Mating-induced central release of oxytocin: Implication for social fear extinction.

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER NATURWISSENSCHAFTEN (DR. RER. NAT.) DER FAKULTÄT FÜR BIOLOGIE UND VORKLINISCHE MEDIZIN DER UNIVERSITÄT REGENSBURG

vorgelegt von Cindy Grossmann

aus Castres, Frankreich

im Jahr 2020

Das Promotionsgesuch wurde eingereicht am:

Die Arbeit wurde angeleitet von: Prof. Dr. rer. nat. Inga D. Neumann

Unterschrift:

Dissertation

Durchgeführt am Institut für Zoologie der Universität Regensburg

Lehrstuhl für Tierphysiologie und Neurobiologie

unter Anleitung von

Prof. Dr. rer. nat. Inga D. Neumann

TABLE OF CONTENTS

ABSTRACT

Anxiety disorders are one of the most common psychiatric disorders. Among them, social anxiety disorder (SAD), or social phobia, are characterized by persistent fear and avoidance of social situations. Unfortunately, treatments for SAD are rather unspecific as patients are generally treated with anxiolytics and/or antidepressant drugs combined with cognitive behavioral therapy. However, a lot of patients are resistant to these treatments or relapse once the treatment is over. This, combined with the high prevalence of SAD, highlights the emergency to develop new treatment strategies more specific to SAD. However, to find new potential targets for SAD treatments, animal models are needed to understand the underlying mechanisms of SAD. One of the major restrictions of SAD research was the lack of animal models. In 2012, the model of social fear conditioning (SFC) has been developed specifically to study SAD. After conditioning, the conditioned mice (SFC^+) develop social avoidance, a sign of social fear, and the main symptom of SAD. This is easily measurable during the social fear extinction training, mimicking the cognitive behavioral therapy. The social fear extinction training consists of several presentations of unknown conspecifics. At the first presentation, the fearful mice show low investigation time (social avoidance). After several presentations of social stimuli, the investigation time of the mice increases, the sign of social fear extinction. Oxytocin, well-known for its prosocial properties, is also a puissant anxiolytic. Previous studies found a crucial role of the neuropeptide oxytocin in social fear. Oxytocin was shown to facilitate social fear extinction when infused within the lateral septum (LS). Moreover, lactating females, which are a model for an upregulation of the OXT system, did not show social fear. Moreover, an infusion of an OXT receptor antagonist (OXTR-A) into the LS of lactating mice induced the expression of social fear during social fear extinction. However, lactation is a model for chronic upregulation of the OXT system as the number of OXT fibers and the OXTR binding are increased. In order to study the effect of an acute endogenous release of OXT, mating in male mice is a more adapted model. Indeed, an acute release of OXT within the paraventricular nucleus of the hypothalamus was found in male rats during mating.

During my PhD thesis, my first aim was then to investigate the effect of mating on social fear extinction in male mice. Male mice were allowed to mate during 1h before to be subjected to social fear extinction. Interestingly, I found a facilitation of social fear extinction exclusively

after successful mating (with ejaculation, Ej^+). The mice that did not ejaculate prior to social fear extinction (Ej^-) did not show this facilitation. I then characterized the effect of mating on cued fear, general anxiety, and social preference/novelty to determine if the benefic effect of ejaculation was specific to social fear. Male mice were allowed to mate during 1h before to undergo cued fear extinction, light-dark box, marbles burying test, and social preference/novelty test. No effect of mating without or with ejaculation has been found on cued fear extinction. I found a general anxiolytic effect of mating (Ej^- and Ej^+) on general anxiety. I found no changes in social preference nor social novelty. Ej^+ mice showed a facilitation of social fear extinction, which cannot be explained by the general anxiolytic effect of mating, with and without ejaculation, or an increased social motivation, as no specific effect of ejaculation was found in the social preference test. Next, I investigated the mechanisms underlying social fear extinction. For that, I conducted an experiment aiming to measure the level of the protein cFos, product of an immediate-early gene and thereby marker for neuronal activity, to evaluate which brain regions are recruited during the different stages of social fear extinction. Male mice were sacrificed either at the first stimulus (SFC^- and SFC^+ mice) or the last stimulus (only SFC^+) of the social fear extinction. I found an increased cFos protein level within the OXT neurons of the PVN and SON, and within the central amygdala (CeA) after the last stimulus of the extinction in SFC^+ mice. I also found a decreased level of cFos in SFC^+ mice at the first stimulus of the extinction in comparison with SFC^- mice in the ventral part of the LS. To assess the involvement of OXT in mating behavior, I measured the release of OXT within the LS and CeA during mating behavior using microdialysis. I found a specific release of OXT during mating behavior within the LS, but not within the CeA. In the next experiment, I aimed to inhibit the effect of the release of OXT during mating by blocking the OXTR with an antagonist. I first infused i.c.v. an OXTR-A immediately after mating before social fear extinction. Both Ej^- and Ej^+ showed an increased social fear expression during extinction. In order to block more specifically the OXTR during mating, I infused an OXTR-A within the LS before mating prior social fear extinction. No effect of the OXTR-A could be found. The OXT release during mating within the LS is then not or not the only reason for the facilitation of the extinction observed after ejaculation.

In the second part of my PhD work, I aimed to investigate the mechanism underlying social fear extinction. The CeA is well known for its role in fear conditioning and expression. In the

context of social fear, I previously found an increased cFos expression in the CeA the last social stimuli of social fear extinction in SFC^+ mice. This implies an involvement of the CeA in the extinction of social fear. Also, an upregulation of the OXTR binding was found after social fear acquisition in SFC^+ mice, suggesting a regulation of the OXT system during social fear acquisition. I then aimed to investigate the involvement of the OXT system within the CeA during social fear extinction. I first infused synthetic OXT within the CeA before social fear extinction. I found a slight facilitation of social fear extinction. In order to confirm this result, I conducted a similar experiment in which I infused a specific OXTR agonist. However, I could not repeat the previously obtained result. In the last experiment, I used chemogenetic to specifically activate the OXTR-positive neurons within the CeA before social fear extinction. However, I could not find any effect of CeA OXTR-positive neurons stimulation on social fear extinction.

Altogether, I showed that ejaculation during mating specifically facilitates the extinction of social fear. However, I could not identify the circuit directly responsible for this effect yet. Independently of mating behavior, I could show the recruitment of the CeA in social fear extinction. More experiments need to be conducted to understand the role of OXT in the CeA in social fear extinction.

INTRODUCTION

1. Emotions

As described by Darwin in "The Expression of the Emotions in Man and Animals" in 1872, emotions can be observed across several species (Figure 1) (Darwin, 1872). However, whether animals experience emotions in the same manner as humans and how to define them is still under debate. Emotions enable the adaptation of the behavioral response towards a specific stimulus. They are essential for survival and they ask the capability to identify an external stimulus, to assess the internal state, and to combine the environmental context and the previous experiences to direct an appropriate behavioral response. Hence, the expression of one emotion is the result of the detection and assessment of the nature of one specific cue combined with the internal body state and the previous experience of the subject. An emotion is expressed by a behavioral, hormonal, and autonomic response (LeDoux, 2000; Anderson and Adolphs, 2014; Barrett and Satpute, 2019). A very prominent section of emotion research is focused on anxiety and fear due to his high relevance for survival.

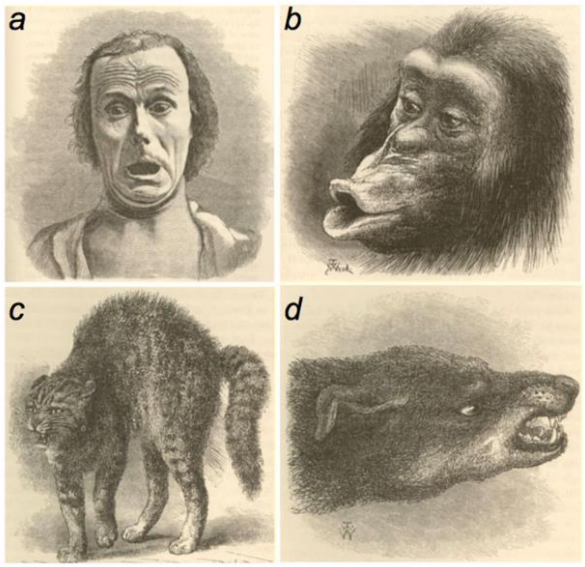

***Figure 1. Expression of emotions observed by Charles Darwin in different species**. a, terror in humans. b, disappointment in chimpanzee. c, hostility in cat. d, hostility in dog (Anderson and Adolphs, 2014).*

2. Anxiety and fear

In the field of emotion, anxiety and fear are probably the most studied as they are highly relevant for survival. The manifestations of anxiety and fear are very similar, however, they are triggered by different stimuli. In 1993, Blanchard et al. attempted to describe the difference between anxiety and fear. Fear is a response to imminent danger, the threat is then physically present and well defined. Anxiety is a response to potential and subjective threats. Fear could then be defined as a response to an immediate threat while anxiety is a response to a distant or future threat. In rodents, anxiety and fear are manifested by different defensive behaviors depending on the context. When the predator or threat is distant, flight or avoidance can be observed. If no escape is possible, a freezing response will be then observed. If the predator is getting closer, this passive behavior change in an active defensive threat or attack. Thus, the behavioral response depends on both the threat proximity and the environment. Other defensive behaviors can also be observed like ultrasonic vocalization, defensive burying, or startle response in response to a threat (Blanchard et al., 1993). Occasional anxiety and fear are beneficial for the survival of an individual, enabling fast responses to possible threats. However, a persistent state of anxiety or a fear response towards non-threatening stimuli can be qualified as pathological. Pathological anxiety and fear are detrimental to the health of an individual, as anxiety and fear are conflicted with other behavior such as feeding, mating, or social interactions among others.

3. Anxiety disorders

Pathological anxiety and fear, i.e. anxiety disorders, cause personal distress, reduce the quality of life, impair social functioning, and increase even the risk of suicide. The term “anxiety disorders” represents a large variety of disorder as general anxiety disorder (chronic apprehension and anxiety), specific phobia (fear about a specific object/situation), social anxiety disorder (SAD, fear of social situations), post-traumatic stress disorder (avoidance and fear of stimuli related with a previous trauma) just to name the most prominent ones (American Psychological Association (APA), 2013). In Europe, the prevalence to develop any kind of anxiety disorder is 14.5% according to the European Study of the Epidemiology of Mental Disorders. Within the broad spectrum of anxiety disorders, specific phobia, generalized anxiety disorder, and SAD are the more common (Baldwin et al., 2013; Bandelow

and Michaelis, 2015). Anxiety disorders can be grouped in two kinds of clinical entities: the ones which are characterized by an elevated fear for specific stimuli (e.g. phobias), and the ones which are characterized by an elevated anxious apprehension. However, anxiety and fear are the major symptoms described in the definition of most of the anxiety disorders. In the DSM V, specific phobia is described as a "Marked fear or anxiety about a specific object or situation (e.g. flying, heights, animals receiving an injection, seeing blood). The phobic object or situation almost always provokes immediate fear or anxiety". Another relevant example is the definition of SAD. In the DSM IV, SAD was described as a "marked and persistent fear of one or more social/performance situations in which the person is exposed to unfamiliar people or possible scrutiny by others". This definition has changed in the DSM V to include also anxiety: "Marked fear or anxiety about one or more social situations in which the individual is exposed to possible scrutiny by others" (American Psychological Association (APA), 2013; Substance Abuse and Mental Health Services Administration, 2016). Even though they have distinct definitions, anxiety and fear cannot be distinguished at a clinical level as both of them are generally expressed in anxiety disorders. SAD is particularly relevant in the context of my thesis, I will now describe it more in detail.

SAD, also called social phobia, is defined by a persistent fear in certain or all social situations. The person suffering from SAD fears being judged, humiliated, or rejected. According to the DSM V, a specific form of SAD is the so-called performance anxiety and is defined by fear of giving a speech or performing on stage (American Psychological Association (APA), 2013). The main symptom of SAD is the avoidance of social situations, which is often accompanied by physical symptoms such as sickness, increased heart rate. Moreover, the majority of SAD patients develop other psychiatric disorders such as depression or substance abuse. The 12-months prevalence for SAD is between 0.6-7.9 % in Europe depending on the country (Wittchen and Jacobi, 2005; Wittchen et al., 2011) and 6.8% in the US (Kessler et al., 2005). The treatments for SAD are non-specifics and have been developed first to treat general anxiety disorder or depression. These include selective serotonin inhibitors, serotonin-norepinephrine reuptake inhibitors, and benzodiazepines for the most prescribed. Other treatments that can be proposed are tricyclic antidepressants, monoamine oxidase inhibitors or, more unusual treatments like β-adrenergic blockers and anticonvulsants. However, 50% of relapse has been observed less than 2 months after the end of the treatment (Blanco et al.,

2013; Williams et al., 2017). That is why pharmacotherapy is often combined with cognitive behavioral therapy, a therapy combining problematic thinking to eliminate negative thoughts and desensitization exposure training, which consists of gradual exposure to anxiety-provoking situations. This combination gives better results and decreases the rate of relapse after treatment (Goldin et al., 2014; Stangier, 2016).

Due to the high prevalence, anxiety disorders are a burden for society. In particular, in the actual context of the COVID-19, the prevalence of anxiety disorders might significantly increase in the post-illness stage (Rogers et al., 2020). Moreover, the prolonged social isolation during the lockdown might increase the probability to develop SAD (Holmes et al., 2020). Anxiety and fear have been extensively studied in preclinical research, however, the question of how dysregulations in the brain lead to anxiety disorder needs a better understanding on a translational level. Moreover, novel treatments are needed to compensate for the lack of specific treatment for anxiety disorder like SAD. That is why, models were developed in rodents in order to investigate the mechanisms underlying fear and anxiety, and their dysregulations, leading to anxiety disorders.

4. Modeling anxiety disorders in rodents

To study anxiety disorders in preclinical studies with laboratory animals and understand their underlying mechanisms, animal models were developed. A good animal model has to fulfill 3 criteria. It should first develop the same symptoms as the human disorder, both at a physiological level and at a behavioral level (face validity). Then, the model should be sensible to pharmacological treatments used in patients (predictive validity). And finally, the causality should be the same, meaning that the neurobiological processes involved in anxiety should be the same in the animal model than in humans (construct validity).

4.1. Measuring anxiety in rodents

Anxiety depends on cues or stimuli that cannot be predicted. In this case, the stimulus elicits a state of anxiety over a long period which decays slowly after the removal of the stimulus (De Jongh et al., 2003).

To measure anxiety, a broad spectrum of tests and paradigms were developed. Most of these tests are based on ethology, as they involved innate anxiety in rodents like exploring new,

bright, and open environments where they are more vulnerable to predators. These tests are conflictual for the rodents, as they oppose their unconditioned general exploratory drive with their innate anxiety of open spaces. Among others, the elevated-plus maze, the open field, and the light/dark box (LDB) are the most commonly used (Campos et al., 2013). The elevated plus-maze consists of two closed arms and two open arms elevated from the ground. Anxious rodents avoid the open arms (Walf and Frye, 2007). Regarding the open field, this paradigm consists of one big open area. Anxious rodents prefer to stay at the edges rather than investigate the bright and unprotected center of the arena (Gould et al., 2009). In the present thesis, I used the LDB to measure anxiety in mice, this paradigm is composed of one dark, i.e. "safe" chamber and one bright, i.e. "anxiogenic" chamber. Thus, anxious animals spend more time within the dark chamber than the bright chamber (Griebel et al., 2000; Hascoët and Bourin, 2009; Miller et al., 2011). All these tests were shown to be sensitive to anxiolytic drugs. However, these tests have the weakness to be sensible to locomotion deficit. Indeed, an animal showing low locomotion can be interpreted as highly anxious while an animal showing high locomotion can be interpreted as non-anxious. Another way to test for anxiety is to use active avoidance tasks, in which rodents try to minimize the threatening stimuli by, for example, burying it. In the shock probe burying (also called defensive burying) paradigm, rats bury an electrified probe after receiving a footshock (Treit et al., 1981; Treit, 1990). This test is mostly used in rats, however, few studies could validate it in mice (Sluyter et al., 1996; Degroot and Nomikos, 2004). In the marbles burying test, the rodent spontaneously buries non-noxious stimuli such as marbles. This test is more common in mice (Deacon, 2006), however, it was shown that rats also bury non-noxious stimuli (Poling et al., 1981). All these tests are sensitive to anxiolytic drugs.

4.2. The Cued Fear Conditioning (CFC)

Based on associative memory, fear can be induced by a short and discrete cue paired with an aversive stimulus. When the cue is presented, it elicits a state of fear that begins and dissipates quickly once the stimulus is removed. In this context, fear conditioning was extensively used to investigate fear behavior and fear-related circuitries in the brain of rodents.

One of the most famous paradigms is the cued fear conditioning (CFC). It is a type of classical conditioning, also called respondent or Pavlovian conditioning paradigm. The principle of CFC is based on the association of two independent stimuli. Before conditioning, one stimulus,

called unconditioned stimulus (US; a footshock), is able to elicit an unconditioned fear response (e.g.freezing). The other stimulus is neutral (for example a tone), it doesn´t lead to any behavioral response. During the conditioning, the neutral stimulus is presented at the same time as the US. The presence of the US is naturally leading to the expression of the unconditioned fear response. After several US-CS pairings, the CS is presented alone and, if the associative learning happened during the conditioning, it should lead to an equivalent response than the unconditioned response, i.e. freezing, which is then called a conditioned response. A procedure of fear extinction then generally takes place. This procedure aims to induce a new learning, where the animal learns that the CS is not linked with the US anymore. During extinction, the CS is then presented alone several times. When extinction is successful, the test animal should present a lower level of fear response at the end of the process than initially. The CFC is mostly used to investigate the basis of fear learning and fear extinction. It is also used to investigate anxiety disorders such as general anxiety disorder or post-traumatic disorder. However, this model is not ideal for investigating a disorder like SAD, as it doesn´t induce social avoidance, the main symptom of SAD.

4.3. The Social Fear Conditioning (SFC)

As previously explained, specific treatment options are lacking for SAD. Studying the underlying mechanisms of SAD is then crucial. However, no appropriate model was available to study this disorder. Several models allow to induce social avoidance in rodents as acute or chronic social defeat or foot-shock exposure (Haller and Bakos, 2002; Louvart et al., 2005; Huhman, 2006). However, these paradigms are unspecific and induce also general anxiety- and depression-like symptoms. That is why the SFC paradigm was developed in 2012 in our lab (Toth et al., 2012a). Based on the operant conditioning or instrumental conditioning, this type of conditioning induces an association between an active behavior and a consequence that can be either positive (reward) or negative (punishment), allowing either to exacerbate or to inhibit a specific behavior. The SFC, which was develop in mice, associates the investigation of a conspecific with a foot shock, leading to a decreased social investigation (social avoidance). Similarly to the CFC, this conditioning is followed by an extinction training procedure where several unknown conspecifics are presented. The mouse then has the choice to investigate the social stimulus or to avoid it. Once the mouse recognizes that the social investigation is no longer paired with the foot shock, the social fear is gradually extinguished

as the investigation time of the social stimulus is increased. The extinction training in socially fearful mice mimics the cognitive behavioral therapy in SAD patients. This procedure allows us to generate generalized social fear in mice, without alteration in anxiety- or depressive-like behavior (face validity). After the acquisition, social fear lasts for at least 2 weeks. Moreover, antidepressant or anxiolytic treatments reverse the SFC-induced social fear (predictive validity) (Toth et al., 2012a). The SFC paradigm is nowadays the best option to study the neuronal substrates of SAD. The mechanisms underlying both SAD in humans and social fear in mice are not well understood. However, the hypothesis of the involvement of the neuropeptide oxytocin (OXT) is growing (Marazziti et al., 2015). Indeed, a study found lower plasmatic OXT levels in SAD patients while they were performing a pro-social task in comparison with healthy controls (Hoge et al., 2012). Using SFC, a release of OXT release within the lateral septum (LS) was found in control mice but not in socially fearful mice during social fear extinction (Zoicas et al., 2014). In contrast, two other studies found elevated plasma OXT in SAD patients in comparison to healthy controls (Hoge et al., 2008; Oh et al., 2018). However, the sampling of OXT was not done during a social task in these last two studies, which might explain the difference in the results. Altogether, both SAD patients and social fear conditioned mice seem to present a dysregulation of the OXT system (construct validity?).

5. Regulation of anxiety and fear: focus on Oxytocin (OXT)

5.1. The OXT system

The nonapeptide OXT was discovered in 1909 by Sir Henry H. Dale, and later, its structure was characterized by Du Vigneaud in 1953 (Du Vigneaud et al., 1953). This highly conserved neuropeptide is synthesized within the paraventricular (PVN), supraoptic (SON) and accessory nuclei of the hypothalamus in mammals. The magnocellular neurons of the PVN and SON project to the neurohypophysis, where OXT is released into the bloodstream. OXT is also released by parvocellular neurons of the PVN. This neuronal population is distinct from the magnocellular neurons as they have a smaller size and do not project to the neurohypophysis. Parvocellular OXT neurons of the PVN project to extrahypothalamic regions including the brainstem and the spinal cord to regulate autonomic function like pain, analgesia and penile erection (Wagner and Clemens, 1993; Ackerman et al., 1997; Gerendai et al., 2001; Eliava et al., 2016). Parvocellular neurons project also to other hypothalamic and extra-hypothalamic

nuclei (Armstrong et al., 1980; Swanson and Kuypers, 1980; de Vries and Buijs, 1983; Sofroniew, 1983; Swanson and Sawchenko, 1983; Knobloch et al., 2012). OXT projections from these nuclei are found in numerous brain areas including the septal area, the bed nucleus of the stria terminalis, the nucleus accumbens, the ventral hippocampus, the medial amygdala (MeA), and the central amygdala (CeA)(Neumann and Landgraf, 1989; Knobloch et al., 2012; Menon et al., 2018; Ferretti et al., 2019) (Figure 2). The central release of OXT within the PVN and SON is due to somato-dendritic release, while the release in the limbic regions is due to axonal release (Ludwig and Leng, 2006). The release of OXT is stimulus- and region-dependent. Using microdialysis sampling, the intracerebral release of OXT was detected in several brain regions (Neumann, 2007) including the PVN/SON in response to swimming in male rats and maternal aggression in female rats (Wotjak et al., 1998; Bosch et al., 2005; Engelmann et al., 2006; Torner et al., 2017), the septum during suckling in lactating rats (Neumann and Landgraf, 1989), social defeat in male rats (Ebner et al., 2000) and social memory retrieval in male rats (Lukas et al., 2013a), social fear extinction in male and female mice (Zoicas et al., 2014; Menon et al., 2018), the dorsal hippocampus during suckling in lactating rats (Neumann and Landgraf, 1989), the CeA during forced swim test and maternal aggression in rats (Bosch et al., 2005; Ebner et al., 2005). Optogenetic stimulation of the hypothalamic OXT terminals in rats was also able to trigger a release of OXT within the CeA (Knobloch et al., 2012; Ferretti et al., 2019). Studies in other species like sheep allowed to identify other regions where OXT is released, e.g. the bed nucleus of the stria terminalis (BNST), the medial preoptic area (MPOA), and the olfactory bulb in parturient ewes, during suckling or, during vaginocervical stimulation (Kendrick et al., 1988, 1992; Lévy et al., 1995). In humans, a peripheral release of OXT has been detected in plasma and saliva in response to stress like running (Landgraf et al., 1982; de Jong et al., 2015). The release of OXT also occurs in response to martial art (Rassovsky et al., 2019) or during a psychosocial test Trier Social Stress Test) (Pierrehumbert et al., 2010). Except during social interactions and stress, the intracerebral release of OXT also occurs following strong physiological stimuli. Well known in females, lactation and birth are two particularly strong stimuli that induce the release of OXT in the PVN and SON (Neumann et al., 1993). In males, a central endogenous release of OXT occurs during mating, like in the PVN for example (Waldherr and Neumann, 2007).

Endogenously release of OXT expresses its effect usually via the OXT receptor (OXTR). The OXTR is a G protein-coupled transmembrane receptor, which is highly expressed throughout the social brain network and many other brain areas like the CeA, basolateral nuclei of the amygdala (BLA), MeA, nucleus accumbens, BNST, PVN, MPOA, hippocampus, periaqueductal gray, LS, or olfactory bulbs (Brinton et al., 1984; De Kloet et al., 1985; Elands et al., 1988; Tribollet et al., 1988) (Figure 2). The broad distribution of the OXTR within the brain, as well as the numerous OXT projections, enable OXT to modulate the expression of social behavior, as well as other behaviors like fear and anxiety.

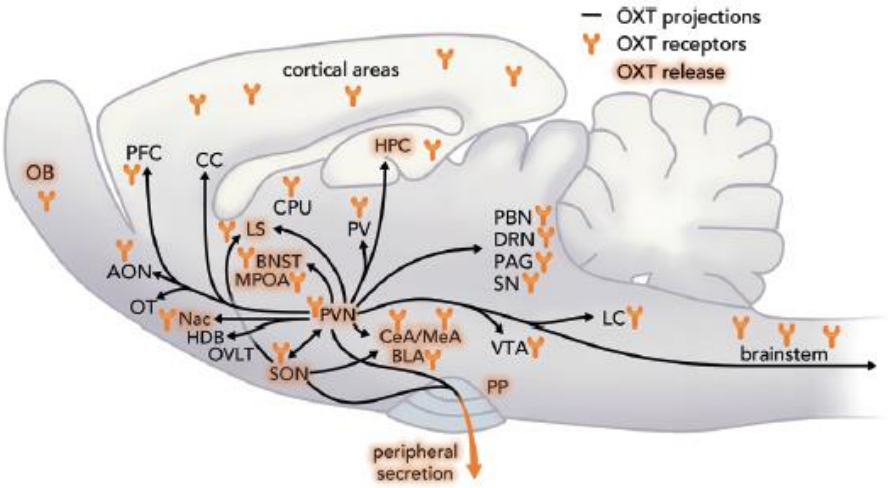

Figure 2. Scheme of OXT projections, sites of OXT release, and OXTR expression within the brain. *AON, anterior olfactory nucleus; OB, olfactory bulb; OT, olfactory tubercle; Nac, nucleus accumbens; OVLT, organum vasculosum laminae terminalis; SON, supraoptic nucleus; PVN, paraventricular nucleus; PP, posterior pituitary; PFC, prefrontal cortex; CC, cingulate cortex; MPOA, medial preoptic area; BNST, bed nucleus of the stria terminalis; LS, lateral septum; CPu, caudate putamen; PV, periventricular nucleus of the thalamus; CeA, central amygdala; MeA, medial amygdala; BLA, basolateral amygdala; VTA, ventral tegmental area; LC, locus coeruleus; PBN, parabrachial nucleus; DRN, dorsal raphe nucleus; PAG, periaqueductal gray; SN, substantia nigra; HPC, hippocampus; HDB, nucleus of the horizontal limb of the diagonal band. Figure from (Grinevich and Neumann, 2020).*

After binding to its receptor OXT modulates social behaviors such as social approach (Lukas et al., 2011), social memory and recognition (Engelmann et al., 1998; Lukas et al., 2013a), maternal aggression (Bosch et al., 2005), aggression (Calcagnoli et al., 2015), male and female sexual behavior (Arletti and Bertolini, 1985; Arletti et al., 1985; Waldherr and Neumann, 2007; Nyuyki et al., 2011) and pair-bonding (Williams et al., 1994; Johnson et al., 2016). Aside from its pro-social effects, OXT also regulates anxiety and fear, this will be discussed in the next section.

