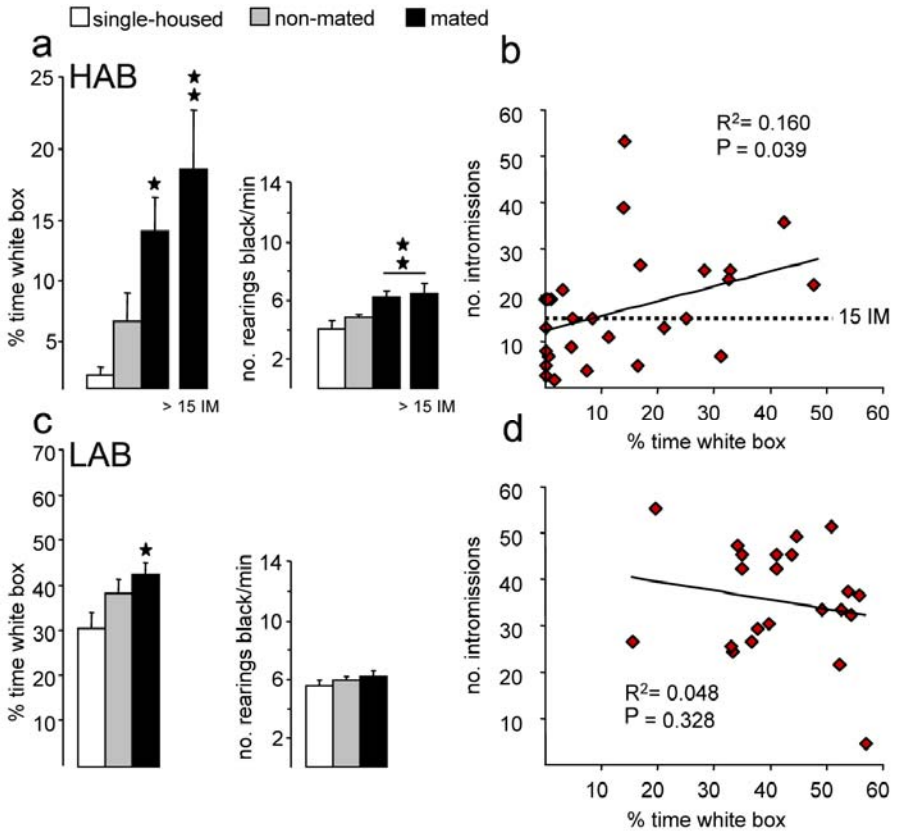

Fig. 25: Anxiety-related behavior of HAB and LAB males 4 h after mating. Male HAB (**a**) and LAB (**c**) rats were tested in the BWB 4 h after termination of a 30-min mating period. Mated HAB males showed decreased levels of anxiety (factor mating: $F_{2,54}$ 4.26, P = 0.019; P < 0.05 versus single-housed males) and increased exploratory behavior ($F_{2,54}$ 12.3, P = 0.02; P < 0.01 versus single-housed males) in the BWB. The level of mating-induced anxiolysis in HAB males was again found to positively correlate with the number of intromissions (IM) achieved (linear regression: $F_{1,25}$ 4.75, P = 0.039, **b**). HAB males with more than 15 IM showed a highly significant reduction of anxiety ($F_{2,39}$ 6.14, P = 0.005; P < 0.01 versus single-housed males, **a**) and increased exploratory behavior ($F_{2,39}$ 10.7, P = 0.006; P < 0.01 versus single-housed males). Mated LAB males showed decreased levels of anxiety ($F_{2,47}$ 4.26, P = 0.026; P < 0.05 versus single-housed males, **c**) but their number of IM did not correlate with their time spent in the white box (**d**). Group size HAB/ LAB: single-housed: n = 13/13; non-mated: n = 16/16; mated: n =28/21; data represent means + S.E.M. ⁑ P < 0.01, ⋆P < 0.05 versus single-housed males.

What are the possible mechanisms behind these findings? Based on their behavior, HAB and LAB animals have been described as a valid animal model of trait anxiety and depression that mimics psychopathological conditions seen in humans (Landgraf and Wigger 2002). Aside from being hyperanxious, HAB animals also show an

elevated HPA axis reactivity upon exposure to a mild emotional stressor (Landgraf et al. 1999). Concerning the role of OT, the brain OT system has been reported to contribute to the regulation of the HPA axis in unselected rats (Gibbs 1986; Neumann et al. 2000a; Neumann et al. 2000b), but central OT release in the PVN of HAB and LAB rats has been shown to be similar in response to different kinds of stressors, thus making an involvement of brain OT in line-divergent HPA axis regulation rather unlikely (Wigger et al. 2004). Additionally, OTR expression in the PVN has been shown to be similar between HAB and LAB males (Wigger et al. 2004) and females (Bosch et al. 2005). In the light of these results, our finding of a more pronounced anxiolytic effect of mating in HAB males can probably not be explained by a general difference in the reactivity of the brain OT system between the two breeding lines, which, however, needs to be verified in future experiments.

