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The role of the estrogen receptor α in the medial amygdala and the ventromedial nucleus of the hypothalamus in sexual motivation, social recognition, aggression and anxiety

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A dissertation for the degree of Philosophiae Doctor

May 2010

Preface and acknowledgments

Since there are many coauthors for Papers in the present thesis, I would like to clarify their participations. I designed and wrote Paper I, II, III and IV under the direction of Professor Anders Ågmo. I performed all behavioral tests, stereotaxic surgery and data analysis in Paper II, III and IV. In Paper II and IV, I carried out immunocytochemistry at the Rockefeller University under the direction of Professor Don Pfaff and Associate Professor Ana Ribeiro. The shRNA against ERα has been designed by Professor Sonoko Ogawa and Associate Professor Sergei Musatov. The virus vector carrying the shRNA against ERα used in our experiments has been provided by Associate Professor Sergei Musatov.

I would like to acknowledge the help of many people during my doctoral project. First of all I would like to express my sincere thanks to Professor Anders Ågmo for taking on the supervision of this thesis under all circumstances. I am deeply grateful to him for all his ideas and criticisms as well as for all discussions.

I am thankful to Professor Floyd Rudmin for reading the manuscripts and assisting with the editing of my English. I would like to express thanks to Professor Gilles LePape for all statistical advices. I wish to thank Associate Professor Darius Arai for helping me in the completion of this thesis. I am thankful the cooperation of the staff of the animal laboratory and the Department of Psychology as well as Truls Traasdahl for all skilful technical support.

I would like to thank Professor Anders Ågmo, Professor Floyd Rudmin, Associate Professor Claudia Rodriguez, and Associate Professor Darius Arai as well as Mahamadou Traoré for providing an excellent social environment and helping me under all circumstances.

Je souhaiterais tout particulièrement, remercier ma femme, Sarah Mondongue qui m'a soutenu contre vents et marées. Sans elle, rien de tout cela n'aurait pu être accompli. Pour tous ses sacrifices et ses efforts, je lui serai éternellement reconnaissant. En dernier lieu, je voudrais dédier ce travail à ma femme et à mon fils venu au monde au beau milieu de la réalisation de cette thèse. Noah, tu as été, sans le savoir, d'un immense soutien.

May, 2010

CONTENTS

Abstract

Estrogens have been shown to be implicated not only in sexual behaviors but also in several cognitive functions, social behaviors, and emotional reactions. Estrogen receptors mediate estrogen actions in the brain and the estrogen receptor α (ERα) has attracted some attention. It is known that copulatory behaviors depend on ERα. However, these behaviors require the presence of a mate; and are, therefore, preceded by search for and approach to a potential mate. This process is labeled sexual incentive motivation. Although the display of female sexual motivation and copulatory behaviors require estrogens, their sites of action in brain are unknown. Social recognition is the ability to recognize a congener. It is an ability that is fundamental for social interaction. However, the effects of estrogens on social recognition are inconsistent and they have never been examined in an habituation-dishabituation procedure. Assuming that estrogens affect social recognition through the ERα, it may be asked at which brain sites ERα may act. Data showed that estrogens, at least in part, through ERα, influence anxiety and aggression. Although the ventromedial nucleus of the hypothalamus (VMN) and the posteriodorsal amygdala (MePDA) are involved in these behaviors, the role of ERα within these structures remains unknown. A review of experiments employing the sexual incentive motivation test used in this work suggests that this procedure provides a precise estimation of sexual motivation in female rats, and it could be used as a preclinical model. Assuming this and taking into account the issues previously presented, we first delineated the role of ERα in the VMN and the MePDA in sexual behaviors and particularly sexual incentive motivation in female rats. To achieve knockdown of ERα in the VMN and the MEPDA of the adult brain, ovariectomized females were injected bilaterally by an adeno-associated virus vector (AAV) expressing a shRNA targeting ERα. Second, we evaluated the role of ovarian hormones on social recognition in the habituation-dishabituation paradigm. Finally, we used the technique previously presented to assess the effect of ERα knockdown in the VMN and the MePDA in social recognition, anxiety, and aggression. Sexual motivation was abolished by ERα knockdown in the VMN. Proceptivity and receptivity were reduced while rejections were enhanced. ERα knockdown in the MePDA have no effect on these behaviors. Consequently, the ERα in the VMN, but not in the MePDA, is crucial for the entire sequence of sexual behaviors from sexual approach to copulation. The second experiment established that ovarian hormones, particularly estrogens, have a facilitatory effect on social recognition, although they are not necessary. To expand this finding, the results of the third experiment showed that ERα knockdown in the MePDA, but not in the VMN, suppressed social recognition and reduced anxiety in the light/dark choice test. In contrast, ERα knockdown in the VMN enhanced aggression against the juvenile but not in the resident intruder test. Thus, ERα in the MePDA contributes to social recognition and modulates anxiety, while ERα in the VMN could partially regulate aggression against juveniles but not against adults. In conclusion, present results represent an important step forward in the understanding of the estrogen-dependent neurobiological mechanisms underlying sexual behaviors, social recognition and emotional reactions. Moreover, they offer the opportunity to understand the indirect role of the ERα in the VMN and in the MePDA for reproductive success.

LIST OF PAPERS

I Spiteri, T., & Ågmo, A. (2006). Preclinical models of sexual desire (Modèles précliniques du désir sexuel). *Sexologies, 15*, 241-249.

II Spiteri, T., Musatov. S., Ogawa. S., Ribeiro. A., Pfaff D.W., & Anders Ågmo (2010). Estrogen-induced sexual incentive motivation, proceptivity and receptivity depend on a functional estrogen receptor α in the ventromedial nucleus of the hypothalamus but not in the amygdala. *Neuroendocrinology, 91*, 142-154.

III Spiteri, T., & Ågmo, A. (2009). Ovarian hormones modulate social recognition in female rats. *Physiology & Behavior, 98,* 247-250.

IV Spiteri, T., Musatov. S., Ogawa. S., Ribeiro. A., Pfaff D.W., & Anders Ågmo (2010). The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behavioural Brain Research, 210,* 211-220.

INTRODUCTION

One of the most ambitious projects of neurosciences is to bridge the chasm between genes and behaviors. In this field, a fascinating focus of research is intended to understand how gene products affect behaviors through the central nervous system. Consequently, it is hoped to delineate the effects of genes in different regions of the brain in order to highlight the neural circuits involved. The study of ovarian hormones, and particularly estrogens, is a good candidate for this type of research. Indeed, estrogens have numerous effects on the central nervous system, and affect a broad range of behaviors (reviewed in McEwen, 2002).

The actions of estrogens are mediated by estrogen receptors (ER) α and β. It has long been known estrogen receptors are transcription factor, i.e. when estrogens bind to them, the receptor is transferred to the nucleus where it activates one or several genes (McEwen & Alves, 1999). In addition, the neuroanatomical distribution of ERs implies that specific regions of the brain are involved in estrogenic effects. In this framework, the present work in female rats aspires to contribute to the knowledge of how estrogens act through the central nervous system to modify certain behaviors.

At least in part, growing interest in estrogens is due to the diversity of their effects. The understanding of the brain process underlying these actions led to a focus on the first discovered and most known estrogen receptor, namely, the ERα (Enmark & Gustafsson, 1999). More specifically, the present thesis focuses on the role of ERα in the posterodorsal medial amygdala (MePDA) and the ventromedial nucleus of the hypothalamus (VMN) in sexual motivation, social recognition, aggression and anxiety.