5.2. OXT in anxiety and fear

The role of OXT in the regulation of stress and anxiety has been well studied. Stressful situations induce the release of OXT both at the periphery and in the brain. For example, in rats, 10 minutes of forced swim induces the peripheral release of OXT into the bloodstream (Wotjak et al., 1998, 2001; Torner et al., 2017), as well as a central release into the PVN/SON (Wotjak et al., 1998, 2001; Torner et al., 2017) and the CeA (Ebner et al., 2000). Also, acute social defeat induces OXT release into the bloodstream, within the hypothalamus (Engelmann et al., 1999) and the LS (Ebner et al., 2000). In accordance, OXT deficient mice showed increased anxiety-like behavior in the elevated plus-maze, a novel environment, and a platform shaker (Mantella et al., 2003; Amico et al., 2004). In addition, OXTRs are expressed in key brain regions involved in the neurocircuitry of anxiety and fear like the prefrontal cortex, the hippocampus, or the amygdala. Hence, OXTR antagonist (OXTR-A) infused either i.c.v. or into the PVN increases the hypothalamic–pituitary–adrenal axis response during stress in the elevated plus-maze, and the forced swim test in male rats (Neumann et al., 2000). Chronic intracerebral infusion of OXT via minipumps (Windle et al., 1997) or OXT infusion into the CeA (Bale et al., 2001) decreases the stress response and anxiety-like behavior in female rats and mice. Moreover, the administration of OXT within the PVN decreases anxiety-like behavior in rats in the elevated plus-maze and the LDB by activating the mitogen-activated protein kinase cell signaling pathway (Blume et al., 2008). In the prelimbic cortex, OXT infusion induces anxiolysis by recruiting GABAergic neurons to modulate downstream regions like the CeA (Sabihi et al., 2014, 2017). In humans, intranasal administration of OXT was also shown to reduce the stress response (Heinrichs et al., 2003; Kirsch et al., 2005) and to reduce aversion to angry faces (Evans et al., 2010). All in all, endogenous OXT is a puissant anxiolytic in both rodents and humans.

Aside from anxiety, OXT also plays a role in the regulation of fear behavior. In CFC, i.c.v. OXT infusion before fear extinction impairs the extinction. However, when infused before the acquisition, OXT does not affect fear acquisition but facilitates fear extinction (Toth et al., 2012b). After contextual fear memory retrieval, microinfusion of OXT, or specific agonists within the infralimbic region of the medial prefrontal cortex facilitates extinction (Lahoud and Maroun, 2013). The OXT infusion within the BLA before fear memory retrieval increases

freezing and impairs fear extinction. Infusions before fear memory retrieval into the CeA does not affect fear extinction. When infused before conditioning into the BLA, OXT decreases the freezing response and facilitates extinction, while, when infused into the CeA, OXT has no effect. However, agonists of the OXTR show different effects than OXT in this study. When infused before fear retrieval into the BLA, OXTR agonists induce a decrease in fear responses during extinction, and, when infused before conditioning into the CeA, they facilitate fear extinction (Lahoud and Maroun, 2013). In another study, an infusion of OXT before extinction reduces fear expression during the extinction. However, OXT impairs the acquisition of context fear when infused into the BLA before the acquisition, reflected by the low level of freezing during extinction. A local infusion or an optogenetically stimulated release of OXT within the CeA suppresses the expression of contextual fear (Viviani et al., 2011; Knobloch et al., 2012). The different effects of OXT into the amygdala sub-regions on fear extinction are puzzling and need more investigation. Moreover, an acute social defeat before context fear conditioning enhanced fear memory, an effect mediated by the OXTR into the LS (Guzmán et al., 2013). However, OXT infusion in the LS decreases fear expression in contextual fear conditioning after positive social exposure (Guzmán et al., 2014). It seems then that OXT effect depends on the valence of the stimulus presented before extinction. In the context of social fear, OXT is released within the LS during social fear extinction and facilitates social fear extinction in both lactating female and male mice (Zoicas et al., 2014; Menon et al., 2018). Moreover, social fear alters OXTR binding in several brain regions. An increase in OXTR binding is found after conditioning within the dorsal LS (dLS) and the right CeA (Zoicas et al., 2014). In SAD patients, the intranasal administration of OXT dampens the amygdala hyperreactivity (Labuschagne et al., 2010; Dodhia et al., 2014; Gorka et al., 2015).

5.3. Brain regions involved in anxiety and fear

5.3.1. Amygdala

The case of the patient S.M. suffering from a bilateral destruction of the amygdala, described in 1994, introduced the relevance of the amygdala for emotions. This patient suffers from bilateral destruction temporal lobes containing the amygdala. The most remarkable change in behavior was the lack of fear (Adolphs et al., 1994). Brown and Schäfer in 1988, followed by Klüver and Bucy in 1937, already described a lack of fear as a consequence of a bilateral ablation of the temporal lobe in Rhesus monkey, the so-called Klüver-Bucy syndrome.

The amygdala can be divided into several sub-nuclei: the MeA, the BLA, and the CeA nucleus. I will here focus on the BLA and CeA as these two regions are participating in the regulation of anxiety and fear. The BLA is mostly constituted of glutamatergic projection neurons and inhibitory interneurons. It receives sensory information from the cortices and the thalamus about the environment but also the hippocampus or the prefrontal cortex. The BLA sends projections to structures like the nucleus accumbens, the BNST, and CeA to then, adapt the behavior in response to a fearful stimulus. Some assume that the CeA plays only the role of a relay between the BLA and the output region, but the CeA also receives direct sensory information and can regulate some behavior via projections to the brainstem independently from the BLA (Pliota et al., 2018). The CeA was strongly investigated concerning its role in defensive behavior and anxiety (LeDoux, 2000). The CeA receives a large variety of inputs including sensory and nociceptive information via projections from the thalamus, the ventral hippocampus, the parabrachial nucleus, or the BLA among others (Figure 3). Thanks to all these connections, the CeA is important for associative learning like conditioning. Even though the CeA is a rather small structure, the microcircuit contained inside is complex. Mainly composed of inhibitory neurons, the CeA can be divided into a medial (CeM) and a lateral (CeL) part (Jolkkonen and Pitkänen, 1998). The CeL is composed of different types of cells, which can be classified depending on their molecular specificity: somatostatin-positive (SOM), corticotropin-releasing factor-positive (CRF) and protein kinase C isoform delta-positive (PKC δ) cells. These cells can be divided also depending on their response to fear stimuli: some cells are activated in response to a conditioned stimulus (CeL^{ON}) while the others are inhibited (CeL^{OFF}) (Ciocchi et al., 2010). Interestingly, the CeL^{OFF}neurons have been identified as the PKC δ-positive cells (Haubensak et al., 2010). All these cells form a micro-inhibitory circuit sending projections to the CeM that modulates fear expression by sending projections to the hypothalamus, the PAG, and the brainstem (Ciocchi et al., 2010; Haubensak et al., 2010; Fadok et al., 2017). The theory of a reciprocal inhibitory circuit would be that the CeL^{ON} (SOM+) and CeL^{OFF} (PKC δ+) are connected in a way that one inhibited the other allowing a rapid behavioral change. For example, the activation of the SOM-positive cells induces a freezing response while the activation of the PKC δ-positive cells suppresses freezing (Fadok et al., 2017). Regarding the CRF-positive cells, they also share reciprocal inhibitory connections with the SOM-positive cells and promote active defensive behavior.

The BLA and CeA receive OXTergic projections from the PVN (Knobloch et al., 2012; Ferretti et al., 2019). Both the BLA and the CeA express in abundance the OXTR. Within the CeA, OXTRs are particularly localized in the CeL. Interestingly, the cells expressing the OXTR have been identified as the CeL^{OFF} or PKC δ-positive cells (Haubensak et al., 2010). Indeed, OXT elicits the activation of approximately 20% of the CeL neurons and inhibits about 50%, leading *in fine* to the suppression of fear expression (Huber et al., 2005). Moreover, the responses of CeA neurons to OXT show a desensitization of the neurons, as more the neurons are stimulated more the response decreases in brain slices from virgin and lactating rats. This effect is particular to the CeA, as it is not present in the MeA (Terenzi and Ingram, 2005). The action of OXT in the amygdala seems to be time- and region-dependent. In the BLA, an infusion of OXT before conditioning enhances context fear expression during the extinction in rats (Lahoud and Maroun, 2013). However, in another study also in rats, OXT infusion into the BLA impairs acquisition, reflected by a decreased context fear expression (Campbell-Smith et al., 2015). When infused before context fear extinction into the BLA, OXT and OXT agonists decrease fear expression (Lahoud and Maroun, 2013; Campbell-Smith et al., 2015), this effect can be blocked by an OXTR-A infusion (Campbell-Smith et al., 2015). OXT infused into the CeA before contextual fear conditioning does not affect fear acquisition. However, OXT agonists infusions impair context fear acquisition, reflected by a decreased contextual fear expression during extinction (Lahoud and Maroun, 2013). Campbell-Smith et al. also shown an impairment of contextual fear acquisition by OXT infusion into the CeA (Campbell-Smith et al., 2015). While no effect was found on contextual fear extinction after OXT infusion before extinction (Lahoud and Maroun, 2013), another study showed an increased expression of contextual fear after pre-extinction OXT infusion. This effect could be blocked by OXTR-A, and an OXTR-A infusion alone abolished the expression of fear during extinction (Campbell-Smith et al., 2015). Optogenetically stimulated release of OXT within the CeA suppresses the expression of contextual fear (Viviani et al., 2011; Knobloch et al., 2012). Several hypotheses have been advanced to explain the multiple effects of OXT in the amygdala, which might vary depending on the protocol, time and doses used. In the context of SFC, OXT is crucial for social fear extinction. Lactating female mice, a model of high activity of the OXT system, do not show social fear (Menon et al., 2018). The possible role of OXT within the CeA in SFC has not yet been studied. However, an increase in OXTR binding was found in the right CeA after conditioning. This might imply a role of the OXTR in social fear conditioning or expression. In

humans, intranasal OXT reduces the activation of the amygdala in response to fear in healthy people and SAD patients (Kirsch et al., 2005; Petrovic et al., 2008; Labuschagne et al., 2010; Dodhia et al., 2014).

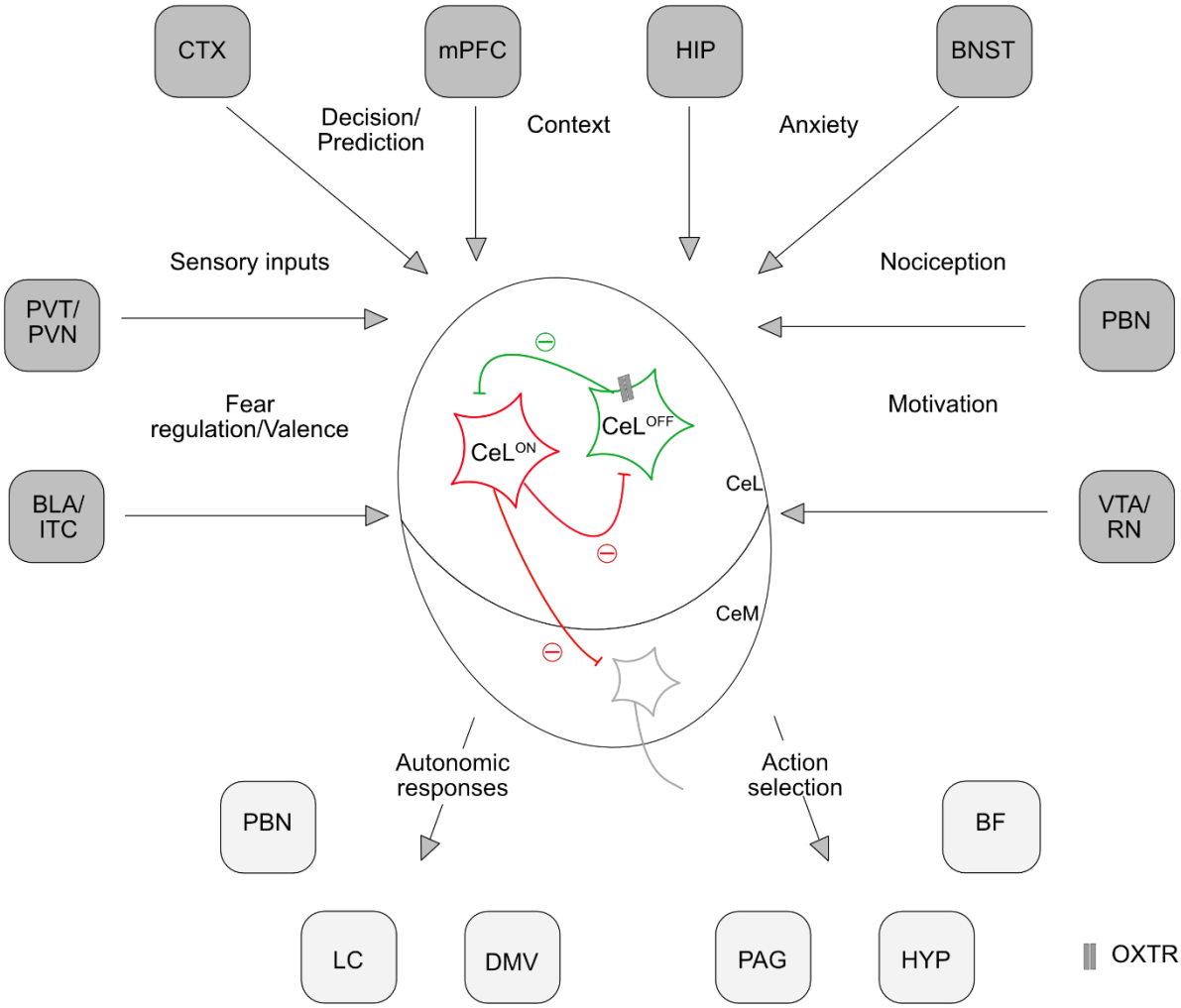

Figure 3. Connectivity of the CeA. *The CeA receives multiple connections from various brain regions to coordinate an appropriate response face to a threat. BF, Basal forebrain; BLA, Basolateral nucleus of the amygdala; BNST, Bed nucleus of the stria terminalis; CTX, cortices; DMV, Dorsal motor nucleus; HIP, Hippocampus; HYP, Hypothalmus; ITC, Intercalated cells of the amygdala; LC, Locus coeruleus; PAG, Periaqueductal grey; PBN, Parabrachial nucleus; mPFC, medial prefrontal cortex; PVN, Paravevntriclar nucleus of the hypothalamus; PVT, Paraventricular nucleus of the thalamus, RN, Raphe nucleus; VTA, Ventral tegmental area. Figure adapted from (Fadok et al., 2018).*

5.3.2. Lateral Septum

The septum is divided into two sub-regions, the medial septum (MS) and LS. The septum is highly connected with the hippocampus, with the MS sending projections to the hippocampus

and the hippocampus sending back projections to the LS, forming the so-called septohippocampal formation. The LS is mainly composed of GABAergic neurons and is highly connected with hypothalamic regions, PAG, amygdala, BNST, and others. The LS receives mostly glutamatergic inputs but also dopaminergic, serotoninergic, cholinergic, and adrenergic (Sheehan et al., 2004). In humans, the famous "septal rage syndrome" is characterized by the disinhibition of fear or an overexpression/inappropriate fear reaction. In rats, lesion studies showed an anxiolytic role of the septum (Brady and Nauta, 1953). cFos studies revealed the activation of the LS after the presentation of a fear conditioned stimulus or after infusion of anxiolytic drugs in male rats (Campeau et al., 1997; Singewald et al., 2003). Using the fear-potentiated-startle and the light-enhances-startle paradigms, measuring fear and anxiety respectively, the ventral (vLS) and the intermediate parts of the LS were shown more activated during anxiety than fear exposure (Veening et al., 2009). It is via connections with structures like the ventral hippocampus and the CeA that the LS can regulate anxiolytic-like behavior. Thus, in male mice, chemogenetic activation of the ventral hippocampus projections to the LS decreased anxiolytic-like behavior in several tests like the elevated plus-maze or the open-field. Conversely, the inhibition of these projections increases anxiety-like behavior (Parfitt et al., 2017). The LS also seems to modulate the activity of CeA. The electrical stimulation of the LS inhibited the firing of CeA neurons. In contrast, the electrical stimulation of the CeA neurons stimulated the activity of the LS neurons, indicating a negative feedback interaction between the LS and the CeA (Thomas et al., 2012). This indicates that the LS decreases the activity of the CeA and hence the fear response. However, it is interesting to note that some lesion studies show an anxiogenic role for the LS. After lesions of the LS, rats display a lower level of anxiety-like behavior as they explore more the open arms of the elevated plus-maze and bury less in the shock-probe burying test (Treit and Pesold, 1990; Menard and Treit, 1996), indicating that the LS could normally be responsible for the anxiety-like behavior expression. Moreover, the activation of the CRF receptor type 2 in the LS induces anxiety-like behavior in the immobilization stress and elevated plus-maze (Radulovic et al., 1999; Henry et al., 2006; Anthony et al., 2014; Lamontagne et al., 2016). These different effects of the LS on anxiety might be due to the heterogeneity of the region. For example, the rostral LS expresses mRNA for neurotensin and encephalin while the caudal LS expresses mRNA for somatostatin and only the vLS expresses mRNA for estrogen receptors (Risold and Swanson, 1997). Hence, different subregions are activated by different stimuli (Degroot and

Treit, 2004; Sheehan et al., 2004). For example, the vLS seems more involved in the freezing response (Mongeau et al., 2003; Veening et al., 2009). The LS is also involved in other behavior, like in social behavior like social memory (Lukas et al., 2013b) and aggressive behavior (Potegal et al., 1981; Borland et al., 2020). In summary, the LS is a complex brain region from the point of view of the network as well as the behavioral output. Focusing specifically on behavior the LS was shown to be involved in anxiety, fear, and social behavior which are also regulated by OXT.

The LS contains OXTR-expressing cells and receives OXT projections, majoritively from the SON (Menon et al., 2018). OXTRs within the LS were shown to potentiate the induction of fear in context fear conditioning following social defeat (Guzmán et al., 2013). Moreover, OXT infusion within the LS decreases fear expression in contextual fear conditioning after positive social exposure (Guzmán et al., 2014). The involvement of the OXT system in the LS in social fear was studied in our lab using the SFC paradigm. Increased OXTR binding is found in socially conditioned animals in the dorsal LS (dLS) (Zoicas et al., 2014). While unconditioned animals have a release of OXT within the LS during social fear extinction, conditioned animals do not show any release. However, the conditioned mice do not investigate the social stimuli at all during this microdialysis experiment, raising then the question if social contact induced the release of OXT during social fear extinction or *vice versa.* The extinction of social fear is also facilitated by a local infusion of OXT into the LS (Zoicas et al., 2014). Moreover, lactating females, a model for enhanced activity of the OXT system, show less fear during social fear extinction than virgin females, an effect that can be blocked by a septal infusion of OXTR-A (Menon et al., 2018). However, lactating females possess an up-regulation of the complete OXT system (increased number of fibers, OXTR binding, etc) due to the long-term adaptation during the peripartum period. It would be then interesting to evaluate the effect of an endogenous release of OXT without any change in fibers and receptor expression. For that, an acute endogenous release of OXT like during mating and ejaculation in males would be a good model.

6. Studying the OXT system: an endogenous model of OXT release in male

To study the role of oxytocin on fear and anxiety, the use of endogenous models of release of OXT is particularly relevant as they allow the release of low and physiological concentration

of OXT, in comparison with a classic pharmacological infusion which is normally well above the normal physiological level. In females, the peripartum period is characterized by many changes in the OXT system for a long period. In males, a prominent release of OXT occurs during mating which is then a brief and acute release of OXT in comparison to the lactating females. In my thesis, I used mating behavior in male mice to trigger the OXT release. I will now briefly review mating behavior in rodents in general and with a special focus on it as a model of endogenous release of OXT.

6.1. Male sexual behavior in mice: from female encounter to ejaculation

During an encounter with a sexually receptive female, male rodents display a series of behavior leading to mating and ejaculation. Sexual behavior can be described as the succession of two different phases: the *precopulatory*/*appetitive* and *copulatory*/*consummatory* phases (Figure 3) (Hull and Dominguez, 2007; Veening and Coolen, 2014). During the *precopulatory*/*appetitive* phase, the male will follow the female, sniffing the anogenital region to determine the sexual receptivity. In mice, the male can often be observed putting his head under the female and lift her to smell, this behavior is the so-called “rooting”. If the female is receptive, the male will then attempt to mount by pressing his forepaws on the side of the female´s abdomen and makes rapid and repeated pelvic movements aiming to penetrate the female. The apparition of mounting behavior is generally considered as the transition into the *copulatory*/*consummatory* phase of sexual behavior. Mounting with intromissions (=the penis successfully penetrate the vaginal canal of the female) are recognizable by deeper and slower pelvic thrusts. Genital grooming is common to observe after each mounting with intromission. This sequence of intromissions and grooming is generally repeated until ejaculation. Ejaculation is characterized by a contraction of the muscles at the base of the penis (Beyer et al., 1981); the male is freezing and falling off on the side for several seconds before separating from the female. The dismount from the female after ejaculation is normally followed by extensive grooming (Coolen et al., 1996; Burns-cusato et al., 2004; Hull and Dominguez, 2007). After ejaculation, the male will not mate with the female for a certain period called post-ejaculatory interval (PEI), which can last from a few minutes up to 24h depending on the species. In mice, several parameters like the number of intromissions before ejaculation, the inter-intromission interval, as well as the PEI can vary depending on the strain of mice (Table 1) (Burns-cusato et al., 2004; Hull and Dominguez,

2007; Liu et al., 2020). The PEI can be shortened by an encounter with a new female, which is characteristic for the so-called "Coolidge effect". After several ejaculations, males reach sexual satiety, when the sexual behavior is inhibited for several hours to several days even during an encounter with a new female. In rats, the first ejaculation facilitates the second ejaculation, as a lower number of intromission is sufficient to trigger ejaculation; thereafter ejaculation will start to have an inhibitory effect, and longer PEI and more intromissions needed to induce ejaculation (Jackson and Dewsbury, 1979; Rodríguez-Manzo and Fernández-Guasti, 1994; Phillips-Farfán and Fernández-Guasti, 2009). The mechanisms regulating PEI are different than the ones regulating sexual satiety (Rodríguez-Manzo et al., 2000b).

Mouse strain	First intromission latency	Number of intromissions	Ejaculation latency
C57BL/6J	46 – 107 sec / 1,5 min	28	888 – 1258 sec / 14 - 20 min
BALB/c	116 sec / 2 min	36	1302 – 3645 sec / 21 – 60 min
CD-1	542,94 sec / 9 min	22	240 – 1824 sec / 4 – 30 min

Table 1. Strain comparison for the average first intromission latency, the number of intromissions before ejaculation, and the ejaculation latency. The CD-1 mice were used during this thesis (Burns-cusato et al., 2004; Liu et al., 2020).

