2. Female reproductive behavior

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List of abbreviations

5-HT	serotonin
ARC	arcuate nucleus of the hypothalamus
ARC-VM	arcuate-ventromedial area of the hypothalamus
BNST	bed nucleus of the stria terminalis
BNSTpm	posteromedial part of BNST
CTF	central tegmental field
DA	dopamine
DRN	dorsal raphé nucleus
E	estrogen
ER	estrogen receptor
Fos-IR	Fos-immunoreactivity
FSH	follicle stimulating hormone
GABA	gamma-aminobutyric acid
GnRH	gonadotropin-releasing hormone
GnSAF	gonadotrophin surge-attenuating factor
Icv	Intracerebroventricular
LH	luteal hormone
LHA	lateral hypothalamic area
MeA	medial amygdala
MeApd	posterodorsal part of MeA
MPN	medial preoptic nucleus
MRN	medial raphé nucleus
NAc	nucleus accumbens

NPY	neuropeptide Y
OT	oxytocin
P	progesterone
PAG	periaqueductal gray
PD	posterodorsal preoptic nucleus
PMV	ventral premammillary nucleus
POA	preoptic area
PR	progesterone receptor
PVN	paraventricular hypothalamic nucleus
SERT	serotonin transporter
SPFp	subparafiscular nucleus
SSRI	selective serotonin reuptake inhibitor
VMN	ventromedial hypothalamus
VMNcv	caudoventral part of VMN
VMNvl	ventrolateral part of VMN
VTA	ventral tegmental area

Abstract

Reproductive behavior is the behavior related to the production of offspring and includes all aspects from the establishment of mating systems, courtship, sexual behavior, parturition, to the care of young. In this chapter, I outline the hormonal regulation of the estrous cycle, followed by a description of the neural regulation of female sexual behavior. Ovarian hormones play an important role in the induction of ovulation and behavioral estrus, in which they interact closely with several neurotransmitters and neuropeptides to induce sexual behavior. This chapter discusses the latest research on the role of estrogen, progesterone, serotonin, dopamine, noradrenaline, oxytocin and GABA in female mating behavior. In addition, the most relevant brain areas, such as the preoptic area and the ventromedial nucleus of the hypothalamus, in which these regulations take place, are discussed.

Keywords: Reproductive behavior, Females, Mating, Sexual behavior, Estrous cycle, Neural regulation, Dopamine, Serotonin, Noradrenaline, Oxytocin, Estrogen, Progesterone

2.1 Introduction

Reproductive behavior can be described as behavior related to the production of offspring. The combination of behavior leading to the union of male and female gametes and behavior facilitating or ensuring the survival and development of the young is required for an optimal reproductive success. Therefore, reproductive behavior includes basically all aspects from the establishment of mating systems, courtship, sexual behavior, parturition, to the care of young (McGraw-Hill Concise Encyclopedia of Bioscience (2005)). The timing and patterning of reproductive activity varies among species and is coordinated by an integration of both overt behavioral and internal physiological events leading to a complex set of behavioral adaptations. Although care of the young is an important part of reproductive behavior, here I will solely focus on the aspects of female mating behavior and the neural mechanisms that are involved in the regulation of these behaviors. In search for explanations to the neurobiological and endocrine bases for behavior, the field has been enormously enriched by the studies carried out on sexual behavior in rodent species. This chapter, therefore, mentions mostly examples of rat sexual behavior, sometimes accompanied by information of some other species.

Female mating behavior is often considered more complicated than male mating behavior, because most animals only start sexual encounters with mates when the females are in a so-called *estrus.*. Females can get highly motivated in this period and will for instance cross a highly charged electrified floor or poke obsessively a lever to gain physical access to a male. In contrast, when the female is in the opposite phase of her estrous cycle, in *anestrus*, no effort will be undertaken to get access to a potential mate. Women, and some other primates, are probably the only mammals in which their sexual drive is not directly associated to their estrous cycle, and mate with their partners throughout the whole cycle (Wallen and Zehr, 2004).

The estrous-phase has several consequences. As already mentioned, females in estrus will seek males, remain in close proximity and initiate copulation. Estrus, thus, affects the attraction female have towards males. However, at the same time, it also influences their own attractivity: males prefer to seek proximity to or mount females in estrus over their conspecifics in anestrus. In the whole process, it is important that the females are able to detect the potential mate and that this elicits particular behavior leading to successful copulation. Reproductive hormones play an important role in the regulation of this process. They are elevated during estrus and cause an enhancement of the acuity, sensitivity and efficiency of the sensory system and thereby improving the detection and response to potential mates when in estrus. In addition, the neuroendocrine changes affect the central nervous system: the higher hormone levels enhance the female's motivation, attention, perception, and behavior. Thus, an environment is created during estrus in which the conditions are most optimal for successful reproduction.

In this chapter, I will first touch upon the concept of the estrous cycle, which leads to the induction of the estrous-phase. It is important to highlight this event first, because the major changes in hormones that are occurring as part of the estrous cycle do not only induce fertility, but also have parallel behavioral consequences. Secondly, I will evaluate the principles of mating behavior and the neural regulation of the female reproductive behavior in more detail.

2.2 The estrous cycle

The basis of the estrous cycle is already present in females at the time of birth, since they are born with a finite number of germ cells that will later become oocytes. When puberty starts, the estrous cycle can be divided into the follicle phase and the luteal phase. The follicle phase occurs prior to ovulation, when the follicles are developing, while the luteal phase is the part of the cycle after ovulation during which the corpus luteum is active. Only when no fertilization of

the oocyte takes place, the corpus luteum degenerates and the estrous cycle starts over. Under normal conditions, this process continues until all germ cells are released and the females will enter the menopause.

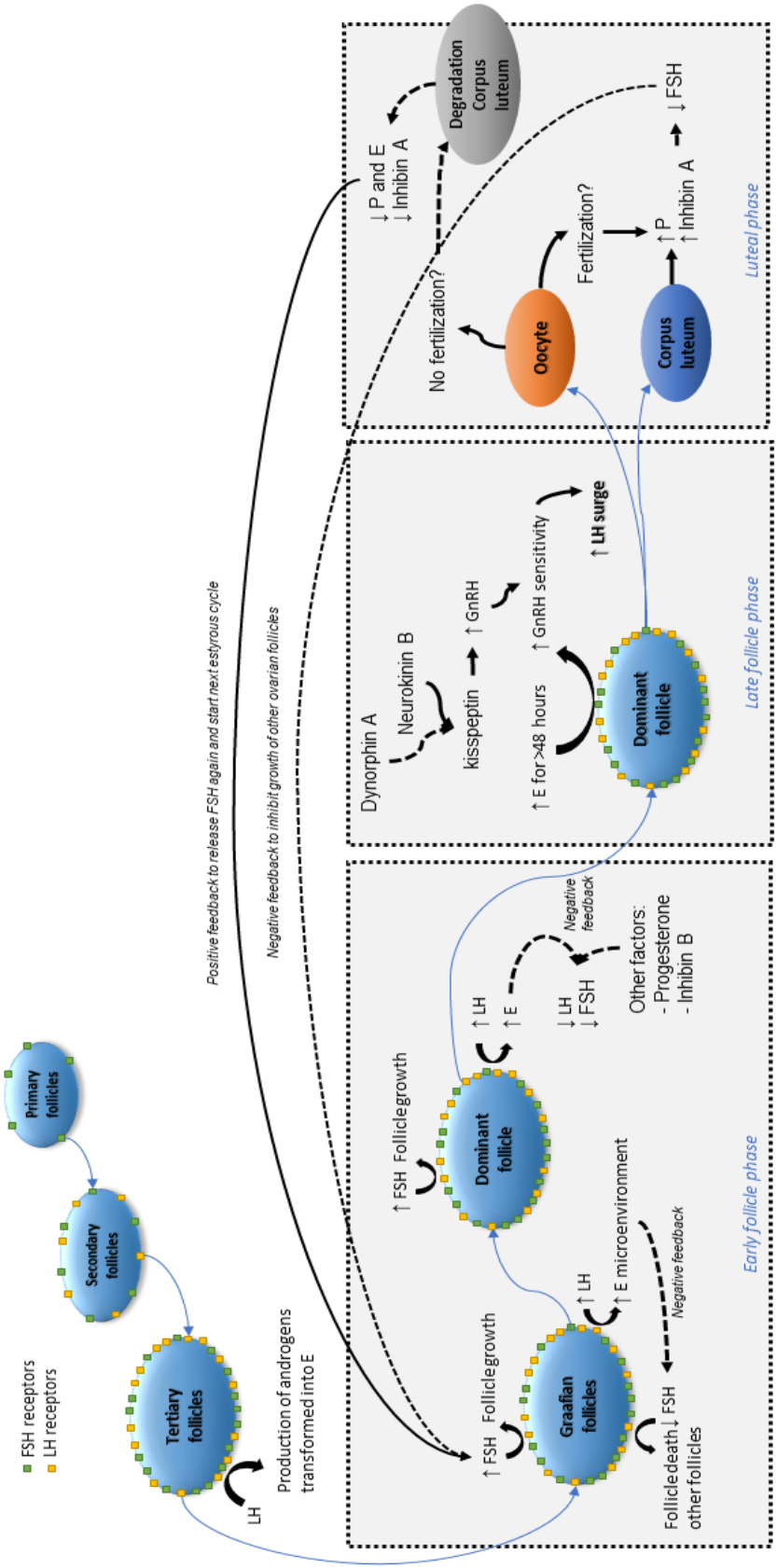
The length of the estrous cycle and the intervals between these cycles vary from species to species. For example, in women this cycle takes about 25-35 days, cows have a cycle of 21 days, and rats and mice complete the phases within 4-5 days. Interestingly, some species, like mice, rats and hamsters, have the capacity to skip the luteal phase when no mating has been taken place. This can be very beneficial because it ensures a rapid return to estrus and thus a new faster opportunity to be impregnated and reproduce.

In all cases, the estrous cycle is under regulation of the well-known hypothalamic-pituitary-gonadal axis. The principle of this axis is that the hypothalamus releases gonadotropin-releasing hormone (GnRH), which induces the pituitary to release follicle stimulating hormone (FSH) and luteal hormone (LH). The release of FSH and LH, in turn, stimulates the ovaries to release steroid hormones, like estrogen (E) and progesterone (P), which subsequently leads to negative or positive feedback mechanisms acting back on the hypothalamus or pituitary. Although E and P are probably the most important regulators of the estrous cycle, other peptides like inhibin, kisspeptin and gonadotrophin surge-attenuating factor (GnSAF) also play a significant role. It is the interplay between endocrine hormones and neuropeptides, causing the release or decline of other substances, which regulates the phases of the estrous cycle and thus the female reproductive behavior. We will discuss the occurring events and the hormonal regulations per phase.