Since estrogens affect numerous behaviors, the reason for which we decided to study these behaviors needs to be explained. Probably the most expected behaviors influenced by estrogens are sexual behaviors (Blaustein & Erskine, 2002). In humans, recent success in pharmacological treatment of sexual dysfunction as well as the recognition of the role of sexual desire in this type of disorders brought attention to animal models of desire. In the

literature, animal sexual motivation is used as an equivalent of human sexual desire. In animals, it is proposed that the intensity of approach behaviors to a potential mate is an estimator of sexual motivation. Since these behaviors represent the urge to establish sexual interaction, they seem to be suitable to provide information on sexual desire. Moreover the arbitrariness and variability of sexual approach behaviors present a similarity with flexible human sexual courting. In contrast, animal copulatory behaviors are very stereotyped making them unsuitable to be compared to human copulatory behaviors. Unfortunately, the role of the ERα has been studied only with regard to copulatory behaviors in female mice and rats. Thus, it is unknown whether, or in which brain structure, ERα acts on sexual approach behaviors.

Growing interest in social neuroscience led to an examination of whether ovarian hormones could be implied in social behaviors other than sexual. The ability to recognize and remember other individuals is called social recognition (Thor & Holloway, 1982). This capacity is fundamental for sexual, affiliative and aggressive behaviors. It is believed that estrogens play a key role in social recognition. Furthermore, mice lacking the genes for ERα and β show social recognition impairment (Choleris, Ogawa, Kavaliers, Gustafsson, Korach, Muglia et al., 2006). However, depending on the paradigms used to infer this capacity in female rats, data are conflicting, ranging from no effect to improvement of this ability. Thus, even if some studies suggest that social recognition depends on estrogens, it remains to explain these discrepant results. Likewise, it could be asked where estrogens act in the brain and through which receptors.

Independently of effects on social recognition, estrogens may modify aggression. Some studies show that a high concentration of estrogens increases the intensity of aggression (Albert, Jonik, & Walsh, 1992b; Albert, Jonik, & Walsh, 1992a); while, their absence reduces aggressive behaviors (Albert, Petrovic, & Walsh, 1989a; Albert, Petrovic, & Walsh, 1989b). Nevertheless, the receptor(s) and brain structures by which estrogens influence aggression are completely unknown.

The estrogen effects on anxiety, mood and fear are among the most prominent. However, the role of estrogens is ambiguous and contradictory findings ranging from anxiogenic to anxiolytic effects have been reported. This variability in emotional responses to estrogens is found in humans as well as in rodents (Arpels, 1996; Morgan, Schulkin, & Pfaff, 2004). Several reasons can be considered to explain these discrepant results. It is possible that ERα and β have opposite effects (Fugger, Foster, Gustafsson, & Rissman, 2000). It has also been proposed that the anxiolytic effect of estrogens is dependent on the safeness of the environment (Morgan et al., 2004). Whatever the explanation, there is no doubt that the study of ERα in specific brain structures could contribute to elucidating the complex neural mechanisms underlying estrogen effects on anxiety.

The present work is not only concerned with the effects of estrogens on the ERα but also with localization of these actions in the brain. Therefore, the choice of structures must be made explicit. There are two main reasons to study the role of ERα in the MePDA and the VMN. First, it is reported that the MePDA and the VMN of female rats have a dense expression of ERα (Shughrue, Lane, & Merchenthaler, 1997). Consequently, it is reasonable to suppose that some estrogenic effects are mediated by these structures. Second, the MePDA and the VMN are known to play a role in the behaviors studied here. We propose to determinate whether this is mediated by ERα in the present thesis.

ERα and the neurobiological basis of sexual motivation

Concept and test of sexual incentive motivation

The recent advent of medical treatment against erectile deficiency attracts attention to the general capacity of drugs to modify sexual functions, and particularly sexual desire. Hypoactive sexual desire has a prevalence of 33% in women but only 15% in men (Laumann, Gagnon, Michael, & Michaels, 1994; Laumann, Paik, & Rosen, 1999). Hyperactive sexual desire in men is also reported as problematic either for themselves or for society. Although this disorder does not exist as a psychiatric disorder in the *DSM-IV*, the fact that most paraphiliacs and sexual offenders have hyperactive sexual desire poses, at least, a social problem (Ågmo, Turi, Ellingsen, & Kaspersen, 2004). Thus, there is a searched for drugs which are able to either enhance or impair sexual desire. Animal models are suitable for this type of research.

From sexual desire in humans to sexual incentive motivation in animals

In order to make inferences from animals to humans, the first step is to operationalize sexual desire. Among the many definitions of desire, two seems relevant in this context: (1) conscious impulse toward something that promises enjoyment or satisfaction in its attainment; (2) sexual urge or appetite (http://www.merriam-webster.com/dictionary/desire). According to them, desire is the result of both an appropriate organismic state and stimuli or events having hedonically positive properties also called positive incentive stimuli. In the theory of Bindra (1974), this interaction is called positive incentive motivation. Thus, desire and positive incentive motivation seem to be conceptually equivalent. Since we discuss only positive effect in this thesis, we will replace the expression “positive incentive” by the word of “incentive”. Moreover both desire and incentive motivation are associated with approach behaviors. Indeed, if an incentive object is distal to an individual in the appropriate state, it is able to activate approach to this object. In the case of sex as incentive stimulus, we can therefore

propose that sexual desire and sexual incentive motivation are equivalent and operationalized by the intensity of approach toward a sexual stimulus (Ågmo, 1999; Ågmo, 2003).

Approach behaviors versus copulatory behaviors

According to the theory of incentive motivation, the contact with an incentive object tends to provoke consummatory behaviors. Consequently, a sexually motivated individual is inclined to perform copulatory behaviors on contact with a mate if the opportunity is given. These behaviors are variable and flexible in humans. In contrast, nonhumans and particularly rodents display stereotyped motor patterns (Ågmo & Ellingsen, 2003). Copulatory behaviors in female rats consist of lordosis and proceptive behaviors both of which activated by tactile stimulation from the male (Pfaff, Lewis, Diakow, & Keiner, 1973; Ågmo et al., 2004; Ågmo, 2007). Consequently, non-human copulatory behaviors appear unsuitable as a model of human sexuality copulation. Unfortunately, most of the research has focused on these stereotyped behaviors, making generalization to humans difficult. In contrast, approach behaviors are variable, depending on the individual animal or human. In other words, approach behaviors are contingent, in contrast to stereotyped copulatory behaviors. The only restraint on the approach behaviors is to reach the mate. With this purpose, humans as well as rats can perform whatever response is fitted to reduce the distance between them and the mate (Ågmo, 2007).

An objection might be made that these two categories of behaviors belong to the same behavioral sequence, and that the separation is artificial. There are two main arguments against this objection. First, the dichotomy between contingent and stereotyped behaviors makes unlikely that nervous processes underlying approach and copulation reflexes are identical. Second, achieving proximity is not necessarily followed by copulatory behaviors. Indeed, some individuals never perform copulatory behaviors although they clearly show approach to the mate (Stone, Barker, & Tomilin, 1935). Furthermore, neuroanatomical studies

demonstrate that lesions can affect differentially approach behaviors and copulatory behaviors. Indeed, lesions of the peridepuncular region abolish copulatory behaviors in female rats without affecting approach to the mate (Pfeifle & Edwards, 1983) while lesions of the nucleus accumbens suppress sexual approach without affecting responses to a mounting male (Rivas & Mir, 1990). Moreover some lesions can even have opposite effects on sexual approach and copulatory behaviors. For example, medial preoptic area damage enhances copulatory behaviors while sexual approach is reduced (Sakuma, 2008). Thus, data suggest that copulatory behaviors and sexual incentive motivation are controlled differently, and although tempting, extrapolation of results between these behaviors is not evident.