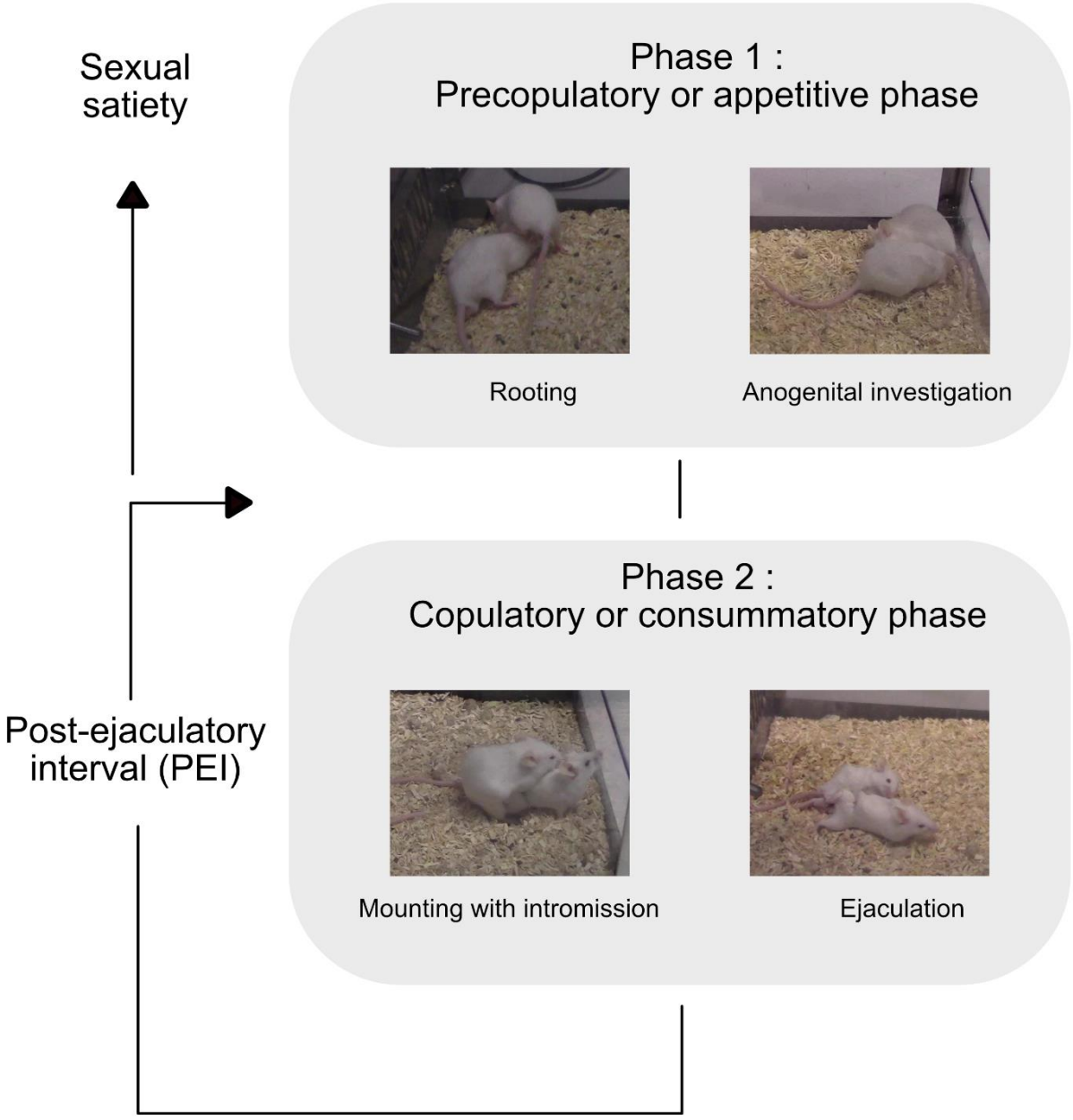

Figure 4. Description of male sexual behavior. During an encounter with a female, the male starts investigating the female to determine the appropriate behavior to adopt. This is the pre-copulatory or appetitive phase (Phase 1). During this phase, the male investigates the female, in particular the anogenital region (anogenital sniffing). If the female is receptive, the male will attempt to mount the female. This marks the passage to the copulatory or consummatory phase (Phase 2), characterized by mounting and intromissions. After several mounts with intromissions, the male will ejaculate. This is followed by a short period of inhibition of mating behavior during the so-called post-ejaculatory interval (PEI). Phase 2 will start again and be repeated until sexual exhaustion or satiety.

6.2. Neuronal circuit of male sexual behavior

The neuronal circuit underlying sexual behavior in rodents was mainly studied in rats, hamsters, and mice using brain regional lesions or assessment of regional neuronal activity patterns. Here, I will briefly describe the neuronal circuit involved in the initiation and the process of mating (Figure 4).

The male sexual behavior circuit can be differentiated in afferent pathways (sensory inputs), integrating pathways that lead to efferents to result in the initiation of motor outputs.

Afferent pathways. The olfactory information about the social stimulus (female or male, receptive or non-receptive) processed by the main and accessory olfactory systems are relayed to the MeA (Kelliher et al., 1999; Baum and Kelliher, 2009; Dhungel et al., 2011; Kunkhyen et al., 2017). Bilateral radiofrequency lesions of the MeA annihilate mating behavior in rats (no intromission nor ejaculation) (Kondo, 1992). The MeA also receives indirect somatosensory information from the genitals (Lehman et al., 1980), via the subparafascicular nucleus of the thalamus (SPF). The MeA receives then all the sensory inputs necessary during mating behavior. The MeA is sending projections to the MPOA. A unilateral radiofrequency lesion of the MeA combined with a contralateral lesion of the MPOA also abolishes sexual behavior in male rats (no intromission nor ejaculation) (Kondo and Arai, 1995). The BNST is functionally connected with the MeA and the MPOA (Simerly and Swanson, 1988). Lesions of the BNST using radiofrequency in rats do not completely block sexual behavior, but only impair it, as a higher number of mounts with intromissions is needed for ejaculation. Moreover, a longer interval between intromissions and the PEI can be observed (Emery and Sachs, 1976; Valcourt and Sachs, 1979). The sensory inputs processed into the MeA are crucial for the initiation of mating behavior. The BNST plays a minor role in the transmission of this information to higher integrative regions like the MPOA.

Integrative areas and efferent pathways. The MPOA is the main integrative area for male sexual behavior, receiving direct chemosensory and genitosensory information from the MeA and the SPF respectively (Baum and Everitt, 1992). The MPOA sends projections to several midbrain regions and brain stem nuclei including the hypothalamus and the PAG to regulate autonomic and motivational states but also somatomotor responses. These projections are crucial for the initiation of sexual behavior (Brackett and Edwards, 1984;

Simerly and Swanson, 1988). The inactivation of the MPOA using an infusion of lidocaine decreases the number of rats displaying mating behavior (mounts, intromissions, and ejaculation) by 50%. Moreover, the latencies to mount and intromit are strongly increased but the ejaculation latency remains the same as the control rats (Hurtazo et al., 2008). The initiation of mating behavior is driven by the MPOA, but not the ejaculation. The hypothalamic PVN is one of the main nuclei involved in male sexual behavior. The PVN receives input from the MPOA (Simerly and Swanson, 1988) and indirectly from the genitals (Marson et al., 1993). Both magnocellular and parvocellular neurons participate in the regulation of mating behavior by either releasing neuropeptides such as OXT into the bloodstream or via extrahypothalamic projections, e.g. to the spinal cord. Projections of parvocellular OXT neurons of the PVN reach the spinal cord, more particularly the thoracolumbar and lumbosacral regions, which innervate the organs of the male reproductive system (Luiten et al., 1985; Wagner and Clemens, 1993). The MPOA and the PVN project both to motor output such as the ventrolateral PAG (Luiten et al., 1985) and the nPGi (Bancila et al., 2002)

Motor output: The PAG forms reciprocal connections with the MPOA (Simerly and Swanson, 1988) and sends projections to the nPGi, which tonically inhibits the spinal cord and the mechanisms controlling penis erection (Normandin and Murphy, 2011). It is the major source of inhibition of genitals reflexes and copulation (Bancila et al., 2002). Bilateral radiofrequency lesions of the nPGi in male rats induce a global increased mating efficiency (decreased mounting and intromission frequency, increased probability of ejaculation) (Yells et al., 1992). The spinal cord, which contains autonomic and somatic nuclei, is the main motor output for male sexual behavior. Within the spinal cord is located the spinal ejaculation generator. Ejaculation is then a spinal reflex and is under the tonic inhibition of the brain (Sachs and Garinello, 1980; Sachs and Bitran, 1990).

Other regions may also participate in the regulation of male sexual behavior. For example, bilateral radiofrequency lesions of the LS but not of the MS significantly decrease the number of mounts and intromissions in male rats (Kondo et al., 1990). The LS projects and send projections to numerous brain regions, including the MPOA and the PVN (Deng et al., 2019). The process of mating behavior recruits various brain regions, which are involved in the regulation of other behavior such as anxiety and fear.

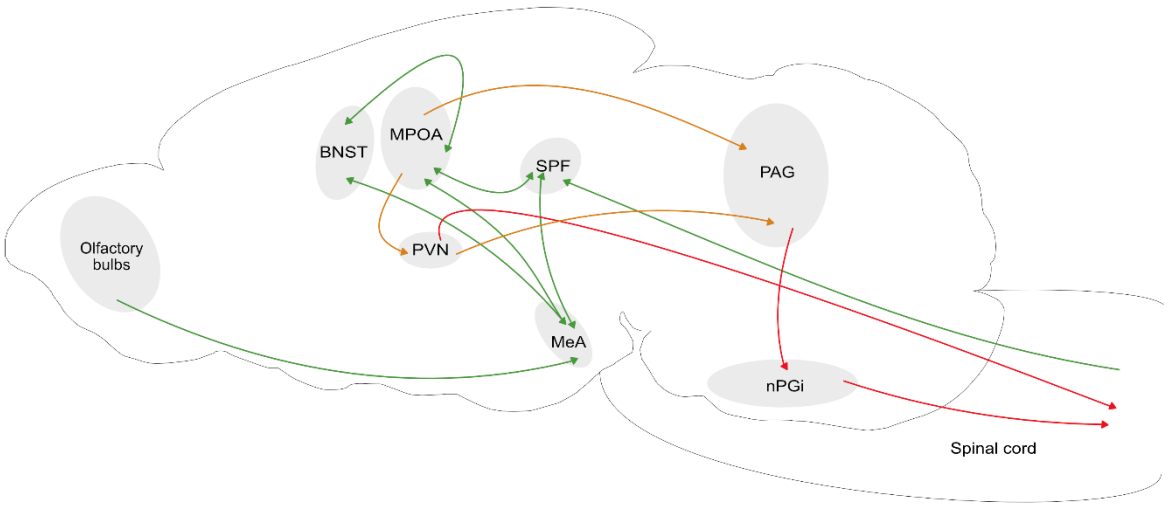

Figure 5. Simplified scheme of the principal brain areas involved in male sexual behavior. In green: ascending pathways. In orange: Integrative pathways. In red: Outputs. Abbreviation: BNST = bed nucleus of the stria terminalis ; MeA = medial amygdala ; MPOA = medial preoptic area ; PAG = periaqueductal grey ; nPGi = nucleus paragigantocellularis of the medulla ; PVN = paraventricular nucleus of the hypothalamus ; SPF = subparafascicular nucleus of the thalamus.

6.3. Effect of sexual behavior on anxiety and fear

Via the involvement of many neurotransmitters, sexual behavior also influences other behaviors. The rewarding effect of sexual behavior is already well known (Pfaus et al., 2001; Paredes, 2009) and has been used to condition rodents. In the conditioned place preference, the rewarding effect of mating can be used to reinforce or induce a preference for a neutral stimulus in mice and rats (Tenk et al., 2009; Quintana et al., 2019). For example, males that develop a conditioned preference for the compartment where they previously mated do not change their preference when the other chamber is paired with morphine, indicating that morphine has a similar or lower rewarding value than mating (Camacho et al., 2009). Moreover, mating, and in particular ejaculation, can affect anxiety- and depression-like behavior. In a test of defensive burying, ejaculation in male rats was shown to exert an anxiolytic effect, whereas intromissions without ejaculation did not induce any change in the burying behavior. This effect was independent of the number of ejaculation (Fernández-Guasti et al., 1989). Pharmacology studies in rats showed that this effect was mediated by GABA, as i.p. application of a GABA antagonist blocked the anxiolytic effect of ejaculation (Fernández-Guasti and Saldívar, 1990). The serotonin system might also play a role in the anxiolytic effect of ejaculation. Indeed, lesion of all serotonin terminals using a selective serotoninergic neurotoxin administrated i.c.v. abolished the anxiolytic effect of ejaculation in the defensive

burying paradigm in male rats (Saldívar et al., 1991). The anxiolytic effect of ejaculation seems to last less than 24h, as no effect was found on anxiety 24h after ejaculation in the defensive burying paradigm. Regarding the male that reached sexual satiety, an ejaculation, even 24h after sexual satiety, did not induce anxiolysis anymore in rats (Rodríguez-Manzo et al., 1999). Ejaculation can also have an anti-depressive-like effect. In rats, males that ejaculated spent less time immobile in the forced-swim test than rats that showed only intromissions (Martínez-Mota et al., 2005).

In the context of fear conditioning, rats were exposed to a female or a male conspecific for 24h immediately after fear conditioning. During the fear memory test, rats that were paired with a female and showed mating behavior had a disrupted contextual fear memory and show less freezing during the fear extinction, both when tested immediately after mating or 7 days after mating in comparison to the male rats exposed to another male or kept single-housed. A systemic or intra-hippocampal (CA1) antagonization of the dopamine (DA) receptor prevented the disruption of the fear memory. While a systemic infusion of a DA agonist after acquisition could mimic the effect of female exposure and disrupt the fear memory during the memory test, the activation of the DA receptors within the CA1 by a selective agonist could not impair the fear memory. The DA receptors expressed into the CA1 of the hippocampus were then necessary but not sufficient to disrupt fear memory formation during mating behavior (Bai et al., 2009). Moreover, mating (without differentiation with or without ejaculation) shows an anxiolytic effect in the elevated plus-maze or the light/dark box (LDB). In this study, male rats mated with a receptive female for 30 min before being tested in the elevated plus-maze and the LDB. Mating rats showed decreased anxiety-like behavior in both tests. Moreover, this beneficial effect of mating was still observable in the elevated plus-maze 2h and 4h after mating. In this context, mating-induced anxiolysis was impaired by the i.c.v. administration of an OXTR-A (Waldherr and Neumann, 2007).

6.4. Regulation of male sexual behavior by the PVN and OXT

The PVN was extensively studied in the context of male sexual behavior. Its connections to several brain regions such as the PAG and the spinal cord but also to the pituitary for hormonal release into the bloodstream make it a potential integration and modulation center for male mating behavior (Argiolas and Melis, 2004; Melis and Argiolas, 2011). The activity of the PVN neurons during mating behavior in male rats was investigated using the expression of the cFos

protein as a marker of neuronal activation. Within the parvocellular region of the PVN, the proportion of OXT neurons expressing cFos was increased by 29% after intromissions in comparison with naïve control rats (Witt and Insel, 1994). In rats, electrolytic lesions of the lateral parvocellular subdivision of the PVN, including the OXT parvocellular neurons, induce an increase in the latencies to mount, without altering the number of mounts or intromissions before ejaculation or the ejaculation latency (Hughes et al., 1987). The OXT parvocellular neurons of the PVN connect directly or indirectly via the nPGi to the spinal cord which controls the muscles responsible for penile reflex (Swanson et al., 1980; Wagner and Clemens, 1991; Marson and Mckenna, 1996; Gerendai et al., 2001; Bancila et al., 2002). Administration of i.c.v. OXT in male rats induces penile erection (Argiolas et al., 1988; Melis et al., 1988) and shortens ejaculation latency and the PEI (Arletti et al., 1992). When infused directly into the PVN of rats, OXT also induces penile erection. This effect of OXT could be reversed by an i.c.v. administration of an OXTR-A (Melis et al., 1988). OXTR-A alone reduced non-contact erections (in presence of an inaccessible receptive female) when infused i.c.v. but not into the PVN of male rats, it also impairs sexual performance (increased intromission and ejaculation latencies and decreased frequency of mounts, intromissions, and ejaculations) but this could be a secondary effect of the lack of penile erection (Argiolas et al., 1989).

Another route of OXT to regulate penile erection is likely to be via magnocellular neurons in the PVN projecting to the pituitary gland, where OXT is released into the bloodstream. Moreover, the OXT magnocellular neurons of the SON are also activated during intromissions until ejaculation (Pattij et al., 2005; Caquineau et al., 2006), and this is likely to reflect the high activity of OXT neurons projecting to the neurohypophysis and increased OXT secretion into blood. Circulating OXT may be important for penile erection as hypophysectomized rats presented a reduced penile erection even after i.c.v. OXT administration (Argiolas et al., 1989). OXTR are highly expressed on the male reproductive organs and participate in the contraction of the muscle inducing penile erection and ejaculation (Filippi et al., 2002, 2005). However, Gupta et al. demonstrated that OXT may act via the vasopressin V1A receptor, and not the OXTR to induce the contraction of erectile and ejaculatory tissues (Gupta et al., 2008).

In addition to its role in penile erection, OXT is also released during mating behavior. Experimental evidences suggest that OXT released during mating is involved in the regulation of male sexual behavior. However, pharmacological experiments showed contradictory

results. In male rats, an elevated release of OXT could be measured in the PVN during mating using microdialysis (Waldherr and Neumann, 2007). In male rats, increased OXTR mRNA expression in the MPOA can be found after 30 min of sexual behavior, independently of sexual experience. However, male rats with a sexual experience display higher levels of OXTR protein measured via western-blot (Gil et al., 2013). OXT administration into the MPOA decreases the latency of ejaculation and decreases the PEI in rats (Gil et al., 2011). Consequently, an infusion of OXTR-A into the MPOA decreases anogenital sniffing, delays mating behavior, and increases the ejaculation latency (Gil et al., 2011, 2013). It seems that OXT acts within the MPOA to facilitate copulation and ejaculation. Within the parvocellular region of the PVN, the number of neurons expressing cFos increased by 124% after ejaculation, in comparison with naïve controls. The proportion of OXT neurons expressing cFos was increased only in the lateral parvocellular subdivision of the PVN. In this region, the expression of cFos in OXT neurons increases by 31% after ejaculation, in comparison with naïve controls (Witt and Insel, 1994). This may imply that OXT afferents to the spinal cord may modulate autonomic regulation of ejaculation (Ackerman et al., 1997; Clément et al., 2008, 2013). Furthermore, the blockade of OXTR in the brain and the spinal cord alters ejaculation in anesthetized rats (Clément et al., 2008), and OXT concentrations considerably increase in CSF after ejaculation (Hughes et al., 1987). Also, in men, an increase in plasma OXT could be found during sexual intercourse and ejaculation in men (Carmichael et al., 1987; Murphy et al., 1987; Krüger et al., 2003; Ückert et al., 2003).

Moreover, there is a possibility of a reciprocal interaction between mating and the OXT system. In mice, Jirikowski et al. found alterations of the OXT-immunoreactivity in the PVN and SON, depending on the duration and the number of mating events (Jirikowski et al., 1991). They sacrificed mice either after one single ejaculation or after 3 weeks of continuous sexual behavior and found that one acute ejaculation in naïve mice decreases OXT-immunoreactivity in the PVN and SON, while 3 weeks of sexual behavior increased it (Jirikowski et al., 1991).

AIMS OF THE PRESENT THESIS

Social fear is a devastating psychopathology with high prevalence. It has been previously shown in a mouse model of SFC that the neuropeptide OXT is essential for social fear extinction. States of high activity of the endogenous OXT system, as seen in lactating females, were found to be accompanied by low levels of social fear. Therefore, in the first major part of my PhD thesis, I aimed to investigate, how sexual behavior in male mice modulates fear and anxiety, focusing on the involvement of the neuropeptide OXT by combining behavioral, microdialysis and pharmacological techniques as well as neuronal activity assessment. In more detail, I aimed to:

1. Study the effects of mating on social fear conditioning, anxiety-like behavior, social preference/novelty and cued fear conditioning,

2. Assess cellular activation patterns within selected brain regions such as the PVN and the LS during the process of social fear extinction and mating and the combination of both,

3. Evaluate the role of OXT in the effect of mating on fear and anxiety using microdialysis and pharmacological approaches.

Using the SFC model, we previously showed in our lab that OXT within the LS can facilitate the extinction of social fear (Zoicas et al., 2014; Menon et al., 2018). However, social fear is without any doubt a complex process, likely involving many other brain areas, such as the amygdala. Therefore, in the second part of my PhD thesis, I studied the involvement of the CeA in the process of social fear extinction, focusing on the OXT system using pharmacological and chemogenetic techniques.

MATERIALS AND METHODS

1. Animals

Male CD1 mice (8-12 weeks; Charles River, Sulzfeld, Germany) and male OXTR-Cre mice (courtesy of Dr. Jan Deussing, Max Planck Institute of Psychiatry, Munich, Deutschland) were used for all mating, social fear conditioning and social preference experiments, while female CD1 mice were used as a mating partner for the mating experiments. Mice were kept under the standard laboratory conditions (12/12 h light/dark cycle, lights on at 06:00, 22°C, 60 humidity, food, and water *ad libitum*). Three days before any experiments, male mice were single-housed in observation cages (30x24x36cm) unless specified otherwise. All experimental procedures were performed between 08:00 and 12:00 hrs in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Oberpfalz and the guidelines of the NIH.

The OXTR-CRE mice are a genetically modified line, originating from the CENSAT consortium. These mice have a CD1 background and express the enzyme Cre recombinase under the OXTR promotor. The Cre recombinase recognizes Lox sequences and recombines the sequence. This means that, when a virus expressing a specific gene surrounded by LoxP sites is infused into the brain, only the cells expressing the Cre-recombinase – the OXTR cells in our case – will be able to recombine and express the sequence of this gene.

2. Behavioral techniques

2.1. Mating experiments

Males underwent a 30 min-training for mating 48h before the mating experiments with a sexually experimented but non-primed female. The aim was to familiarize the animals with the mating procedure and to give them a sexual experience. To avoid any long-term effect of mating on the future experiments, no ejaculation was achieved during the mating training. Indeed, the training was stopped if the male achieved a successful mounting (one or more intromissions). Female mice were primed 38h before the mating experiment with estrogen (ß-estradiol 3-benzoate, 25µg/0.05ml of oil, sub-cutaneous.; Sigma-Aldrich) to increase sexual

receptivity. 24h after priming, females were trained for mating. The female was introduced into the cage of a sexually experienced male. Moreover, the estrous cycle of the females was evaluated by sampling a vaginal smear. After observation under the microscope, the females in proestrus/estrus were classified as receptive while the females in the metestrus/diestrus phases were non-receptive. On the day of the experiment, a primed and sexually experienced female was introduced for 1h in the home cage of the male. The female mice that were receptive during the mating training and which were in a receptive phase of the estrus cycle were used in priority.

All mating experiments were video recorded for later analysis. The latencies of the first intromission and ejaculation, as well as the number of successful mounting (mount of the female + intromissions), were manually scored. The males were then separated in three groups: non-mating (NM), control mice that did not undergo the mating protocol, mating without ejaculation (Ej^-) and mating with ejaculation (Ej^+). After the mating experiment, an interval of 10 min was given before further behavioral testing.

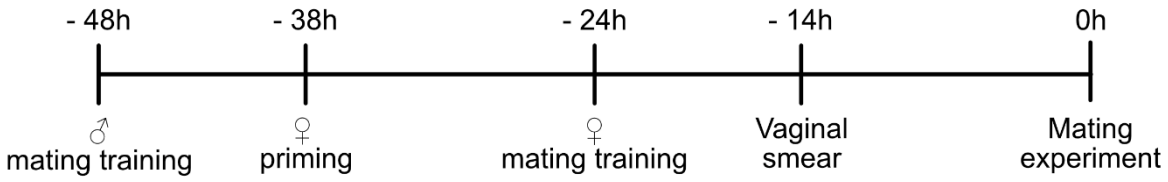

Figure 6. Experimental design of the mating experiments. The male mice were trained for mating 48h before the mating experiment. The females were primed 38h before the experiment and trained for mating 24h before. The estrous cycle of the females was checked 14 h prior experiment.

2.2. Experimental design of the behavioral experiments

Social and cued fear conditioning. In order to investigate the effect of mating on social and cued fear conditioning, male mice were kept single-housed for 2 days in observation cages. Then, male mice underwent 30 min of mating training with a non-primed and sexually experienced female, 24h before social (Figure 7A), or cued fear conditioning (Figure 7B). 23h after the fear acquisition, a receptive female was introduced in the home cage of the male and left undisturbed for 1h. Control mice were left single-housed for 1h. 10 min after the removal of the female, the fear extinction took place. The recall was 24h after fear extinction.

Anxiety-like behavior tests and social preference/novelty test (SPT/SNT). In order to investigate the effect of mating on anxiety-like behavior and social preference/novelty. Male mice were kept single-housed for 2 days in observation cages. They then underwent 30 min of mating training with a non-receptive experienced female. 48h after mating training, a receptive female was introduced in the home cage of the male and left undisturbed for 1h. Control mice were left single-housed for 1h. 10 min after the removal of the female, mated and control mice were subjected to either the SPT/SNT, the LDB, the marbles burying test (Figure 7C).

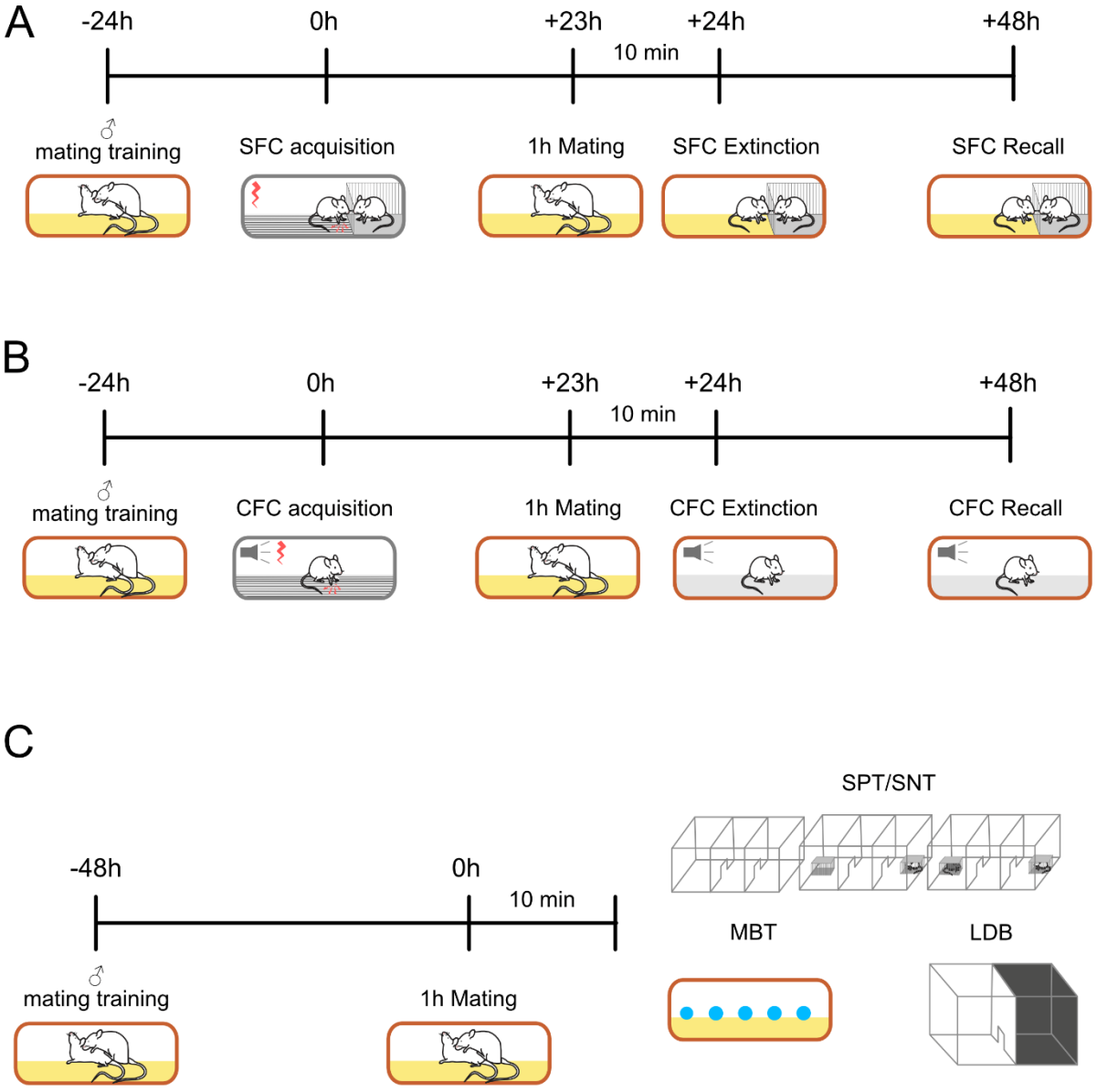

Figure 7 Experimental design of the behavioral experiments. A. Mating before social fear extinction. B. Mating before cued fear extinction. C. Mating before either anxiety-like behavioral tests or social preference/novelty test.