2.2.1 Follicle growth - premenstrual phase

As mentioned before, females have a finite number of *immature oocytes* at birth that are packed in a layer of granulosa cells and form the so-called *primordial follicles*. Only the surviving follicles will finally enter the estrous cycle as *tertiary follicles.* At the primordial stage, follicles are biologically inactive which can last for about 50 years (in women). After an initial recruitment, some primordial follicles are "awaken" during puberty and enter the primary follicle stage. In this stage, the granulosa cells change shape and the oocyte genome is activated and starts transcribing genes. An important step in this phase is that these primary follicles start expressing FSH receptors (as shown in Fig. 2.1), although they remain gonadotropin-independent until later. As soon as the follicle develops a theca-layer in addition to the granulosa cells, the follicle is called a *secondary follicle*. These theca-cells will now express LH receptors. The presence of LH induces these cells to produce androgens, which will be transformed into estrogens by the granulosa cells: the follicle is now called a *tertiary follicle*.

Fig. 2.1. (Simplified) schematic drawing of the estrous cycle.



2.2.2 Early follicle phase

The follicle phase is the start of the estrous cycle. Only a fraction of the follicles have survived and developed into tertiary follicles, containing granulosa and theca-cells surrounding the mature oocyte. The follicles now also contain a fluid-filled cavity adjacent to the oocyte, also called *antrum*: the follicle is now called *antrum follicle* or *Graafian follicle*. Besides the support function for the oocyte, the granulosa and theca cell layers play an important role in the establishment of a high estrogen microenvironment needed for the development of the follicle. The follicles now express more FSH receptors and become responsive to FSH which in turn stimulates follicle growth. At the same time, increased LH levels stimulate the theca-cells to produce androgens, which are transformed into E by the granulosa cells. Together, this creates the high estrogen microenvironment that is necessary for follicle development. The high levels of E now trigger a negative feedback loop downregulating the release of FSH from the anterior pituitary. The decrease in FSH levels causes the death of follicles that express less FSH receptors leading to the selection of one *dominant follicle*.

It should be mentioned, though, that this is a very simplified description of the process. In reality, it is much more complicated (reviewed in (Messinis et al., 2014)). The role of E in this negative feedback system is well known (Messinis and Templeton, 1987, Messinis et al., 1994, Messinis et al., 1998), but more factors are involved. When both E and P are administered to healthy post-menopausal women (who normally have elevated LH and FSH levels), LH levels decrease in response to these steroids, while FSH decreases but remains higher than usual during the normal follicular phase. This suggests that the gonadotrophins LH and FSH are differentially controlled by the ovaries that release E and P, and that FSH is also regulated by other substances. In addition, LH must also have other regulators, since the decline in LH is not sustained by estrogens administration alone (Dafopoulos et al., 2004). A candidate for this control function is

progesterone. Progesterone levels increase during the luteal phase, but also in the follicular phase some, although a low levels of, progesterone is present. The administration of anti-progesterone drugs also increases LH levels during the follicular phase (Kazem et al., 1996), suggesting that P is indeed controlling the LH levels in this phase as well.

Another factor that is involved in this whole regulation is the nonsteroidal substance inhibin. Inhibin B is mainly released by the growing follicles during the early follicular phase of the cycle, while inhibin A is secreted especially by the corpus luteum in the luteal phase. The precise role of these substances are not yet known, but inhibin B seems to be involved in the negative feedback effect of the ovaries on the FSH secretion, because increased levels of inhibin B are related with decreased levels in FSH (de Koning et al., 2008).

2.2.3 Late follicle phase

Now the follicles produce high levels of E, the late follicle phase starts, in which the role of E changes from a negative feedback regulator into a positive feedback controller. The earlier slight increase in E resulted in a decrease in FSH and LH secretion, but now the E levels are increased dramatically for a longer time (when it exceeds a certain threshold and last for more than 48 hours), the pituitary instead starts to secrete a large amount of LH (Lasley et al., 1975).

The *LH surge* is most likely caused by a combination of GnRH release and sensitization of the pituitary. The high levels of E trigger the pituitary to express more GnRH receptors, thereby sensitizing the pituitary to hypothalamic GnRH (Laws et al., 1990)**.** At the same time, the high levels of E (and P) stimulate the release of GnRH in the hypothalamus. This effect, thought, is regulated via an indirect action because GnRH neurons do not express estrogen and progesterone receptors (Huang and Harlan, 1993). An important mediator in this process is the neuropeptide kisspeptin, co-released with neurokinin B and dynorphin A (Clarke et al., 2015).

The kisspeptin neurons situated closely to the GnRH neurons in the hypothalamus, do express estrogen receptors (Franceschini et al., 2006), and therefore, it is believed that kisspeptin is the direct trigger of GnRH secretion. Kisspeptin most likely bind to the KISS1 receptors expressed on the cell body of most GnRH neurons to induce this effect (Clarke et al., 2015, Herbison et al., 2010). This hypothesis is strengthened by the fact that selective deletion of KISS1 receptors on GnRH neurons does indeed induce a hypogonadal phenotype (Novaira et al., 2014). In addition, kisspeptin levels rise during the preovulatory phase (Latif and Rafique, 2015, Smith et al., 2006), and kisspeptin neurons get activated around ovulation (Clarkson et al., 2008), suggesting a direct role of kisspeptin in the LH surge. Inhibition of kisspeptin action abolished the pro-estrous LH surge and inhibits the estrous cycle in rats (Adachi et al., 2007, Kinoshita et al., 2005).

Interestingly kisspeptin is not only involved in the regulation of the LH-surge. A very recent study provided evidence that kisspeptin neurons in the hypothalamus are an essential part of a motivational neural circuit as well, that is triggered by male olfactory cues and leads to female lordosis behavior. Deletion of KISS1 receptors did not disrupt lordosis itself, but it did cause changes in modulating mate preferences (Hellier et al., 2018). This suggests that kisspeptin does not only regulate the estrous cycle by inducing the LH-surge, but is also involved in the next step of the behavioral consequences of receptivity.

As mentioned above, kisspeptin is actually co-released with neurokinin B and dynorphin A in the so-called KNDy neurons (Clarke et al., 2015). An increase in neurokinin B is thought to initiate a positive feedback loop resulting in activation of neurokinin-3 receptors on the KNDy neurons, and thereby releasing kisspeptin (Goodman et al., 2014). Dynorphin A, on the other hand, is thought to regulate the inhibitory control on the GnRH pulse by acting directly on the KNDy neurons. Thus, neurokinin B and dynorphin A control the synchronized activity of KNDy

neurons and kisspeptin release, which in turn can activate GnRH neurons to regulate pulsatile GnRH secretion (Goodman et al., 2014).

Interestingly, the LH surge in response to the GnRH release and sensitivity does occur solely during the late follicular phase, and not during the early phase, suggesting that additional factors must be involved in the regulation of this specific effect. Interestingly, administration of GnRH during the early phase, in normal cycling women, does not induce an increase in LH (Messinis et al., 1994, Messinis et al., 1998). This suggests that the factors that are involved must have a suppressing effect on the pituitary.

One potential candidate for this antagonizing effect could be the nonsteroidal substance gonadotropin surge-attenuating factor (GnSAF), which is mainly produced by the granulosa cells in the ovaries in response to FSH (and not inhibin, E, P or any other steroid hormone) (reviewed in (Fowler et al., 2003)). The production of GnSAF in the follicles is clearly related to the follicle size, in which the smaller follicles contain the highest levels of GnSAF (Fowler et al., 2001). As soon as the number of small follicles declines, as happens towards the late follicle phase, the bioactive levels of GnSAF decline. Whereas GnSAF normally negatively affects the pituitary responsiveness to GnRH and keeping LH levels low, it is thus, the low of absent negative feedback by GnSAF that could contribute to increase LH secretion during the late follicle phase (de Koning et al., 2001), providing E the possibility to overcome the inhibitory GnSAF effects. E levels increase already during the middle follicle phase, but only in the late follicle phase, after which FSH levels have declined and a dominant follicle has been selected, GnSAF production stops. GnSAF, thus, seems to regulate the timing of the LH surge.

Another important players in the regulation of the LH surge is P, in which P probably also functions via sensitizing the pituitary to GnRH (Kazem et al., 1996). The concentrations of P remain low during the largest part of the follicle phase, until the onset of the LH surge. The

increase in P functions as an amplifier of the positive feedback mechanism of E on the pituitary. P seems to advance the onset of the LH surge and augments the amplitude of the LH surge (Messinis and Templeton, 1990, March et al., 1979), but only in the presence of certain concentrations of E. One study suggested that it is actually the neuroprogesterone synthesized in the hypothalamus that plays a role as obligatory mediator for the onset of E-induced LH surge (Micevych et al., 2003).

At last, GABA and glutamate seem also to be involved in the regulation of the GnRH/LH surge. Subpopulations of estrogen receptor-expressing cells in the hypothalamus are GABAergic or glutamatergic (Cheong et al., 2015). It is hypothesized that these E-related GABAergic and glutamatergic transmission is important for both the negative and positive feedback mechanism on the GnRH release. GABA levels are increased in the hypothalamus during the moment of negative feedback (Herbison and Dyer, 1991), but then fall just before the GnRH/LH surge (Jarry et al., 1992, Robinson et al., 1991), suggesting that estrogen modulates GABAergic signaling to suppress GnRH neuron activity and thereby regulate the negative feedback (Petersen et al., 2003, Herbison, 1998). For positive feedback, on the other hand, an increase in glutamate levels close to the GnRH neuron cell bodies occurs at the time of the GnRH/LH surge (Ping et al., 1994, Jarry et al., 1995) and glutamate receptor antagonists abolish the surge (Ping et al., 1997), suggesting the glutamate is involved in the positive feedback loop to release GnRH.

In conclusion, the amplitude of the LH surge is a result of a balance between the positive actions of E and P, and the negative action of GnSAF. In addition, the role of kisspeptin in the regulation of this LH surge should not be forgotten ultimately resulting in the rupture of the ovarian follicle, causing the oocyte to be released from the ovary via the oviduct towards the uterus for fertilization: *ovulation*.

2.2.4 Luteal phase

To induce the start of the luteal phase, the LH surge should now be terminated. This process is probably controlled by ovarian hormones as well, because a decrease in LH is seen before but not after ovariectomy (Dafopoulos et al., 2006). This time it is P that is mostly involved in the regulation via a negative feedback system. When the oocyte is expelled from the follicle, the remaining follicle cells become luteinized and form the corpus luteum. In this luteal phase, the corpus luteum secretes progesterone and inhibin A which act on the pituitary to suppress the release of FSH and thereby inhibit the growth of other ovarian follicles. Only when no fertilization of the oocyte takes place, the corpus luteum degenerates and the levels of inhibin, estrogen and progesterone decline. Now FSH can be released again which results in the start of the next estrous cycle (reviewed in (Hawkins and Matzuk, 2008)).