The positive correlation between the number of IM and reduced anxiety after mating we saw exclusively in HAB males is difficult to interpret. One possible explanation is a direct correlation between the intensity of the sexual interactions and the activation of the central OT system. In this case, a low number of IM would only elicit a small increase in OT release in the PVN in HAB males, whereas a high number of IM and, therefore, a more pronounced increase in extracellular OT, may mediate the anxiolysis seen in sexually active HAB males. This theory is supported by studies in male Syrian hamsters, where the expression of c-Fos protein in distinct brain regions was found to depend on the number of ejaculations achieved (Fernandez-Fewell and Meredith 1994; Kollack-Walker and Newman 1997). We know from lactating female HAB rats, that they react with a more pronounced OT release in the PVN and central amygdala (CeA) as compared to LAB and unselected females when confronted with an emotionally stressful situation (Bosch et al. 2005). This finding points towards a

stronger activation of the OT system in HAB rats in response to a similar environmental stimulus. If this finding in females is also valid in males, a similar amount of IM should elevate the intra-PVN release of OT more in HAB than in LAB or unselected males. This theory is supported by our results, as a comparable (high) amount of IM has stronger anxiolytic effects in HAB than in LAB males. However, evidence for a differential release of central OT in response to mating between the two breeding lines is lacking.

Another possible explanation for the differential effect of mating in HAB and LAB males arises from the results of chapter III, namely that mating is a stressful stimulus itself and activates the HPA axis. As HAB animals are hypersensitive to emotional stress (Landgraf et al. 1999; Neumann et al. 2005), it can be speculated that sexual activity is more stressful for these animals than for LAB or unselected males and might elicit a more severe hormonal and/or central stress response and thereby, act anxiogenic. This anxiogenic effect of mating in HAB animals would counteract its anxiolytic properties. Therefore, only a prolonged or more intense mating period, as reflected by a higher number of IM, may be sufficient to overcome these concomitant negative effects and, thus, lead to a measurable reduction in anxiety in HAB males. It has been shown that LAB males show a higher level of intermale aggression and a hypersensitive HPA axis compared to HAB males in this context (Frank et al. 2006; Veenema et al. 2007). This is not in contrast to the aforementioned theory of a higher stress-reactivity during mating in HAB males, as LAB males show a generally lower stressor susceptibility than HAB rats (Landgraf et al. 1999; Salome et al. 2004; Neumann et al. 2005), and the finding of a hypersensitive HPA axis in LAB animals is clearly linked to aggressive behavior. Therefore, sexual activity, which is not predominantly an aggressive interaction, but requires the male to show proactive

behavior, may be a more stressful social context for HAB males. However, data on HPA axis reactivity during sexual activity is lacking in HAB and LAB animals.

In summary, the anxiolytic effect of sexual activity has not only been confirmed in this additional study, but has been proven to be effective in reducing anxiety in a psychopathologically relevant animal model.

However, we have to be careful when trying to derive insight into human sexuality and its emotional implications from results as those gained here. As Beach said in 1979: “The validity of interspecific generalization can never exceed the reliability of intraspecific analysis;” (Beach 1979). The opinion that animal sexual behavior, as well as emotionality, stress coping and anxiety are homologous and analogous to the one of humans and are controlled by similar or identical neurochemical and hormonal systems is widely accepted among neuroscientists (Pfaus et al. 2003). Therefore, our finding of positive effects of mating on trait and state anxiety, and the involvement of brain OT, may provide a model of stress-protection for other species as well. Or, to say it provocatively, sexual activity and mating may be a possible remedy for anxiety and depression disorders.

Another aspect to be discussed here is the fact that the interaction with a non-receptive female already tended to reduce anxiety in male HAB and LAB rats (Fig. 24 a, Fig. 25 a, c). This effect could also be observed in part of the experiments presented in chapter II (Fig. 6 b, c, Fig. 7 a). In this context it is of interest to note that OT release within the PVN tended to rise during visual and olfactory contact with a receptive but not a non-receptive female (Fig. 8 a). Therefore, we have to consider that other central neurotransmitter systems, including serotonin, DA and opioids are activated during both types of social contact (Pfaus 1999; Argiolas and Melis 2004; Devidze et al. 2006) and may contribute to the anxiolytic effect observed. Another

possible explanation for the decreased anxiety after contact with a non-receptive female could be the general increase in arousal triggered by the interaction with a conspecific after the pre-experimental period of single-housing that all animals undergo before behavioral testing in our lab.