2.3. Social Fear Conditioning paradigm (SFC)

The SFC paradigm was first developed in 2012 in order to model social anxiety disorder in mice. This paradigm, based on the principles of operant conditioning, induces social avoidance, and social fear, without affecting anxiety- or depressive-like behaviors in mice (Toth et al., 2012a).

The SFC protocol was performed on 3 consecutive days as previously described (Toth et al., 2013; Menon et al., 2018). On day 1, the social fear acquisition was performed in a rectangle-conditioning chamber (45x22x41cm) with black Perspex walls and electric grid floor (TSE system GmbH, Bad Hamburg, Germany). The social fear extinction (day 2) and extinction recall (day 3) took place in the home cage of the test mouse. Each trial was video- recorded for later analysis.

Social fear acquisition (day 1). Animals were introduced into the conditioning chamber. After 3 min of habituation, a non-social stimulus (empty wire mesh cage, 7x7x6 cm) was introduced into the chamber and placed on the lower right corner for 3 min. The non-social stimulus was then replaced by a social stimulus, a wire mesh cage containing a conspecific of the same strain, sex, and age. Unconditioned mice (SFC^-) were allowed to explore this social stimulus for 3 min before they were returned to their home cage. The conditioned mice (SFC^+) were given an electric foot (0.7mA, pulsed) every time they directly contacted the social stimulus. The duration of the foot shocks was equivalent to the time that the animals needed to escape the social stimulus (~1sec.). Mice received on average between 1 and 3 foot shocks, the inter-shock interval was dependent on when the social contact was made. Mice receiving 7 or more foot-shocks were excluded. Mice were returned in their home cage when no further social contact was made after 6 min if they previously received only 1 shock, or after 2 min if they received 2 or more shocks.

Social fear extinction (day 2). In their home cage, mice were exposed to 3 non-social stimuli (empty wire mesh cage, 7x7x6) for 3 min with 3 min inter-stimulus interval, allowing to assess non-social fear. They were then exposed to 6 unknown and different social stimuli (wire mesh cage containing a conspecific) for 3 min each with 3 min inter-stimulus interval to assess social fear and social fear extinction.

Social fear extinction recall (day 3). In their home cage, mice were exposed to 6 unknown and different social stimuli for 3 min each with 3 min inter-stimulus interval.

Scoring of the social fear. During the social fear extinction and extinction recall, 5 parameters were manually scored to measure social fear using the JWatcher program (V 1.0, Macquarie University, and UCLA) (See Table 2). Social fear is reflected by the percentage social investigation i.e., the more the mice investigate the social stimulus the less they are socially fearful.

Behavior	**Definition**
Investigation	Direct contact with the non-social or social stimulus.
On the cage	Standing on the stimuli cage without investigation.
Freezing	Passive fear behavior. Complete immobility except the movement needed to breathe for >1sec.
Burying	Active fear behavior. Throwing the bedding towards the stimulus cage to bury it.
Exploration	Any other behavior unrelated to the stimulus (eating, sleeping, exploration …)

Table 2. Description of the different behaviors scored during the SFC.

2.4. Cued-Fear Conditioning (CFC)

The CFC procedure was adapted from Toth et al., 2012 (Toth et al., 2012b). The cued fear experiments were performed on 3 consecutive days in a conditioning box (TSE system GmbH, Bad Hamburg, Germany) using two different contexts A and B. The context A, which consists of a transparent Perspex box (29.5x29.5x34.5 cm) with an electric grid floor, was used for the cued fear acquisition. Before each trial, the box was cleaned with water containing a neutral-smelling detergent. Context B, which consists of a black Perspex box (29.5x29.5x34.5 cm) with a smooth black floor, was used for both cued fear extinction and recall. Before each trial, the box was cleaned with a lemon-smelling detergent. Background noise was constantly present. Illumination was provided by a white-light (310 lx) for the context A or yellow light (40 lx) for the context B. Auditory stimuli were delivered by a speaker integrated into the ceiling of the conditioning chamber.

Cued fear acquisition (day 1). Animals were placed in the conditioning chamber (context A). After 5 min of habituation, they were subjected to 5 CS-US pairings with a 2 min inter-stimulus interval. The CS was a 30 sec sound (80 dB, 8 kHz) which was paired for the last 2 sec with the US, a mild electric foot-shock (pulsed, 0.7mA). 5 min after the last CS-US pairing, the mice were returned to their home cage.

Cued fear extinction (day 2). Animals were placed in the chamber (context B). After 5 min of habituation, the mice were exposed to 20 CS presentations (sound, 30 sec, 80 dB, 8 kHz) with 5 sec inter-stimulus presentation. 5 min after the last CS presentation, the mice were returned to their home cage.

Cued fear extinction recall (day3). Animals were placed in the chamber (context B). After 5 min of habituation, the mice were exposed to 2 CS presentations (sound, 30 sec, 80 dB, 8 kHz) with 5 sec inter-stimulus presentation. After the last CS presentation, they were directly returned to their home cage.

Scoring of the cued fear. Freezing was manually quantified as a measure of fear and defined as at least 1 sec of complete immobility but respiratory movements using JWatcher (V 1.0, Macquarie University, and UCLA). During the cued fear extinction, the CS presentations are shown in 10 blocks, each block representing the mean freezing percentage during two CS presentations.

2.5. Social Preference test (SPT) and Social Novelty test (SNT)

The apparatus consists of a rectangular three-chambered box divided by transparent Plexiglas walls with small circular openings allowing access to each chamber. 24h before the test, the mice were habituated to the three-chambered box. The day before the experiment, the mice were habituated to the three-chambered test. A mouse was placed into the middle compartment and was allowed to explore the chambers for 5 min. At the end of the 5min, the mouse was removed and 2 identical non-social stimuli (empty wire mesh cages) were placed into the right and left chambers for 5 min. The mouse was then removed and returned to its home cage. On the day of the experiment, the mouse was placed in the middle compartment and allowed to explore for 5 min. After the habituation period, the mouse was removed and a non-social stimulus (empty wire mesh cage) and a social stimulus (wire mesh cage containing an unfamiliar conspecific) were placed in the two lateral chambers. The test mouse was then

reintroduced in the middle compartment and allowed to explore all 3 chambers freely for 5 min. After the SPT, the test mouse was again removed and the unsocial stimulus was replaced by a new social stimulus, following which the test mouse was introduced in the middle compartment and allowed to explore all 3 chambers freely for 5 min. At the end of the test, the mouse was returned to its home cage. The apparatus was cleaned with water containing a very small amount of detergent and dried after each animal. To avoid a place preference bias, the compartments containing the non-social and the social stimuli were exchanged for each mouse. Each session was video recorded and the time spent in each compartment was evaluated with the software Ethovision XT, a video tracking system (Noldus Information Technology, Wageningen, The Netherlands). The animals that did not enter in all the compartments within the first 150 sec were excluded from the analysis.

2.6. The light/dark box (LDB)

The LDB is a classic test, which was originally established to test the efficacy of anxiolytic, was in our case used to evaluate anxiety-like behavior in rodents, using their innate aversion for brightly lit areas. The LDB constitutes of two compartments, one is enclosed and dark (1/3, 0-1 lx) and the other one is open and bright (2/3, 300 lx). During this test, we measure the natural exploratory behavior of the mice. We measure here the time spent in the light compartment, as a measure of anxiety-like behavior, and the number of entries in the light compartment and the locomotion, as measures of exploratory behavior (Hascoët and Bourin, 2009). The mice were introduced into the light compartment and allow to explore for 10 min before to be returned in their home cage. The box was cleaned before each animal using water containing a small amount of lemon-smell detergent. The LDB has been evaluated using the software Ethovision XT (Noldus Information Technology, Wageningen, The Netherlands).

2.7. The marbles burying test

In opposition to other classical anxiety-like behavioral tests, the marbles burying test is not based on a negative experience, but on their innate fear of unknown objects. Digging and burying are two innate behaviors in rodents. In the marbles burying test, mice show both digging and burying behaviors, these behaviors were shown to be sensitive to anxiolytic drugs but also antidepressant drugs (Deacon, 2006). The marbles burying test consists of a transparent box filled with bedding at a depth of 5 cm and 20 marbles (1.5 cm of diameter).

Animals were introduced in the lower right corner, without any contact with the marbles. They were left undisturbed for 30 min into the cage. The total of marbles buried was counted at 5, 10, 15, 20 and, 30min. A marble was considered buried when ¾ were non-visible.

3. Pharmacology and stereotactic techniques

3.1. Experimental design of the pharmacological and chemogenetic experiments

Manipulation of the OXT system after or during mating. In order to assess the role of the OXT release during mating in social fear extinction, male mice underwent surgery for intracerebroventricular (i.c.v.) or local cannula implantation into the LS and were left for recovery for 3 or 7 days respectively. Meanwhile, they were handled every day to habituate them to the i.c.v. or local infusion procedure. As described before, males underwent the mating training 24h before to be conditioned. 24h later, the males were then infused with either OXTR-A or a vehicle (Veh) 10 min after (i.c.v.) or before (local) the mating procedure. As described above, the mice were then subjected to the social fear extinction. The recall took place 24h after extinction (Figure 8A and B).

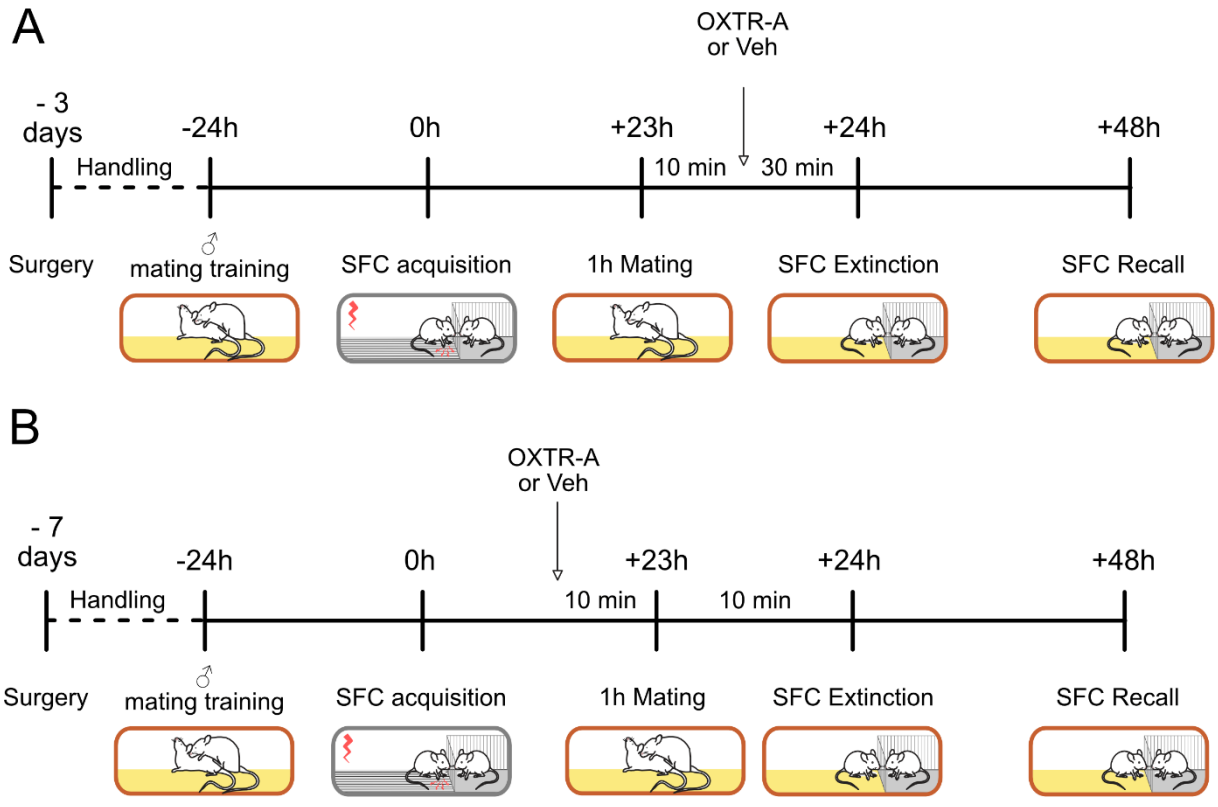

Figure 8. Experimental design of the pharmacological experiments aiming to block the OXTR i.c.v. after mating (A) or locally within the LS (B) before mating prior to social fear extinction.

Manipulation of the OXT system during social fear extinction. To assess the involvement of the OXT system within the CeA during social fear extinction, male mice underwent surgery for local cannula implantation or virus microinfusion into the CeA and were left for recovery for 7 or 3 weeks respectively. They were handled every day for 7 days before social fear acquisition to habituate them to the local infusion or intraperitoneal (i.p.) procedure. For the pharmacological experiments, 24h after the acquisition, the males were infused with either synthetic OXT or Veh, Carbetocin or Veh, 10 min before social fear extinction (Figure 9A). For the chemogenetic experiment, 24h after the acquisition, the males received an i.p. injection of Clozapine N-oxide dihydrochloride 40 min before social fear extinction. The recall took place 24h after extinction (Figure 9B).

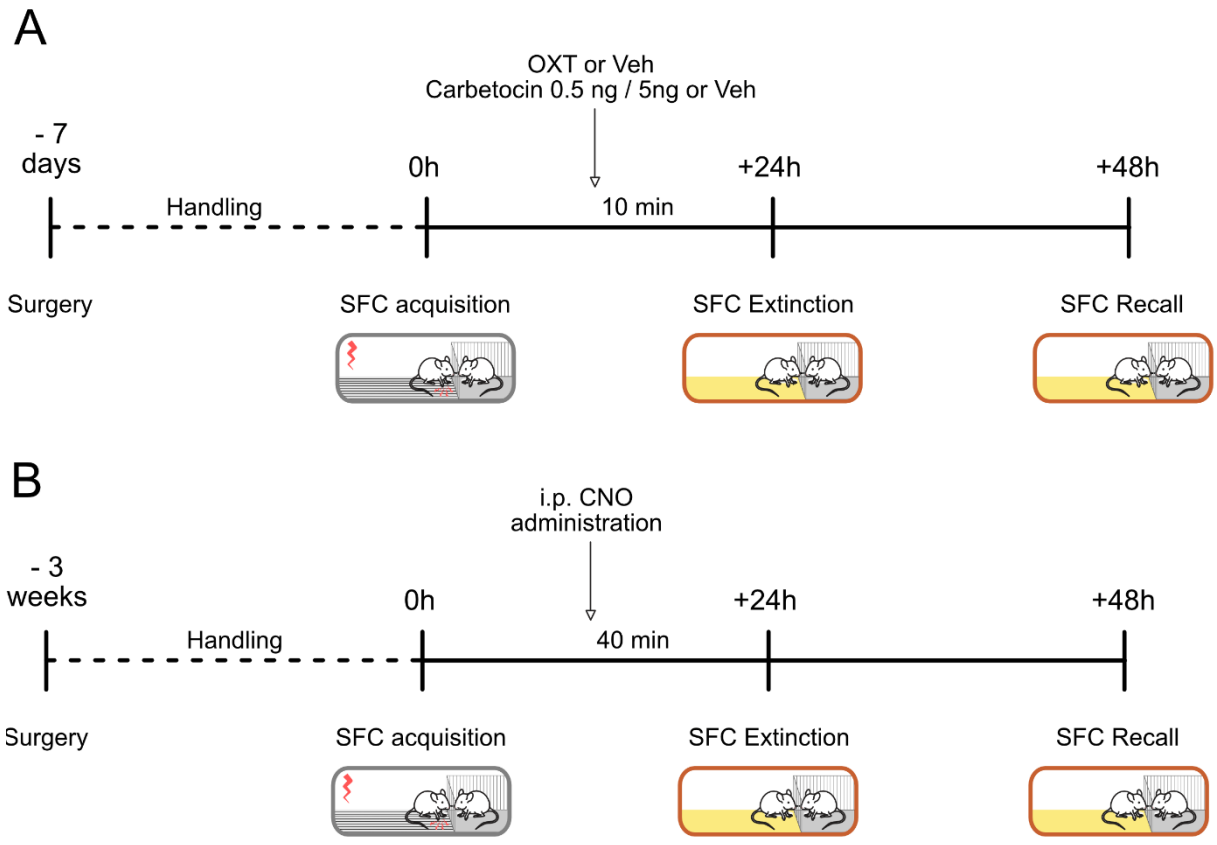

Figure 9. Experimental design of the manipulation of the OXT system within the CeA using OXT or carbetocin infusions (A) or chemogenetic (B).

3.2. Stereotactic guide cannulas and microdialysis probe implantations and intracerebral infusion

All surgeries have been conducted in semi-sterile conditions. Mice were anesthetized with Isoflurane (Forene, Abbott GmbH, Wiesbaden, Germany) and received an i.p. injection of an analgesic (Buprenovet, 300µl/kg, Bayer, Germany) before to be fixed on the stereotaxic frame. Guide cannulas (i.c.v. 21G unilateral, local 23G bilateral, 8mm, Injecta GmbH, Germany) or microdialysis probe (unilateral, 2mm, self-made, molecular cut-off 18 kDa) were implanted. The coordinates of the target regions were determined according to mouse brain atlas by Paxinos and Watson (A or P, anterior (+) or posterior (−) to Bregma; L or R, left or right to midline; V, ventral to the surface of skull; Franklin and Paxinos, 2008). To target the ventricle and the LS, the guide cannulas have been implanted 2 mm above the target region (from Bregma, i.c.v. : AP + 0.2 mm, ML + 1.0 mm, V: + 1.4 mm ; LS: AP + 0.3 mm, ML ± 0.5 mm, V: + 1.6 mm). For the CeA, which is located more ventrally, the guide cannulas have been implanted 2mm above (Experiment 1) or 3 mm above (Experiment 2) the target region (from Bregma, AP -1.4mm, ML ±3.1mm, V -2.9mm or -1.9mm). For the microdialysis experiment, the probe was implanted directly into the targeted region (CeA: AP -1.5 mm, ML ±3.1 mm, V -5.0mm). The cannulas were fixed to the scalp using two jeweler's screws and dental cement (Kallocryl, Speiko-Dr. Speier GmbH, Muenster, Germany). A stylet (26G, 8mm) was inserted into the guide cannula to avoid any contact with the external environment. After surgery, the mice received an injection of antibiotics to avoid post-surgical infections (3 mg/30 µl Baytril, Bayer GmbH, Leverkusen, Germany). The weight of the mice was measured prior and after surgery until recovery (i.e. gain of weight compared to the weight one day after surgery). Mice which lost 20% of their weight between the surgery and the day of the experiment were excluded from the experiment. The mice were handled and the stylet was exchanged every day after surgery until the first day of experiment. Experiments were conducted either 3 days (microdialysis and i.c.v.) or 7 days (local infusions) after surgery.

3.3. Intracerebral infusions

All the infusions were performed in awake but slightly restrained animals by a blind experimenter. The vehicle groups received a sterile Ringer solution (i.c.v.: 2µl, LS/CeA: 0.2µl/side). At the end of the experiments, a blue dye was infused to verify histologically the correct location of the implantations. The doses and the time of infusion were selected based on

previous studies in the lab (Zoicas et al., 2014; Menon et al., 2018). For the OXTR agonist Carbetocin, the doses were selected based on a prior experiment in the lab (unpublished, Dr. Rohit Menon).

i.c.v. infusion. A stainless steel needle (26G; 10 mm) connected via polyethylene tubing to a Hamilton syringe (Hamilton Company, Bonaduz, Switzerland) was directly inserted into the guide cannula. 30 min prior extinction, the animals received the OXTR-A desGly-NH2,d(CH2)5[Tyr(Me)2,Thr4]OVT (i.c.v.: 2 µg/2 µl, kindly provided by Dr. Maurice Manning, Toledo, Ohio).

CeA infusion. In a first experiment (Experiment 1), the animals were implanted bilaterally 2 mm above the CeA with guide cannulas (23G, 8mm) and the infusions were made with an infusion system made of a stainless steel needle (26G; 10mm) connected via a polyethylene tubing to a Hamilton syringe. However, the behavior of these animals in the SFC paradigm seemed impaired (See Results Section 2.1.). That is why a second experiment (Experiment 2) was performed with an implantation of the guide cannulas (21G, 8 mm) 3 mm above the CeA and an infusion system made of glass fiber (11 mm) connected to a Hamilton syringe via a polyethylene tubing. This allowed me to minimize the damage into the brain as the guide cannulas were less penetrating into the tissue and the diameter of the infusion system was comparatively smaller. For these experiments, mice received either synthetic OXT (Sigma-Aldrich Chemie GmbH; 0.1ng/0.2µl/side) or Carbetocin (0.5 or 5ng/0.2µl/side) 10 min before the extinction of the SFC.

LS infusion. Based on the CeA infusion issues, the bilateral infusions into the LS have been also made using a glass fiber infusion system (10 mm long). The animals were implanted bilaterally using 23G guide cannulas. The implantations were 2 mm above the target region. As the LS is a brain structure that is relatively dorsal to the CeA, implanting the cannulas 3 mm above was not possible because the majority of the cannula would have been outside the brain, impairing then their stability and increasing the risk that the mice remove the cannulas while grooming. The animals received an OXTR antagonist (OXTR-A; desGly-NH2,d(CH2)5[Tyr(Me)2,Thr4]OVT; 20ng/0.2 µl/side) 10 min before mating.

3.4. Virus microinfusion and chemogenetic experiments

Following a procedure similar to the guide cannulas implantations, a recombinant adeno-associated virus (AAV) with a hSyn-DIO-hM3D(Gq)-MCherry construct coding for an excitatory Gq-DREADD (Designer Receptor Exclusively Activated by a Designer Drug) was infused directly into the CeA of OXTR-Cre mice (70 nl/side, AP -1.4 mm, ML ±3.1 mm, DV -4.9mm). After infusion, the skin was sutured and the mice were single-housed for 1 week. After recovery, the mice were group-housed for 2 more weeks, the time necessary for the virus to be expressed in the targeted cells i.e., neurons according to the viral construct with the hSyn promoter. Moreover, this virus possesses 2 loxP sites, meaning that only cells expressing the Cre recombinase will be able to express the Gq-DREADD in its active form. Thus, only the OXTR-positive neurons will express the Gq-DREADD. 40 min before social fear extinction, mice received an i.p. injection of Clozapine N-oxide dihydrochloride (CNO; 3mg/kg; HelloBio, Bristol, United Kingdom), which is the ligand to activate the Gq-DREADD. The mice were sacrificed at the end of the experiment and the brains were removed for verification of the virus expression using the expression of reporter protein mCherry as a proxy for viral expression.

3.5. Microdialysis for measuring OXT release

Intracerebral microdialysis allows monitoring local extracellular concentrations of neuropeptides, including OXT. This technique is performed *in vivo*, enabling to measure the release of OXT in behaving animals. The implanted probe possesses a U-shaped semi-permeable membrane (molecular cutoff 18 kDa) and is perfused with a Ringer solution. Based on the principle of passive diffusion, OXT will diffuse from the extracellular medium (higher concentration) to the probe (lower concentration). The microdialysis probe was implanted into the right CeA as described above (AP -1.5 mm, ML ±3.1 mm, DV -5.0mm). One day after surgery, the mice were habituated to the weight of the collecting cup. On the next day, the probes were connected to the microdialysis pump. The microdialysis probes were rinsed for 2h with a sterile Ringer solution (pH 7.4, rate: 3.3µl/min). Then, 2 basal measures were taken before the introduction of a female into the cage. 3 measurements were made during mating behavior, followed by 1 basal measurement after the removal of the female. Each dialysate was collected after 30 min of behavior into 200 µl microfuge tubes containing 10 µl of 0.1M HCl. The samples were immediately frozen and stored at -20°C before quantification by radioimmunoassay (sensitivity 0.3 pg/sample, cross-reactivity 0.7%; RIAgnosis, Germany). The

experiment has been repeated once at the start of the light phase and a second time during the start of the dark phase to evaluate the effect of the light cycle on the release of OXT during mating behavior. As no difference was found, the result shown in this thesis included both light and dark measurements. Later, the animals were sacrificed and the correct implantation of the probe was verified.

4. Histological and microscopy techniques

4.1. Sacrifice and transcardial perfusion

For histological analysis, the animals were sacrificed with CO2. The brains were removed and directly frozen in dry ice-cold 2-methylbutane and conserved at -20°C.