Box 2.1 Vaginal estrus

The vaginal lumen also undergoes cellular changes during the estrous cycle. When observing vaginal smears under the microscope, four different stages can be differentiated: vaginal estrus, diestrus I, diestrus II and proestrus. The first vaginal estrous stage is characterized by cornified epithelial cells, which look like cornflakes under the microscope. In rats, this stage last for about 36 hours, after which the cornified cells reduce in number and are replaced by leukocytes (white blood cells) and a few nucleated epithelial cells. This stage, called vaginal diestrus, persists for approximately 48 hours and is divided in diestrus I (the first day) and diestrus II (the second day). After diestrus, the vaginal lumen enters the vaginal proestrous stage. This stage (which takes about 12 hours) can be recognized by the presence of many nucleated epithelial cells, in addition to a dramatic reduction in the number of leukocytes.

These stages of the vaginal cycle are driven by ovarian hormones and therefore coincide with the phases of the ovarian estrous cycle. The vaginal estrus is correlated with the presence of recently ruptured follicles following ovulation and the formation of the corpora luteum. In addition, tertiary follicles begin to develop from secondary follicles at this time. As soon as the tertiary follicles become larger and the granulosa cells become more numerous, the diestrous stage started. The tertiary follicles are now called Graafian follicles and the corpus luteum is fully formed from the postovulatory follicle. At the time the Graafian follicles are about to ovulate, the vaginal proestrous stage has started.

It is important to mention that the term estrus is rather confusing here, because the behavioral estrus (the moment in which female are ready to mate) coincides with the night of the vaginal proestrous stage, and not with the vaginal estrous stage. Therefore, we always refer to the vaginal estrus and behavioral estrus to clarify this difference.

2.3 Mating behavior

As soon as females enter behavioral estrus, they are ready to participate in mating behavior. In order to understand the neurobiological regulation of this mating behavior, it is important to recognize the elements and phases of the sexual interactions. Interestingly, there is an enormous similarity in the patterns of mating behavior among species. The typical behaviors could be different between species, but the goals of the behaviors are comparable. Mating behavior in general can be divided into three different phases: the introductory (precopulatory), copulatory, and executive phase (in males ejaculations, in females orgasms). The completion of all phases is needed to have a chance for reproductive success. In human males, sexual activity normally ends at ejaculation: multiple ejaculations are quite infrequent. Some women, on the other hand, are able to reach multiple orgasms. In other species, like rats and mice, ejaculation is not the end of the sexual encounter, resulting in that most rats display multiple ejaculations in rapid succession before ceasing to copulate (Ågmo, 1997).

The interplay between males and females starts with behaviors like approaching and sniffing each other's anogenital regions to obtain pheromonal cues of sexual readiness to mate: *the introductory phase*. Without an intrinsic state of sexual motivation, no approach behavior will occur. Sexual motivation could thus be seen as another important component of the introductory phase. In order to have reproductive success, individuals must be more attractive within a population or preferring more attractive partners to produce offspring. Intrasexual competition for access to a mate is believed to be common among many mammals (Darwin, 1859), with the exception for e.g. wild rats (Barnett, 1975, McClintock, 1984). Though, even if intrasexual competition were unusual, also rats still have to make a choice of partner with whom to initiate copulatory activity whenever there is more than one potential partner available. It was already in 1976 that Beach proposed that *attractivity* was an important component of female sexual

behavior (Beach, 1976). He defined attractivity as a female's value as a sexual stimulus, and included behavioral as well as nonbehavioral components such as olfactory cues that stimulated the male to engage in sexual behavior with the female.

The introductory phase is followed by the *copulatory phase* in which female rats in behavioral estrus display a variety of paracopulatory behaviors (also called solicitation or proceptive behavior); e.g. hopping and darting (reviewed in (Heijkoop et al., 2018)). Hopping can be recognized by a typical jumping behavior of the female with four legs off the ground, while darting is a runaway behavior that suddenly stops with the female presenting her body to the male rat for mounting. Darting and hopping occur frequently and in a random order, and seem to have a similar implication. Therefore, these behaviors are scored together as 'paracopulatory behavior', which are species-typical and signal the readiness to mate, thereby functioning as an index of feminine sexual motivation (Beach, 1976). However, in order to distinguish this motivation from the intrinsic motivation of the introductory phase, it could be hypothesized that the paracopulatory behaviors might represents the motivational level of keeping participating in the sexual intercourse rather than of the female's intrinsic sexual motivation to start sexual interaction.

In close relation to the paracopulatory behaviors of females, male rats show repeated intromissions and mounts during the copulatory phase. Intromissions are characterized as mounts including pelvic thrusting. It was always believed that copulation occurred upon initiation of the female rats (McClintock and Adler, 1978). However, a recent study by Bergheim et al. (2015), performed in a seminatural environment, showed that the copulatory acts were a consequence of a subtle interaction between the male and female. This indicates that the behavior of both rats are equally important in the initiation of copulation, and thus not controlled by solely the female.

In response to the male copulatory behaviors, the females display lordoses in which a posture of a hollow back and deflected tail is presented to give the male access to her vagina. The reflexive behavior lordosis reflects the female's receptivity, and is very much depending on the hormonal state of the female. The presence of estrogen alone is sufficient to induce receptivity, but progesterone facilitates the estrogen-induced lordosis response (Edwards et al., 1968). Another important female sexual behavior shown in response to the male copulatory behaviors is "pacing" or solicitation. This is the ability of the female rat to control the timing of the receipt of sexual stimulation, as a pattern of approach and withdrawal from the male. The display of this behavior is directly dependent upon the intensity of the coital stimulation (mounts, intromissions and ejaculations) received immediately prior to the solicitation behaviors. The rate of approaches toward the male is negatively correlated with the intensity of the stimulus from the male (Erskine, 1985).

After a series of mounts and intromissions, ejaculation (the executive phase) is reached. In rats, usually 10 to 20 intromissions are needed during a short period (ca. 2-10 minutes) to reach an ejaculation. After an ejaculation, male rats need a break for about 5 minutes (the post ejaculatory interval) before preceding with the next ejaculatory cycle. The copulatory and executive phase can be repeated until the rats obtain sexual satisfaction.

The different phases of the sexual cycle are probably regulated via different mechanisms, because sexual motivation and copulatory behavior can vary independently upon a variety of treatments (Kondo and Sachs, 2002, Ågmo, 2002). However, the phases are not independent. When β-endorphin is infused in the medial preoptic area of the hypothalamus before introduction to a mate, only introductory behavior in terms of investigating the anogenital region of the female, is displayed. But when β-endorphin is infused after the first mounts, and thus after the start of the copulatory phase, copulation until ejaculation succeeded normally (Stavy and Herbert,

1989). This suggests the existence of different mechanisms in the sexual behavioral cycle that are closely interconnected with a specific transition point (threshold) preceding the mounts and intromissions. Similar findings do not exist in females yet, but we hypothesize that comparable mechanisms also exist in females.

Unfortunately, most studies on female sexual behavior have solely focused on lordosis behavior; sexual motivation and paracopulatory behaviors were often forgotten. The use of lordosis as a simple laboratory measure of sexual behavior has been useful in addressing many questions regarding sexual responsiveness. However, the approach and intrinsic motivation, and paracopulatory behaviors displayed by the female during mating may be equally important for the understanding of the mechanisms behind behavior (Heijkoop et al., 2018). In order to draw conclusions on the neural basis of female sexual behavior, it is important to evaluate the full spectrum of behaviors shown by females. Still, most results described in this chapter are from previous articles in which only lordosis behavior was investigated. This means that this chapter is incomplete in describing the full picture of female sexual behavior.. Fortunately, the additional behaviors gain interest among scientists; more and more investigators evaluate paracopulatory behaviors alongside to lordosis behavior. Hopefully, future studies will lead to additional insights into the neural regulation of all facets of female sexual behavior.

2.4 Brain regions involved in female sexual behavior

Sexual behavior is regulated by several brain areas. Studies with lesions, electrical stimulation, tract-tracing, and Fos-immunoreactivity (Fos-IR), performed in different species, give a nice overview of these functional regions (previously reviewed in (Snoeren et al., 2014a)). Fos-IR activation studies have shown that several brain areas are activated after mating behavior in female rats; e.g. the preoptic area (POA), the bed nucleus of the stria terminalis (BNST), the

medial amygdala (MeA), the central tegmental field (CTF), the ventromedial hypothalamic nucleus (VMN), and the periaqueductal gray (PAG) (Figure 2, (Erskine, 1993, Pfaus et al., 1993, Polston and Erskine, 1995, Rowe and Erskine, 1993, Tetel et al., 1993). Additional studies showed in more detail that different subregions of the brain areas become activated during the different phases of sexual behavior. Chemosensory investigation (introductory phase), for instance, induced Fos-IR in the female posteromedial part of the BNST (BNSTpm) (Coolen et al., 1996), and posterodorsal part of MeA (MeApd) (Coolen et al., 1996, Tetel et al., 1993), whereas other brain subregions become active during the copulatory phase. The VMN is known as the major site for the regulation of lordosis behavior. Lesions of this area dramatically reduce lordosis (Pfaff and Sakuma, 1979a), while electrical stimulation facilitates the expression of lordosis (Pfaff and Sakuma, 1979b). Fos-IR techniques have shown that lordosis behavior in response to mounts activates especially the ventrolateral part of the VMN (VMNvl) (Coolen et al., 1996), while intromissions cause stronger induction of Fos-IR in the same brain area without a higher expression of lordosis behavior (Pfaus et al., 1993, Rowe and Erskine, 1993). This suggests that the intensity of Fos-IR induction in the VMNvl is not exclusively a reflection of vaginocervical sensory stimulation nor a translation of motor activity related to lordosis behavior, but might reflect some aspects of the internal motivational state of the female concerning the display of lordosis behavior. In mice, it was shown with single-unit recording that VMNvl neurons are indeed more activated in the presence of a conspecific with preference for the male stimulus when hormonally receptive (internally motivated) compared to the non-receptive state (Nomoto and Lima, 2015).

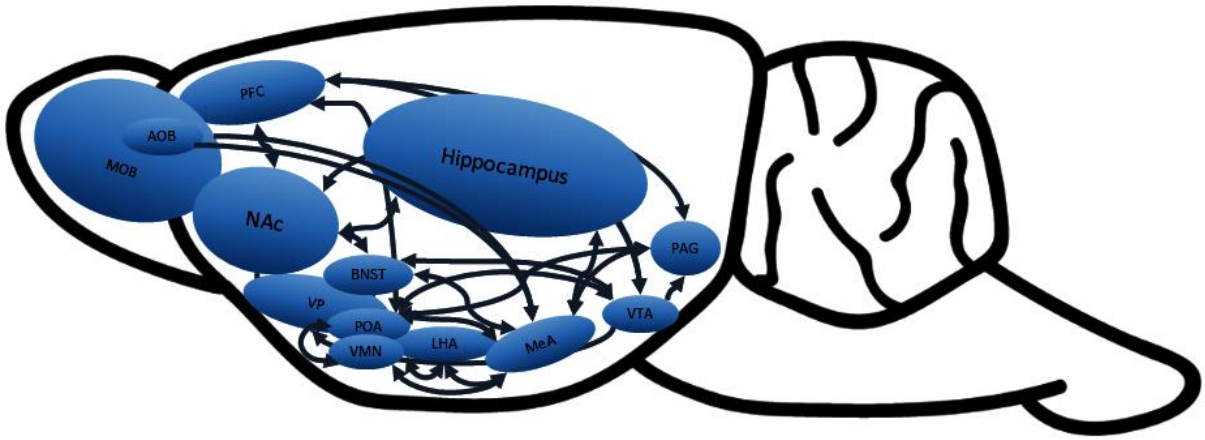


Fig. 2.2. A schematic drawing of the most important brain areas involved in female reproductive behavior and their connectivity. NAc = nucleus accumbens, BNST = bed nucleus of stria terminalis, POA = preoptic areas, LHA = lateral hypothalamus, VMN = ventromedial nucleus of the hypothalamus, MeA = medial amygdala, VTA = ventral tegmental area, PAG = periaqueductal gray, MOB/AOB = olfactory bulb, PFC = prefrontal cortex, VP = ventral pallidum.