3 OT release in distinct brain regions during sexual activity in male rats

The microdialysis study performed in chapter II revealed that OT release within the PVN is increased during mating in male rats (Fig. 8). When looking at the time course of OT release, it is noteworthy that OT already increases during olfactory and visual contact with a receptive female (sample 4), peaks during mating (sample 5), then starts to drop in the second half of the mating period (sample 6) and quickly reaches basal level after removal of the female (sample 7). The fact that icv administration of the OT-A abolished the reduced anxiety after mating even though the infusion was performed after the termination of the mating period and, therefore, the peak OT release within the PVN, leads to the suggestion that the transient OT release is not directly responsible for the anxiolytic effect of mating. Local release of OT in the PVN has been suggested to be important for positive feedback and autoexcitation of OT neurons and may facilitate remote axonal release in the neurohypophysis (Kombian et al. 1997; Landgraf and Neumann 2004; Neumann 2007) and possibly also in other brain regions that receive oxytocinergic innervations from the PVN (Gimpl and Fahrenholz 2001; Melis et al. 2007). To elucidate if OT is released in other brain regions during mating we performed additional microdialysis experiments within the CeA, the hypothalamic supraoptic nucleus (SON) and the VTA of male rats, according to the experimental setup of chapter II. In the CeA, OT fibers and receptors

are abundant in female and male rats (Sofroniew 1983; Barberis and Tribollet 1996; Gimpl and Fahrenholz 2001), local OT has been shown to exert anxiolytic effects (Bale et al. 2001; Neumann 2002), to inhibit autonomic fear responses (Huber et al. 2005) and to promote sedation (Ebner et al. 2005).

The SON is the second major site of OT synthesis within the brain and known to regulate the activity of oxytocinergic neurons and neurohypophyseal release of OT (Lambert et al. 1993; Neumann et al. 1994a; Kombian et al. 1997). In male mammals OT is released into the bloodstream during sexual activity and at the time of ejaculation (Stoneham et al. 1985; Kruger et al. 2003). Local release of OT has been demonstrated in both hypothalamic nuclei in response to various stimuli, including suckling, parturition and exposure to physical or emotional stressors (Moos et al. 1989; Neumann et al. 1993; Wotjak et al. 1998; Wigger and Neumann 2002). In addition, the fact that recent imaging studies revealed centrally projecting oxytocinergic neurons originating from the SON (V. Grinevich, unpublished data) implicates a role of the SON also in central oxytocinergic neurotransmission.

The VTA is a region known to receive oxytocinergic innervations from the PVN (Buijs 1978; Sofroniew 1983; Roeling et al. 1993) and contains OTR and its mRNA (Freund-Mercier et al. 1987; Vaccari et al. 1998). Importantly, the VTA also contains the cell bodies of mesolimbic DA neurons and is thought to play a key role in the motivational and rewarding properties of natural reinforcing stimuli, such as food and sexual activity (Fibiger and Phillips 1988; Wise and Rompre 1989; Everitt 1990).

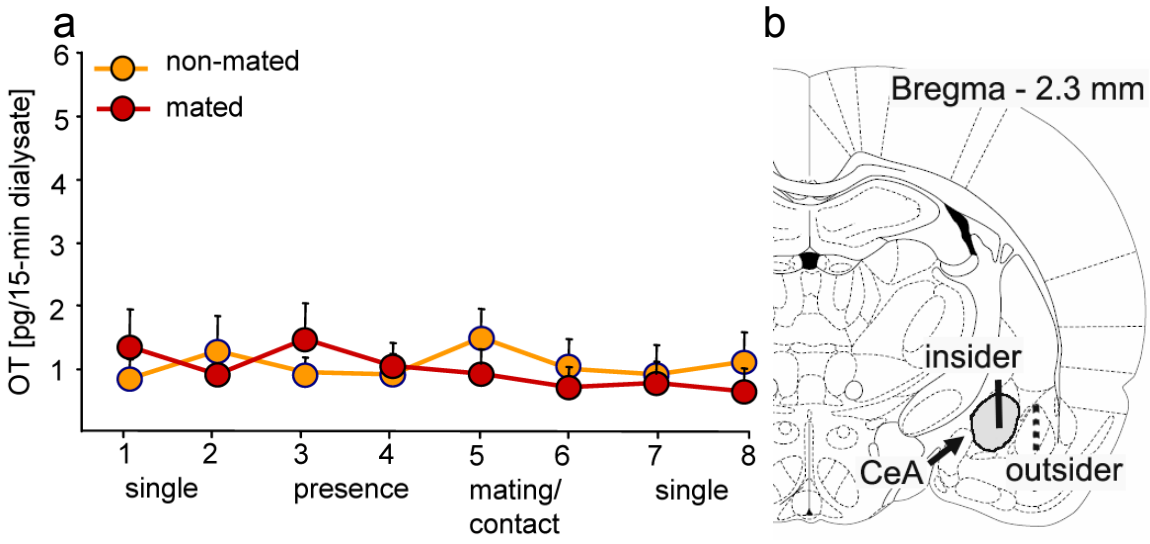

Fig. 26: OT release within the CeA during sexual activity in male rats. Mating does not trigger increased OT release into the extracellular fluid of the CeA of male Wistar rats. (**a**). Two 15-min microdialysates were sampled during single-housing (dialysates 1, 2), during the presence of a non-receptive (non-mated group, orange; n = 8) or receptive (mated group, red; n = 5) female behind a perforated wall (dialysates 3, 4), during physical contact (non-mated) or mating with the respective female (dialysates 5, 6) and after removal of the female from the male's home cage (dialysates 7, 8). Data represent means + S.E.M. (**b**) Schematic drawing of the rat brain and representative positions of microdialysis probes within (insider) and outside (outsider) the CeA (2.3 mm caudal to Bregma, 3.8 mm lateral to midline, 8.5 mm beneath the surface of the skull, Paxinos and Watson 1998).