Measuring and testing sexual incentive motivation

Rats are gregarious, and the presence of a conspecific initiates approach behaviors (Latane, 1969; Latane & Glass, 1968). In the laboratory, it is possible to exclude other possible reasons for approach toward other rats such as protection against predator or low temperature. Since rats like to approach and remain close to a conspecific in sexual and non-sexual situations, a test of sexual incentive motivation needs to differentiate between sexual incentive motivation and social incentive motivation. Following this reasoning, a conspecific showing no sexual activities indicates a social incentive; while, a conspecific showing sexual activities indicates both a social and a sexual incentive. Thus, the intensity of sexual incentive motivation can be measured by the difference between the time spent by rats close to an individual being both a sexual and social incentive, and another individual being only a social incentive. Consequently, the sexual motivation of female rats is assessed by the comparison between time spent close to an intact male (sexual and social incentive) and a castrated male (social incentive). For male rats, a receptive female is both a sexual and a social incentive while an ovariectomized or non-receptive female is only a social incentive.

A procedure for determining the intensity of sexual incentive motivation has been developed by Meyerson and Lindström (1973) and adapted by Ågmo et al. (Ågmo, 2003; Ågmo et al., 2004). In this test (Fig. 1), the subject is given the choice to spend time close to a social incentive conspecific or to a sexual and a social incentive conspecific. A set of experiments manipulating gonadal hormones must performed in order to validate this test (Ågmo et al., 2004). A summary of the validated results are presented in this thesis.

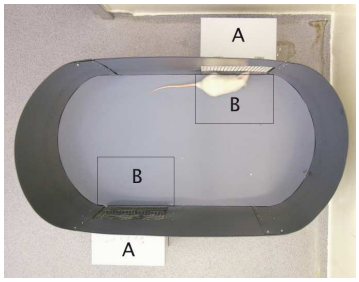

Figure 1: A photograph of the sexual incentive motivation test arena. The incentive animal cages (marked with an A on the photograph) are located on the outside of the oval arena . The side facing the arena is made of a wire mesh that allows the experimental subject to see, smell and hear the incentive. A virtual zone (marked with a B on the photograph) is defined outside each incentive animal cage. A computerized video-track system determines the experimental subject's position, the time spent in the incentive zones, the number of visits to them, the distance moved during the test, the mean speed of movement while moving, and the duration of immobility.

Criteria of a good preclinical model

The quality of a preclinical model should be judged by 3 criteria. Since the main purpose of an animal model of sexual desire is to predict effects of drugs, the first criterion is the correlation. This criterion is in reality a set of criteria since the goal is to establish a similarity between drug effects in humans and animals. Thus, therapeutically effective drugs in humans should also be effective with the similar magnitude in the animal model. Likewise, humans and the animal model should share the same ineffective drugs. The second criterion is

the isomorphism. This means that the motor patterns behaviors in humans and in the animal model are similar. It is worth noting that the motor patterns do not need to be the same but they should share the same purpose, that is, they should be functionally equivalent. A preclinical model should also be homologous. This third criterion refers to the similarity of underlying causes or behavioral process between humans and the animal model. According to these criteria, the possibility that the sexual incentive motivation test can serve as preclinical model of sexual desire is examined in this thesis.

Concept of central motive state

In the process of operationalization of the concept of sexual motivation, we insisted on the proprieties of incentives. However, a sexual incentive does not always produce approach behaviors. As mentioned previously, a non-receptive female rat does not prefer an intact male (sexual and social incentive) compared to a castrated male (social incentive). Thus, the effect of incentives on the organism depends on some internal mechanism. This mechanism is called *central motive state* in the incentive motivation theory of Bindra (1974). The concept of an internal mechanism is of particular importance in this study since we are interested in the neural circuit underlying sexual motivation.

The role of ovarian hormones and ERs in sexual behaviors

Since female sexual behaviors are dependent on ovarian hormones, the presence of ERs in a specific area is a necessary but not sufficient condition for speculating about brain structures involved. It is known that progesterone, through progesterone receptors (PRs), amplifies the effect of estrogens on sexual behaviors (Ogawa, Olazabal, Parhar, & Pfaff, 1994). Hence, the distribution of PRs in the brain is also an important clue to focus on one specific area rather than on others. Nonetheless, ERs and PRs are widely distributed in the mammal brain (Sar & Stumpf, 1973; Stumpf, Sar, & Keefer, 1975; Pfaff, 1968) and additional clues are necessary for speculations about brain structures important for sexual behaviors. The MePDA and the VMN of female rats are two areas in which ERs and PRs are localized. The next subsection will present data suggesting their implication in sexual behaviors. Thus, this work will be focused on these regions of the female rat's brain.

The role of the ERα in the VMN and the MePDA in sexual behaviors

Background on copulatory behaviors

Copulatory behaviors are easy to identify and quantify. Moreover they are strictly dependent on ovarian hormones (reviewed in Blaustein & Erskine, 2002). It is not, therefore, surprising that these behaviors are chosen in most studies trying to understand how estrogen-dependent neural circuits affect behaviors. However, the understanding of copulatory behaviors is not our main goal. To understand the arguments on other aspects of sexual behaviors, it is necessary to possess some knowledge of copulatory behaviors.

The first issue is the respective actions of the two ERs in mediating estrogenic effects on copulatory reflexes. In rodents, transgenic techniques allow us to suppress a specific gene and, in turn, eliminate a specific molecule coded by this gene. These techniques are used to produce female mice, called "knockouts", which lack ERα and/or β. Lordosis is completely abolished in female mice lacking ERα. Moreover they are very rejective toward males attempting to mount (Ogawa, Eng, Taylor, Lubahn, Korach, & Pfaff, 1998). In contrast, those lacking ERβ show similar copulatory reflexes to wild-type mice (Ogawa, Chan, Chester, Gustafsson, Korach, & Pfaff, 1999). At least in mice, ERα is therefore thought to be necessary for copulatory reflexes.

The second issue is the neural circuit underlying these effects. Pfaff and colleagues described the hypothalamic control of the lordosis circuit (Ogawa et al., 1999; Pfaff, 1980; Pfaff, 1999). Among hypothalamic structures, the VMN is a crucial site of action for ovarian hormones. Indeed, the injection of estrogens and progesterone within the VMN is sufficient for the display of sexual receptivity in female rats. Moreover, the activation of estrogen and progesterone receptors within the VMN is necessary (Pleim, Brown, Maclusky, Etgen, & Barfield, 1989). A reduced number of these receptors within that area decreased the ability estrogens and progesterone to induce sexual receptivity (Ogawa et al., 1994; Etgen & Barfield, 1986). More accurately, a brilliant recent study in mice demonstrated that the

administration of a ribonucleotic acid, called RNA interference, directed against the ERα gene within the VMN abolishes lordosis and proceptive behaviors (Musatov, Chen, Pfaff, Kaplitt, & Ogawa, 2006). We must recall that lordosis and proceptive behaviors are triggered by the close proximity of the male and mainly by tactile stimulation. Thus, it is possible to assume that the activation of ERα in the VMN is necessary for responses to proximal sexual stimuli.