Other brain (sub) areas that are activated during the copulatory phase are the POA, lateral septum, BNST, MeA, subparafiscular nucleus (SPFp), and PAG (Coolen et al., 1996, Pfaus et al., 1993, Tetel et al., 1993, Tetel et al., 1994, Pfaus et al., 1996). After receiving ejaculations, neuronal activation was observed in the medial preoptic nucleus (MPN), BNSTpm, MeApd and SPFp (Pfaus et al., 1993, Coolen et al., 1996, Polston and Erskine, 1995, Rowe and Erskine, 1993). But also in the VMNvl, the caudoventral part of VMN (VMNcv) and ventral premammillary nucleus (PMV) (Coolen et al., 1996). Interestingly, most of the areas activated after mating (MPN, BNSTpm, posterodorsal preoptic nucleus (PD), VMNcv and PMV) also

showed Fos-IR after treatment with estrogen and progesterone (Coolen et al., 1996), suggesting a close link between hormonal treatment and sexual behavior.

These Fos-IR studies show which brain areas become activated under certain circumstances, but it does not explain how essential these regions are in the control of female sexual behavior. For this kind of information, lesion and electrical stimulation studies are more useful. Unfortunately, only a few brain regions have been extensively studied in females. Several studies demonstrate that lesions of the VMN result in dramatic decreases in lordosis and paracopulatory behaviors (Mathews and Edwards, 1977, Rajendren et al., 1991), while electrical stimulation results in a facilitation of lordosis (Pfaff and Sakuma, 1979b). VMN lesions also abolished sexual incentive motivation, expressed as approach to a potential mate, in the female rat (Emery and Moss, 1984). The suggests that the role of the VMN in the regulation of sexual motivation and behavior in female rats is mainly stimulatory.

In contrast to the VMN, the POA might play an inhibitory role in the control of lordosis behavior, because POA lesions cause an increase in lordosis responses in females (Powers and Valenstein, 1972a, Hoshina et al., 1994). Electrical stimulation of this brain area, on the other hand, reduces lordosis behavior (Moss et al., 1974). It should be mentioned, though, that some studies did not find these facilitating effects on receptive behavior after POA lesions (Guarraci et al., 2004, Yang and Clements, 2000). The regulatory role of the POA on lordosis might be context specific, in which the lesions only affect lordosis behavior when the females are in a non-paced mating situation (Whitney, 1986). In contrast to the stimulatory effects on lordosis, paracopulatory behavior seems to get abolished by POA lesions (Hoshina et al., 1994, Guarraci et al., 2004), just as paced mating behavior seen as prolonging contact-return latencies and percentage of exits decline (Yang and Clements, 2000, Guarraci et al., 2004). Together, this indicates that the POA plays a dual role in the copulatory phase of sexual behavior; an inhibiting

role in the reflexive lordosis response and a stimulating role in paracopulatory behaviors. During the introductory phase, the POA plays a stimulatory role as well, since POA lesions abolished the female's preference for sexually receptive males (Guarraci and Clark, 2006).

Single-cell recordings showed that different subsets of neurons in the POA are involved in the regulation of the different behaviors (Kato and Sakuma, 2000). Nevertheless, neuronal links for both paracopulatory and receptive components have been found to originate in the POA, indicating an important role of this hypothalamic nucleus in the regulation of sexual motivation and behavior.

The VMN and POA receive intensive neural inputs from the MeA and the BNST (Canteras et al., 1995, Gu et al., 2003, Shimogawa et al., 2015), which in turn receive projections from both the main and accessory olfactory systems (Baum and Cherry, 2015). It is thought that the olfactory stimulation will reach the VMN and POA via the MeA (Canteras et al., 1995, Gu et al., 2003), mainly through the BNST. Olfactory stimuli are crucial for the activation of approach behaviors (Bergvall et al., 1991, Thor and Flannelly, 1977), and without approach copulation will never occur. It would, therefore, be logic that these brain areas play an important role in female sexual behavior as well. In mice, neural activation in the MeA is found in response to pheromonal stimuli (Samuelsen and Meredith, 2009), with a different response to same sex and opposite sex chemosensory stimuli (Bergan et al., 2014). In line with this finding, lesion studies have shown that the MeA reduces approach behavior of sexually receptive females towards male rats (Kondo and Sakuma, 2005), suggesting that the MeA plays a stimulatory role on the introductory phase of female sexual behavior. Interestingly, in terms of effects on paracopulatory or receptive behaviors, MeA lesions studies show conflicting results. Some have shown no changes in darts and lordosis behavior upon MeA lesions (Guarraci and Clark, 2006, Kondo and Sakuma, 2005), while others found increases in lordosis intensity and paracopulatory behaviors

(Polston and Erskine, 2001, Masco and Carrer, 1980, Rajendren and Moss, 1993), thereby suggesting an inhibitory role of the MeA during copulation. To cause even more confusion, when the state-of-the-art technique optogenetics was used in mice to silence the specific projections from the olfactory system to the MeA, sexually experiences female mice showed reduced levels of lordosis responses (McCarthy et al., 2017a). This would suggest that chemosensory inputs from the olfactory areas are required to show full receptivity in female mice. When all neurons in the MeA were inhibited with a chemogenetic approach, a decrease in lordosis responses was found in CNO-treated females versus controls, suggesting that the MeA plays a stimulatory on receptive behavior. Interestingly, when the females were tested multiple time during inhibition of the MeA, the lordosis responses returned to a normal level after 6 tests, suggesting that it was more the 'learning curve' in improvement of receptivity after obtaining experience that was attenuated rather than the ability to perform a lordosis (McCarthy et al., 2017b). They hypothesized that the combination of hormone priming and the experience of the sensory input of a mounting male is required for a progressive improvement in lordosis responsiveness. In that perspective, it could be hypothesized that a minimal number of MeA neurons should be inhibited in order to completely block the lordosis throughout sexual experience (McCarthy et al., 2017b). This idea is supported by a previous lesion study in which bilaterial deletion of the MeA indeed attenuated the expression of lordosis in mice (DiBenedictis et al., 2012). These differences in findings could be caused by the use of different species, either mice or rats, which show complete different patterns of sexual behavior. However, more research is needed to understand the function of MeA on female reproductive behavior.

For the BNST, it was found that this brain area is not involved in the regulation of sexual behavior, whereas lesions did not change lordosis, paracopulatory and pacing behavior in females (Guarraci and Clark, 2006, Gray et al., 1978). This was in line with a study performed in

hamsters that showed that BNST lesions do not affect male-odor preference and lordosis behavior in females (Martinez and Petrulis, 2011). However, other studies did suggest a stimulatory role for the BNST in approach behavior (Jenkins and Becker, 2001, Yang and Clements, 2000), but this was then probably caused by collateral damage to other brain regions. Although surprising, the BNST does not seem to play an essential role in the execution of female sexual behavior. The expression of c-Fos in this area could instead reflect the integration of sensory information and not the execution of sexual behavior.

A few studies have been evaluating the role of some other brain regions in female sexual behavior: e.g. the anterior hypothalamus, nucleus accumbens (NAc), ventral tegmental area (VTA) and periventricular gray. Lesions in the NAc seem to increase the number of rejections from the female rat, while leaving the lordosis behavior intact (Rivas and Mir, 1990). Still, conclusions could not really be drawn from this studies, whereas the author claimed that increase in rejections might just as well be a consequence of an increase in general hyper-reactivity reducing the female's tolerance to sexual interactions from the male. The results of the studies investigating the other brain regions are too limited or controversial to outline them any further, but an overview can be found in (Paredes and Ågmo, 2004).

Unfortunately, these is still an important gap in knowledge when exploring the neural regulatory circuitry of female reproductive behavior. The studies mentioned above have explored the role of different brain areas in regulating the different phases of female sexual behavior, but they have not investigated the connection between these brain areas. As mentioned above, and shown in Fig 2.2, all brain areas are closely connected to one and another, meaning that they are should work together to get from the introductory phase to a successful executive phase. None of the studies mentioned above was able to explore the role of specific projections between brain areas on female sexual behavior. In the last decade, many new state-of-the-art techniques have

entered the field of behavioral neuroscience which could be very helpful in answering these questions: e.g. optogenetics, chemogenetics, and calcium imaging. Lesion studies might have revealed the structures and projections needed for a behavior, but is has proven to be extremely difficult to manipulate the precise connections that are involved: lesions tend to affect both the direct and indirect pathways. Fiber photometry, as example, works by injecting adeno-associated viral (AAV) vectors expressing calcium indicators in neurons locally in brain area A, these indicators will be expressed across all axons of neurons originating in this area, including the downstream projections to other areas (Girven and Sparta, 2017). Activation of neurons corresponds with increasing levels of intracellular calcium. This calcium then binds to the calcium indicators in the axons, resulting in the emission of a fluorescent signal that can be detected by the implanted fiber (Girven and Sparta, 2017). By implanting an optic fiber into the projection area B, fiber photometry can measure the neural activity of the specific projections from the area A to area B within a millisecond timescale. Using the same principle with AAV vectors, but now designed to express clozapine-N-oxide (CNO)-sensitive receptors in neurons originating in the brain area, chemogenetics can turn on or off (depending on the type of AAV vector) the specific brain projections by inserting CNO intracranially in the area B. In summary, fiber photometry can study the neural activity linked to certain behaviors, whereas chemogenetics can be used as proof of concept to show that disinhibition/activation of the brain projections have direct consequences for certain behaviors (Roth, 2016). As one can imagine, this kind of novel techniques have been important for the understanding of the neural regulation of behavior. These methods could contribute to a large extend to the understanding of the complexity of female sexual behavior, and give insight into the connectivity between brain areas. Unfortunately, the use of these state-of-the-art techniques have not yet entered the field of reproductive behavior, and especially not in

female sexual behavior. Except for the few example that were given above, all our knowledge still comes from lesion and pharmacological studies only, but hopefully future studies will also implement the new techniques to unravel the role of specific projections in female reproductive behavior.