In our experiments, we could confirm basal levels of OT release within the CeA (Bosch et al. 2004; Bosch et al. 2005; Ebner et al. 2005). However, local OT concentrations did not change in response to female contact or mating (Fig. 26 a). OT infused into the CeA exerts anxiolytic effects (Bale et al. 2001) and OT release is increased within the CeA in response to stressor exposure (Bosch et al. 2005; Ebner et al. 2005). Therefore, we expected mating-induced changes in OT release either in the context of anxiolysis or the stressful component of mating, as reflected by increased HPA axis activation (chapter III). However, OT release did not change during mating or, it might be possible that the fluctuations in extracellular OT content are at submicromolar level, which still acts neuromodulatory but is below the detection limit of our methodical capabilities (Landgraf and Neumann 2004).

As the 15-min dialysates obtained from the amygdala contained OT concentrations near the detection limit we decided to increase the sampling interval to 30 min for the subsequent experiments.

Similar to the CeA, OT release stayed unchanged in the SON during contact and mating with a receptive female (Fig. 27 a). The tendency of increased OT release during the presence of an inaccessible receptive female in the mated group (dialysate 3) is based on a single animal that showed elevated OT release at this time, and can therefore not be seen as a general trend towards an activated brain OT system in the SON. It is known that OT is released from the posterior pituitary during sexual activity and especially at the time of ejaculation in rats (Stoneham et al. 1985; Hughes et al. 1987) and men (Kruger et al. 2003). Our finding of unchanged OT release within the SON suggests that peripheral release in this context is not dependent on autoexcitatory actions of OT within the SON as found, for example, during suckling (Neumann et al. 1994a). Additionally, our finding suggests that locally released OT within the SON is not contributing to the anxiolytic effect of sexual activity.