Background on sexual incentive motivation

In the previous subsection, it was demonstrated that the VMN is necessary for female copulatory behaviors and that the ERα mediates this control in female mice. Evidence exists that the VMN is also important for sexual incentive motivation. Indeed, lesions of the VMN eliminate a female's sexual approach to a male (Clark, Pfeifle, & Edwards, 1981; Emery & Moss, 1984). Thus, ERα in the VMN may be important for sexual approach in female rats. However, the role of ERα in sexual incentive motivation is less apparent in female rats than in female mice. First, studies regarding the role of the ERα in mice have unfortunately never tested sexual incentive motivation. As was shown above, data on copulatory behaviors cannot be easily extended to sexual incentive motivation. Second, many observations in mice suggest that sexual approach to a male is independent of ovarian hormones; whereas, that is not the case in female rats. Sexual incentive motivation does not vary during the estrus cycle of female mice while female rats present a peak of attraction in proestrus (Clark, Kelton, Guarraci, & Clyons, 2004; Scott & Pfaff, 1970; Spiteri & Ågmo, 2006). Consequently, the extrapolation from results in mice to rats should be made with caution. Third, the ER subtype involved in sexual incentive motivation cannot be inferred from lesion data. All together, these arguments suggest that a direct investigation of the role of the ERα in the VMN in sexual incentive motivation in female rats is necessary.

By definition, stimuli which are able to enhance sexual incentive motivation and initiate approach behaviors should act at distance. It is well known that odors are very

relevant clues in rodents (reviewed in Halpin, 1986). Therefore, it is not surprising that the role of olfactory systems in sexual incentive motivation has been the focus of many studies. Thus, it has been demonstrated that olfactory stimuli acting upon both the main and accessory olfactory systems are crucial for approach to a potential mate (Hernandez-Gonzalez, Guevara, & Ågmo, 2008). The MePDA is an important structure receiving fibers from both olfactory systems. Likewise, the amygdala sends a number of projection to the VMN (Canteras, Simerly, & Swanson, 1996). Since lesion of the MePDA decrease sexual incentive motivation in female rats (Kondo & Sakuma, 2005; Romero, Beltramino, & Carrer, 1990), this region of the brain is believed to be important for sexual approach to a male. These data suggest that the MePDA could be an important structure for integration of olfactory information associated with approach behaviors to a sexually active male. Although this structure is rich in ERα, it remains unknown whether this subtype of ERs is involved in these behaviors. This thesis proposes to investigate this issue.

ERα and the neubiological basis of social recognition

Definition and paradigms of social recognition

The construction of social organization is based on what animals know about each other. This knowledge of the social world requires at least the ability to recognize social categories (for example, kin, hierarchical position etc.) and the ability to differentiate one individual from another (Colgan, 1983). Both abilities have been demonstrated in mammals (Halpin, 1986). However, this thesis focuses only on the identification of individuals. Curiously, this ability is named social recognition in the basic definition (Thor & Holloway, 1982). It is noteworthy that the ability to identify and recognize other conspecifics is crucial for the regulation of several social behaviors, from affiliative to aggressive. Consequently, social recognition is fundamental for many aspect of social life.

The behavioral investigation of the way by which rats and mice recognize individuals is critical in the operationalization of social recognition. Ethological studies showed that rats and mice explore spontaneously a new individual, and this exploration is strongly reduced if the same individual is presented a short time later. The timing is critical since the reduced exploration when the same individual is presented does not occur if the interval between exposures is too long (Ferguson, Young, & Insel, 2002). Thus, social recognition is inferred from decreased investigation of a previously encountered individual. Paradigms used in rodents reflect this feature.

Two main paradigms coexist in the field of social recognition: social discrimination and the habituation-dishabituation procedure. The social discrimination procedure consists of two exposures. At the first exposure, the subject is exposed to a juvenile. The same juvenile and a novel juvenile are simultaneously presented at the second exposure. Differential time spent investigating the familiar and the novel juvenile is interpreted as evidence that juveniles are discriminated by the subject (Engelmann, Wotjak, & Landgraf, 1995). The habituation-dishabituation procedure is based on the same spontaneous tendency but the habituation phase

consists of 4 consecutive exposures to the same juvenile with usually 10 or 15 min between exposures. Then, the subject is exposed to a novel juvenile at the 5th exposure (dishabituation phase) (reviewed in Gheusi, Bluthe, Goodall, & Dantzer, 1994). This test offers a sensitive evaluation of social recognition in two ways: First, the decreased investigation of the juvenile during the multiple exposures to the same juvenile, second, the increased investigation of the juvenile during the dishabituation phase.

The role of ovarian hormones and ERα in social recognition

Estrogens are believed to improve social recognition. However, estrogenic regulation of social recognition appears unclear. Recognition does not vary during the estrus cycle and this ability is not improved by a high-dose treatment with estradiol in ovariectomized rats (Markham & Juraska, 2007; Reyes-Guerrero, Vazquez-Garcia, Elias-Vinas, Donatti-Albarran, & Guevara-Guzman, 2006). In contrast, it is reported that estrogens replacement improves social recognition in ovariectomized rats and mice (Hlinak, 1993; Tang et al., 2005). At least two reasons can be proposed to explain the inconsistent data. The paradigm used in the studies mentioned above is the social discrimination procedure. This paradigm uses only two exposures to the same juvenile, thereby offering a less sensitive test of social recognition than the habituation-dishabituation procedure. This possible explanation is reinforced by the fact that the latter procedure is used to show that ERα knockout mice are deficient in social recognition (Choleris, Gustafsson, Korach, Muglia, Pfaff, & Ogawa, 2003). The absence of progesterone could also constitute another reason for these discrepant results. Since many effects of estrogens are enhanced by progesterone, it is conceivable that progesterone maximizes the effects of estrogens on social recognition (Frye, Duffy, & Walf, 2007; Gibbs, 2003; Sandstrom & Williams, 2001). In order to address these issues, ovariectomized rats are given estradiol + progesterone, and they are tested in the habituation-dishabituation procedure in one of the studies of this thesis.

The role of the ERα in the medial amygdala in social recognition

As important as estrogens and ERα are, the neuroanatomical area in which they act turn out to be crucial for the understanding of the neurobiological control of social recognition. Anatomical regions involved in social recognition by rats are those that mediate olfaction, learning and memory. Although the neural pathway remains uncertain, all studies agree that the medial amygdala is a fundamental structure for social recognition (Bielsky & Young, 2004; Choleris, Little, Mong, Puram, Langer, & Pfaff, 2007; Ferguson et al., 2002). The fact that this structure is also rich in ERα (Osterlund, Kuiper, Gustafsson, & Hurd, 1998) suggests that the facilitation of social recognition by estrogens may be mediated by ERα in the medial amygdala. It is noteworthy that estrogens through ERα up-regulate progesterone receptors in the medial amygdala (Greco, Allegretto, Tetel, & Blaustein, 2001; Kudwa, Michopoulos, Gatewood, & Rissman, 2006; Moffat, Rissman, Shupnik, & Blaustein, 1998). Thus, the administration of progesterone following estradiol might facilitate the observation of ERα effect on social recognition.