For immunostaining analysis, the animal was first deeply anesthetized. Once the state of the animal is such that it has stopped breathing but the heart is still beating, the thoracic cavity was delicately open to reveal the heart. A 26G needle connected to the perfusion pump was introduced into the right ventricle and, after starting the perfusion pump, the left atrium was opened. The bloodstream was then flushed with a solution of 1X PBS pH 7.4 at a rate of 19 ml/min. Only when the blood has been removed from the bloodstream, the fixation solution can be perfused (4% Paraformaldehyde, PFA, pH 7.4). This solution allows the fixation of all cells permanently for further immunostaining to detect and mark specific protein, explaining the importance of flushing the blood cells. After 2 min of PFA perfusion, the brain was removed and stored in a 4% PFA solution overnight to insure a complete fixation of the tissue. The brain was then transferred in a 30% sucrose solution for 48h to remove the water molecules from the tissue before being flash-frozen with dry ice-cold 2-methylbutane. The brain was then stored at – 20°C.

4.2. Immunohistochemistry

For the investigation of the activation pattern during social fear extinction and/or mating, the cFos protein and the phosphorylated state of the kinase Erk (pErk) have been chosen as markers of cellular activation. cFos is the product of the immediate-early gene fos gene which is one of the first genes being translated following cell activation. The peak of cFos expression is approximatively after 90 min (Curran and Morgan, 1995). pErk is immediately measurable after stimulation (Trainor et al., 2010).

Neuronal activation during the social fear extinction To reveal the process of social fear extinction, I used cFos expression as a marker of neuronal activity. Male mice were subjected to the social fear acquisition, in which a set of the mice was unconditioned while the rest was conditioned. On the following day, the extinction took place. 6 SFC^- and 6 SFC^+ mice were sacrificed 90 min after exposition to the first social stimulus (SS1) of the extinction in order to compare the different cFos expressions into targeted brain regions depending on the conditioning. 7 SFC^+ mice were sacrificed 90 min after the exposition to the sixth social stimulus (SS6) in order to compare the different cFos expressions into targeted brain regions depending on the social fear extinction. Then, the groups SFC^-/SS1 and SFC^+/SS1 were compared to each other to assess the conditioning effect on cFos expression at the start of the extinction procedure, and the groups SFC^+/SS1 and SFC^+/SS6 were compared to each other to assess the effect of the extinction process on cFos expression.

After the removal and fixation of the brain, the frozen brains were sliced using a cryo Leica CM3050S (Leica Microsystems). 30 μm-slices were collected in series from the LS, PVN, SON, and CeA. The slices were stained following different protocols in order to detect the cFos-positive neurons in the LS, the OXT neurons cFos-positive in the PVN and SON, or the cFos-positive cells into the CeA. In general, the slices were washed thrice for 10 min in PBS, followed by a 15 min treatment against the endogenous peroxidase (3% H_2O_2 and 10% Methanol in 1X PBS - 0,3% Triton). The slices were then washed 3 times for 10 min in 1X PBS – 0,3% Triton before to be incubated in a blocking solution (5% Normal Goat serum in 1X PBS – 0,3% Triton) for 1h at room temperature. After blocking, these slices were then incubated with the primary antibodies diluted in blocking solution for 48h at 4°C (See table 2 for dilution). For the LS and the PVN/SON slices, an immunofluorescence staining was performed to allow the detection of several targets on the same slice. The next steps were then all performed in the dark to preserve the fluorescence. The slices were washed for 3 times 10 min in 1X PBS – 0,3% Triton to remove the primary antibodies which did not bound before to be incubated with the secondary antibodies coupled with fluorescent protein diluted in blocking solution for 2h at room temperature (1:1000, conjugated with a CF dye 488, 561 or 647, Biotum). The slices were once again washed three times 10 min and mounted on a slide and covered. For the CeA slices, a chromogenic or DAB immunostaining was performed as only one target needed to be detected. This method is more stable and easier to conserve than a fluorescence

immunostaining. After incubation with the primary antibody, the slices were washed 3 times for 10 min in 1X PBS – 0,3% Triton and incubated with a biotinylated secondary antibody for 2h at room temperature. They were washed then again 3 times for 10 min and were incubated an ABC solution (ABC kit, PK-4000, Vector Labs) for 1h at room temperature. After 3 times 10-min washes, they were incubated with a DAB solution containing nickel (DAB kit, SK-4100, Vectors Labs), the reaction was stopped after approximately 2 min. The slices were washed 6 times for 10 min before being mounted and covered.

Differential neuronal activation during mating and social fear extinction. In order to understand the effect of ejaculation on social fear extinction and what makes the difference between a male that mated and one that ejaculated, an experiment aiming to compare the different patterns of neuronal activation in between mice that mated with or without ejaculation and their respective social fear extinction has been conducted. 10 CD1 mice were conditioned with the SFC protocol. 24h later, the mice were allowed to mate with a primed female. Each mouse that ejaculated was separated from its female partner. At precisely the same time point another mouse that had not yet ejaculated was also separated from its mating partner. This protocol allowed me to control for an influence of the duration of the social contact with the female on social fear extinction and cFos or pErk expression. The extinction of the social fear was started 1h03min after ejaculation (or after the removal of the female mating partner). The extinction protocol was stopped after the second social stimulus, as this is the time point where visible behavioral differences emerge between the mice that did ejaculate and the other ones. Thus, I chose this time point to assess if the behavioral differences were reflected in the pErk expression level. The duration of this shortened social fear extinction was 27 min. After the second social stimulus, the mice were immediately deeply anesthetized before to be perfused, meaning that the sacrifice and perfusion were approximately 90 min after mating/ejaculation (peak of cFos expression) and within 10 min (peak of pErk expression) after the extinction of social fear. After the perfusion, the brains were stored in a 4% PFA solution pH 7.4 overnight and moved into a 30% sucrose solution for 48h. They were then frozen and conserved at -20°C.

30 µm-slices were collected in series from the LS, PVN, SON, and MPOA. The slices were stained in order to detect the cFos- and pErk-positive neurons as well as OXT neurons. In general, the slices were washed 3 times for 10 min in PBS, followed by 10 min in 100% ice-cold

methanol. The slices were then incubated for 20 min in a glycine buffer 0.1M and washed 3 times for 6 min in 1X PBS – 0,3% Triton before to be incubated in a blocking solution (5% Normal Goat serum in 1X PBS – 0,3% Triton) for 1h at room temperature. After blocking, the slices were incubated for 64h with the primary antibodies diluted in blocking solution at 4°C (See table 3 for dilution). The next steps were all performed in the dark to preserve the fluorescence. The slices were washed for 3 times 10 min in 1X PBS – 0,3% Triton to remove the unbound primary antibodies before incubation with the secondary antibodies coupled with a fluorescent protein diluted in 1X PBS – 0,3% Triton for 2h at room temperature (1:1000, conjugated with a CF dye 488, 561 or 647, Biotum). The slices were once again washed 3 times 10 min before to be mounted on a slide and covered.

Primary antibodies	Dilution
Rabbit anti-cFos (Abcam)	1:20 000, incubation 48h 4°C
Mouse anti-neurophysin-OXT (p38), kindly provided Dr. Harold Gainer (NIH, Bethesda)	1:10 000, incubation 48h 4°C
Rabbit anti-pERK	1:1000, incubation 64h 4°C
Sheep anti-cFos (Abcam)	1:10 000, incubation 64h 4°C

Table 3. List of the primary antibodies used during this thesis.

4.3. Image Analysis and Cell Quantification

Systematic random sampling was used through the different target regions by counting the cells on both hemispheres of each section in 1:4 series (40 µm apart).

Neuronal activation during the SFC extinction. The pictures from the LS and the PVN were obtained using a Leica SP8 Confocal Laser Scanning Microscope system (Leica Microsystems). Because of technical issues, a Leica AF6000LX fluorescence microscope equipped with a Leica DFC7000 GT digital camera (Leica Microsystems; courtesy of Prof. Eugen Kerkhoff, Department of Neurology, Regensburg, Germany) was used to image the SON. Z-stack images with a 2.41 µm (LS) or 1 µm (PVN/SON) step size were collected using a 20x

objective. For bright-field images, a Leica THUNDER Imager Tissue upright microscope (Leica Microsystems) was used with 10X objective.

Differential neuronal activation during mating and social fear extinction. The pictures from the LS, the MPOA and, the PVN were obtained using a Leica THUNDER Imager Tissue upright microscope, 20x objective (Leica Microsystems).

Cell quantification was performed using the image processing software package, ImageJ (National Institute of Health, USA; (Schneider et al., 2012)). Systematic random sampling was employed for cFos-positive cell quantifications in the LS, the unbiased Physical Paired Dissector method (Mayhew, 1992) was performed for unbiased stereological estimation of the number of each immunopositive cell phenotype. The total number of both OXT neurons and OXT-positive neurons expressing cFos were counted. All cell numbers are expressed as an average per section.

For area quantification, the area of interest was outlined manually and its area calculated using the image processing software ImageJ.

5. Statistical analysis

Statistical analysis was done using IBM SPSS Statistics 25. Data were analyzed by independent t-tests, paired t-tests, one- and two-way analysis of variance (ANOVA) for repeated measures, corrected with Greenhouse-Geisser when the sphericity was not assumed and followed by a Bonferroni posthoc when appropriate or other when specified.

RESULTS

1. Investigation of the effect of sexual behavior on social fear in male mice: involvement of the OXT system

1.1. Establishment of the priming and mating experiment

The initial protocol for the mating experiment consisted of priming the females with estrogen 48h prior to the experiment and, on the day of the experiment, allowing the male to mate for 1h with a receptive female. In the case the female was not receptive, it was replaced by another female. However, I realized that this protocol was not optimal, as approximately 33% of males were not mating with the females (Figure 10B), as the females were either not in a receptive phase of the cycle (proestrus/oestrus) or too stressed to mate. After trying several protocols, I selected the most efficient one (Figure 10A): the males were "trained" for mating 48h before the mating experiment, to avoid any long-term effect of the training on the experiment. During the male "mating training", a sexually experienced female was introduced into the cage of a male for 30 min. 38h prior to the experiment, I primed the female with estrogen, and 24h after priming, I assessed the estrous cycle of the females by sampling a vaginal smear, and "trained" them to mate. After observation under the microscope of the vaginal smear, the females in proestrus/estrus were classified as receptive (R; ~75%) while the females in the metestrus/diestrus phases were non-receptive (nR; ~25%; Figure 10C). For the female "mating training", a test female is introduced into the cage of a sexually experienced male. However, most females refused to mate during the training, but those who did were selected as suitable for the next day mating experiments. For both male and female training, the female was removed from the male cage when a mounting with intromissions was observed. Then, no ejaculation occurred during the training. The aim of the "mating training" is to familiarize the animal with the mating procedure and to give them a mating experience. On the day of the experiment, the females that were receptive when the vaginal smear has been taken are used in priority. With this protocol, the rate of males that mated during the experiment raised to ~98% in comparison to ~67% with the old protocol. Accordingly, the rate of males that did not mate at all decreased to ~2% in comparison to ~33% with the old protocol (Figure 10B).

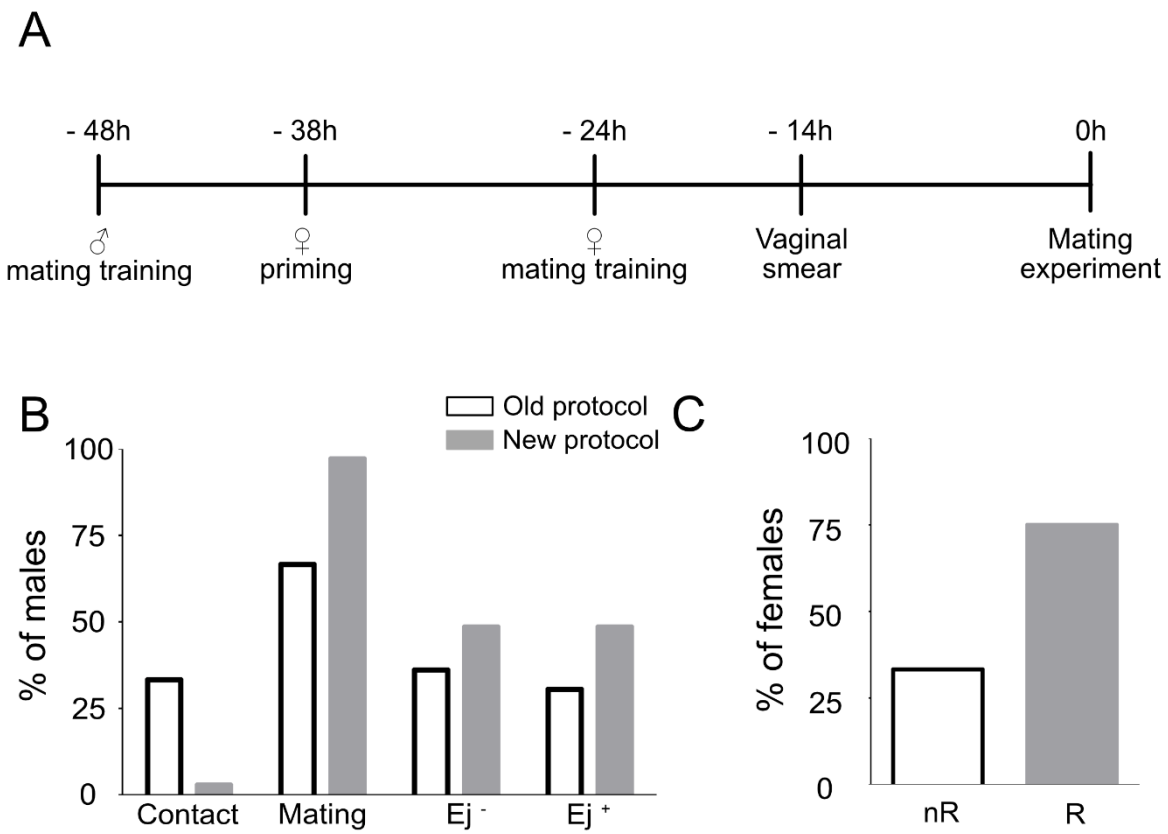

Figure 10. Improvement of the protocol for mating experiments. The protocol for mating experiments is now composed of 3 steps, the training of the males 48h prior experiment, the priming of the females 38h prior experiment, and the training of the females (- 24h) plus the analysis of the vaginal smear (-14h) prior experiment (A). This protocol increased the percentage of males mating (B) and females in a receptive state (R) vs. in a non-receptive state (nR) (C). Mating = all mice that mated with or without ejaculation; Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation.

1.2. Behavioral characterization of the effect of mating on anxiety-like, fear and social behavior

Mating effect on social fear extinction

To determine whether mating affects social fear extinction, male mice were allowed to mate for 1 h before being subjected to the social fear extinction. This experiment was performed in 2 sets. As no significant difference could be found between the preliminary experiment performed by a previous student and the experiment performed within this thesis, the social fear extinction and recall results have been pooled and are presented here (Figure 11C and D). The data presented on the figures 11B and C were only obtained from the repeated experiment, as no record from the previous experiment could be found (Figure 11A and B). Mice received an equivalent number of shocks during the social fear acquisition (Figure 11B).

It is interesting to note that even though the mice have been properly conditioned (as reflected by the low social contact at the first social stimulus); the mating behavior seems to be intact (Figure 11C). During social fear extinction, mice showed a similar investigation of the non-social stimuli. At the presentation of the first social stimulus, all mice showed a low time of social investigation, reflecting social fear. Over the presentation of the 5 other stimuli, the level of social investigation increased for all conditioned groups, however, the Ej^+ mice showed a higher amount of social investigation over the social fear extinction, indicating that these mice extinguished social fear faster than the NM and Ej^- groups (Figure 11D). During the recall, a difference can still be found between the Ej^- and Ej^+ groups, only at the first stimulus (Figure 11E). For detailed statistics, see table 4.

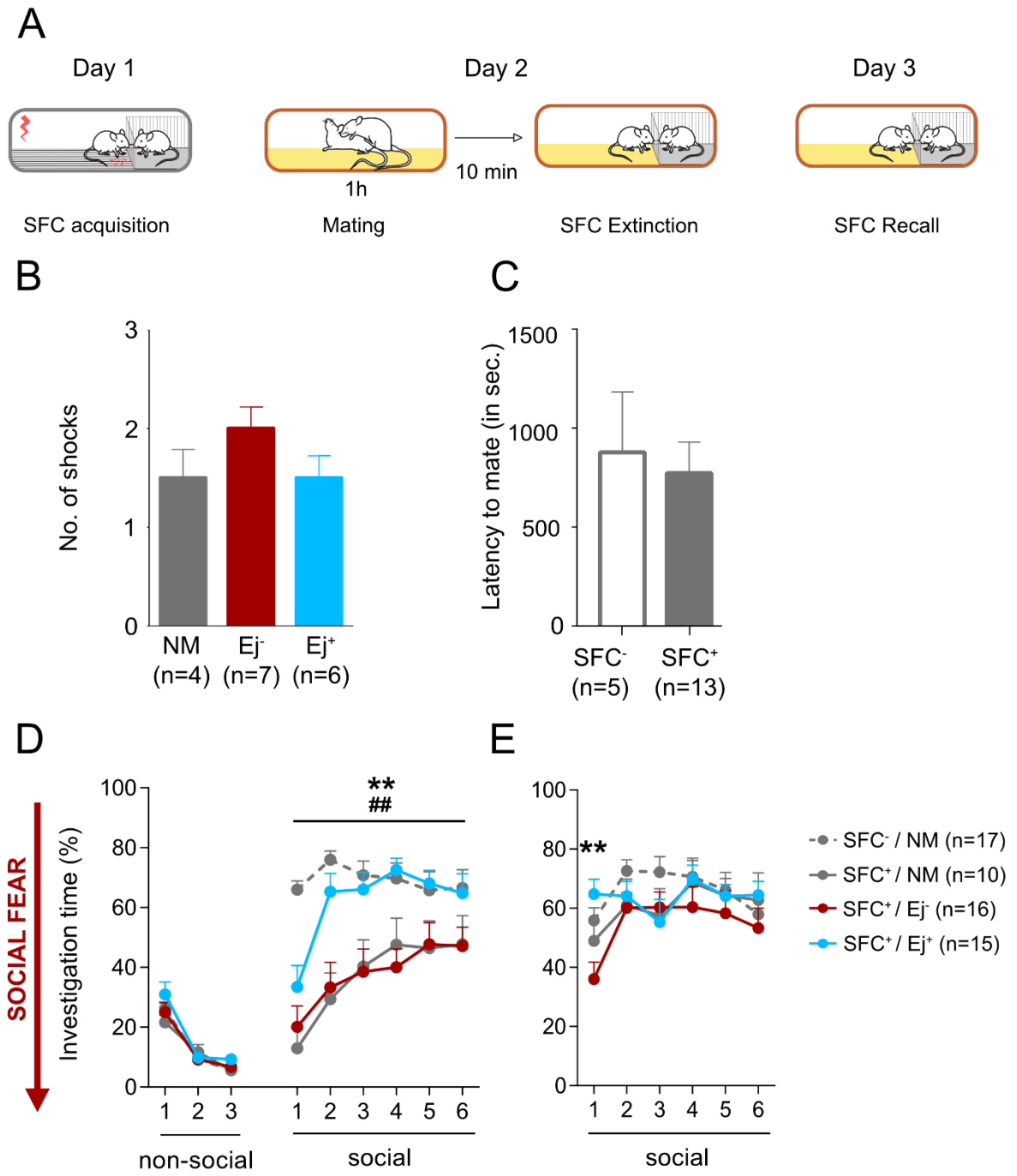

Figure 11. Ejaculation prior social fear extinction facilitates extinction. (A) Experimental design: 24h after the acquisition, the mice were allowed to mate for 1h before to perform the SFC extinction. 24h later, the SFC recall was performed. (B) Social fear acquisition and (C) Mating behavior. The mice did not differ in the number of shocks received during the acquisition, neither in the latency to mate. During social fear extinction (D), the mice that ejaculated extinguished the social fear faster than the two other groups. During the recall (E), the mice that did not ejaculate are still fearful at the first stimulus. SFC^- = unconditioned; SFC^+ = conditioned; NM =non mating; Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation. Data presented in mean ± SEM. ** $p<0.01$ Ej^+ vs. Ej^- ; ## $p<0.01$ Ej^+ vs. NM.

Figure 11	Test	Effect	Pair-wise comparisons
B	No. of shocks *One-way ANOVA*	Mating F(2,1.601)=0.236	
C	Latency mating *t-test for equality of means*	T(6.283)=0.304, p=0.771	
D	SFC extinction (non-social stimuli) *Two-way ANOVA repeated measures*	**Stimuli** **F(2,76)=73.890, p=0.0001** Mating F(2,38)=1.182, p=0.318 Stimuli*mating F(4,76)=1.791, p=0.139	
D	SFC extinction (social stimuli) *Two-way ANOVA repeated measures*	**Stimuli** **F(5,190) = 16.510, p=0.0001** **Mating** **F(2,38)=7.089, p=0.002** Stimuli*mating F(10,190) = 0.865, p= 0.567	Social Stimulus 1-6 NM vs. Ej^- p=1.000 **NM vs. Ej^+ p=0.012** **Ej^- vs. Ej^+ p=0.005**
E	Recall *Two-way ANOVA repeated measures*	**Stimuli** **F(5,190) = 4.393, p=0.001** Mating F(2,38)=0.957, p=0.393 **Stimuli*mating** **F(10,190) = 1.842, p= 0.056**	Social Stimulus 1 NM vs. Mating p=0.405 NM vs. Ej^+ p=0.221 **Ej^- vs. Ej^+ p=0.002** Social Stimulus 2-6 p>0.05 for all comparisons

Table 4. Statistic tables for the effect of mating on SFC.

Effect of mating on CFC

As ejaculation had no specific effect on anxiety-like and social behavior, I decided to evaluate the effect of mating on a non-social fear conditioning paradigm. The mice were allowed to mate for 1h before the cued fear extinction (Figure 12A). No significant difference could be found between NM, Ej^- and Ej^+, neither during the acquisition, extinction, and recall (Figure 12B, C, and D). For detailed statistics, see table 5.

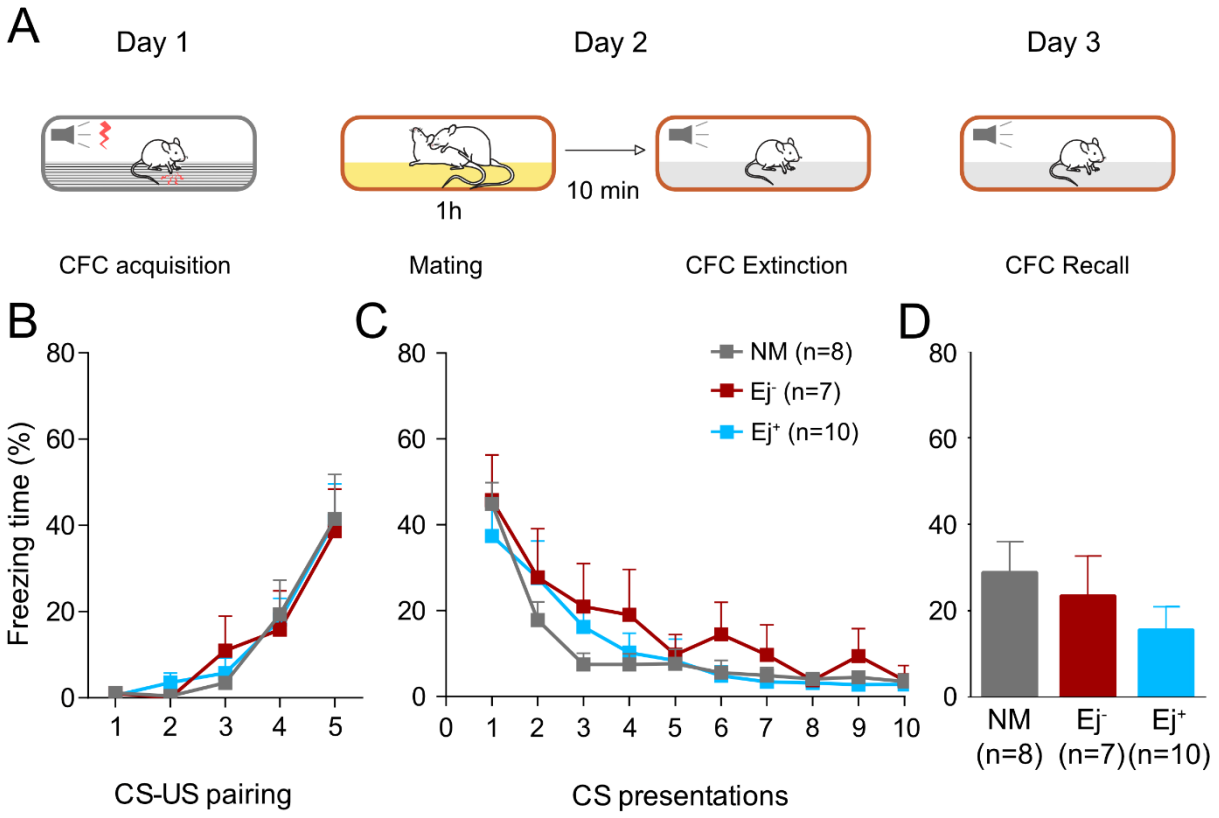

Figure 12. Mating does not affect CFC. (A) Experimental design: the mice were conditioned on day 1. On day 2, the mice were allowed to mate for 1h before to perform the CFC extinction. On day 3, the mice went through the CFC recall. During the CFC acquisition (B), extinction (C) and recall (D), no difference was found between the groups. NM =non mating; Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation. Data presented in mean ± SEM. *** p<0.001.

Figure 12	Test	Effects	
B	Acquisition *Two-way ANOVA repeated measures*	**CS** **F(4,88)=32.073, p=0.0001** Mating F(2,22)=0.009, p=0.991	Side*Mating F(8,88)=0.217, p=0.928
C	Extinction *Two-way ANOVA repeated measures*	**CS** **F(9,198)=26.819, p=0.0001** Mating F(2,22)=0.569, p=0.574	Side*Mating F(18,198)=0.659, p=0.686
D	Recall *One-way ANOVA*	F(2,22)=0.928, p=0.410	

Table 5. Statistic tables for the effect of mating on CFC.