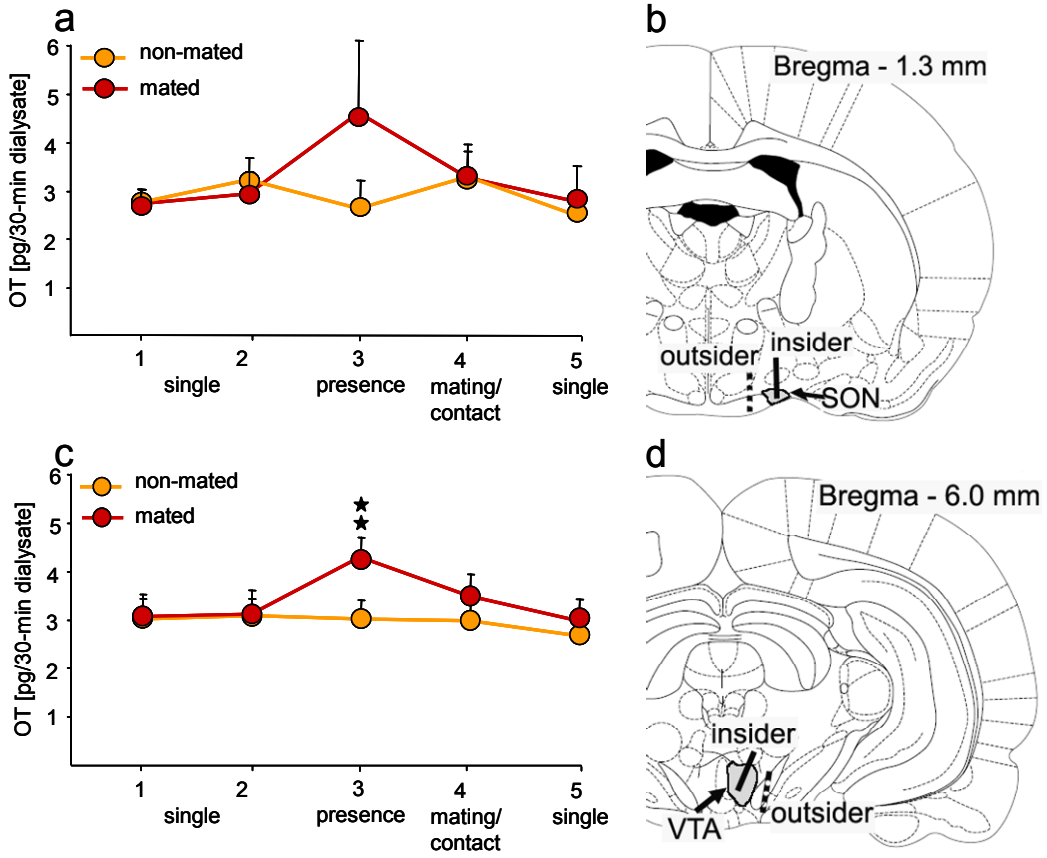

Fig. 27: OT release within the brain during sexual activity in male rats. Mating does not significantly increase OT release into the extracellular fluid of the SON (**a**), but increased release of OT is found in VTA (**c**) during the presence of an inaccessible receptive female. Thirty-min microdialysates were sampled during single-housing (dialysates 1, 2), during the presence of a non-receptive (non-mated group, n = 8 / 6) or receptive (mated group, n = 8 / 10) female behind a perforated wall (dialysate 3), during physical contact (non-mated) or mating with the respective female (dialysate 4) and after removal of the female from the male's home cage (dialysate 5). (**c**) OT release in the VTA was found to depend on mating ($F_{1,14}$ 8.68, P = 0.011) and time ($F_{4,56}$ 3.06, P = 0.024), with increased OT release during the presence of an inaccessible receptive female (P < 0.01). Data represent means + S.E.M. ⁑ P < 0.01 versus basal values (dialysates 1, 2 and 5) and non-mated males (dialysate 3). (**b, d**) Schematic drawings of the rat brain and representative positions of microdialysis probes within (insider) and outside (outsider) the SON (**b**, 1.9 mm caudal to Bregma, 1.8 mm lateral to midline, 9.4 mm beneath the surface of the skull) and the VTA (**d**, 6.0 mm, 2.2 mm, 8.8 mm) (Paxinos and Watson 1998).

The VTA, as part of the mesolimbic DA system, has been associated with male sexual behavior since increased local neuronal activity was shown in early *c-fos* studies (Robertson et al. 1991). More recently, the role of the mesolimbic system in sexual behavior and its rewarding properties have been studied in detail (Melis et al. 2003; Melis et al. 2007; Succu et al. 2007; Succu et al. 2008). Local injections of OT into the VTA have been found to induce penile erections and increase DA release in the NAcc (Melis et al. 2007). However, release of endogenous OT in the VTA during sexual activity was measured for the first time in the present study.

We found increased OT release during the presence of a receptive female behind a perforated polycarbon wall, but not during sexual contact with the female (Fig. 27 c). In contrast, neither the presence of an unprimed female behind the dividing wall nor physical contact with this female affected OT release within the VTA. This finding provides further evidence for the involvement of OT in the motivational aspects underlying sexual behavior. It also strongly supports the proposition of Argiolas and co-workers about a neuronal circuit that connects the PVN with the VTA and the NAcc and plays a role in both anticipatory and consummatory aspects of male sexual behavior (Martino et al. 2005; Melis et al. 2007; Succu et al. 2007; Succu et al. 2008). However, as shown in chapter II, the mere presence of a receptive female without mating does not decrease anxiety in males. From this we conclude that the increased OT release found in the VTA, and the tendency seen in the PVN during olfactory and visual contact, is not directly involved in the anxiolytic effect of mating.

In summary, these microdialysis experiments clarify the mechanisms behind the OT-mediated anxiolytic effect of mating to some extent, as OT release was found to be unchanged in the CeA and SON during mating, and to increase in VTA during

olfactory and visual contact with a receptive female. The CeA and SON seem to be uninvolved in the oxytocinergic mediation of reduced anxiety after mating, while OT release in the VTA is likely to mediate the increased motivation in response to a receptive female, likely via the mesolimbic DA system.

There are several other brain regions that have been shown to contain moderate to high levels of OT mRNA and are activated by sexual behavior, for example the BNST and MPOA (Greco et al. 1996; Greco et al. 1998; Gimpl and Fahrenholz 2001). Therefore, OT release in these regions could be involved in the modulation of anxiety-related behavior after mating. Additionally, OT may diffuse to remote brain regions after its dendritic release in the PVN (Landgraf and Neumann 2004) and reach distant receptors at low but still neuromodulatory efficient concentrations (Lambert et al. 1994; Brussaard et al. 1996). In this case, our microdialysis procedure would not be able to detect submicromolar changes in the extracellular OT concentration. This hypothesis could be verified by local injection of the OT-A directly after mating, for example into the CeA, as this would inhibit any type of local OT actions and therefore inhibit possible anxiolytic actions of OT.

4 Conclusion and outlook

The present thesis provides evidence that sexual activity and mating can positively affect the response to subsequent anxiogenic and stressful stimuli in male Wistar rats. These effects of mating could be verified in rats selectively bred for extremes in anxiety-related behavior, namely the HAB and LAB breeding lines. Further, we could show that mating stimulates the brain OT system, as elevated release of OT was measured during sexual activity within the PVN and in anticipation of mating within the VTA of male rats. The activated brain OT system is causally linked to the anxiolytic effect of mating, as blockade of OTR abolished the anxiolytic effect of mating. In contrast, brain OT seems to be uninvolved in the attenuated neuronal reactivity found in the PVN of mated male rats after stressor exposure.

In females, it could be shown that sexual activity also increases brain OT release within the PVN, but only under paced mating conditions. Paced mating, however, did not change anxiety-related behavior in female rats. Females became even more anxious if they were mated in unpaced conditions.