ERα and neurobiological basis of anxiety

The role of estrogens and ERs in anxiety

Estrogens are well known to affect emotions, and particularly anxiety. However, in both rats and mice, estrogen effects are not uniform and range from increased to decreased anxiety (Morgan & Pfaff, 2001; Morgan et al., 2004). In female rats, anxiolytic effects of estrogens have been demonstrated in certain studies utilizing the elevated plus-maze (Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001; Walf & Frye, 2005; Koss, Gehlert, & Shekhar, 2004; Pandaranandaka, Poonyachoti, & Kalandakanond-Thongsong, 2006); whereas, no effects are found in other studies using the same paradigm (Fernandez-Guasti, Martinez-Mota, Estrada-Camarena, Contreras, & Lopez-Rubalcava, 1999; Mora, Dussaubat, & DiazVeliz, 1996; Nomikos & Spyraki, 1988). In contrast, anxiogenic effects of estrogens have been reported in the social interaction test (Koss et al., 2004) as well as in the fear-potentiated startle paradigm (Hiroi & Neumaier, 2006).

Two main assumptions are proposed to explain these discrepant results. First, recent studies suggest that ERα and β could have opposite effects. In fact, selective stimulation of ERβ reveals anxiolytic effects in many studies; whereas, stimulation of ERα produces either no effect or an anxiogenic effects (Lund, Rovis, Chung, & Handa, 2005; Walf, Ciriza, Garcia-Segura, & Frye, 2008; Hughes et al., 2008). However, it is worth noting that neural mechanisms by which estrogen receptors influence anxiety are not well understood. Second, it is proposed that estrogen effects depend on the safeness of the environment. Indeed, data converge to suggest that estrogens are anxiogenic in threatening context and anxiolytic in safe context. These effects are considered to enhance the likelihood of reproductive success since anxiolytic effects in safe environment facilitate sexual behaviors; whereas, anxiogenic effects in threatening environment prevent reproductive activities in unsuitable environment (Morgan et al., 2004).

The role of the ERα in the VMN and the medial amygdala in anxiety

Anxiety is increased by electrical stimulation of the medial amygdala indicating that this structure is important for this emotional response (Adamec & Morgan, 1994; Morgan, Watchus, Milgram, & Fleming, 1999; Rasia-Filho, Londero, & Achaval, 2000). Moreover, anxiolytic effects are reported after injection of estradiol in this structure. These effects are similar to those observed after systemic estradiol administration (Frye & Walf, 2004). Thus, these data suggest that the medial amygdala is prominent among structures in which estrogens affect anxiety.

The role of the VMN in anxiety has been addressed in only a few older studies. However, data suggest that this structure may be implicated in anxiety. Indeed, enhanced anxiety (Milani & Graeff, 1987), hyperirritability (Sclafani, 1971) as well as increased defensiveness (Grossman, 1972a; Grossman, 1972b) have been observed after electrical stimulation of this nucleus. To our knowledge the role of estrogens or estrogen receptors in the VMN in anxiety has never been investigated. Their roles are unknown in this nucleus. The exitability of some neurons of the VMN is increased by estrogens (Lee et al., 2008; Lee, Devidze, Pfaff, & Zhou, 2006; Zhou et al., 2007). In sum, it is possible that estrogens and estrogen receptors are involved in VMN effects in anxiety.

ERα and neurobiological basis of aggression

The role of estrogens and ERs in aggression

Aggression in rodents covers a broad range of behaviors in varied situations, including food competition, aggression toward opposite or same sex conspecifics or interspecifics, and aggression toward foster pups. Although it was demonstrated that female rats are able to become aggressive (Dejonge & Vandepoll, 1984; Vandepoll, Dejonge, Vanoyen, Vanpelt, & Debruin, 1981; Vandepoll, Smeets, Vanoyen, & Vanderzwan, 1982), aggression is commonly considered to be a predominantly male activity. Probably for this reason, most studies on aggressive behaviors in rats have focused on males. However, female aggressive behaviors have been assessed in specific situations, mainly those related to hormonal changes. For instance, females are more aggressive toward conspecific intruders during the postpartum period than at other periods (Flannelly & Flannelly, 1987). In this framework, the role of ovarian hormones is questioned. The intensity of aggressive behaviors is reduced in ovariectomized females (Albert, Walsh, Gorzalka, Siemens, & Louie, 1986; Albert et al., 1989a; Barfield, Busch, & Wallen, 1972) while a high concentration of estrogens increases aggression toward opposite sex conspecifics or unfamiliar females (Albert, Jonik, & Walsh, 1992a; Albert, Jonik, & Walsh, 1992b). Although these data suggest that estrogens facilitate aggression in female rats, it is also known that females in proestrus or estrus display less aggression toward opposite sex conspecifics or unfamiliar females than those in diestrus (Ho, Olsson, Westberg, Melke, & Eriksson, 2001; Swanson, Vandepoll, & Vanpelt, 1982). Likewise, female mice who lack ERα showed either reduced or enhanced aggressive behaviors according to circumstances (Ogawa et al., 1998). Thus, it appears reasonable to propose that estrogens may increase aggression while the administration of estradiol followed by progesterone may reduce it.

The role of the ERα in the VMN and the medial amygdala in aggression

In female rats, both the medial amygdala and the VMN modify aggression in different situations. Fighting induced by food competition as well as defensiveness to non-painful stimuli are reduced by lesions of the medial amygdala (Kemble & Davies, 1981; Kemble, Blanchard, Blanchard, & Takushi, 1984). In the same way, lesions of the VMN decrease aggression toward foster pups; while, aggression toward an intruder is increased by electrical stimulation of this structure (Hansen, 1989; Kruk et al., 1984). Although estrogen receptors, particularly ERα, are abundant in the medial amygdala and the VMN, their roles in aggression are unknown.

OBJECTIVES OF THE THESIS

The main purpose of this work is to better understand the neural mechanisms which underlie the influence of estrogens on sexual incentive motivation, social recognition, and emotional reactions related to aggression and anxiety in female rats. For this, I directed my study towards the role of ERα in the VMN and the MePDA. Hence, the goals of this thesis are:

1. To address the issue of measurement of sexual incentive motivation in rats. Presented is a test of sexual incentive motivation which could serve as a preclinical model (paper I).

2. To explore the role of ERα in the VMN and in the MePDA vis-à-vis estrogen-induced sexual incentive motivation (paper II). According to the findings that ERα in the VMN is necessary for copulatory behaviors in female mice, it is expected that ERα in the VMN could be important for sexual incentive motivation as well as copulatory behaviors in female rats. The role of ERα in the MePDA could not be predicted.

3. To investigate the influence of estrogens on social recognition in the habituation-dishabituation paradigm (paper III). Data from other paradigms are inconsistent. However, social recognition is impaired in mice lacking ERs in the habituation-dishabituation paradigm. Hence, it is expected that estrogens have an effect on social recognition in female rats.

4. To study whether the influence of estrogens on social recognition, anxiety and aggression is mediated by the ERα in the VMN or the MePDA (paper IV). Female mice lacking ERα are deficient in the habituation-dishabituation paradigm, and

neuroanatomical studies show that the medial amygdala is a crucial structure for social recognition in female mice and rats. Consequently, it is expected that ERα in the MePDA contributes to social recognition. Since female mice whose gene for ERα is disrupted, are more anxious, the anxiolytic effects of estrogens in the medial amygdala could be mediated by ERα in the MePDA. In contrast, no prediction could be made regarding aggression. The reproductive success is heightened by the fact that estrogens enhance anxiety and aggression in the threatening environment and reduce them in safe environment. Hence, the crucial and facilitatory roles of ERα in the VMN in sexual interaction lead us to expect that ERα in the VMN enhances anxiety and aggression in a threatening environment.

GENERAL METHODS

Subjects

All experiments use Wistar rats obtained from Charles River WIGA (Sulzfeld, Germany); all experimental subjects are females. All subjects (250 g upon arrival to the animal facilities) were ovariectomized under isofluorane anesthesia.