2.5 Chemical messengers involved in female sexual behavior

The brain regions involved in female sexual behavior use several different types of chemical messengers to regulate mating behavior. We have already discussed how ovarian hormones such as E and P regulate the estrous cycle and thereby induce behavioral estrus, but these hormones also play an important role in mating behavior itself. In addition, several types of neurotransmitters and neuropeptides are involved in the regulation mechanisms behind mating behavior; e.g. serotonin, dopamine, noradrenaline, oxytocin and gamma-aminobutyric acid (GABA). A selection of the most important chemical messengers will be discussed in more detail.

2.5.1 Estrogen and progesterone

Female sexual behavior is highly dependent on the ovarian hormones E and P (Pfaff and Schwartz-Giblin, 1988). Ovariectomy causes a robust disruption of lordosis and paracopulatory behaviors in females, but this decline can be partly restored by treatment with E, or completely following sequential treatment with E and then P (Powers and Valenstein, 1972b, Fadem et al., 1979, Jones et al., 2013). Several studies have shown that there is a dose-dependent effect of E on female sexual behavior, in which the lordosis and paracopulatory behavior increase with a rising dose of E (Snoeren et al., 2011a, Powers and Valenstein, 1972b, Davidson et al., 1968, Meyerson, 1964, Jones et al., 2013). P has a similar dose-dependent effect when added to E in

terms of paracopulatory behavior and time spend with a male (Powers and Valenstein, 1972b, Snoeren et al., 2011a, Meyerson, 1964). The lordosis quotient seem to be less dose-dependent of P, but requires a minimal hormonal level to be induced.

The mechanisms behind the hormonal regulation are not yet completely understood, but the behavioral effects of E and P rely, at least in part, on their regulatory actions on neurotransmitter synthesis, release, and/or receptors (reviewed in (Kow et al., 1994)). When E levels increase, the expression of additional P receptors (PR) is seen in the POA and the VMN (MacLusky and McEwen, 1978). Changes is co-localization of E receptors (ER) and PR are also found during the estrous cycle with high co-localization at proestrus (Sa and Fonseca, 2017). Classically, it was though that E and P affect transcription mechanism in the nucleus, but with the recent discovery of membrane-associated steroid receptors it now is assumed that E and P have also neurotransmitter-like actions activating intracellular events and influence transcription (reviewed in (Micevych et al., 2015)). It is a complicated mechanism in which many peptides play a role (Micevych et al., 2015), but principally within minutes of treatment with E, an active inhibition of lordosis is initiated in the arcuate nucleus (ARC). In this brain region, E induces the release if neuropeptide Y (NPY) that act on neurons expressing NPY-Y1 and $GABA_B$ receptors (Mills et al., 2004, Sinchak et al., 2013). The activated neurons project to the POA where they release another peptide called ß-endorphin that, in turn, activates and induces internalization of μ-opioid receptors. These μ-opioid neurons subsequently innervate VMN neurons regulating the descending output of the hypothalamus that controls lordosis behavior (reviewed in (Micevych and Dewing, 2011)). For approximately 20 hours, this activation of μ-opioid receptors inhibits the display of lordosis behavior, until P is added to the system and reverses the E-induced inhibition, allowing for lordosis (Sinchak and Micevych, 2001). How this P-regulated process works is still unclear, but P-knockout mice are not able to show lordosis (Lydon et al., 1995), and

PR antagonists and PR antisense oligonucleotide block the induction of lordosis by P (Pfaff and Schwartz-Giblin, 1988). The mechanism that P uses for the stimulating effect in lordosis is probably a different mechanism than the E-circuit.

Besides the direct relationship between E and P, there is also a link between ovarian hormones and neurotransmitters and peptides. Both E and P, for example, change the levels of GABA and glutamate in the VMN. Whereas E causes a rise in GABA and glutamate levels in the VMN, P results in a decline in neurotransmitter levels (Luine et al., 1997). This indicates that ovarian hormones regulate sexual behavior via a control on side-specific neurotransmitter levels. A similar relationship exist between ovarian hormones and other neurotransmitters. ER and PR agonists, for instance, also modify noradrenaline levels (Lubbers et al., 2010, Nagle and Rosner, 1980, Janowsky and Davis, 1970), and an increase in E levels within the VMN causes a rise in oxytocin binding in this region (Schumacher et al., 1989). Levels of serotonin levels, in addition, vary during the estrous cycle (Gundlah et al., 1998). In addition, P treatment results in a serotonin turnover in the VMN of rats treated with E directly in the VMN (Gereau et al., 1993). Important in this situation is that P does not have an effect when E is missing, suggesting that there is a direct link between both hormones and serotonin and that the facilitating effects of P on female sexual behavior could be modulated via a serotonin turnover. This suggests the existence of a mechanism by which two ovarian hormones act synergically in a specific brain region to promote the right conditions to induce a behavioral response.

The E-induced lordosis behavior is depending on ER activation. Estrogens act via two different estrogen receptors, the estrogen receptor α (ERα) and the estrogen receptor β (ERβ). Studies in both rats and mice have shown that the ERα is important for the activation of sexual behaviors (Spiteri et al., 2010a, Spiteri et al., 2012, Ogawa et al., 1999, Ogawa et al., 1998, Mazzucco et al., 2008). The ERβ, on the other hand, is not necessary to induce receptivity

(Mazzucco et al., 2008), and might be more involved in other behaviors in which E plays a role, such as fear and anxiety (Spiteri et al., 2012, Spiteri et al., 2010b), social recognition (Spiteri and Ågmo, 2009, Spiteri et al., 2010b), and aggression (Albert et al., 1992, Spiteri et al., 2010b).

Estrogen in specific brain areas

One of the main sites of action of E is the VMN. The ERα plays an important role in the stimulatory control of the VMN on female sexual behavior, since the VMN shows a high expression of this receptor (Simerly et al., 1990) and has many ERα positive neurons (Yamada et al., 2009). Besides, E treatment promotes the development of axodendritic synapses in the VMN (Frankfurt and McEwen, 1991). Infusion of E directly in the VMN facilitate lordosis behavior in female rats (Barfield and Chen, 1977), which is probably regulated via ERα. This was shown by several studies using site-specific silencing of ERα (via the infusion of adeno-associated viral vector directed against the ERα gene) within the VMN. Reduced levels of ERα in the VMN cause a decline in sexual receptivity and paracopulatory behaviors in rats and mice (Spiteri et al., 2010a, Musatov et al., 2006, Snoeren et al., 2015). In addition, local infusions of antiestrogens in the VMN decrease lordosis in rats (Meisel et al., 1987).

Another important area for E-mediated effects is the POA. As mentioned before, POA lesions have been shown to abolish paracopulatory behavior, while promoting lordosis (Hoshina et al., 1994). The role of the ERα in the POA, however, is rather confusing. Site-specific silencing of ERα in the POA resulted in increased levels of lordosis responses, while paracopulatory behaviors remained unaffected (Spiteri et al., 2012). Sexual motivation for a sexually attractive male rat was also reduced in these females with reduced ERα levels in the POA (Spiteri et al., 2012). This suggests that ERα could play a role in the inhibitory function of the POA in lordosis, but not in the regulation of paracopulatory behaviors.

However, my study in which we used a seminatural environment to study the effects of ERα silencing in the POA and VMN on female sexual behavior revealed more surprising results. Females with fewer ERα in the POA and VMN showed lower levels of both lordosis responses and paracopulatory behaviors compared to the control females (Snoeren et al., 2015). However, the lordosis quotients were left unaffected, meaning that the reduction in lordosis responses was caused by a decrease in received mounts and intromissions, and not by the incapability to perform lordosis (Snoeren et al., 2015). In conclusion, ERα in both the POA and VMN are *not* essential to induce lordosis responses. The decline in lordosis responses and paracopulatory behaviors could instead reflect a reduction in sexual motivation.

A reduction in ERα levels in the MeA or BNST did not affect the number of paracopulatory behaviors or lordosis responses compared to controls (Spiteri et al., 2010a, Snoeren et al., 2015). As mentioned before, the MeA might play an inhibitory role on female copulatory behavior, but ERα expression seems to be not essential in this regulation.

2.5.2 Serotonin

The sexual behavior system is under constant inhibitory control to assure that copulation occurs only under the proper circumstances. Serotonin is involved in this inhibition and in the disinhibition to induce the sexual behaviors. Therefore, serotonin release is regulated via a negative feedback mechanism (Aghajanian, 1978, Gothert and Weinheimer, 1979) that is controlled by different serotonergic receptors. The serotonergic neurotransmitter system consists of the endogenous ligand, 5-hydroxytryptamine (5-HT, serotonin) and 14 functionally distinct 5-HT receptor subtypes. The receptors can be divided into seven families, namely 5-HT_{1-7}, with all different and limited distributions in the nervous system (Hoyer et al., 1994). Except for the 5-

HT_3 receptor subtype, which is a ligand-gated ion channel, 5-HT receptors are 7-transmembrane receptors and act via G-proteins. The last mechanism involved in the maintenance of serotonin levels is the serotonin transporter (SERT) which is responsible for the active transport of serotonin into neurons (Murphy et al., 2004).

5-HT neural activity is most likely tonically elevated during the execution of behavior, thereby facilitating the whole behavior instead of subparts like specific muscle groups or motor programs (Jacobs and Fornal, 1997, Muller and Jacobs, 2010). Therefore, 5-HT probably acts as modulator or facilitator in sexual behavior, rather than playing a role as central mediator. The increased firing of 5-HT neurons during sexual behaviors is under control of several feedback systems located somatodendritically (5-HT_{1A} receptors) or presynaptically (5-HT_{1B} receptors), and also postsynaptic (5-HT_{1A}, 5-$HT_{2A/2C}$ and 5-HT_4 receptors (Sharp, 2010). The role of the 5-HT system in sexual behavior is mostly studied in male rats (reviewed in (Snoeren et al., 2014b)), although this neurotransmitter is regularly studied in female sexual behavior as well (reviewed in (Snoeren et al., 2014a).

5-HT seems to play a dual role in the control of female sexual behavior with 5-HT_{1A} receptors acting to inhibit, and 5-HT_2 receptors to facilitate female sexual behavior upon activation. It, therefore, depends on which receptor subtype becomes activated on what kind of effects are induced. Many studies have shown an inhibiting effect of 5-HT_{1A} receptor agonists on paracopulatory behavior and lordosis in female rats (Mendelson and Gorzalka, 1986, Kishitake and Yamanouchi, 2003, Ahlenius et al., 1989, Ahlenius et al., 1986, Fernandez-Guasti et al., 1987, Snoeren et al., 2011b, Snoeren et al., 2011c). The inhibiting effects were found in both ovariectomized females primed with E alone or with E in combination with P, although some studies could not find the effects in females primed with only E (Snoeren et al., 2011c, Ahlenius et al., 1986). The inhibiting effects of 5-HT_{1A} receptor agonists are antagonized by specific 5-

HT_{1A} receptor antagonists (Snoeren et al., 2010, Johansson et al., 1991). Likewise, 5-HT_{1B} receptor agonists also induce inhibiting effects on lordosis behavior (Uphouse et al., 2010, Uphouse et al., 2009). In conclusion, 5-HT_{1A} and 5-HT_{1B} receptors are involved in the inhibition of female sexual behavior during the copulatory phase.