Mating effect on anxiety-like behavior

To understand this effect of ejaculation on social fear extinction, I decided to investigate the effect of ejaculation on anxiety-like behavior. I hypothesized that a less anxious animal would approach the social stimulus much faster than the more anxious one. As mating is known to

reduce anxiety-like behavior in male rats, I investigated the effect of mating without or with ejaculation on anxiety-like behavior in mice using two tests: the LDB and the marbles burying test. I chose these two tests based on the literature. Mating has been shown to have an anxiolytic effect in the LDB in male rats in our lab (Waldherr and Neumann, 2007). However, the studies of the group of Fernández-Guasti used the defensive probe burying test to highlight a particular anxiolytic effect in male rats (Fernández-Guasti et al., 1989). This test requires a special apparatus that we don´t have in our lab. However, I tried to recreate this test in our lab with the available equipment and chose to perform the marbles burying test. Male mice were then allowed to mate for 1h before they underwent either the LDB or the marbles burying test (Figure 13A). In the LDB, Ej^- and Ej^+ mice showed a reduced anxiety-like behavior, reflected by a significant increase in the time spent in the light compartment (Figure 13B). No significant difference was found in the number of entries in the light compartment and the distance moved, indicating that the locomotion of these mice was not changed following mating (Figure 13C and D). In the marbles burying test, no statistical significance was found but a trend ($p=0.057$), indicating that mice which ejaculated might burry less than the non-mating and mating without ejaculation mice (Figure 13E). For detailed statistics, see table 6.

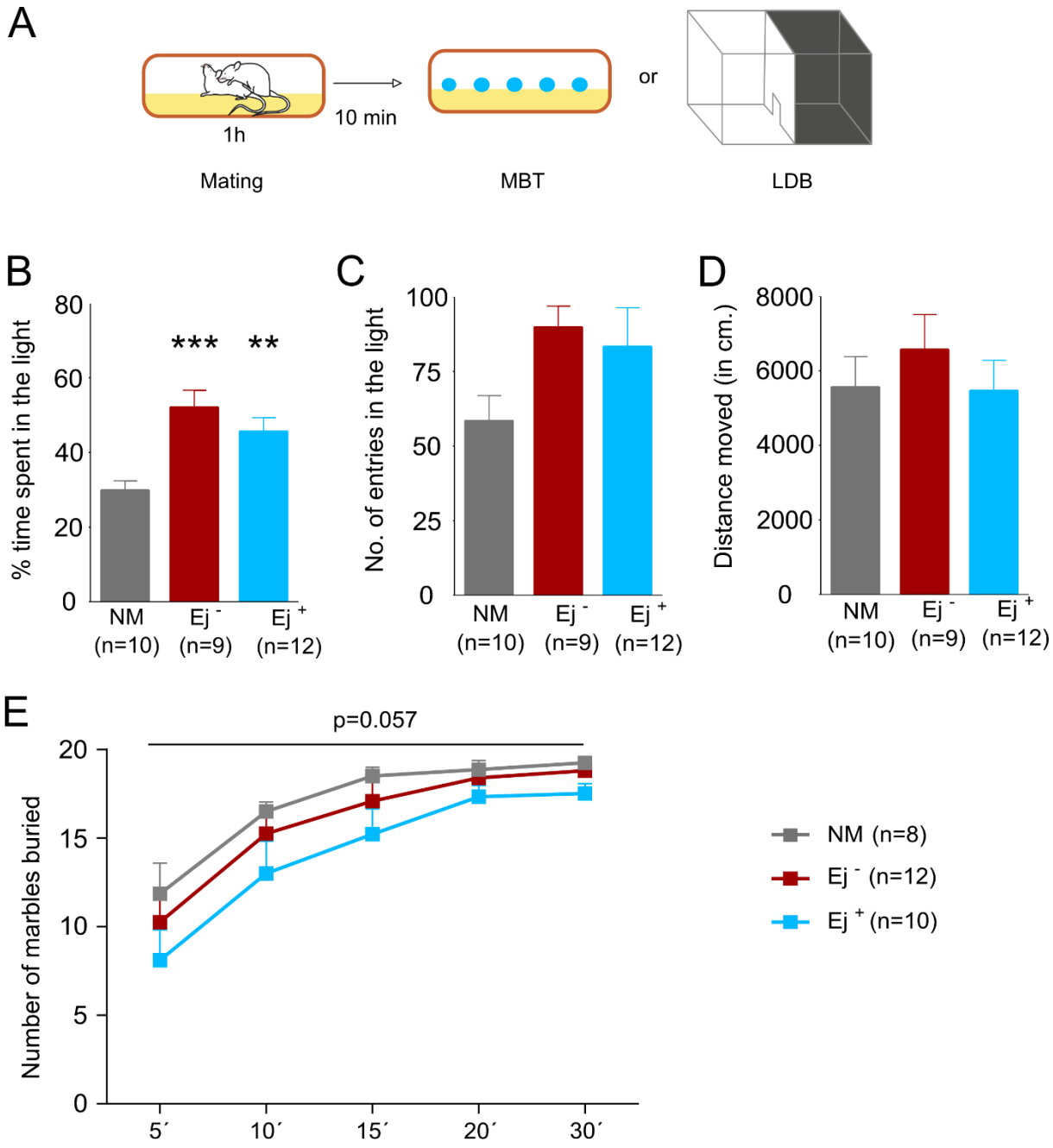

Figure 13. Mating has an anxiolytic effect in male mice. (A) Experimental design. Mice were allowed to mate for 1h before to undergo either the LDB or the marbles burying test. In the LDB, Ej^- and Ej^+ showed a decrease in anxiety-like behavior (B: time spent in the light compartment), and without modification of the locomotor behavior (C: number of entries in the light compartment and D: distance moved in the LDB). In the marbles burying test (E), no statistical difference could be found but a strong trend (p=0.057) indicating that Ej^+ mice tended to burry less marbles. NM =non mating; Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation. Data presented in mean ± SEM. *** $p<0.001$ Ej^- vs. NM; ** $p<0.01$ Ej^+ vs. NM.

Figure 13	Test	Effects	Pair-wise comparisons
B	LDB (% of time) *One-way ANOVA*	**Mating** **F(2,28)=8.528, p=0.001**	**NM vs. Ej$^-$ p=0.001** **NM vs. Ej$^+$ p=0.016** Ej$^-$ vs. Ej$^+$ p=0.719
C	LDB (no. of entries) *One-way ANOVA*	Mating F(2,28) = 2.256, p=0.124	
D	LDB (distance moved) *One-way ANOVA*	Mating F(2,28) = 0.459, p= 0.637	
E	Marbles burying test *Two-way ANOVA repeated measures*	**Time** **F(4,27) = 94.444, p=0.0001** Mating F(2,27) = 3.194, p=0.057 Time*Mating F(2,27) = 0.337, p=0.717	LSD post-hoc test NM vs. Ej$^+$ p=0.026 Ej$^-$ vs. Ej$^+$ p= 0.061

Table 6. Statistic tables for the effect of mating on anxiety-like behavior.

Mating effect on social behavior in the SPT and SNT

The social fear extinction possesses also a social component, which might contribute to the effect of the ejaculation on social fear extinction. Hence, I decided to test the mice in the SPT and SNT after mating (Figure 14A). The hypothesis was that, after mating, mice might be more attracted by a social stimulus, which may lead to facilitated social fear extinction in the SFC paradigm. Mating did not affect social behavior, as evidenced in the SPT (Figure 14C) or SNT (Figure 14D). During the habituation, all groups spent equivalent time in the left and right compartment (Figure 14B). In the SPT, all groups showed social preference (Figure 14C) and all groups were able to recognize the novel social stimulus in the SNT (Figure 14D). For detailed statistics, see table 7.

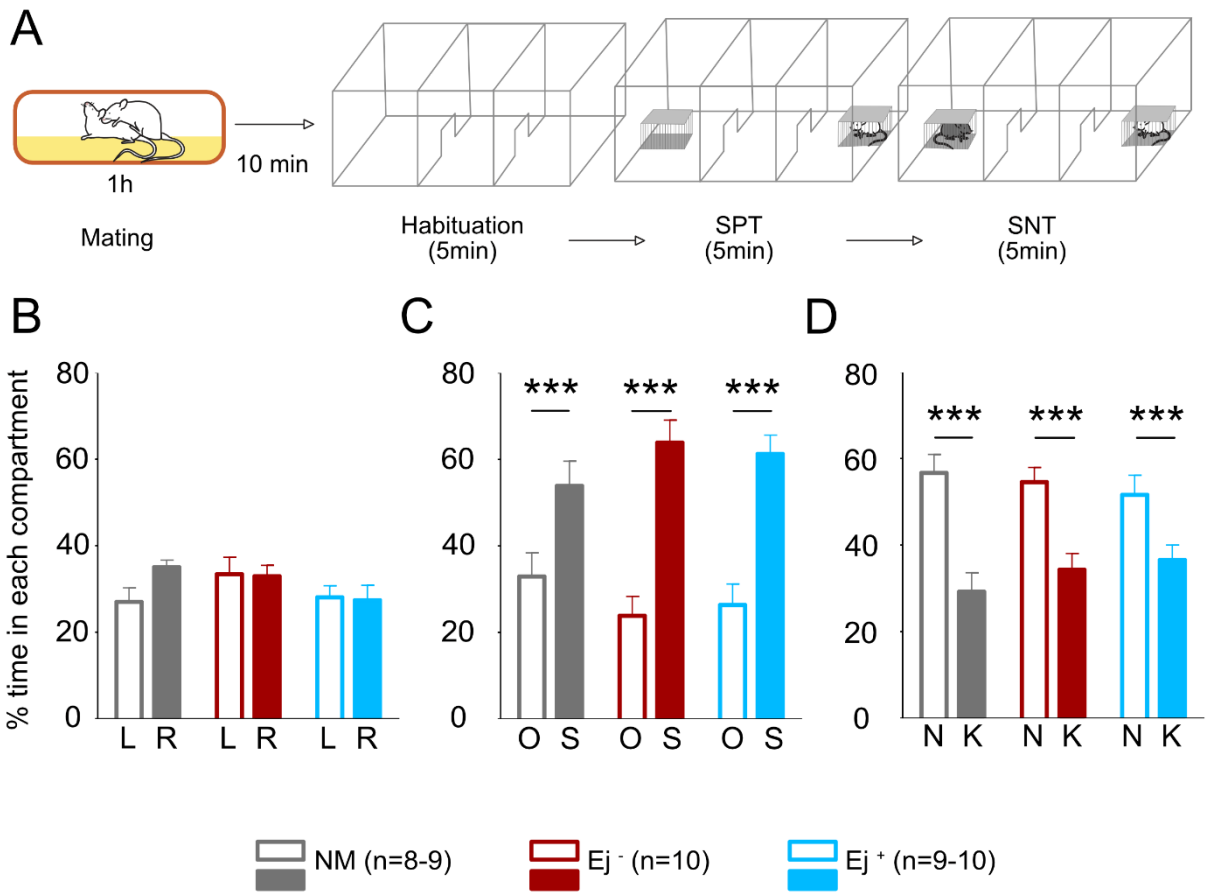

Figure 14. Mating does not modify social behavior in the SPT and SNT. (A) Experimental design. Mice were allowed to mate for 1h before to be introduced into the three-chambered box for 5 min habituation, followed by 5 min SPT and 5 min SNT. (B) Habituation. (C) SPT, O=Object and S=social. (D) SNT, N=novel and K=known. No difference have been found between NM, Ej^- and Ej^+ in the SPT (C) or SNT (D). NM =non mating; Ej^- = mating without ejaculation; Ej^+ = mating with ejaculation. Data presented in mean ± SEM. *** p<0.001.

Figure 14	Test	Effects	
A	Habituation *Two-way ANOVA repeated measures*	Side F(1,25)=0.538, p=0.470 Mating F(2,25)=1.653, p=0.212	Side*Mating F(2,23)=1.467, p=0.250
B	SPT *Two-way ANOVA repeated measures*	**Side** **F(1,23)=33.776, p=0.0001** Mating F(2,23)=0.020, p=0.980	Side*Mating F(2,23)=1.081, p=0.356
C	SNT *Two-way ANOVA repeated measures*	**Side** **F(1,25)=22.032, p=0.0001** Mating F(2,25)=0.668, p=0.522	Side*Mating F(2,25)=0.634, p=0.539

Table 7. Statistic tables for the effect of mating on social behavior in the SPT and SNT.

1.3. Summary of the behavioral experiments

I could find a specific enhancing effect of ejaculation on SFC extinction, with no effect on CFC. Moreover, I could confirm that mating behavior has an anxiolytic effect in LDB, which was already founded in rats. However, it seems that the mice that ejaculated bury less than the other groups. No modification of the social behavior in the SPT nor SNT could be found.

1.4. Neuronal activation pattern during SFC extinction

The neuronal circuitry underlying sexual behavior is quite well known. However, the mechanisms allowing social fear extinction are still under investigation. As the effect of ejaculation was specifically observed on social fear extinction, I aimed to reveal the process of social fear extinction and used cFos expression as a marker of neuronal activity at the start and the end of the social fear conditioning procedure. With this experiment, I wanted to have an indication as to which brain regions might be involved in this process. As it is known that social fear extinction is dependent on the OXT system within the LS (Zoicas et al., 2014; Menon et al., 2018), I targeted the PVN and SON - where the OXT neurons are located -, the LS and the CeA – brain region known to be involved in fear acquisition and extinction. The mice went through the social fear acquisition, during which some were unconditioned (SFC^-) and the others conditioned (SFC^+). On the second day, the social fear extinction was performed. In order to visualize a modification of the cFos expression pattern during extinction, the mice were sacrificed either after the first social stimulus (SS1, the start of the extinction process), either after the sixth social stimuli (SS6, end of the extinction process). In either case, the sacrifice and perfusion of the animals were 90 min after exposure, the peak of cFos protein expression. Consequently, I analyzed the effect of the conditioning by comparing the SFC^- and SFC^+ mice at the first stimulus, and the effect of the extinction process by comparing SFC^+ after the first or the last stimuli (Figure 15A). During the social fear extinction, the SFC^+ mice showed social avoidance at the first stimulus, which is a sign of social fear. On the contrary, SFC^- mice showed a high level of social investigation. For the group SFC^+/SS6, the mice showed social fear extinction, as the level of social investigation increased over time. However, two animals have been excluded from this group, as they did not extinguish the social fear (Figure 15B). Within the PVN and SON, the percentage of OXT neurons that are expressing the protein cFos has been counted (Figure 15C). No statistical difference could be found. However, the high variation in the SFC^+ groups suggested that a higher number of animals might be necessary to

find a statistical difference. Within the LS, a differentiation between the ventral and the dorsal was made, as these two heterogeneous parts of the LS might be regulated by different processes (Figure 15D). In general, the dorsal LS showed a higher amount of cFos-positive cells. However, only in the ventral part, the SFC^+/SS1 mice presented a lower number of cFos-positive cells in comparison to the SFC^-/SS1 mice, indicating that the SFC^+ mice have reduced ventral LS activation when compared to the SFC^- mice. Within the CeA, no difference was observed between the SFC^-/SS1 and the SFC^+/SS1 mice. However, the SFC^+/SS6 showed a significantly higher number of cFos-positive cells than the SFC^+/SS1 mice (Figure 15E). For detailed statistics, see table 8.

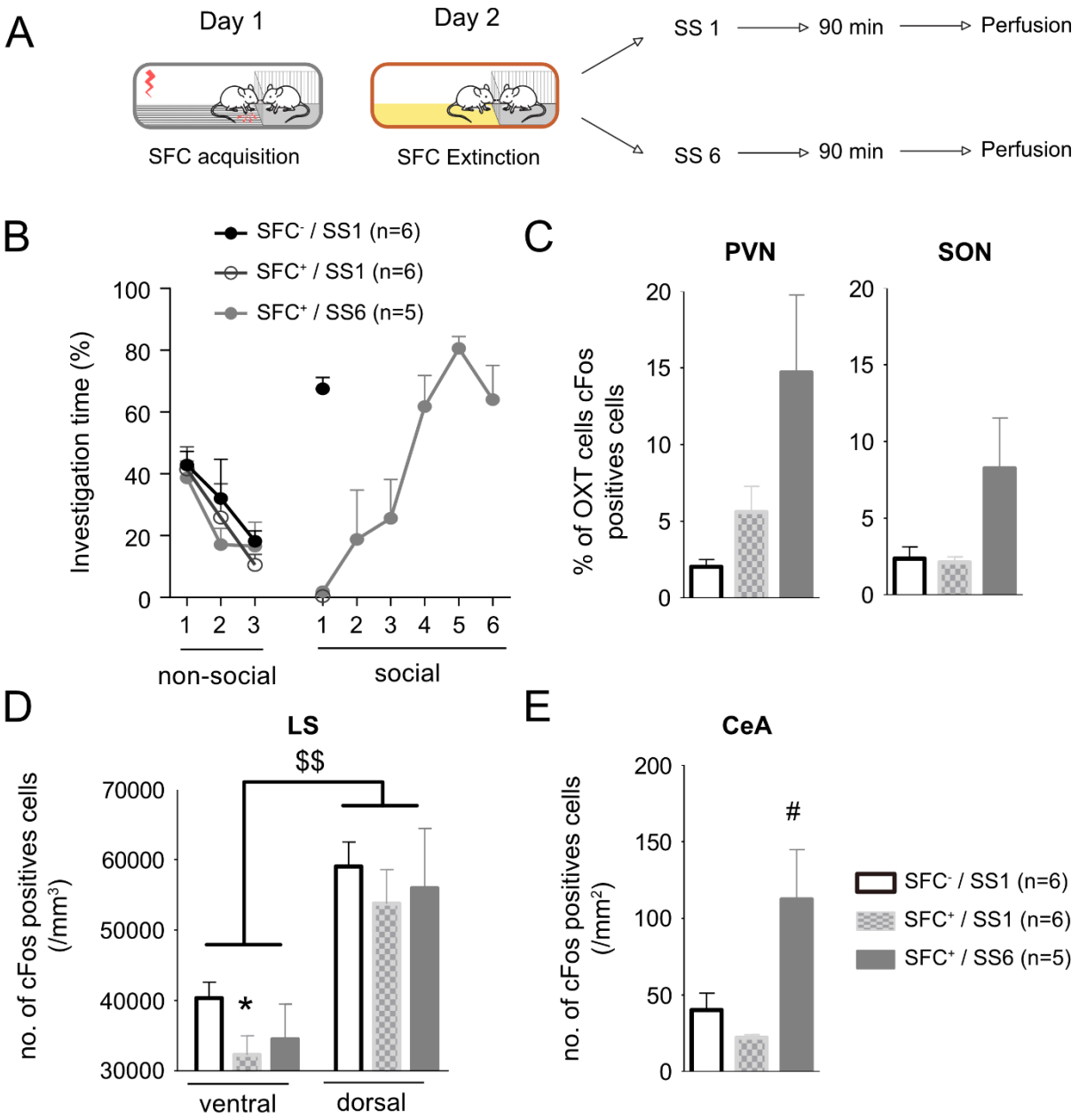

Figure 15. Activation of different brain regions during social fear extinction. (A) Experimental design: the mice were conditioned on day 1. On day 2, the mice underwent the social fear extinction where they were exposed to either one (SS1) or six (SS6) stimuli. 90 min after social exposure, the mice sacrificed. (B) Social fear extinction. The SFC^- mice show a high level of social investigation, while the SFC^+ mice show social avoidance. At the end of the extinction procedure, the mice that received six stimuli show a high level of investigation, a sign of social fear extinction. (C) Percentage of OXT neurons expressing the cFos protein within the PVN and the SON. No statistical difference between the groups could be found. (D) Number of cFos-positive cells within the ventral and dorsal LS. The dorsal part of the LS presented, in general, a higher number of positive cells. In the ventral part, the SFC^+/SS1 group showed less positive cells than the SFC^-/SS1 group. (E) In the CeA, the SFC^+/SS6 mice presented a higher number of positive cells than the SFC^+/SS1. Data presented in mean ± SEM. * $p<0.05$ SFC^-/SS1 vs. SFC^+/SS1. # $p=0.05$ SFC^+/SS1 vs. SFC^+/SS6. $$ $p<0.01$ ventral vs. dorsal.

Figure 15	Test	Effects	
C	cFos within the PVN *Independent t-test*	Conditioning (SFC^- /SS1vs. SFC^+/SS1) T(6.925)=2.035, p=0.082	Stimuli (SFC^+ /SS1vs. SFC^+/SS6) T(-4.9)=-1.711, p=0.149
C	cFos within the SON *Independent t-test*	Conditioning (SFC^- /SS1vs. SFC^+/SS1) T(11)=-0.315, p=0.759	Stimuli (SFC^+ /SS1vs. SFC^+/SS6) T(4.102)=-1.861, p=0.134
D	cFos within the ventral LS *Independent t-test*	Conditioning (SFC^- /SS1vs. SFC^+/SS1) T(11)=-0.854, p=0.411	Stimuli (SFC^+ /SS1vs. SFC^+/SS6) T(10)=--0.241, p=0.814
D	cFos within the dorsal LS *Independent t-test*	**Conditioning (SFC^- /SS1vs. SFC^+/SS1)** **T(11)=-2.248, p=0.046**	Stimuli (SFC^+ /SS1vs. SFC^+/SS6) T(10)=-0.447, p=0.677
D	cFos within the LS *Paired t-test (ventral vs. dorsal)*	**SFC^- /SS1** **T(5)=-5.896, p=0.002** **SFC^+ /SS1** **T(6)=-6.397, p=0.001**	**SFC^+ /SS6** **T(4)=-4.812, p=0.009**
E	cFos within the CeA *Independent t-test*	Conditioning (SFC^- /SS1vs. SFC^+/SS1) T(5.313)=-1.639, p=0.159	**Stimuli (SFC^+ /SS1vs. SFC^+/SS6)** **T(4.028)=-2.773, p=0.05**

Table 8. Statistic tables for the expression of cFos during SFC extinction.

1.5. OXT release during mating behavior

The previous results indicate that the activity of the LS and CeA is regulated during social fear extinction. Knowing that OXT plays a key role during social fear extinction (Zoicas et al., 2014; Menon et al., 2018), I wanted to know if one of these regions is also involved in mating behavior. More particularly, I wanted to know if OXT is released during mating in one of these regions. It is already known from the literature that OXT is released within the PVN during mating (Waldherr and Neumann, 2007), however, this information is still not available for the LS and the CeA. To obtain this information, I performed a microdialysis experiment, where I measured the release of OXT during mating. Unfortunately, the dialysates had to be collected every 30 min to ensure a sufficient quantity for analysis. No differentiation between Ej^- and Ej^+ mice could be done, as ejaculation is an extremely brief moment and cannot be targeted with this technique. Before mating, two dialysates have been sampled under basal conditions. Then, a female was introduce into the cage of the male for 1h in the case of the LS experiment or 1h30 for the CeA experiment. The mating duration has to be extended for the later experiment, as the ejaculation latency was longer for this experiment. Two dialysates for the septum, or three for the amygdala, were collected during mating behavior. After the removal of the female, another dialysate under basal condition was sampled. The microdialysis within

the septum was performed by a previous PhD student. The measurement exclusively in the LS was not possible due to the size of the probe. The sampling was done within the septal area, including lateral septum and medial septum. The analysis of the microdialysis during mating revealed that OXT is specifically released during mating in the septum. No release was found in the male that only had social contact with the female (Figure 16A). For the same reasons, the sampling within the amygdala included not only the CeA but also a part the dorsal MeA and the ventral BLA. In the amygdala, OXT was released in both groups, indicating that the contact with a female also induces the release of OXT in this brain region (Figure 16B). For detailed statistics, see table 9.

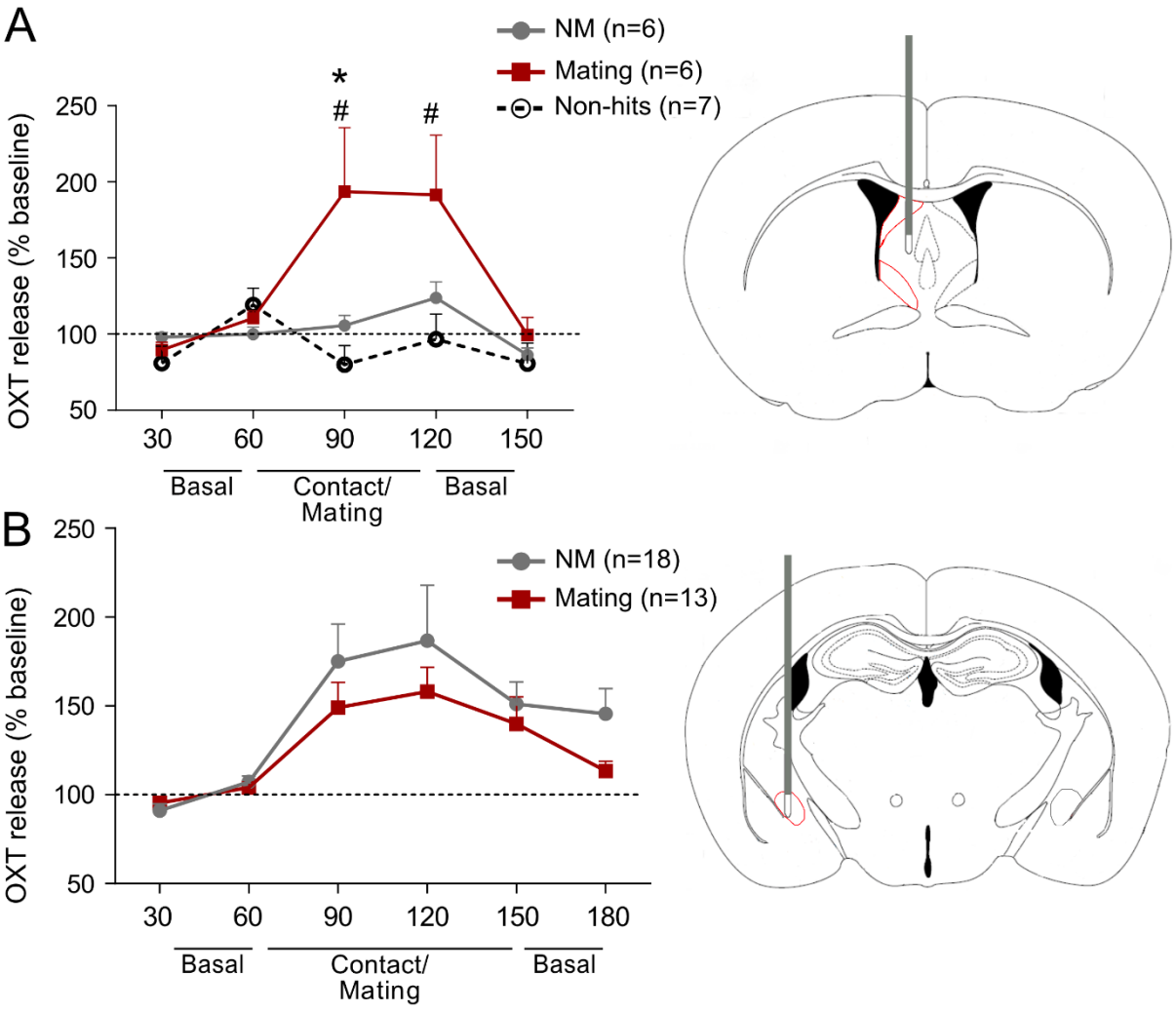

Figure 16. Mating-induce release of OXT in the septum but not in the amygdala. Release of OXT in the LS specifically during mating (A). Within the amygdala, the presence of the female induces release of OXT (B). Data presented in mean ± SEM. Mating = mating with or without ejaculation, NM = in presence of a female but do not show mating behavior. * p<0.05 Mating vs. NM; # p<0.05 Mating vs. Non-hits.