In Paper II, males are used as incentive. Some males (300 g upon arrival) are castrated under isofluorane anesthesia.

In Paper III and IV, intact females are used for reproduction thereby assuring an adequate supply of juvenile rats for the social recognition test. The juveniles are used between 23 and 26 days of age.

The shRNA technique and ERα knockdown in Papers II and IV

Although the generation of receptor knockout rodents advanced our knowledge of their function, gene deletions with this method is global and potentially confounded by the developmental consequences. Indeed, the studies that identified a receptor gene essential for mammalian behaviors are unclear as to which effects are due to a mere absence of this receptor in the mature neuron and which deficits result from the lack of receptor expression during development or as a consequence of genetic compensation. For instance, recent findings indicate a potential confound in the design of estrogen receptor knock out mice because these animals express an abnormal splicing variant (Moffat et al., 1998). This truncated form of the receptor retains both the DNA and ligand-binding domains and is able to mediate, albeit far less efficiently, some estrogenic effects.

The availability of a new technique provides a valuable alternative to conventional transgenic techniques and allows addressing this problem. This technique uses a somatic gene knockdown in individual nuclei of normally developed brain through short hairpin RNA (shRNA). The shRNA will become incorporated into the neurons adjacent to the injection

site, and as soon as it starts to be expressed, it will eliminate the target for which it is designed for the rest of the animals' life. The shRNA in the study of the brain makes it possible to specifically delineate the effects of a gene (by suppression of its expression) in a discrete region of an adult brain and provide a valuable alternative to conventional transgenic techniques.

Using this technique, researchers developed a shRNA against the ERα. In this thesis, we use this shRNA to block specifically the ERα gene expression in the VMN and the MePDA of female rats. It is possible to determine the magnitude as well as the spread of ERα knockdown by immunochemistry.

Behavioral tests: Principles and interests

For paper II and IV, tests are performed 3 – 4 weeks after intracerebral infusion of the shRNA. All experimental females are given estradiol benzoate (18 µg/kg) about 52 hrs before behavioral testing is begun. Progesterone (1 mg/rat) is injected 48 hr after estradiol and 4 hrs before the beginning of behavioral tests. These doses of the ovarian hormones have previously been reported to produce receptivity and a sexual incentive motivation of the same magnitude as that observed at proestrus/estrus in intact, cycling rats (Spiteri & Ågmo, 2006; Ågmo,Turi, Ellingsen, & Kaspersen., 2004).

Test for sexual incentive motivation (paper I and II). This test is extensively described in the introduction. Briefly, copulatory behaviors are displayed only in contact with a mate. Consequently, they are preceded by search for and approach to the potential partner. Thus, the intensity of approach represents the sexual incentive motivation. This test infers the sexual incentive motivation from the time spent close to an unreachable sexual and social incentive (intact male) compared to an unreachable social incentive (castrated male).

Test for copulatory behavior (paper II). In this test, the receptivity of females is quantified by the number of lordosis in response to mount (until they received 10 mounts with

pelvic thrusting) and the number of proceptive behaviors (hop-darting and ear-wiggling) as well as rejections (kicking, boxing, fleeing).

Social recognition test (paper III and IV). This test is described in details in the introduction. Briefly, the habituation-dishabituation paradigm consists of 4 exposures of 5 minutes to the same juvenile (habituation) following by a fifth exposure in which a novel juvenile is presented (dishabituation) with 15 min interexposure interval. Social recognition is inferred from the spontaneous tendency to investigate a novel individual and from a marked reduction of this exploration after exposure to the same individual a short time later. The dishabituation phase rules out the possibly that reduced response during the habituation phase is a consequence of fatigue.

Light/dark choice test (paper IV). The principle of this unconditioned test is to give the animals a choice between a safe and a threatening environment. The time spent in each kind of environment serves as criterion to assess its safeness or anxiogenic aspect. In this test, females are given the choice between a dark part (safe) and a lighted part (threatening). They usually spend less time in the lighted part compared to the dark part of the light/dark box.

Resident-intruder test (paper IV). This test is based on the spontaneous display of aggressive behaviors in female rats against an unknown intruder of the opposite sex. Therefore, a castrated male is introduced into their home cage and left there for 10 min. A castrated male is used to avoid sexual interaction with receptive females.

SUMMARY OF THE PAPERS

Paper I:

This article aims to present and validate a test of sexual incentive motivation which could be used as a preclinical model. For this purpose, it is demonstrated that existing tests based on motor execution of learned responses are inadequate. Indeed, the existing tests make it difficult to know whether experimental effects are due to the modification of learning or to motivation. Moreover, motor functions are affected by pharmacological and hormonal treatment. Therefore, motor and motivational effects are confounded. Instead, we propose a test based on approach behaviors which is relatively insensitive to motor activity and does not require learning. In fact, this test uses the duration close to potential mate (sexual and social incentive) compared to sexually neutral conspecific (social incentive) as the measure of sexual motivation. Thus, no learning and a very low level of activity are necessary to explore the area close to the incentives. It is well known that both females and males need gonadal hormones to show sexual activities. Consequently, gonadectomized rats used as experimental subjects, should spend equal time close to both sexual and social incentives and social incentive. As expected in the sexual incentive motivation test, they do not demonstrate preference for a sexual and social incentive compared to a purely social incentive. Moreover, the restoration of sexual activities by injection of gonadal hormones in both male and female rats leads to preference for the sexual incentive. Thus, sexual incentive motivation depends on gonadal hormones. Moreover, in female rats, this motivation is dependent on the dose of estradiol. Prolonged sexual activities are known to reduce sexual incentive motivation. Thus, sexually exhausted females or males are not expected to display preference for a sexual incentive, an intact male or a receptive female, respectively. This is what is observed in the sexual incentive motivation test. Sexually exhausted females or males spend similar time

close to a social incentive and a sexual and social incentive. All together, these experiments suggest that this procedure provides precise estimation of sexual incentive motivation.

Paper II:

This study seeks to determine the function of the ERα in the VMN and the MePDA vis-à-vis sexual incentive motivation, proceptivity and receptivity of female rats. An adeno-associated virus vector containing a shRNA against either the sequence specific of the ERα gene or the sequence specific to luciferase as control, is injected bilaterally into the VMN or the MePDA. All females are given estradiol benzoate and progesterone 48 h later and 4 h before behavioral tests. Results show that sexual incentive motivation is abolished in females with 80% reduction of ERα in the VMN. They also show a reduction of proceptivity and receptivity while they display an increase of rejection. The elimination of ERα gene expression in the MePDA as well as the infusion of control AAV vector fail to modify these behaviors. Therefore, the ERα in the VMN, but not in the MePDA, is crucial for sexual incentive motivation, proceptivity and receptivity. These data suggest that the ERα in the VMN is important for the entire sequence of sexual behaviors from approach behaviors to potential mate until the accomplishment of copulatory behaviors.

Paper III:

The aim of this study is to investigate the effect of estradiol and progesterone on social recognition in the habituation-dishabituation procedure. To this purpose, two groups of ovariectomized female rats are employed. The treated group is given estradiol benzoate followed by progesterone 48h later, and the control group is given oil with the same timing. The habituation-dishabituation procedure is constituted by 4 exposures to the same juvenile (habituation phase) and a 5^{th} exposure to a novel juvenile (dishabituation phase). Both groups show habituation to repeated exposure to the juvenile and dishabituation when replaced by a

novel juvenile. This indicates that all females recognized the juvenile. However, the reduction of time spent investigating the juvenile after repeated exposures is larger in the group treated with ovarian hormones than in the control group. Moreover, the same effect is observed when within-exposure investigation time is analyzed. The decrease of the investigation from the first to the last minute is faster in the treated group than in the control group. Thus, data reveal that ovarian hormones facilitate social recognition although they are not necessary for this. Using multiple exposures to the same individual, the habituation-dishabituation procedure offers a sensitive test of social recognition. Since ovarian hormones have subtle effects, it is suggested that this procedure could be particularly suitable. It is even possible that the use of a less sensitive procedure (social discrimination procedure) in some earlier studies may account for the discrepant results reported in the literature.