5-HT_2 and 5-HT_3 receptors, on the other hand, seem to play a stimulatory role on sexual functioning. Agonists of these receptors facilitate lordosis behavior in female rats (Mendelson and Gorzalka, 1985, Wolf et al., 1998a), while antagonists for 5-HT_2 and 5-HT_3 receptors inhibit lordosis and paracopulatory behavior (Gonzalez et al., 1997, Maswood et al., 1997, Miryala et al., 2013). Agonists for the 5-$HT_{2A/2C}$ receptor subtypes stimulate lordosis and paracopulatory behavior (Nedergaard et al., 2004, Rossler et al., 2006, Wolf et al., 1999), while antagonists inhibit sexual behavior (Sinclair-Worley and Uphouse, 2004, Uphouse et al., 2003, Kaspersen and Ågmo, 2012).

Drugs that increase serotonin levels, like selective serotonin reuptake inhibitors (SSRIs), could disrupt the balance between activation of 5-HT receptors that inhibit and those that facilitate sexual behavior. The SSRI fluoxetine, for instance, is known to cause sexual dysfunctions in women (Clayton, 2002) and female rats (Ventura-Aquino and Fernandez-Guasti, 2013, Guptarak et al., 2010). This inhibition in sexual behavior could be caused by activation of the 5-HT_{1A} receptor (Guptarak et al., 2010), or its blocking effect on 5-HT_2 receptors (Palvimaki et al., 1996). Interestingly, studies with chronic treatment of another SSRI (paroxetine) did not affect lordosis and paracopulatory behavior in estrous females (Snoeren et al., 2011c, Kaspersen and Ågmo, 2012), although it did reduce the sexual incentive motivation (Kaspersen and Ågmo, 2012). In females primed with only E, however, 7 days of paroxetine treatment induced a decrease in sexual behavior (Snoeren et al., 2011c), but this effect disappeared after chronic treatment with the antidepressant. The same lack of effects on copulatory behavior was found in

SERT knockout female rats (Snoeren et al., 2010). In all studies it was shown that the 5-HT_{1A} receptor was desensitized, which might explain the lack of sexual dysfunctions (Snoeren et al., 2010, Snoeren et al., 2011c). The 5-HT_{1B} and 5-HT_{2C} receptors, on the other hand, do not play a role in the paroxetine effects (Kaspersen and Ågmo, 2012).

Serotonin in specific brain areas

The inhibiting role of 5-HT_{1A} receptors in female sexual behavior is without doubt, but the specific location of the responsible 5-HT_{1A} receptors is unknown. The dorsal raphé nucleus (DRN) is one of the brain areas with an intense distribution of 5-HT_{1A} receptors. Studies have shown that lesions in the DRN increase lordosis (Kakeyama et al., 1997, Kakeyama and Yamanouchi, 1996, Arendash and Gorski, 1983), suggesting an inhibiting role for this brain area. Nevertheless, local injections of 5-HT_{1A} receptor agonists in the DRN do not affect lordosis or solicitation behavior (Uphouse et al., 1992a), suggesting that the 5-HT_{1A} receptors do not play a role in the inhibiting effects of the DRN. Infusion of the 5-HT_{1A} receptor agonist in the medial raphé nucleus (MRN), on the other hand, decreases lordosis responses.

The MRN projects to the VMN, which is another brain area with high levels of 5HT_{1A} receptors. Local 5-HT_{1A} receptor agonist injections in the VMN suppress lordosis and paracopulatory behavior (Uphouse et al., 1992b, Uphouse et al., 1996a, Wolf et al., 1998b, Uphouse et al., 1993, Gonzalez et al., 1997, Trevino et al., 1999, Uphouse et al., 2000). These inhibitory effect can be attenuated by several 5-HT_{1A} receptor antagonists (Uphouse et al., 1996a). Together, this suggests a role for 5-HT_{1A} receptors in the VMN in the regulation of lordosis and paracopulatory behavior in females.

However, the VMN contains also other 5-HT receptor subtypes which could play a role on the regulation of female sexual behavior. Local infusions of 5-$HT_{2A/2C}$ receptor antagonists,

for instance, inhibit lordosis behavior (Uphouse et al., 1996b, Wolf et al., 1998a), while a 5-HT_2 receptor agonist increases lordosis responses in suboptimally hormone-primed females (Maswood et al., 1997, Wolf et al., 1998a). In the VMN, it is the 5-HT_{2C} that have been implicated in lordosis modulation (Wolf et al., 1999), while 5-HT_{2A} receptors may be involved more in the POA (Gonzalez et al., 1997). It was also shown that bilateral infusions of a 5-HT_3 receptor antagonist in the VMN disrupts the lordosis responses, an effect that could be attenuated by the administration of a receptor agonist (Maswood et al., 1997). The expected stimulatory role of the VMN is thus probably regulated via the 5-$HT_{2A/2C}$ receptors.

Another possible brain area that is involved in female sexual behavior is the POA. As mentioned before in 2.4, the POA is assumed to be inhibitory in the control of lordosis behavior, while playing a stimulatory role in the regulation of paracopulatory behavior. That this effect might be regulated via 5-HT_{1A} receptors in the POA was suggested by a reduction in lordosis behavior in response to local 5-HT_{1A} receptor agonist infusions (Uphouse and Caldarola-Pastuszka, 1993). Interestingly, the local infusions did not change the amount of paracopulatory behavior, suggesting that these different sexual behaviors might be regulated via the interplay of different neurotransmitters and/or brain areas.

In conclusion, 5-HT seems to play a dual role in the control of female sexual behavior with 5-HT_1 receptors playing an inhibitory, and 5-$HT_{2/3}$ receptors a stimulatory role in female sexual behavior. The hypothalamus is an essential player in the serotonergic control of sexual functioning. The VMN and POA are already studied, but hopefully more brain areas and precise brain projections will be investigated in the future. In addition, very few studies are available upon the role of 5-HT in the introductory phase. I hope that this gap in research will be acknowledged and explored in future studies as well.

2.5.3 Dopamine

Dopamine (DA) has received extensive attention on the role it plays in male and female sexual behavior. Regrettably, most studies on dopamine and female sexual behavior have been focusing solely on lordosis behavior and neglected the paracopulatory behaviors. Despite the single focus, though, the role of dopamine in lordosis remains rather controversial (also reviewed in (Melis and Argiolas, 1995)). DA receptor agonists and antagonists have been reported to have both inhibitory and facilitatory effects on female receptivity. On one hand, DA receptor agonists have been shown to suppress lordosis behavior in hormonally primed females, while antagonists stimulate receptivity (Everitt and Fuxe, 1977, Everitt et al., 1975, Michanek and Meyerson, 1977b, Michanek and Meyerson, 1982, Fernandez-Guasti et al., 1987). In addition, agents that destruct dopaminergic neurons cause stimulation of lordosis (Ahlenius et al., 1972, Everitt et al., 1975). To the contrary, however, some other studies showed beneficial effects of dopamine on receptive behavior (Foreman and Moss, 1979, Hamburger-Bar and Rigter, 1975), or no effect on lordosis (Ellingsen and Ågmo, 2004). Infusion of dopaminergic agonists in the 3rd ventricles in the brain also resulted in increased lordosis behavior (Ma et al., 2010). The few studies that did investigate the effects of non-selective DA receptor agonists on paracopulatory behaviors reported an inhibitory effects for dopamine in hormonally fully primed females (Ellingsen and Ågmo, 2004, Snoeren et al., 2011b).

Two main details should be considered to explain the differences in the dopaminergic effects: hormones and dosage. As mentioned before, ovarian hormones play an important role in female sexual behavior. In general, it is easier to facilitate female sexual behavior from a low baseline of receptivity, and to inhibit fully primed females, than the other way around. In addition, besides the direct role of ovarian hormones on sexual functioning, they also play an indirect role by changing the balance and sensitivity of receptor expressions. Ovariectomy

changes the dopamine content and turnover, as well as the density in DA receptors and their affinity for agonists and antagonists in different brain areas (Gunnet et al., 1986, Levesque and Di Paolo, 1988, Hruska, 1986, Hruska and Nowak, 1988). This suggests that differences in hormonal priming in the experiments could influence the outcomes of dopaminergic agent priming. When analyzing the studies mentioned above, they suggest that low doses of DA receptor agonists facilitate lordosis in low primed females, while high doses inhibit receptivity in fully primed females.

The actions of dopamine are mediated by five different receptor subtypes, which are members of the large G-protein coupled receptor superfamily. The dopamine receptor subtypes are divided into two major subclasses: the D1-like and D2-like receptors, which typically couple to Gs and Gj mediated transduction systems. In the brain, the various receptor subtypes display specific anatomical distributions, with D1-like receptors being mainly post-synaptic and D2-like receptors being both pre- and post-synaptic (reviewed in (Jaber et al., 1996). The different receptors could also explain the different role of dopamine on female sexual behavior.

Grierson et al. suggested that low doses of dopaminergic agents act via presynaptic receptors, and therefore inhibit dopamine release and stimulate female sexual behavior, while high doses inhibit lordosis via postsynaptic receptors (Grierson et al., 1988). When agents were administered that cause a small increase in DA levels, lordosis behavior was stimulated, while the induction of large amounts of dopamine resulted in inhibitory effects (Stoof and Kebabian, 1984). Similar differences in effects with low and high doses of agents were found with selective D_2 receptor agonists. It was suggested that these effects acted solely on presynaptic receptors (Titus et al., 1983). Interestingly, no effects were seen with D_1 receptor agonists and antagonists (O'Connor and Brown, 1982). In addition, the stimulating effects of dopaminergic agents could only be inhibited by D_2 receptor antagonists and not by antagonists for D_1 receptors (Grierson et

al., 1988). This suggests that dopamine D_2 receptors are more (or only) involved in lordosis behaviors than dopamine D_1 receptors.

Dopamine in specific brain areas

When dopamine or DA receptor agonists are administered directly in the hypothalamic area, it exerts mostly stimulatory effects on female sexual behavior, while DA receptor antagonists inhibit lordosis (Foreman and Moss, 1979). These effects were found in the POA and ARC, but not the lateral hypothalamus (LHA), and suggest that these brain regions play a role in the dopaminergic regulation of female sexual behavior. Another important brain region with dopaminergic control on female sexual behavior is the VMN. Dopaminergic agents that were locally infused in the VMN had also a stimulatory effect on female sexual behavior (Mani et al., 1994).