We can conclude that sexual activity beneficially affects the response to emotional and physical/ psychological stressors in male rats. The consequences of sexual activity in females need further investigation, especially in context of the activated brain OT system, likely to be involved in the rewarding experience of paced mating. This may provide an interesting basis for future studies.

Several directions for future experiments arise from the results presented in this thesis.

In male rats, it would be interesting to investigate possible effects of mating on other behavioral parameters besides anxiety. Of major interest in this regard are social behaviors like social phobia or aggression, as they have a high translational value

concerning human social interactions. There are several established testing conditions in rats that allow the estimation of social anxiety and social avoidance. Additionally, consequences of mating on normal or abnormal intermale aggression would be an interesting direction to follow. Therefore, an interesting approach will be to mate male rats and investigate the impact of sexual activity on the subsequent level of aggressive intermale behavior.

The possible involvement of other neurotransmitter systems is also of interest. Our finding of anxiolytic mating effects in the HAB and LAB breeding lines points towards a possible involvement of the brain VP system, or even direct interactions between OT and VP in the control of anxiety-related behavior. Furthermore, the microdialysis results of an increased OT release in the VTA of unselected males suggests a role of the activated mesolimbic DA system in mating induced reward and maybe also anxiolysis. Therefore, a pharmacological manipulation of the central DA transmission, for example by blocking DA receptors in the NAcc, will reveal possible mechanisms behind our findings.

In the same line, the paced mating-induced OT release in the PVN of females did not affect anxiety-related behavior, but could convey other beneficial effects. A blockade of OTR directly after paced mating will reveal the involvement of brain OT in the rewarding aspects of sexual activity in females.

Finally, with regard to the reduced neuronal stress reactivity of mated male rats, we are currently working on the characterization of other transmitter systems and brain regions involved in this stress-protective effect of mating. Of a major interest in this context is the locus coeruleus and its noradrenergic innervation of the PVN, as this brainstem region has been shown to directly influence the stress-induced synthesis and release of CRH in the PVN.

Summary in German

Zusammenfassung auf Deutsch

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Sex ist eine der wichtigsten Interaktionen zwischen weiblichen und männlichen Tieren einer Art. Im Vordergrund steht dabei der Zweck der Reproduktion. Es gibt aber noch andere positive Auswirkungen auf den Organismus, zum Beispiel eine erhöhte Widerstandsfähigkeit gegenüber belastenden Umwelteinflüssen. Diese Eigenschaften sexueller Aktivität stehen aber erst am Anfang ihrer Erforschung.

Lange Zeit wurde in der Wissenschaft die Meinung vertreten, dass Sex einzig der Fortpflanzung und damit dem Erhalt der Spezies dient. In Modelorganismen, wie z.B. Mäusen oder Ratten, konnte allerdings bereits früh gezeigt werden, dass Sex eine Tätigkeit ist, die über eine Aktivierung des zentralen Dopamin- und Opioidsystems belohnend wirkt. Somit wäre im Prinzip das Auftreten sexueller Interaktionen erklärt: Der erfüllte Drang sich fortzupflanzen wirkt befriedigend. Folglich wird das einzelne Individuum durch die zentrale Ausschüttung von Neurotransmittern motiviert, die sexuelle Aktivität zu suchen.

Die Wissenschaft lieferte in den letzten 20 Jahren vereinzelt immer wieder Hinweise darauf, dass sowohl in Menschen, als auch in anderen Säugetieren, zusätzliche positive Effekte nach der Kopulation beobachtet werden können, die entweder das Verhalten oder die hormonelle Reaktivität beeinflussen können.

Dass Ruhe und Entspannung weit verbreitete Phänomene nach sexueller Aktivität darstellen, ist aus menschlicher Erfahrung bekannt. In kürzlich veröffentlichten Studien wurde gezeigt, dass Sex und Intimität zwischen Paaren die Probanden weniger anfällig für psychische Stressoren macht. Diese verminderte Reaktivität ist sichtbar in geringerer Cortisolfreisetzung und vermindertem Blutdruckanstieg. Ähnliche Ergebnisse, eine geringere Ausschüttung von Stresshormonen in Folge einer körperlichen oder psychischen Belastung, konnten auch in Ratten gezeigt werden, wenn diese vor der Stressbelastung die Möglichkeit zur Verpaarung

erhielten. Basierend auf diesen Hinweisen, die einen vergleichbaren positiven Effekt von Sexualverhalten, über die reine Reproduktion und das „Glücksgefühl“ hinaus, in Menschen und Tieren nahe legen, war es das Ziel dieser Arbeit, weitere positive emotionale, hormonelle und neuronale Folgeeffekte zu untersuchen. Zudem sollten die zentralen Mechanismen die dabei im Gehirn eine Rolle spielen und vor allem der Einfluss des Neuropeptids Oxytocin (OT) untersucht werden.