Figure 16	Test	Effects	Pairwise comparisons
A	Microdialysis in the septum *Two-way ANOVA, repeated measures*	**Time** **F(4,64)=5.802, p=0.0001** **Mating** **F(2,16)=5.836, p=0.012** **Time*Mating** **F(4,64)=3.495, p=0.002** **Time*Mating** **F(4,64)=3.495, p=0.002**	At 90min: Mating vs. NM p=0.074 Mating vs. Non-hits p=0.013 At 120 min: Mating vs. Non-hits p=0.041
B	Microdialysis in the amygdala *Two-way ANOVA, repeated measures*	**Time** **F(5,65)=9.719, p=0.0001** Mating F(1,13)=0.013, p=0.912 Time*Mating F(5,65)=0.596, p=0.703	

Table 9. Statistic tables for the microdialysis within the septum and the amygdala during mating.

1.6. i.c.v. administration of OXTR-A after mating prior social fear extinction

According to the previous results, OXT seems to be a common link between mating behavior and social fear extinction. Indeed, a central release of OXT occurs during both social fear extinction and mating behavior, particularly in the LS. Before to perform any local experiment, I performed an i.c.v. experiment to block the OXT system by infusing a specific OXTR-A. With this experiment, I aimed to highlight the involvement of the OXT system in a general manner. After surgery, the mice were kept single-housed until the social fear acquisition. 24h after, the mice were allowed to mate for 1h. The infusion of Veh or OXTR-A was done 10 min after the end of the mating procedure. The social fear extinction started 30 min after infusion (Figure 17A). The i.c.v. infusion of the OXTR-A has to be done after mating since central OXT is needed for penile erection and mating behavior. Preliminary results of this experiment showed a loss of the beneficial effect of ejaculation on extinction in Veh animals. In this protocol, the mice were single-housed for 7 days after surgery in order to get a full recovery. I hypothesized that this social isolation might be the reason for the loss of the ejaculation effect (Han et al., 2018). Thus, for this experiment, the mice were single-housed for only 3 days after the surgery. During the social fear acquisition, all the groups received the same amount of shocks (Figure 17B). No difference was found in the non-social stimuli investigation (Figure 17C). The OXTR-A groups showed a reduced investigation of the social stimuli, which indicates an impairment of the social fear extinction. However, no difference was found between the SFC^{+}/Ej^{-}/Veh and

Ej⁺/Veh. (Figure 17C). It seems then that the loss of the ejaculation effect was not due to the social isolation but might be due to either the surgery or the infusion procedure. The extinction recall is under analysis. For detailed statistics, see table 10.

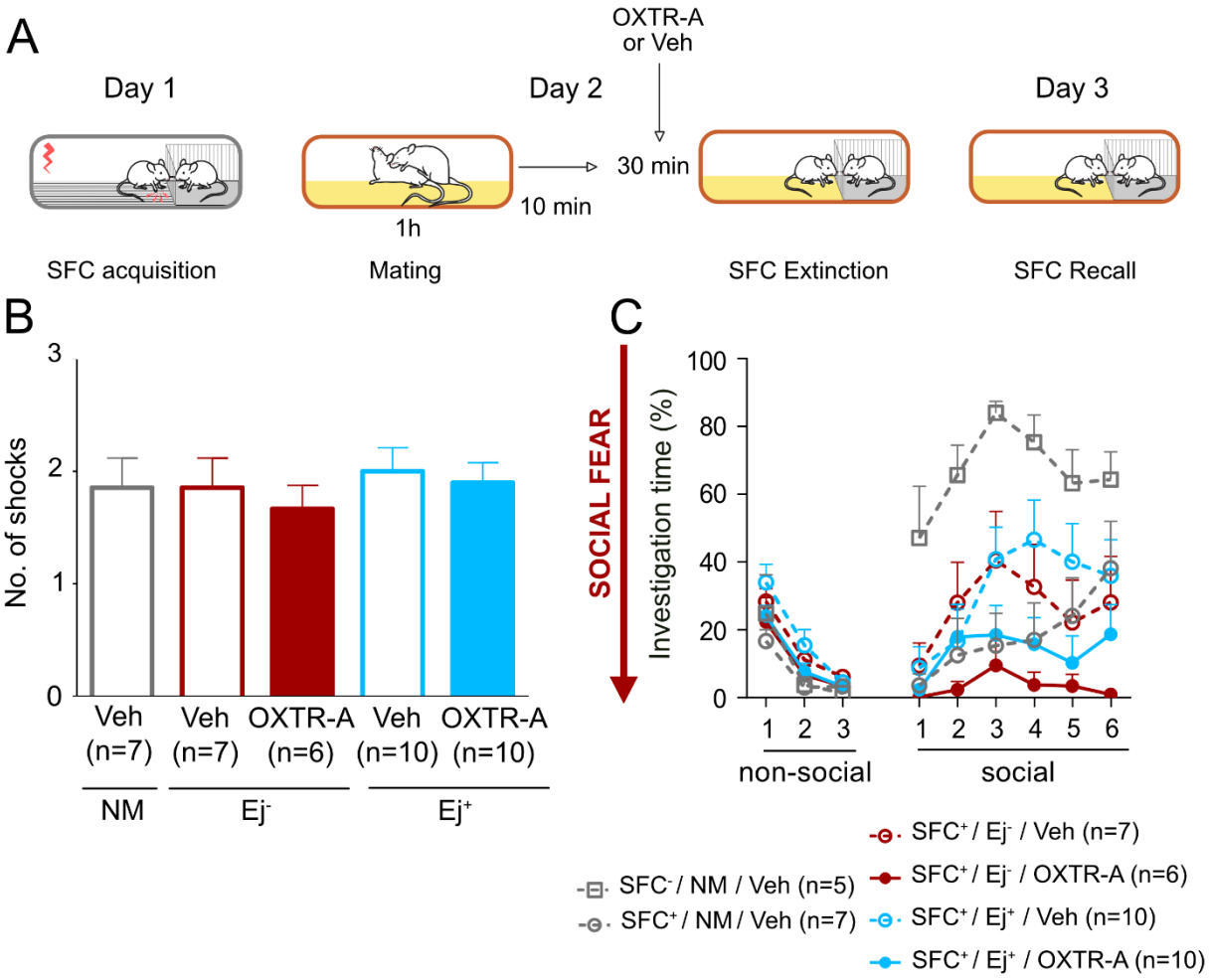

Figure 17. i.c.v. OXTR-A impairs social fear extinction independently of mating behavior. (A) Experimental design: 24h after the acquisition, male mice were allowed to mate for 1h. 10 min after mating, they received either an infusion of Veh or OXTR-A. The extinction took place 30 min after infusion. 24h later, the recall was performed. (B) SFC acquisition, no difference was found between the groups. (C) SFC extinction. No difference was found between SFC⁺/Ej⁻/Veh and SFC⁺/Ej⁺/Veh groups. A main effect of the OXTR-A was found, showing an impairment of the SFC extinction. Data presented in mean ± SEM.

Figure 17	Test	Effect	
B	No. of shocks *One-way ANOVA*	Mating F(1,29)=0.726, p=0.401 Treatment F(1,29)=0.433, p=0.516	Mating*Treatment F(1,29)=0.042, p=0.839
C	SFC extinction (non-social stimuli) *Two-way ANOVA repeated measures* *(Only Ej⁻ and Ej⁺ groups)*	**Stimuli** **F(2,58)=62.342, p=0.0001** Mating F(1,29)=0.469, p=0.499 Treatment F(1,29)=3.916, p=0.057 Mating*Treatment F(1,29)=0.127, p=0.724	Mating*treatment F(2,58)=0.625, p=0.512 Stimuli*mating F(5,145)=0.740, p=0.532 Stimuli*treatment F(5,145)=1.680, p=0.177 Stimuli*mating*treatment F(5,145)=0.925, p=0.433
C	SFC extinction (social stimuli) *Two-way ANOVA repeated measures* *(Only Ej⁻ and Ej⁺ groups)*	**Stimuli** **F(5,145)=5.491, p=0.002** Mating F(1,29)=1.007, p=0.324 **Treatment** **F(1,29)=7.331, p=0.011** Mating*Treatment F(1,29)=0.151, p=0.701	Mating*treatment F(1,20)=0.005, p=0.946 Stimuli*mating F(5,100)=1.230, p=0.303 Stimuli*treatment F(5,100)=0.401, p=0.667 Stimuli*mating*treatment F(5,100)=1.036, p=0.363
C	SFC extinction (social stimuli) *Two-way ANOVA repeated measures (Only Veh groups)*	**Stimuli** **F(5,105)=5.794, p=0.0001** Mating F(2,21)=0.603, p=0.556	Stimuli*mating F(10,105)=1.302, p=0.276

Table 10. Statistic tables for the i.c.v. infusion of OXTR-A after mating prior extinction.

1.7. Septal administration of OXTR-A before mating prior social fear extinction

As i.c.v. OXTR-A was able to impair social fear extinction when administrated after mating in the same manner in both Ej⁻ and Ej⁺ mice, it can be hypothesized that OXT might be involved in the facilitatory effect of ejaculation on social fear extinction. However, the fact that there was no difference between SFC⁺/Ej⁻/Veh and SFC⁺/Ej⁺/Veh might negatively influence this hypothesis. As the main region involved in the effect of OXT in social fear extinction is the LS and OXT is also released in this region during mating behavior. I then decided to inhibit the OXT system within the LS during mating before social fear extinction by locally infusing an OXTR-A. The aim of this experiment was to causally link the OXT release within the LS during mating with the facilitatory effect of ejaculation on social fear extinction. For this purpose, guide cannulas were surgically implanted bilaterally above the LS. The mice were kept single-housed for 7 days after the surgery. As the i.c.v. experiment showed that social isolation was

not responsible for the loss of the ejaculation effect during social fear extinction, I chose to let the mice recover from the surgery for a longer period, which is the standard protocol in our lab and ensures full recovery of the mice. The mice underwent social fear acquisition. 24h later, the mice received either an infusion of Veh or OXTR-A. 10 min after the infusion, the mice were allowed to mate for 1h. 10 min after the end of the mating, the social fear extinction started (Figure 18A). The analysis of the mating behavior showed that the OXTR-A infusion within the LS does not impair sexual behavior (Figure 18B, C, and D). During social fear acquisition, the mice received the same amount of shocks (Figure 18E). No difference has been found during social fear extinction (Figure 18F). As for the i.c.v. experiment, the effect of ejaculation on social fear extinction is lost in the Veh group. Regarding the effect of OXTR-A, its infusion into the LS before mating does not impair social fear extinction. The extinction recall of this experiment is under analysis. For detailed statistics, see table 11.

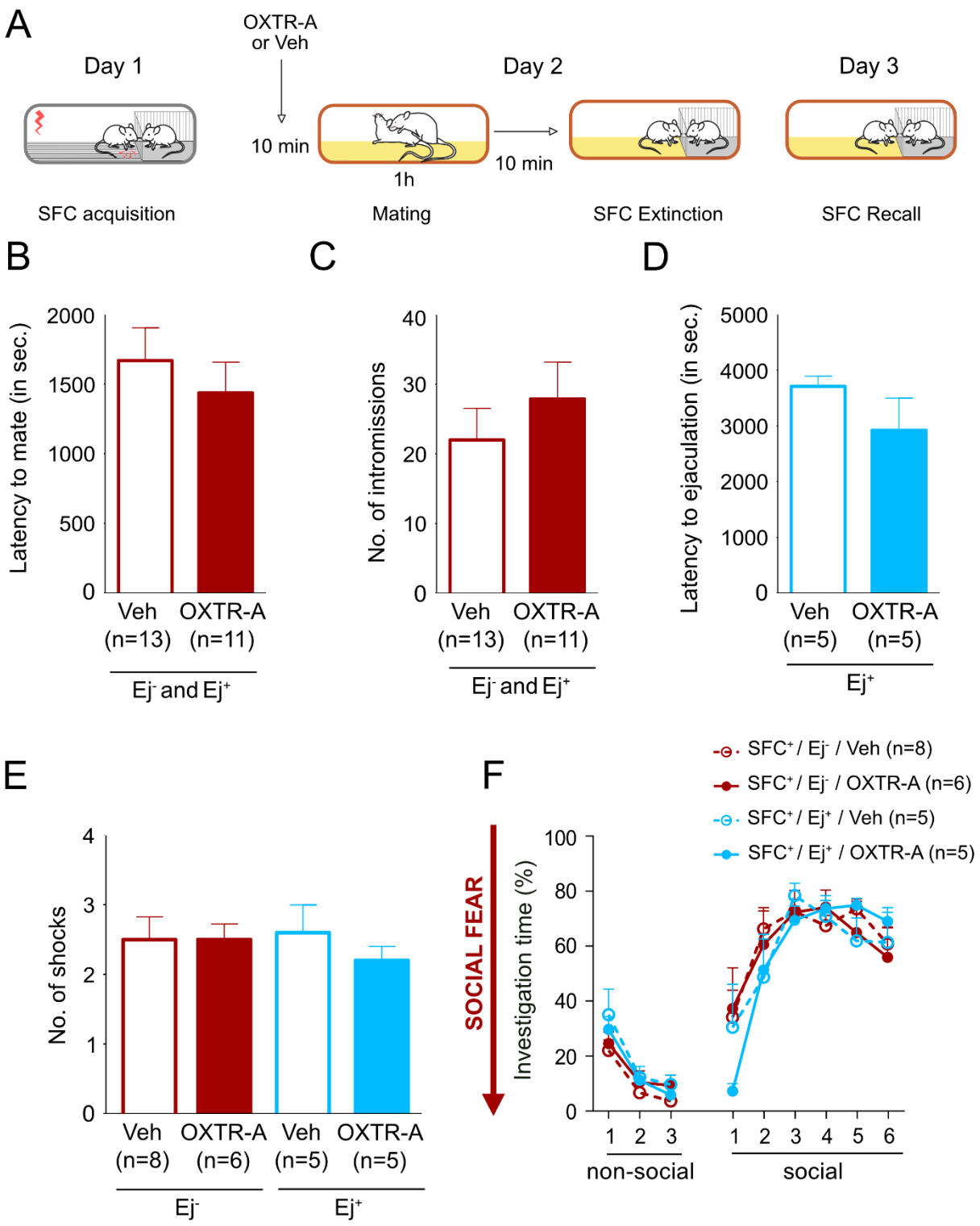

Figure 18. OXTR-A infusion within the LS before mating does not impair social fear extinction. (A) Experimental design: 24h after acquisition, male mice received either an infusion of Veh or OXTR-A 10 before to mate for 1h. 10 min after mating, the SFC extinction started. 24h later, the recall was performed. (B) Latency to mate, this graph combined the Ej^- and Ej^+ mice. (C) Number of intromissions, this graph combined the Ej^- and Ej^+ mice. (D) Latency to ejaculate, only Ej^+ mice. (E and F) No difference was found between the groups. Data presented in mean ± SEM.

Figure 18	Test	Effect	
A	Latency mating *t-test for equality of means*	T(23)=0.473, p=0.641	
B	No. of intromissions *t-test for equality of means*	T(23)=-0.709, p=0.486	
C	Latency ejaculation *t-test for equality of means*	T(9)= 0.907, p=0.388	
D	No. of shocks *One-way ANOVA*	Mating F(1,20)=0.101, p=0.754 Treatment F(1,20)=0.402, p=0.533	Mating*Treatment F(1,20)=0.231, p=0.533
E	SFC extinction (non-social stimuli) *Two-way ANOVA repeated measures*	**Stimuli** **F(2,40)=68.788, p=0.0001** Mating F(1,20)=1.976, p=0.175 Treatment F(1,20)=0.007, p=0.934	Mating*treatment F(1,20)=1.319, p=0.264 Stimuli*mating F(2,40)=2.273, p=0.137 Stimuli*treatment F(2,40)=0.287, p=0.765 Stimuli*mating*treatment F(2,40)=0.180, p=0.748
E	SFC extinction (social stimuli) *Two-way ANOVA repeated measures*	**Stimuli** **F(5,100)=16.455, p=0.0001** Mating F(1,20)=0.704, p=0.411 Treatment F(1,20)=0.091, p=0.766	Mating*treatment F(1,20)=0.005, p=0.946 Stimuli*mating F(5,100)=1.230, p=0.303 Stimuli*treatment F(5,100)=0.401, p=0.667 Stimuli*mating*treatment F(5,100)=1.036, p=0.363

Table 11. Statistic tables for the septal infusion of OXTR-A before mating prior extinction.

1.8. Differential neuronal activation during sexual behavior and SFC extinction

As the blockage of the OXTR-A during mating did not lead to any conclusive result during the social fear extinction, I aimed to understand what is the difference between the mice that mated without and with ejaculation. I performed an experiment that aims to measure the expression of the cFos protein, product of the immediate-early gene *cfos,* during mating and ejaculation, and the level of pErk, also a marker of neuronal activation, during social fear extinction in the same animals. Thanks to the comparison of the cFos expression level between Ej^- and Ej^+ mice, I would be able to identify a potential brain region specifically recruited during ejaculation. Furthermore, the comparison of the pErk expression level

between Ej^- and Ej^+ mice, I would be able to identify a potential brain region differently recruited during social fear extinction, which might be responsible for the social fear extinction facilitation induced by ejaculation. For that, animals have been sacrificed immediately after the second social stimulus of the social fear extinction (90 min after mating), the time point where the Ej^- and the Ej^+ mice show the greatest difference in the social investigation (Figure 19A and B). I focused on brain regions known to be involved in social fear extinction: the PVN and the SON (source of OXT neurons) and the LS (Zoicas et al., 2014; Menon et al., 2018). As our knowledge about the social fear circuit is limited, I decided to also investigate the MPOA, a brain region known to be involved in mating behavior (for more details see Introduction). This experiment is currently under analysis.

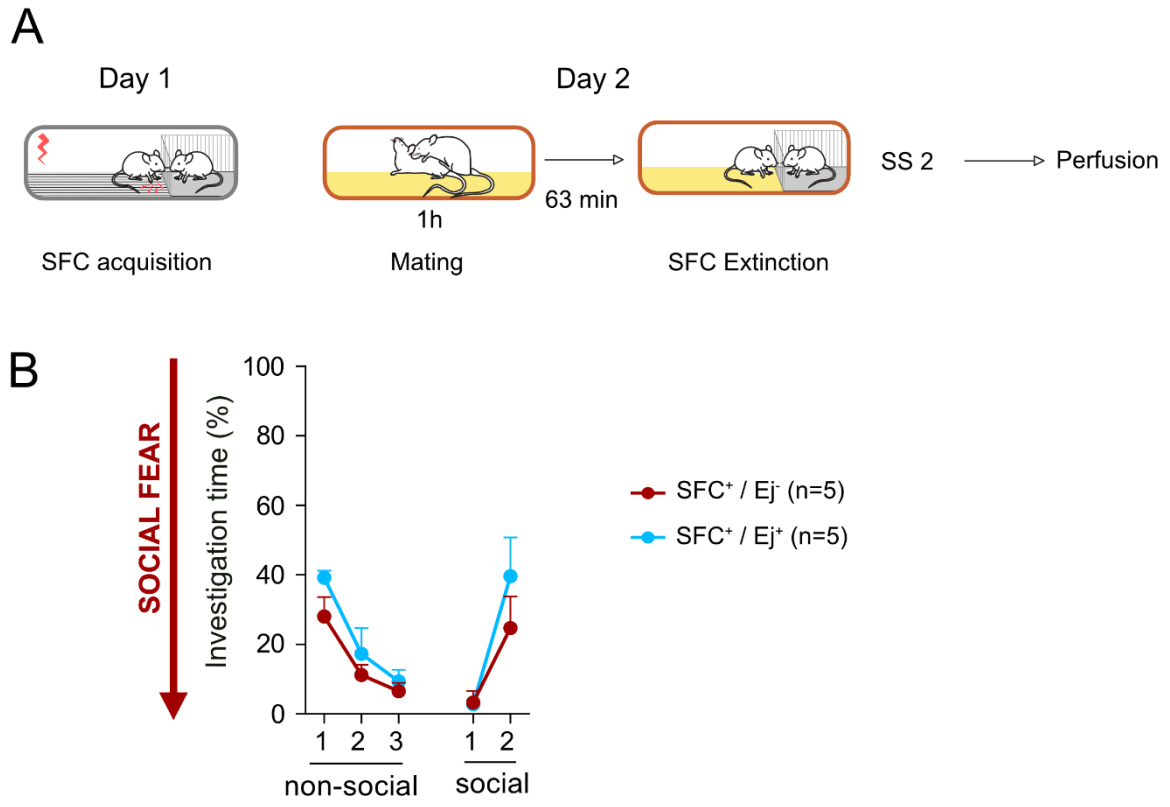

Figure 19. Activation of different brain regions during mating and social fear extinction. (A) Experimental design: the mice were conditioned on day 1. On day 2, the mice were allowed to mate for 1h before to undergo the social fear extinction where they were exposed to 3 non-social stimuli and 2 social stimuli. The mice sacrificed immediately after the second social stimulus. (B) Social fear extinction. Both SFC^+/Ej^- and SFC^+/Ej^+ mice show a social avoidance at the first stimulus. However, at the second stimulus, SFC^+/Ej^+ mice seem to have a higher social investigation time than the SFC^+/Ej^- mice.

1.9. Summary

In summary, I could confirm that mating has an anxiolytic effect in male mice. However, in the marbles burying test, only ejaculation seems to have a positive effect. Accordingly, ejaculation also facilitates social fear extinction. In an effort to investigate the process of social fear extinction, I could highlight a modulation of the activity of the LS and the CeA following either conditioning or extinction, respectively. Unfortunately, the low number of animals could not allow me to find statistically significant effects within the PVN or the SON. I found a release of OXT within the LS specifically during sexual behavior. However, male sexual behavior seems to not depend on the OXT system within the LS as a local infusion of OXTR-A did not impair it. To finish, even though OXT may still be involved in the effect of ejaculation in social fear extinction, it seems that this effect does not depend on the OXTR within the LS.

2. Involvement of the OXT system within the CeA in social fear extinction

As previously explained in my major project, I aimed to assess the involvement of specific brain regions, including the CeA, in the process of social fear extinction. The mice went through the social fear acquisition, during which some were unconditioned (SFC^-) and the others conditioned (SFC^+). On the second day, the extinction was performed. In order to visualize a modification of the cellular activity pattern during extinction, the mice were sacrificed either after the first social stimulus (SS1, the start of the extinction process), either after the sixth social stimuli (SS6, end of the extinction process). In either case, the sacrifice and perfusion of the animals were 90 min after exposure, the peak of cFos protein expression. Consequently, I analyzed the effect of the conditioning by comparing the SFC^- and SFC^+ mice at the first stimulus, and the effect of the extinction process by comparing SFC^+ after the first or the last stimuli (Figure 20A). During the social fear extinction, the SFC^+ mice showed social avoidance at the first stimulus, which is a sign of social fear. On the contrary, SFC^- mice showed a high level of social investigation. For the group SFC^+/SS6, the mice showed social fear extinction, as the level of social investigation increased over time. However, two animals have been excluded from this group, as they did not extinguish the social fear (Figure 20B). I found an increase of the cFos protein expression after social fear extinction in SFC^+ mice in the CeA. This change in the expression of cFos in the CeA implies the recruitment of the CeA during social fear extinction. For detailed statistics, see table 12.