The results of these studies, however, are rather confusing. Infusion of low doses of the nonselective dopamine receptor agonist apomorphine directly in the POA cause an increase in paracopulatory behavior (Graham and Pfaus, 2010) in E-primed females. High doses of the same drug, however, seem to have no effect on this behavior. Other components of female sexual behavior such as lordosis, solicitation and pacing behavior are also not affected by both the low and high doses of the agonist (Graham and Pfaus, 2010). Another study, however, did show an increase in lordosis behavior in females primed with low doses of hormones (Foreman and Moss, 1979). In fully-primed females, on the other hand, the lordosis responses were left unaffected by the DA receptor agonist (Foreman and Moss, 1979). Administration of nonselective DA receptor antagonists, on the other hand, resulted in disrupted lordosis (Foreman and Moss, 1979), solicitation, pacing and paracopulatory behaviors in fully primed females (Graham and Pfaus, 2012).

Whether these effects are regulated via the dopamine D_1 or D_2 receptors remains unclear. On one hand, it was suggested that the dopaminergic effects in the POA are also regulated via the dopamine D_2 receptors, because administration of a selective D_2 receptor agonist induced the same stimulatory effects in low primed females (Graham and Pfaus, 2010). However, when the data was analyzed in more detail, this effect was only found on solicitation behaviors and not in the number of darts and hops, lordosis or pacing behavior (Graham and Pfaus, 2010). Locally infused D_1 receptor agonists, on the other hand, resulted in normal lordosis behavior (Apostolakis et al., 1996, Graham and Pfaus, 2010), but a decrease in paracopulatory behaviors (only in low doses of the dopaminergic agent) (Graham and Pfaus, 2010). While the D_2 receptor agonist solely affected solicitation behavior, the D_1 receptor agonist affected only darts and hops. This indicates that both receptors might play a role in the regulation of female sexual behavior, with each receptor being involved in different aspects of behavior. Graham & Pfaus suggested that the ratio of DA receptor subtypes within the POA is critical for the display of sexual behavior (Graham and Pfaus, 2010).

Surprisingly, when DA receptor *antagonists* were injected within the POA, unexpected results were found. Whereas one would expect that antagonists would have no or opposing effects to agonists, high doses D_2 receptor antagonist increased solicitation behavior in fully-primed females (Graham and Pfaus, 2012), just as agonists have shown to do in low primed females. The D_2 receptor antagonist did also increase pacing behavior, but had no effect on lordosis and paracopulatory behavior (Graham and Pfaus, 2012). Local administration of a D_1 receptor antagonist, on the other hand, caused a slight decrease in solicitation and pacing behavior, while leaving other components of female sexual behavior unaffected (Graham and Pfaus, 2012).

Could we, therefore, conclude that infusions of DA receptor antagonists in the POA have similar effects as DA receptor agonist? The elements of female sexual behavior were unequally affected by the dopaminergic agents: sometimes the pacing behavior was affected, while another time paracopulatory behavior was influenced. Since no clear pattern was found, it can be suggested that the different elements were not regulated by a certain DA receptor.

A possible explanation for the differences in results is that the stimulatory effects of the antagonists were found in E+P primed females, whereas the effects of the agonists were found in E primed females. The authors, therefore, suggested that female primed with E + P show a tilt towards D_1 receptor stimulation and thereby increasing sexual behavior. In E primed females, on the other hand, shifting activity towards the D_2 receptors has the same effect (Graham and Pfaus, 2012). These effects are in line with previously described studies of Grierson et al. (1988), who showed that D_2 receptor activation facilitates lordosis in E-primed females, but inhibits sexual behavior in E+P-primed females (Grierson et al., 1988). The switch in D_1/D_2 balance in the POA after different hormonal priming was later confirmed by using immunohistochemistry, western blots, and autoradiography: E and P affected the dopaminergic receptors in opposite ways in the POA in which E causes a lower and P a higher D_1:D_2 ratio, respectively (Graham et al., 2015). This suggests that E act via a D_2 receptor mediated system, while E+P work via the D_1 receptors.

Extracellular DA levels in the POA fluctuate in response to circulating hormones. P injections increase extracellular DA levels and stimulate sexual behavior in females primed with a low dose of E, while it does not affect de levels and behavior of females primed with higher doses of E (Matuszewich et al., 2000). This suggests that DA in the POA may indeed be important for the facilitation of sexual behavior by P. As mentioned above, DA seem to interact with a P-dependent mechanism in the VMN to promote lordosis via the D_1 receptor (Mani et al., 1994). Therefore, a possible mechanism could exist in the POA in which E upregulates P

receptors in the POA and other brain areas, which in turn increases D_1 receptor activation (like previously shown in the ventral tegmental area (VTA) (Petralia and Frye, 2006)). In this perspective, D_1 receptor activation becomes only relevant when P is added to the circulation and causes facilitation for paracopulatory behaviors in female rats. D_2 receptor activation, on the other hand, would have an inhibitory role under the control of P. DA could, thus, have two different effects on female sexual behavior by acting on different receptors on certain neuron populations with an altered balance in D_1 and D_2 receptors (Graham and Pfaus, 2012).

More research is needed to determine whether these hypotheses on the role of dopamine in female sexual behavior are correct. According to a very elegant review written by Paredes and Ågmo (2004), the role of dopamine might be less essential than always assumed. They nicely describe how the effects of the dopaminergic agents on female sexual behavior might be linked more to the dopaminergic effect on motoric aspects than sexual behavior (Paredes and Ågmo, 2004). Dopamine is very important in the control of movement, and many effects of dopaminergic agents can be explained by the effects on locomotor activity: e.g. amphetamine, a dopaminergic agonist, is shown to produce inhibitory effects on lordosis behavior in female rats (Michanek and Meyerson, 1977a). However, in the same study it was shown that the same dose that affected the lordosis, also increased stereotyped activity, which could be blocked by a DA antagonist. In addition, Parades and Ågmo described a study in which a DA receptor antagonist could induce prolonged lordosis responses, which could be interpreted as an increase in lordosis intensity. However, the drug produced at the same time a dramatic decrease in motor execution (Paredes and Ågmo, 2004). They recognized that there might be a link between the effects on lordosis and motor activity, an idea that is also supported by a study in males in which it was shown that whenever motor execution was impaired, sexual behavior was disrupted as well

(Agmo et al., 1987). In summary, caution is needed when drawing conclusions on the role of dopamine in female sexual behavior.

2.5.4 Noradrenaline

The role of noradrenaline (NA) in female sexual behavior is not yet clear. Most studies performed in this field are studies that administered adrenoceptor agonists and antagonists locally in different brain areas. It is, therefore, difficult to determine what general effect NA has on female sexual functioning.

The NA system consists of different receptor types, including α_1, α_2, β adrenoceptors, and noradrenaline transporters. Adrenoceptors are located in the brain, spinal cord and periphery (Frankhuyzen and Mulder, 1982, Nasseri and Minneman, 1987), and are localized both post- and presynaptically, as inhibitory receptors on non-adrenergic neurons (heteroceptors) and on the terminals and dendrites of the noradrenergic neurons themselves (autoreceptors) (Frankhuyzen and Mulder, 1982, Nasseri and Minneman, 1987). Again, the different adrenoceptors seem to play diverse roles in female reproductive behavior (reviewed in (Snoeren, 2015).

Unfortunately, no studies are available in which the effect of systemically administered NA was investigated on female sexual behavior. Only a few studies are known in which agents acting on specific adrenoceptors have been investigated. Systematic administration of an α2-adrenoceptor agonist, for instance, does not have an effect on lordosis in female rats (Davis and Kohl, 1977). Similar lack of results were found with the administration of non-selective and selective α2-adrenoceptor antagonists: no effects were found on sexual incentive motivation, paracopulatory behavior and lordosis (Snoeren, 2015, Gonzalez et al., 1996, Davis and Kohl, 1977, Ventura-Aquino and Fernandez-Guasti, 2013). This suggests that, systemically, α2-adrenoceptors are not involved in the regulation of female sexual behavior.

Thus, if NA is involved in the regulation of sexual behavior in females, it must involve other adrenoceptors, like the α_1- or β-adrenoceptors. Studies in which α1-adrenoceptor agonists were administered in the cerebral ventricles show that α_1-adrenoceptors might play a stimulatory role on lordosis (Kow et al., 1992). α_1-Adrenoceptor antagonists, on the other hand, attenuate lordosis and paracopulatory behavior in females when administered in the ventricles (Gonzalez-Flores et al., 2007). The role of the β-adrenoceptors is still rather unclear. While one study found that the β-adrenoceptor agonist isoproterenol facilitated lordosis (Kow et al., 1992), another study did not find any effects (Gonzalez-Flores et al., 2007).

In summary, these few studies suggest that NA has in general a stimulatory effect on lordosis behavior, an effect that is probably regulated via α_1- and/or β-adrenoceptors, and definitely not via α_2-adrenoceptors. However, all these studies were performed in ovariectomized females primed with only E. Therefore, we can only conclude that α_1- and/or β-adrenoceptors are involved in sexual behavior of hormonally low primed females. In addition, it is unknown how these adrenoceptors are involved in the introductory phase of female sexual behavior.

Noradrenaline in specific brain areas

Interestingly, it seems that the adrenoceptors play more defined roles in specific brain areas. Several studies have been performed in which adrenergic agents were administered locally in the POA, VMN, arcuate-ventromedial area of the hypothalamus (ARC-VM), lateral hypothalamic area (LHA) and median eminence.

The role of NA and adrenoceptors in the POA on female sexual behavior is rather unclear: both, a stimulatory and an inhibitory function on sexual behavior have been suggested. On one hand, it was suggested that NA had a stimulatory effects on lordosis, an effect that was regulated via β-adrenoceptors (Foreman and Moss, 1978), while on the other hand, inhibitory effects on

lordosis behavior were found when NA was injected in the POA (Caldwell and Clemens, 1986). The inhibition was probably regulated via the α_2-adrenoceptors, instead of the α1- and β-adrenoceptors, since administration of α_1- and β-adrenoceptor antagonists did not affect lordosis behavior (Etgen, 1990), or attenuated the inhibitory effects on NA in the POA (Caldwell and Clemens, 1986). Only α_2-adrenoceptor antagonists attenuated the effect of NA in the POA (Caldwell and Clemens, 1986).

These differences in results are pretty peculiar, but might be caused (again) by the hormonal state of the females. The stimulatory effects were found in ovariectomized females treated with only E, while the inhibitory effects were seen in females primed with both E and P. Because natural cycling females have both E and P, it is most reasonable to conclude that NA in the POA plays an inhibitory role on lordosis behavior. This inhibitory effect is most likely regulated via α2-adrenoceptors and not α_1- or β-adrenoceptors in the POA. It should be mentioned, though, that α_2-adrenoceptor antagonists by itself do not affect lordosis when locally injected into the POA (Gonzalez et al., 1996, Etgen, 1990)). This indicates that under normal basal circumstances α_2-adrenoceptors in the POA do not play a crucial role in sexual behavior, but with elevated levels of NA, α_2-adrenoceptors become more important.