Paper IV:

This study explores the role of the ERα in the MePDA and the VMN in social recognition, anxiety, and aggression. A shRNA encoded within an adeno-associated viral vector against either the sequence specific of the ERα gene or the sequence specific to luciferase as control, is injected bilaterally into the VMN or the MePDA. Estradiol and progesterone treatment are given to all females. Social recognition is eliminated in females with 81% reduction of ERα in the MePDA while anxiety in the light/dark choice test is reduced. In contrast, social recognition is not modified by the similar reduction of the ERα in the VMN; while, aggression against the juvenile and anxiety-like behaviors are increased. No treatment affects the resident-intruder test using a castrated male as intruder. Thus, the ERα in the MePDA contributes to social recognition and it modulates anxiety in threatening context. Oppositely, the ERα in the VMN could be important for anxious responses in social contexts and for aggression against juveniles but not against adults. Even if the ERα knockdown in the MePDA did not directly affect sexual behaviors, we propose that the ERα in this structure

could indirectly influence reproductive behaviors by acting on social recognition such as recognition of a mate. The role of the ERα in the VMN could be to allow the display of either aggression or sexuality, depending on the context.

GENERAL DISCUSSION

Sexual incentive motivation test as a preclinical model of sexual desire

A potential application of this research is the possibility to extend the results on sexual motivation obtained in Paper II to human beings. The data presented in Paper I plead in favor of the fact that the sexual incentive motivation test in rats can serve as preclinical model of sexual desire in humans. The comparison between data in rats and humans allows us to determine that the criteria of a preclinical model are partially fulfilled by this test. First of all, the choice of sexual approach behaviors in the procedure fulfills the criterion of isomorphism. Since approach behaviors to a potential mate are the result of sexual desire as well as sexual incentive motivation, it is possible to state that the purpose is similar in humans and in the animal model. Moreover, these behaviors, preceding stereotyped copulatory behaviors, are variable and contingent making them suitable to be compared to the flexible human sexual behaviors.

As indicated in the introduction, the correlation is a group of criteria concerning the matching between the effect of treatment in humans and in the animal model. Unfortunately, only few drugs are known to act on human sexual desire. Consequently, it is difficult to make any conclusions concerning correlational validity of our preclinical model.

The last criterion, named homology, is that fundamental causes of behaviors are similar for animals and humans. The model of rodents, and in particular rats, can be considered to be homologous because studies of neural and behavioral mechanisms determining sexual desire and motivation tend to demonstrate their similarity (Ågmo et al., 2003; Pfaff, 1999). All together, results from the preclinical test presented above suggest that this model could be isomorphic and homologous to human sexual desire. Although there is no way to validate correlational criteria, we propose that this procedure is an acceptable preclinical model of sexual desire.

Efficiency and specificity of ERα knockdown

Immunocytochemistry in Paper II and IV reveals that the shRNA employed in these experiments produced a drastic reduction of ERα in the VMN and the MePDA. Moreover, the number of this receptor in adjacent structures is unaffected. Since the shRNA used here does not affect the ERβ expression (Musatov et al., 2006), it is reasonable to propose that the behavioral effects observed in the Paper II and IV were caused by the reduced number of ERα in the VMN and in the MePDA. Since the few remaining ERα could have some effect, a total suppression of this receptor might amplify the effects reported here.

Effects of ERα knockdown on copulatory behaviors

Results of Paper II do show that ERα within the VMN are *necessary* for lordosis and proceptive behaviors in female rats as it is the case in female mice (Musatov et al., 2006). The action of estrogens through ER within the VMN is not only *necessary* but also *sufficient* for copulatory behaviors. Indeed, implants of ER antagonists into the VMN reduce these behaviors (Meisel, Dohanich, Mcewen, & Pfaff, 1987); while, copulatory behaviors are displayed by infusion of estradiol into this structure (Barfield & Chen, 1977; Pleim et al., 1989). Thus, it is possible that the activation of ERα within the VMN is both *necessary* and *sufficient* for these behaviors. However, modulatory effects of estrogens through ERα are conceivable outside the VMN. Although lesion studies have shown that the MePDA can inhibit lordosis and proceptive behaviors (Masco & Carrer, 1980; Polston & Erskine, 2001), the present data suggest that this effect is unrelated to the ERα.

Effects of ERα knockdown on sexual motivation

Results obtained in Paper II argue that the activation of ERα within the VMN of female rats is *necessary* for sexual approach to a potential mate. Present data coincide with

lesion studies of the VMN (Clark et al., 1981; Emery et al., 1984). In contrast, our study does not allow for concluding whether this action is *sufficient* for these behaviors. Our results also suggest that estrogen actions through ERα in the MePDA are not *necessary*. Since the process of sexual approach behaviors begins with the detection of volatile olfactory stimuli by the main olfactory system (Romero et al., 1990; Bakker, vanOphemert, & Slob, 1996; Hosokawa & Chiba, 2007; Keller, Douhard, Baum, & Bakker, 2006; Carr, Loeb, & Dissinge, 1965; Xiao, Kondo, & Sakuma, 2004; Xiao, Kondo, & Sakuma, 2005), an important issue is whether or not the activation of ERα by estrogens is required for sexual approach behaviors in structures involved in olfactory detection in addition to the VMN. Olfactory epithelial tissue (Parfenova, 1986), primary olfactory neurons (Shinoda, Shiotani, & Osawa, 1989), main and accessory olfactory bulbs and the amygdala (Pfaff, 1968; Shughrue et al., 1997; Simerly, Chang, Muramatsu, & Swanson, 1990) are rich in estrogen receptors. Consequently, these structures are good candidates to be required for sexual approach behaviors.

The ensemble of data from copulatory and sexual approach behaviors suggests that the activation of ERα in the VMN is crucial for sexual behaviors. Main efferent projection of the VMN is to the periaqueducal gray (Canteras, Simerly, & Swanson, 1994; Krieger, Conrad, & Pfaff, 1979). The periaqueducal gray is reported to mediate estrogens action involved both in lordosis (Barfield et al., 1977) and sexual partner preference (Pfeifle et al., 1983). Thus, another concern for future studies is whether or not ERα in this structure can modulate the entire sequence of sexual behaviors.

Effects of ovarian hormones on social recognition

Paper III demonstrated that ovarian hormones exert a facilitatory and modulatory role on social recognition in which the magnitude of recognition is crucial. It is worth noting that social recognition is more efficient in female than in male rats (Gheusi et al., 1994). These

results suggest that the sexual dimorphism in social recognition could be due to ovarian hormones.

Effects of ERα knockdown in the MePDA in social recognition test

The previous data in Paper III allow assumptions that ovarian hormones and particularly estrogens have effects on social recognition. We ask in Paper IV, in which structures of the brain may estrogens act, through the ERα. Paper IV reveals that ERα in the MePDA contributes to the ability to recognize a conspecific juvenile. This result is consistent with social recognition deficiency shown by ERα knockout mice and with the specificity of social recognition process compared to the one of object recognition.