Zu diesem Zweck wurden männliche Ratten verpaart und anschließend zu verschiedenen Zeitpunkten in Verhaltenstests auf ihre Ängstlichkeit hin untersucht. Verglichen wurden sie zum einen mit einzeln gehaltenen Tieren, zum anderen mit Männchen, die Kontakt zu einem nicht paarungswilligen Weibchen hatten. Ausschließlich die vorher verpaarten männlichen Ratten zeigten eine geringere Ängstlichkeit und dieser Effekt hielt bis zu vier Stunden nach der Verpaarung an. In weiteren Versuchsgruppen wurde mithilfe der Mikrodialyse-Technik die Freisetzung von OT im hypothalamischen *nucleus paraventricularis* (PVN) während der Verpaarung gemessen. Dies zeigte, dass während der Verpaarung eine erhöhte Ausschüttung dieses Neuropeptids im PVN stattfindet. Um einen kausalen Zusammenhang zwischen der zentralen Freisetzung von OT und dem beobachteten Verhalten herzustellen, wurden dessen Rezeptoren pharmakologisch besetzt und dadurch inaktiviert. Die so behandelten Tiere wurden erneut auf ihre Ängstlichkeit hin untersucht. Dieses Experiment ergab, dass die verpaarten männlichen Ratten, die direkt nach dem 30-minütigen Paarungsintervall den Rezeptorantagonist intracerebroventrikulär verabreicht bekamen, nun wieder eine mit den Kontrollgruppen vergleichbare Ängstlichkeit zeigen. Somit wurde der direkte Zusammenhang zwischen der OT-Freisetzung im Gehirn und der verminderten Ängstlichkeit von Männchen nach dem Sex bewiesen.

Weiterführend wurden die hormonelle Stressantwort und die neuronale Stressreaktivität von verpaarten männlichen Ratten untersucht. Hierzu wurden die Tiere verpaart und 45 Minuten beziehungsweise vier Stunden später gezwungen eine Minute lang zu schwimmen. Dieser physische und psychische Stressor ist dafür bekannt, dass er sowohl peripher als auch im Gehirn eine deutlich messbare Stressantwort auslöst. Bei den Tieren wurden sowohl vor und nach der Verpaarung, beziehungsweise dem Schwimmstress, Blutproben über chronisch implantierte Jugularvenen-Katheter entnommen. Im so gewonnenen Blutplasma wurde die Konzentration der stressrelevanten Hormone ACTH und Corticosteron bestimmt. Das Experiment zeigte, dass die Verpaarung selbst eine starke hormonelle Stressantwort auslöst, jedoch die Reaktivität auf die zusätzliche Stressbelastung weder 45 Minuten noch vier Stunden später beeinflusst. Alle Versuchsgruppen zeigten einen vergleichbaren Anstieg von ACTH und Corticosteron im Plasma als Reaktion auf den Stressor. In einem weiteren Experiment wurden die Männchen nach dem 30-minütigen Paarungsintervall zehn Minuten zum Schwimmen gezwungen. In ihren Gehirnen wurde anschließende die neuronale Aktivität anhand der mRNA des Protoonkogenes *c-fos*, sowie dem Vorhandensein von CRH-mRNA bestimmt. Im Gegensatz zur unveränderten hormonellen Stressantwort als Folge des 1-minütigen Schwimmstresses, zeigte sich hier eine verminderte stressbedingte Synthese der beiden mRNAs im PVN von verpaarten Tieren. Um den möglichen Einfluss des Neuropeptids OT auf die zentral Stressantwort nach der sexuellen Aktivität näher zu untersuchen, wurde bei einer weiteren Gruppe von Tieren ebenfalls das zentrale OT-System pharmakologisch blockiert. Allerdings zeigte sich hierdurch keine Veränderung der geringen Synthese von *c-fos* mRNA als Folge von Schwimmstress. OT ist in diesem Fall also nicht an der veränderten neuronalen Stressantwort im PVN beteiligt.

Im letzten hier vorgestellten Projekt wurde der Einfluss der Verpaarung auf die Ängstlichkeit von weiblichen Ratten untersucht. Zu diesem Zweck wurden Gruppen von ovarektomierten und hormonbehandelten Weibchen unter zwei verschiedenen Bedingungen verpaart. Entweder war es ihnen möglich die Häufigkeit der sexuellen Interaktion mit dem Männchen zu kontrollieren, indem sie sich in einen dem größeren Männchen nicht zugänglichen Teil des Käfigs zurückziehen konnten. Im anderen Fall fand die Verpaarung in einem kleineren Käfig statt. Hier konnte das Männchen dauerhaft Kopulationsversuche unternehmen. Anschließend wurden die Weibchen zu verschiedenen Zeitpunkten und unterschiedlichen Lichtverhältnissen auf ihre Ängstlichkeit hin untersucht. Als Kontrollen dienten hierbei ovarektomierte, sowie ovarektomierte und hormonbehandelte Weibchen. Hierbei ergab sich, dass bei Weibchen, die die Verpaarung kontrollieren konnten, die Ängstlichkeit unverändert blieb. Die unausweichliche Interaktion mit dem Männchen erhöhte dagegen die Ängstlichkeit der so verpaarten Weibchen. In den Kontrollgruppen zeigte sich interessanterweise eine deutlich verminderte Ängstlichkeit aufgrund der für die Verpaarung erforderlichen Hormonbehandlung. Analog zu den Männchen wurde auch bei weiblichen Versuchstieren die Freisetzung von OT im PVN während der Verpaarung, hier in beiden verwendeten Versuchsanordnungen, gemessen. Es zeigte sich, dass nur wenn das Weibchen die Häufigkeit der Interaktion kontrolliert, ein starker Anstieg in der Freisetzung des Neuropeptids messbar war. Da dieser Befund nicht mit einer Veränderung der Ängstlichkeit einherging, wird es ein zukünftiges Ziel sein, die mögliche Bedeutung dieser Aktivierung des zentralen OT Systems zu erklären.