The stimulating effect of NA could then be regulated, for instance, via the ventromedial nucleus of the hypothalamus. Local NA injections in this area stimulate lordosis behavior in E primed females (Fernandez-Guasti et al., 1985a). The role of α_2-adrenoceptors in this region is rather unclear, but α_1- and β-adrenoceptor seem to be involved in the stimulatory effects in the VMN. Systemic co-administration of both an α_1-adrenoceptor antagonist and nonselective β-adrenoceptor antagonist prevent the effects of locally injected NA in the VMN (Fernandez-Guasti et al., 1985a). α_1-Adrenoceptor antagonists by itself decrease lordosis quotients in most studies (Etgen, 1990, Fernandez-Guasti et al., 1985b, Kow et al., 1992).

Other brain areas that are involved in the noradrenergic system regulating female sexual behavior are the ARC-VM and the median eminence. However, it remains unclear via which receptors NA regulates sexual behavior in the ARC-VM. β-Adrenoceptors might be involved in the stimulatory effects, while α_1-adrenoceptors might inhibit lordosis in this brain area (Foreman and Moss, 1978). In the median eminence, β-adrenoceptors, and not the α_1-adrenoceptors, are involved in the stimulatory effects of NA (Scimonelli et al., 2000). The adrenoceptors in the LHA, on the other hand, are clearly not involved in the regulation of female sexual behavior (Foreman and Moss, 1978).

As mentioned before, hormones play an important role in the role of NA on sexual behavior. To date, it appears that inhibitory effects can only be found in rats primed with both E and P, while stimulatory effects are mainly found in females primed with only E (as reviewed in (Snoeren, 2015). As discussed previously under 2.5.1, it is obvious that the hormonal status of the females is important for their sexual functioning. Interestingly, there seems to be a close relation between hormones and the adrenergic system. For example, P has a direct stimulating effect on NA levels (Nagle and Rosner, 1980, Janowsky and Davis, 1970), but also ER agonists modify NA levels in the rat brain (Lubbers et al., 2010). Interestingly, E modifies activity of both β- and α_1-adrenoceptors in the hypothalamus and POA, attenuating β-adrenoceptors while augmenting α_1-adrenoceptor responses (Etgen et al., 1992, Petitti et al., 1992, Ungar et al., 1993). It is tempting to speculate that attenuation of NA action at hypothalamic β-adrenoceptor along with the potentiation of NA action at the α_1-adrenoceptors are functionally related to E priming of lordosis behavior. More research is needed to discover the exact relationship between ovarian hormones and NA.

2.5.5 Oxytocin

Oxytocin (OT) is a neuropeptide with a remarkable variety of physiological functions, especially in pair bonding, reproductive behavior and conditions during and after childbirth. OT is produced in magno- and parvocellular neurons of the paraventricular hypothalamic nucleus (PVN), in the supraoptic hypothalamic nucleus as well as in the BNST and POA (reviewed in (Veening et al., 2014). OT plays most likely a stimulatory role in sexual desire, part of the introductory phase, and the expectancy of future reward (Bancroft, 2005, Pfaus, 2009). Though, the presence of OT is not essential for the coordination and performance of sexual behavior in female rodents: normal patterns of sexual behavior was seen in OT-knockout mice (Nishimori et al., 1996).

However, when OT is injected in the cerebral ventricles, it induces an increase in paracopulatory behaviors and lordosis in females (Pedersen and Boccia, 2006). Intracerebroventricular (icv)-infusions of OT receptor antagonists, on the other hand, have an inhibitory effect on female sexual behavior (Pedersen and Boccia, 2002). Mice and rats could be a little bit different in these aspects. The studies in mice are not conclusive. Some claim that OT may be unnecessary for the induction of lordosis in mice (Lee et al., 2010), others showed impaired lordosis behavior in oxytocin gene knockout mice (Zimmermann-Peruzatto et al., 2016). In rats, though, OT clearly facilitates lordosis (Arletti and Bertolini, 1985, Caldwell et al., 1986, Schumacher et al., 1989). Interestingly, these stimulatory effects seem to depend on the light/dark schedule, whereas rats respond stronger on OT during the dark period (Schumacher et al., 1991).

In addition, the timing of OT receptor antagonists infusion is relevant. Both lordosis and paracopulatory behavior are suppressed by OT receptor antagonists icv infusions before P administration (Pedersen and Boccia, 2002). However, when infused after P administration,

female sexual behavior was not affected at first (4-6 hours after P), but was reduced 8-12 hours after P (Pedersen and Boccia, 2002). This suggests that OT receptors are involved in mediating the onset of female sexual behavior during the first hour following P treatment. In addition, OT contributes in the maintenance of sexual behavior many more hours.

The VMN and POA have been shown to be involved in the oxytocinergic control of female sexual behavior. Infusion of OT into the VMN and POA result in increased lordosis in females treated with E and P (Schulze and Gorzalka, 1991, Schumacher et al., 1989). In the VMN, an interesting interplay takes place between OT, E and P. An increase in E levels within the VMN causes a 4-fold rise in OT binding in this region. Additionally, within 4 hours of co-treatment of P, OT receptors are distributed over a zone surrounding the ventrolateral part of the VMN (Schumacher et al., 1989), on the location of the neuroactive substance. This suggests a mechanism by which two ovarian hormones act synergically in a specific brain region to promote the right conditions to induce a behavioral response.

2.5.6 GABA

GABA is known as an inhibitory neurotransmitter in the brain. Increased GABA activity in the brain results in less lordosis responses (McGinnis et al., 1980), suggesting a inhibitory role for GABA in female sexual behavior. Interestingly, results from infusions of GABAergic agents near the VMN or mid central grey is associated with facilitating effects on lordosis, which probably is regulated via $GABA_A$ receptors (Donoso and Zarate, 1981, McCarthy et al., 1991, Kow and Pfaff, 1988). When $GABA_A$ receptor agonists were infused in the VMN, they also facilitated sexual behavior (McCarthy et al., 1990), whereas local infusion of antagonists had inhibitory effects (Luine et al., 1999). The hypothesis is that GABA might facilitate behavior via inhibiting the action of another inhibitory transmitter, a disinhibitory mechanism. Serotonin might be a good candidate for this regulation (Luine et al., 1997, Ogawa et al., 1991).

To the contrary, infusion of GABAergic antagonists in the POA seem to enhance lordosis (McCarthy et al., 1990), suggesting an inhibitory role of GABA in the POA. Thus, GABA seems to have an opposite effect in the POA and the VMN. GABA levels in the VMN are significantly higher during proestrus than diestrus, while in the POA the levels are lowest at proestrus (Frankfurt et al., 1984). This suggest that the GABAergic facilitatory effect of lordosis are regulated mostly in the VMN via a disinhibitory mechanisms, whereas GABA in the POA might be involved in the termination of the behavior.

2.6 General discussion

In summary, it is clear the female reproductive behavior is regulated via a close interplay between different hormones, neurotransmitters and neuropeptides, of which estrogen, progesterone, serotonin, dopamine and oxytocin have received most attention in research. In addition, several brain areas are important in this regulation. The VMN and POA are mostly studied, but more brain areas must be involved in female sexual behavior. It is therefore very crucial that more research will be done of the neural regulation of female sexual behavior in order to unravel the complete mechanism by which paracopulatory behaviors and lordosis are regulated. In addition, it would be helpful if future research would also focus on the communication and interplay between brain areas in regulating the different phases of sexual behavior.

Females are often considered difficult to study, because ovarian hormones play an important role and make the results rather inconclusive. In this chapter we have often seen different effects of agents on sexual behavior tested in females with different levels of receptivity. It is not clear how an agent in the same dose can facilitate female sexual behavior in E primed rats and inhibit this behavior in E and P primed females. A logic explanation would be an

interaction effect of the agent with P, but the question remains whether this effect is only pharmacological and if they have any physiological relevance. In any case, it should be clear that the baseline level of female sexual behavior is a crucial factor that needs to be considered when evaluating the effects of any agent upon this behavior. It is, therefore, very disappointing that many studies in this field have only explored one or the other condition. When the effects of agents would have been investigated in low and high primed females within the same study, the hypothesis could have evolved into a real proven theory. When more research will be done on the interactions between ovarian hormones and neurotransmitters, we might be able to unravel this complexity and come to clear conclusions of how female sexual behavior is regulated. At least we are able to control for the hormonal effects in females by using ovariectomy and manually change the hormonal level. Females could, therefore, also been seen as an excellent study object, in comparison to males where testosterone levels might also influence results but are neglected.

The context in which females are tested seems to be very relevant as well. In this chapter, we have described several studies in which they have shown that different results were found in pace versus non-paced mating conditions, or in a standard testing situation versus a seminatural environment. These differences in effects are clearly seen in the POA. As mentioned before, Whitney (1986) showed that lesioned females allowed fewer copulatory contacts, exhibited less paracopulatory behaviors and spent less time with the males than controls in a paced mating set-up, while it increased lordosis under non-paced mating conditions (Whitney, 1986). Similar results were found after deletion of ERα in the POA: in a regular test set-up females show normal sexual behavior, whereas they show declined sexual activity in a seminatural environment in which they can pace their interactions (Snoeren et al., 2015). This leads to the conclusion that when given the opportunity, complete or partial POA lesioned females will not interact with a male. Using a different and more unnatural test set-up can lead to different conclusions and

should, therefore, be avoided as much as possible. For sure, more attention should be given to the type of test set-ups that are used to produce certain results.

However, the scientists worst enemy it is probably the complexity of the neural regulation of female sexual behavior. The behavior is clearly regulated via a close interplay between different neurotransmitter systems and brain regions, and the current research methods were not sufficient to unravel this complex system. Until recently, brain areas were seen as structures that could be divided into a few sub regions that probably could play different role in regulating behaviors. However, nowadays, new research can prove that even within such subregions, different subtypes of neurons can be differently involved in the same mechanisms, or that neurons co-release different neurotransmitters. Besides, there is a tight line between different behaviors: e.g. Lee et al. (2014) showed how neurons in the VMN of male mice can easily switch from stimulating mounting and attacking another mouse (Lee et al., 2014). Interestingly, they have used the state-of-the-art technique optogenetics for this study.

So far, only lesion and pharmacological approaches have been used to study female sexual behavior. As discussed before, these methods are limited in their interpretations. Lesion studies will not only affect a certain brain area, but will also affect the direct and indirect pathways. Pharmacology, on the other hand, is a ‘dirty’ method in that drugs can act on multiple receptors in multiple cell types and brain areas at the same time. They are, therefore, not sufficient for the understanding of the complexity of the neural regulation. Therefore, it is highly recommended that researchers in the field of female reproductive behavior would modernize their research and start to use the novel technique like fiber photometry and optogenetics to study the neural circuitries involved in regulating female sexual behavior. This would open up for opportunities to study the precise interplay of brain areas and neurotransmitters in regulating sexual behaviors. In addition, the temporal resolution of these techniques would also allow us to

study the neural regulation of the different phases in more detail, and to study what is needed to the switch from one phase to the other.

2.7 References

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