We speculate that an indirect effect of ERα knockdown in the MePDA could explain social recognition impairment in this study. Oxytocin and oxytocin receptors have been demonstrated to be necessary for social recognition (Bielsky et al., 2004; Engelmann, Ebner, Wotjak, & Landgraf, 1998; Ferguson et al., 2000; Winslow & Insel, 2002). It is also well known that estrogens enhance the number of oxytocin receptor in the medial amygdala (QuinonesJenab et al., 1997). Since this effect does not depend on the ERβ (Patisaul, Scordalakes, Young, & Rissman, 2003), it is possible that the estrogens regulate the expression of oxytocin receptors through ERα in the medial amygdala. Thus, social recognition deficiency could be an indirect effect of down-regulation of oxytocin receptor expression in the MePDA after the reduction of ERα in this structure. This explanation would concur with the proposal that estrogens affect social recognition through their actions both on oxytocin synthesis and on the oxytocin receptor (Choleris et al., 2003).

The social recognition test in Paper IV demonstrated the additional result that the reduction of ERα in the VMN enhances aggression towards a conspecific unfamiliar juvenile while anxiety-related behaviors, i.e. self-grooming and freezing (Spruijt, Vanhooff, & Gispen, 1992; Gray, 1988), is increased when it becomes familiar. Since ERα knockdown in the VMN

nucleus does not affect social recognition, these observations suggest that the familiar juvenile loses its ability to provoke aggression and instead produce anxiety. Thus, ERα in the VMN modulates anxious reactions in social context.

Results of Paper IV also show that the activation of ERα in the MePDA is anxiogenic in threatening environment. However, the mechanism that underlies the effect of estrogens on anxiety through ERα in the MePDA remains unknown.

Such females also move more distance in both light and dark chambers than controls. We propose that estrogens heighten arousal and thereby increase locomotor activity in safe environments. Also, the locomotion would be reduced in threatening environments since the heightened arousal would provoke anxiety (Pfaff, 1980) which would counteract the locomotor stimulating effect. Indeed, female rats treated with estrogens show an enhancement of locomotion in safe environments (Beatty, 1992; Wade, 1972); while, they reduce their locomotor activity in threatening environments (DiazVeliz, Soto, Dussaubat, & Mora, 1989). The present data coincide with these earlier studies since females showing a low anxiety also displayed increased locomotor activity.

The possible role of progesterone receptors

In Papers II, III, and IV, estradiol administration is followed by progesterone. Consequently, it is not possible to exclude a contribution of progesterone to the observed behavioral changes. It is well known that estrogens enhance the expression of the progesterone receptor and data suggest that this effect is mediated by the ERα (Jacob et al., 2001). For this reason, it is possible that ERα knockdown in the VMN and the MePDA considerably reduces the number of progesterone receptors in these structures. Nonetheless, this would be a direct result of the lack of ERα receptors, and does not challenge the conclusion in this thesis. In any case, there are no relevant data on this issue in the present studies since we did not quantify the number of progesterone receptors.

Overview of ERα knockdown in the VMN and the MePDA

The overall conclusion of these studies of female rats is that the ERα in the VMN appears to be crucial for the entire sequence of behavioral events from the processes leading to the establishment of sexual contact until the accomplishment of copulatory behaviors. The ERα in the VMN could also facilitate sexual behaviors by modulating aggression and anxiety-related behaviors in social contexts. Although the ERα in the MePDA is not involved in sexual behaviors, this receptor could indirectly influence these behaviors by its participation in other cognitive and emotional functions. Indeed, the ERα in the MePDA contributes to social recognition and this ability may have consequences for sexual behaviors. For example, the recognition of sexual partners can influence the choice of a novel male to reinitiate copulation (Ågmo, Ellingsen, Turi, & Larsson, 2002). Likewise, it is possible that effects on anxiety in nonsocial context of the ERα in the MePDA ease sexual interactions. For the purpose of reproductive success, it is probably more relevant to show aggression and anxiety in a threatening environment and sociability in a safe context. All together, the present results suggest that the ERα in the VMN and the MePDA may assure the display of adaptive behaviors during sexual interactions.

Several times in the course of these thesis studies, we have noted the possible importance of our results for humans. The closest phylogenetic animal for which we have data about consequences of lesions and stimulations of the VMN is the monkey, and there is evidence that the VMN is of importance for phenomena related to sexual incentive motivation and copulatory behaviors (Koyama, Fujita, Aou, & Oomura, 1988). There are no experimental data about consequences of lesions and stimulations of the VMN in human females. There are only few studies using imaging data measures of blood flow in the brain during exposure to sexual incentives like pornographic movies. Even if the hemodynamic response of the hypothalamus is shown (Stark, Schienle, Girod, et al, 2005), the poor anatomical resolution

prevent determination of the exact site of activation. Thus, we are forced to conclude that human data are too few for any conclusion. However, the VMN is important for mammalian species studied, including non-human primates. Consequently, there is little reason to doubt that the VMN is important for human female sexual behavior also. Thus, it appears reasonable to propose that present results obtained in female rats could be useful for the understanding of sexual behavior in women.

The findings regarding knockdown of ERα in the MePDA could also have human applications. As presented in the discussion, social recognition deficiency could be an indirect effect of down-regulation of oxytocin receptor expression in the MePDA after the reduction of ERα in this structure. Oxytocin is suggested to be involved in human psychiatric disorders with social deficits like social phobias and autism. For example, autism spectrum disorders include failure to recognize the uniqueness of others. Moreover, this disorder is more frequent in males compared to females due to lower oxytocin level in males (Young, 2001). This sex difference leads to speculation about the role of sexual hormones. Thus, our results further suggest that the ERα in the medial amygdala could be implicated in autism. More directly, estrogens are known to influence anxiety, mood and affective disorders in women. For example, clinical observations show that 46% of women admitted to psychiatric hospitals are admitted at or immediately before menstruation (Fink, Sumner, Rosie, Grace, & Quinn, 1996), and starting at puberty, women are twice more likely to develop anxiety and depressive disorders than men (Pigott, 1999). The fall in estrogen levels during the menstrual and reproductive cycles can be associated with depression, irritability, panic and anxiety (Epperson, Wisner, & Yamamoto, 1999). However, high levels of estrogens can provoke increased feeling of general well-being as well as anxiety (Arpels, 1996). Thus, estrogen effects are not uniform and range from improved well-being to anxiety. These variable emotional responses to estrogens are also found in rodents. Data presented in this thesis show that activation of the ERα in the medial amygdala has an anxiogenic effect in nonsocial and

threatening environment while the ERα in the VMN has an anxiolytic effect in social context. This could constitute, at least, one way by which estrogens act on anxiety, mood and associated disorders.

CONCLUSIONS

To answer the issues raised in the goals of the present work, the main findings can be summed up as follows:

1. The test presented in the Paper I provides a relevant measure of sexual incentive motivation in rats, and it can serve as an acceptable preclinical model.

2. The ERα in the VMN is *necessary* and may be *sufficient* for sexual motivation as well as copulatory behaviors in female rats. In contrast, the ERα in the MePDA does not influence any aspect of female rat sexual behaviors.

3. Ovarian hormones improve social recognition in the habituation-dishabituation paradigm even if they are not *necessary* for this.

4. The ERα in the MePDA contributes to social recognition and has anxiogenic effect in a threatening environment. The ERα in the VMN decreases aggression and anxiety-related behaviors in social context.

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ISBN xxx-xx-xxxx-xxx-x