Um einen Hinweis auf den Einfluss der Steroidhormone Estradiol und Progesteron auf das hier beobachtete Ängstlichkeitsverhalten zu erlangen, wurde die

Konzentration der subkutan verabreichten Hormone im Blut gemessen. Hierbei zeigte sich, dass nach der jeweiligen subkutanen Applikation eine rasche Erhöhung der Konzentration dieser Hormone im Blutplasma nachweisbar ist. Auch nach 27 Stunden waren die Werte noch nicht wieder zu basalen Werten zurückgekehrt. Es ist bemerkenswert, dass circa vier bis sechs Stunden nach der Injektion die höchste Konzentration von Progesteron im Blutplasma gefunden wurde. Dies stimmt mit dem Zeitraum überein, der unabhängig von diesem Ergebnis für die Verpaarung und auch die Verhaltenstests gewählt wurde. Deshalb ist ein Einfluss der peripheren Konzentration von Progesteron auf die gemessene Ängstlichkeit, wenn auch hier nicht kausal nachgewiesen, sehr wahrscheinlich.

Zusammenfassend kann man sagen, dass in dieser Arbeit positive Einflüsse von Sex gezeigt werden konnten. Vor allem in Bezug auf das Ängstlichkeitsverhalten und die zentrale Stressreaktivität von männlichen Ratten konnten deutliche Effekte gezeigt werden. Im Falle der Ängstlichkeit wird diese Veränderung durch eine erhöhte Aktivität des OT-Systems im Gehirn bedingt. Eine erhöhte Ausschüttung dieses Neuropeptids konnte auch in Weibchen während der Paarung nachgewiesen werden, hier aber ohne Beeinträchtigung des Verhaltens. Verpaarung und Sex führen folglich zu positiven Adaptationen des Organismus. Allerdings ist die Ausprägung dieser Anpassungen sowohl geschlechts- und mit großer Wahrscheinlichkeit auch artspezifisch. Dennoch liefert diese Arbeit für die Humanforschung wichtige, grundlegende Einblicke, da in Menschen ähnliche Veränderungen durch Sexualverhalten beobachtetet werden können und es wahrscheinlich ist, dass dabei ebenfalls die hier aufgedeckten Mechanismen beteiligt sind.

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Abbreviations

Abbreviations

α-MSH	α-Melanocyte Stimulating Hormone
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis Of Variance
BNST	Bed Nucleus of the Stria Terminalis
BWB	Black-White Box
CRH	Corticotropin-Releasing Hormone
DA	Dopamine
E	Estradiol
	Enzyme-Linked Immuno Sorbant
ELISA	Assay
EPM	Elevated Plus-Maze
	functional Magnetic Resonance
fMRI	Imaging
FS	Forced Swimming
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
HAB	High Anxiety-related Behavior
HPA	Hypothalamo-Pituitary-Adrenal
HPG	Hypothalamo-Pituitary-Gonadal
icv	Intracerebroventricular
IM	Intromissions
LAB	Low Anxiety-related Behavior
M	Mated
MeA	Medial Amygdala

MPOA	Medial Preoptic Area
mRNA	messenger Ribonucleic Acid
NAcc	Nucleus Accumbens
NM	Non-Mated
OT	Oxytocin
OT-A	Oxytocin Antagonist
OTR	Oxytocin Receptor
OVX	Ovariectomized
P	Progesterone
PET	Positron Emission Tomography
PVN	Paraventricular Nucleus
s.c.	Subcutaneously
S.E.M.	Standard Error of the Mean
SH	Single-Housed
T	Testosterone
VEH	Vehicle
VMH	Ventromedial Hypothalamus
VP	Arginine Vasopressin
VP-A	Vasopressin Antagonist
VTA	Ventral Tegmental Area

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitates gekennzeichnet.

Weitere Personen als die angegebenen waren an der inhaltlich-materiellen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe eines Promotionsberaters oder anderer Personen in Anspruch genommen. Niemand hat von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation stehen.

Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Regensburg, __.03.2009

(Martin Waldherr)

Then, with your permission, we will leave it at that. The temptation to form premature theories upon insufficient data is the bane of our profession.

Sherlock Holmes