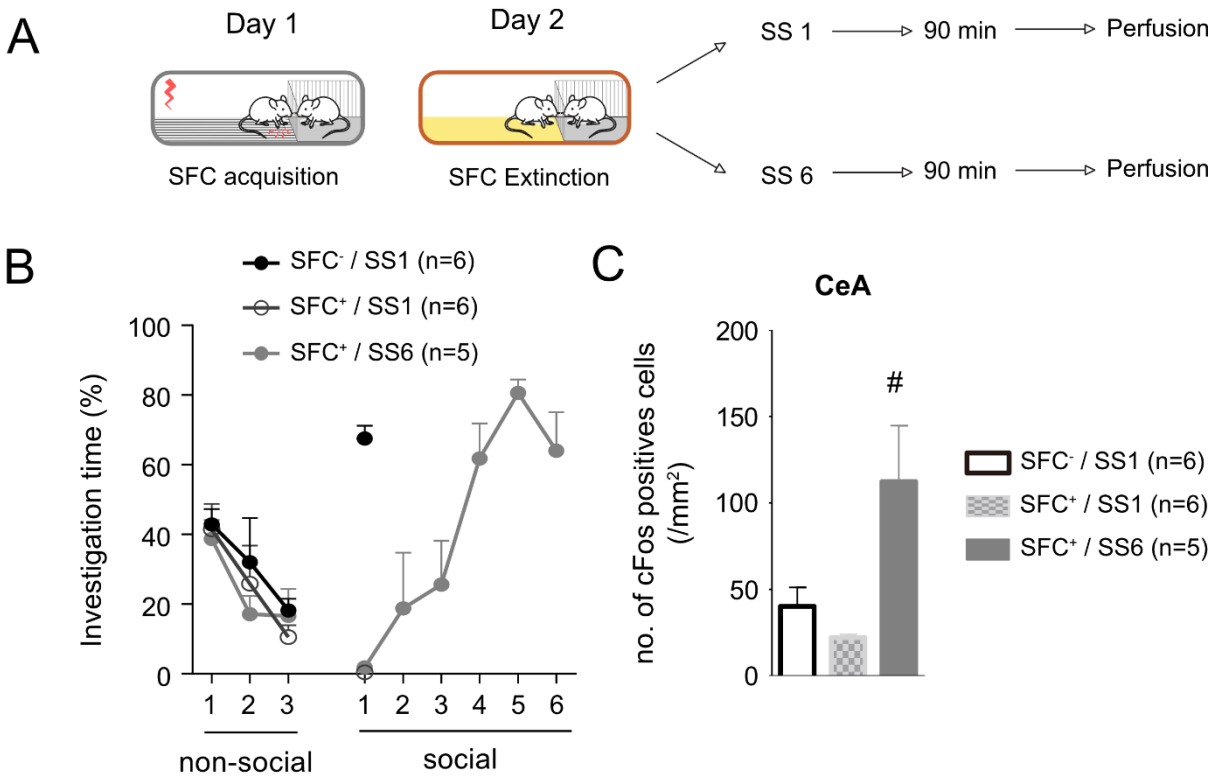

Figure 20. Activation of the CeA during social fear extinction. (A) Experimental design: the mice were conditioned on day 1. On day 2, the mice underwent the SFC extinction where they were exposed to either one (SS1) or six (SS6) stimuli. 90 min after social exposure, the mice sacrificed. (B) Social fear extinction. The SFC^- mice show a high level of social investigation, while the SFC^+ mice show social avoidance. At the end of the extinction procedure, the mice that received six stimuli show a high level of investigation, a sign of social fear extinction. (C) In the CeA, the SFC^+/SS6 mice presented a higher number of positive cells than the SFC^+/SS1. Data presented in mean ± SEM. # p=0.05 SFC^+/SS1 vs. SFC^+/SS6.

Figure 20	Test	Effects	
C	cFos within the CeA *Independent t-test*	Conditioning (SFC^- /SS1vs. SFC^+/SS1) T(5.313)=-1.639, p=0.159	**Stimuli (SFC^+ /SS1vs. SFC^+/SS6)** **T(4.028)=-2.773, p=0.05**

Table 12. Statistic tables for the expression of cFos during social fear extinction.

2.1. Pharmacological manipulation of the OXTR into the CeA

Infusion of synthetic OXT into the CeA

In order to assess the involvement of the OXT system within the CeA on social fear extinction, I infused synthetic OXT directly into the CeA. A first experiment has been performed (Experiment 1, Figure 21A, and B) where the guide cannulas have been implanted 2mm above the CeA. 7 days after surgery, the mice were submitted to the social fear acquisition during

which they received an equivalent number of shocks (Figure 21A). 24h after the acquisition, the mice received either Veh or OXT. For this experiment, I used a classic infusion system composed of a 27G needle connected to a Hamilton syringe via tubing. 10 min after infusion, the mice underwent the social fear extinction. During the investigation of the non-social stimuli, an effect of the conditioning could be observed. Indeed, the SFC^- groups investigated the non-social stimuli significantly more than the SFC^+ mice. Moreover, during the exposition to the 6 social stimuli, the analysis of the SFC^- groups only shows a main effect of the stimuli and a significant linear within-subjects contrast, indicating that the SFC^- mice exhibited decreased social investigation during the social fear extinction (Figure 14 B). I hypothesized that this abnormal behavior from the SFC^- mice might be caused by the surgery. Thus to circumvent this, in a second experiment (Experiment 2, Figure21 C, D, and E), I modified the implantation site to 3mm above the CeA and used a different infusion system made of glass fiber to limit the damage into the brain. During the acquisition, the mice received the same number of shocks (Figure 21C). 24h after the acquisition, the extinction was performed. An interaction between the stimuli and the treatment could be found, however, the posthoc analysis did not reveal any statistically significant differences. During the social stimuli exposure, the analysis of only the SFC^- groups showed no statistical difference within the different stimuli, indicating that the modification of the procedure prevented the disruption of the behavior. A significant difference could be found when a two-way ANOVA for repeated measures was run on the 2 first social stimuli, showing that OXT allowed to slightly facilitate social fear extinction (Figure 21D). During the recall, a treatment effect could still be found with a statistical significance on the last stimulus within the SFC^+ which received Veh and OXT, the Veh group investigating less than the OXT. For detailed statistics, see table 13.

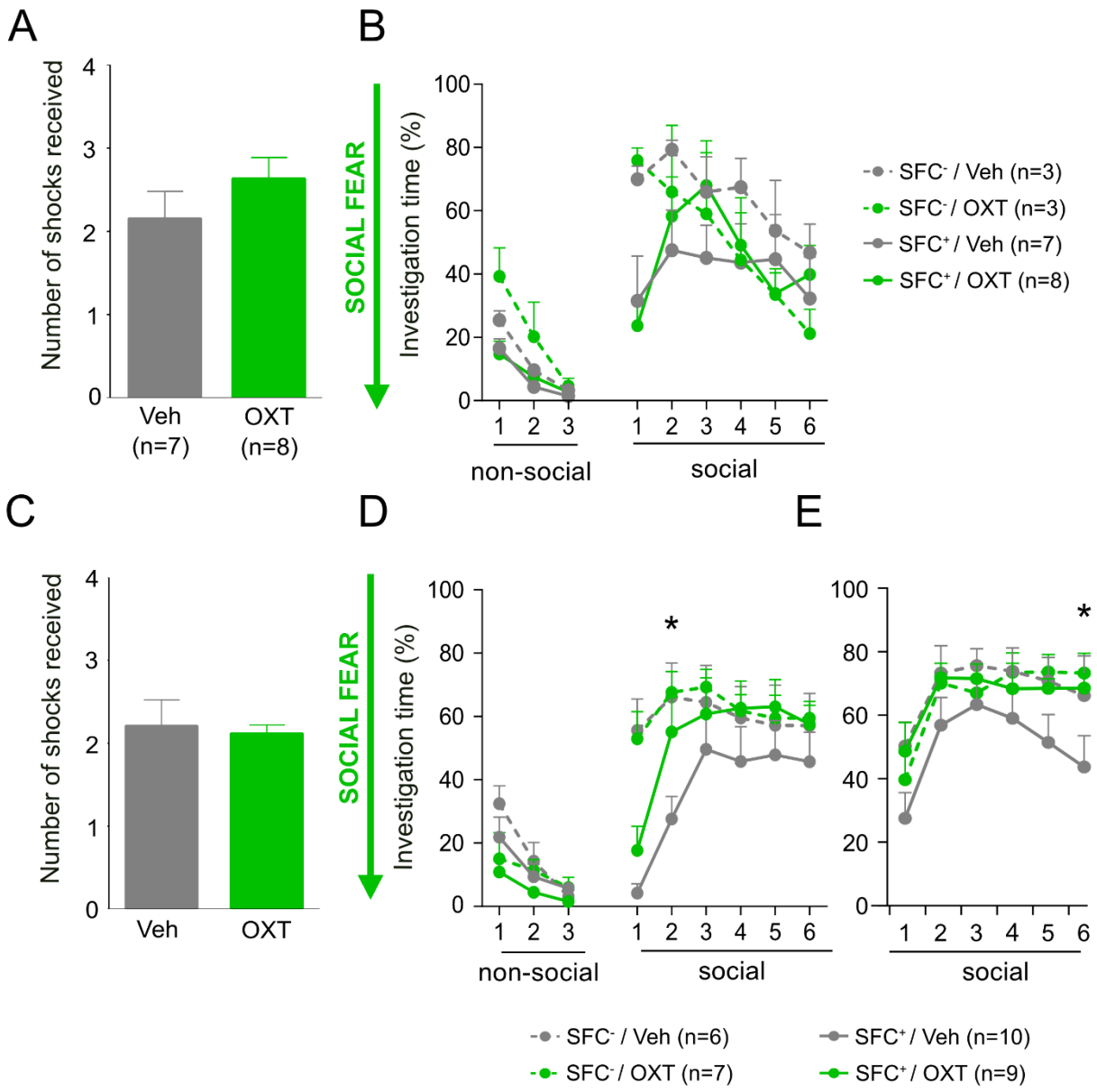

Figure 21. OXT infusion within the CeA slightly facilitates social fear extinction. (A and B) Experiment 1: implantation 2mm above the CeA. The mice received the same number of shock during SFC acquisition (A). However, the behavior of the SFC⁻ groups was impaired during social fear extinction (B). (C, D, and E) Experiment 2: implantation 3 mm above the CeA. The mice received the same number of shock during SFC acquisition (C). During the extinction, the behavior of the SFC⁻ was restored. Moreover, the SFC⁺/OXT mice extinguished faster than the SFC⁺/Veh mice (D). Extinction recall (E). Data presented in mean ± SEM.

Figure 21	Tests	Effects	Pairwise comparisons
A	No. of shocks (implantation 2mm above) *Independent T-test for equality of means*	T(13)=-0.775, p=0.452	
B	SFC extinction (non-social stimuli) (implantation 2mm above) *Two-way ANOVA repeated measures (Stimuli*Conditioning*Treatment)*	**Time** **F(2,34)=44.279, p=0.0001** **Conditioning** **F(1,17)=13.30, p=0.002** Treatment F(1,17)=3.498, p=0.079 **Time*Conditioning** **F(2,34)=5.354, p=0.018** Time*Treatment F(2,34)=0.875, p=0.400 Time*Conditioning*Treatment F(2,34)=1.450, p=0.250	
B	SFC extinction (social stimuli) (implantation 2mm above) *Two-way ANOVA repeated measures* *Only SFC$^-$ (Stimuli* Treatment)*	**Time** **F(5,20)=5.602, p=0.002** Treatment F(1,4)=0.986, p=0.377 Time*Conditioning F(5,20)=0.796, p=0.566 **Linear within-subjects contrast** **Time(1,4)=49.167,p=0.002**	
C	No. of shocks (implantation 3mm above) *Independent T-test for equality of means*	T(17)=-0.246, p=0.808	
D	SFC extinction (non-social stimuli) (implantation 3mm above) *Two-way ANOVA repeated measures (Stimuli*Conditioning*Treatment)*	**Time** **F(2,56)=26.309, p=0.0001** Conditioning F(1,28)=1.810, p=0.189 Treatment F(1,28)=3.149, p=0.087 Time*Conditioning F(2,56)=1.137, p=0.311 **Time*Treatment** **F(2,56)=5.017, p=0.022** Time*Conditioning*Treatment F(2,56)=1.145, p=0.309	Stimulus 1 SFC$^-$ Veh vs. OXT p=0.080 p>0.05 for all other pairwise comparisons
D	SFC extinction (social stimuli) (implantation 3mm above)	Time F(5,55)=1.216, p=0.314 Treatment F(1,11)=0.038, p=0.850	

	Two-way ANOVA repeated measures *Only SFC⁻ (Stimuli* Treatment)*	Time*Treatment F(5,55)=0.071, p=0.996 Linear within-subjects contrast F(1,11)=0.067,p=0.800	
D	SFC extinction (social stimuli) (implantation 3mm above) *Two-way ANOVA repeated measures* *Only SFC⁺ (Stimuli* Treatment)*	**Time** **F(5,85)=17.033, p=0.0001** Treatment F(1,17)=2.344, p=0.144 Time*Treatment F(5,85)=0.509, p=0.678	**Stimulus 2** **SFC⁺ Veh vs. OXT** **p=0.48** p>0.05 for all other pairwise comparisons
D	SFC extinction (social stimuli) (implantation 3mm above) *Two-way ANOVA repeated measures* *Only SFC⁺- stimuli 1/2 (Stimuli* Treatment)*	**Time** **F(1,17)=28.152, p=0.0001** **Treatment** **F(1,17)=5.253, p=0.035** Time*Treatment F(1,17)=1.491, p=0.239	Stimuli 1 SFC⁺ Veh vs. OXT p=0.098 **Stimulus 2** **SFC⁺ Veh vs. OXT** **p=0.048**
E	SFC recall (implantation 3mm above) *Two-way ANOVA repeated measures* *Only SFC⁺ (Stimuli*Treatment)*	**Time** **F(5,80)=5.579, p=0.0001** **Treatment** **F(1,16)=5.371, p=0.034** Time*Treatment F(5,80)=0.525, p=0.757	Stimuli 6 SFC⁺ Veh vs. OXT p=0.035 p>0.05 for all other pairwise comparisons

Table 13. Statistic tables for the infusion of OXT into the CeA before SFC extinction.

Infusion of the specific OXTR agonist Carbetocin into the CeA

OXT is known to interact with the OXTR but also with the vasopressin receptors. To confirm that the effect of OXT was mediated via the OXTR, I chose to infuse a selective agonist of the OXTR i.e., Carbetocin. The doses have been chosen depending on a previous experiment done in the lab in a different brain region (unpublished, Dr. Rohit Menon). Another appropriate experiment would have consisted of inhibiting the OXTR or vasopressin receptors before infusion of OXT and see if the effect of OXT infusion in the CeA shown in the previous experiment remained. However, based on the issue encountered in the previous experiment (Experiment 1, Figure 21A, and B), I assumed that a double-infusion within the CeA might disturb the normal social fear extinction behavior. During the acquisition, the mice received the same number of shocks (Figure 22A). The SFC⁻ group represents the SFC⁻ mice that received either Veh or Carbetocin. These groups have been pooled as no difference was found between

them. The different groups investigated the 3 non-social stimuli in the same manner. No difference was found during the investigation of the social stimuli between the SFC$^+$ mice which received either Veh, Carbetocin 0.5ng, or Carbetocin 5ng. No main effect of the stimuli could be found during the extinction, sign that the mice did not extinguish the social fear (Figure 22B). During the recall, a stimuli main effect has been found, signifying that the mice started to extinguish the fear only during the recall. No difference has been found between the groups (Figure 22C). For detailed statistics, see table 14.

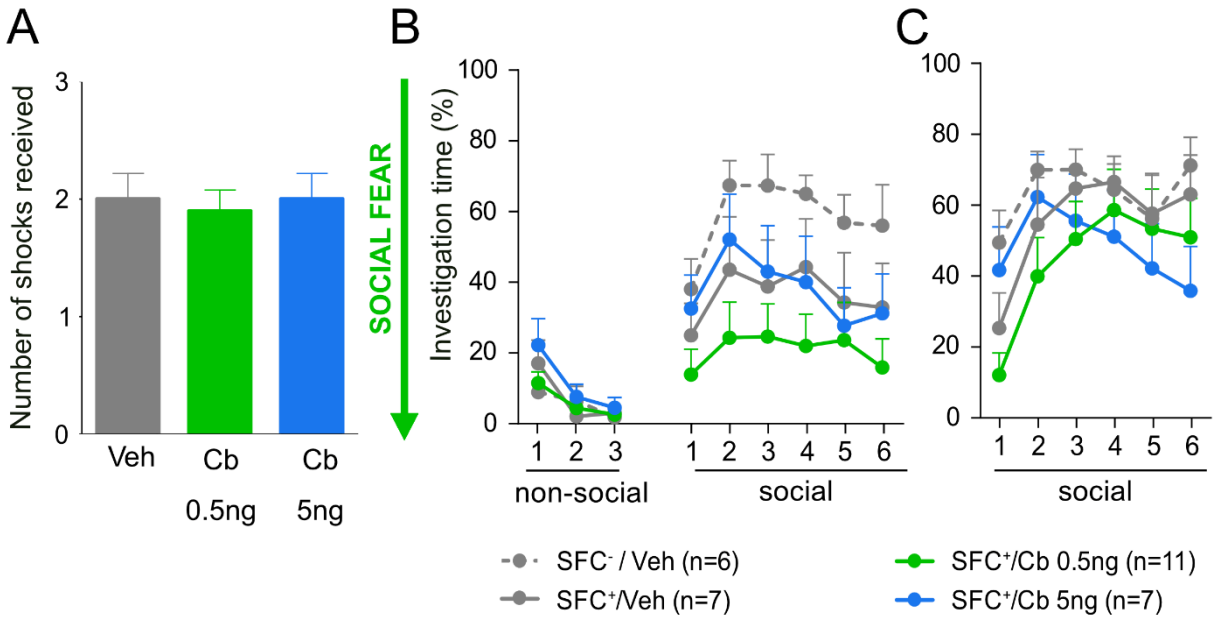

Figure 22. Carbetocin (Cb) infusion within the CeA does not affect social fear extinction. (A) The mice received the same number of shocks during SFC acquisition. (B and C) SFC extinction and extinction recall. No effect of Carbetocin was found. Data presented in mean ± SEM.

Figure 22	Test	Effects	
A	No. of shocks *One-way ANOVA*	Treatment F(2,21)=0.089, p=0.915	
B	SFC extinction (non-social stimuli) *Two-way ANOVA repeated measures (Stimuli*Conditioning*Treatment)*	**Time** **F(2,52)=13.199, p=0.0001** Conditioning F(1,26)=0.177, p=0.678 Treatment F(1,26)=0.960, p=0.396	Time*Conditioning F(2,52)=2.022, p=0.143 Time*Treatment F(4,52)=0.917, p=0.461

B	SFC extinction (social stimuli) *Two-way ANOVA repeated measures* *SFC^+ only (Stimuli* Treatment)*	Time F(5,105)=2.947, p=0.051 Treatment F(2,21)=0.785, p=0.469	Time*Treatment F(10,105)=0.782, p=0.461
C	SFC recall *Two-way ANOVA repeated measures* *SFC^+ only (Stimuli* Treatment)*	**Time** **F(5,110)=7.128, p=0.001** Treatment F(2,21)=0.785, p=0.469	Time*Treatment F(10,110)=1.775, p=0.139

Table 14. Statistic tables for the infusion of Carbetocin into the CeA before social fear extinction.

2.2. Chemogenetic activation of the OXTR neurons into the CeA

As the Carbetocin seemed to not affect social fear when infused into the CeA, I decided to confirm the involvement of the OXT system within the CeA using chemogenetics. I infused a virus coding for a Gq-DREADD into the CeA of OXTR-Cre mice in order to directly activate the neurons expressing this receptor. The surgery took place 3 weeks before the acquisition. The mice received the same number of shocks (Figure 23A). Before extinction, the mice received an i.p. injection of either Veh or CNO. To control that the infusion of CNO alone does not affect the behavior, a control group that did not express the Gq-DREADD received an i.p. CNO infusion 40min before extinction (data not shown on the graph for more clarity). No effect of CNO on social fear extinction could be found. The mice investigated all the non-social stimuli in the same manner. No treatment effect was found during the investigation of the social stimuli (Figure 23B). However, after histology, the diffusion of the virus seems to be only partly into the CeA. Most of the virus seems to be located into the dorsal MeA (Figure 23C).

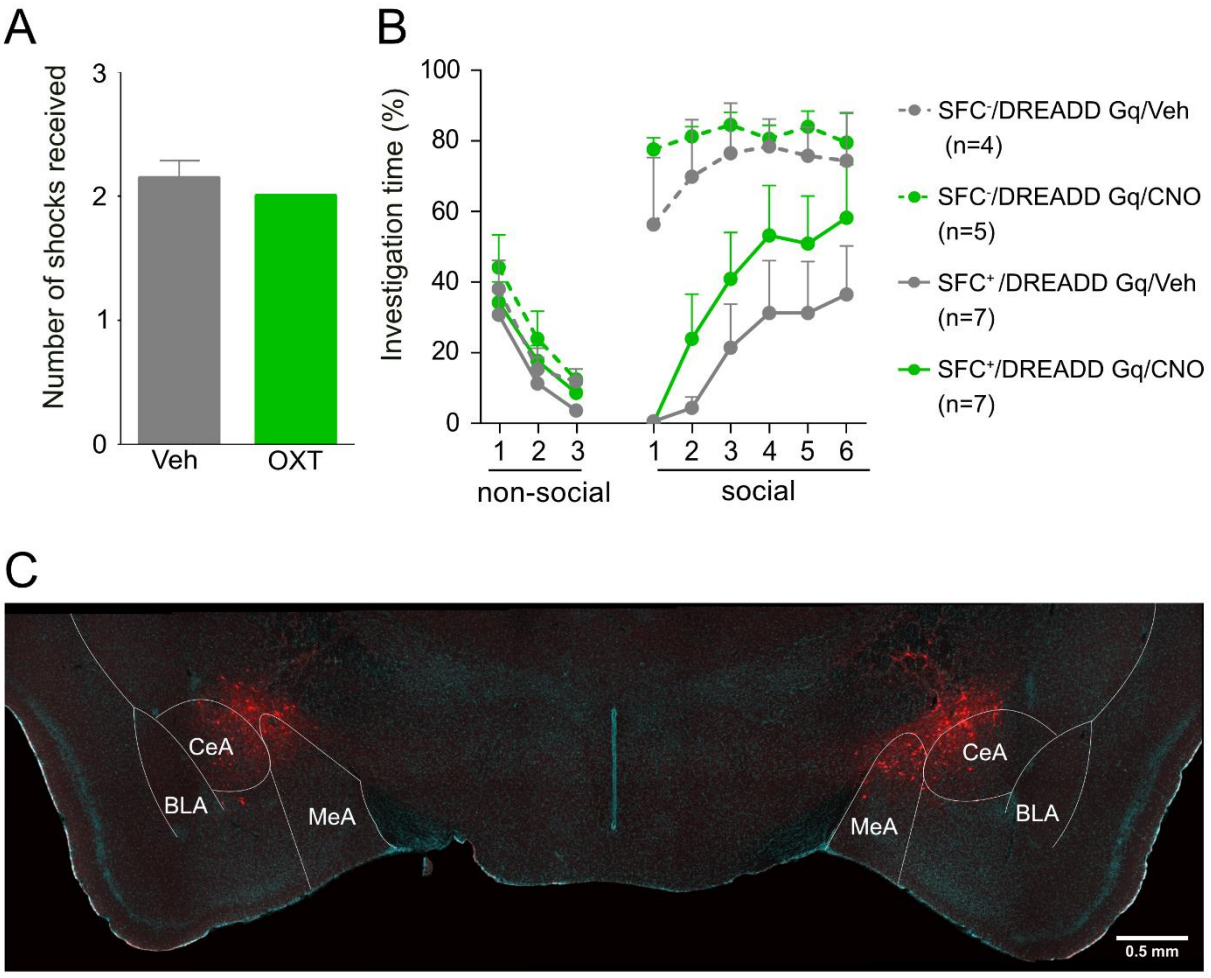

Figure 23. Specific activation of OXTR-positive cells within the CeA via a Gq-DREADD does not affect social fear extinction. (A) The mice received the same number of shocks during SFC acquisition. (B) Social fear extinction. No difference was found between SFC^+/Gq-DREADD/Veh and SFC^+/Gq-DREADD/CNO. C) Example of the Gq-DREADD expression. Data presented in mean ± SEM.

Figure 23	Test	Main effects	Pairwise comparisons
A	No. of shocks *Independent t-test for equality of means*	T(12)=-1.0, p=0.356	
B	**B/** SFC extinction (non-social stimuli) Two-way ANOVA repeated measures (Stimuli*Conditioning*Treatment)	**Time** **F(2,38)=41.967, p=0.0001** Conditioning F(1,19)=1.9, p=0.184 Treatment F(1,19)=1.106, p=0.306 Time*Conditioning F(2,38)=0.177, p=0.839 Time*Treatment F(2,38)=0.295, p=0.746 Time*Conditioning*Treatment F(2,38)=0.204,p=0.816	
B	SFC extinction (social stimuli) *Two-way ANOVA repeated measures (Stimuli*Conditioning*Treatment)*	Time F(5,95)=10.492, p=0.0001 Conditioning F(1,19)=21.942, p=0.0001 Treatment F(1,19)=1.708, p=0.207 **Time*Conditioning** **F(5,95)=4.290, p=0.018** Time*Treatment F(5,95)=0.060, p=0.952 Time*Conditioning*Treatment F(5,95)=1.132,p=0.336	**Stimuli 1 to 6** **SFC^- vs SFC^+** **p<0.05**

Table 15 Statistic table for Gq-DREADD expression into the CeA before social fear extinction.

2.3. Summary

I could show that an infusion of OXT into the CeA allows a slight facilitation of social fear extinction. However, I was not able to reproduce this result by infusing a specific OXTR agonist into the CeA. The result obtained using chemogenetic are inconclusive due to a bad diffusion of the virus.

GENERAL DISCUSSION

1. Effect of sexual behavior on social fear in male mice: involvement of the OXT system

In the first part of my PhD thesis, I aimed to investigate, how sexual behavior in male mice modulates fear and anxiety, focusing on the involvement of the neuropeptide OXT by combining behavioral, microdialysis, and pharmacological techniques as well as neuronal activity assessment. In more detail:

1. I was able to show that ejaculation allows the facilitation of social fear extinction. This cannot be explained by a decrease in anxiety-like behavior or a modulation of natural social preference behavior.

2. I confirmed the involvement of the LS and the CeA in the process of social fear extinction.

3. I identified the specific release of OXT within the LS during mating behavior. However, I found that a blockage of the OXTR in the LS during mating doesn´t affect social fear extinction.

1.1. Effect of mating on emotional, social and socio-emotional behavior

Based on the anxiolytic effect of the mating-induced release of OXT within the PVN (Waldherr and Neumann, 2007), I hypothesized that the central release of OXT during mating would facilitate socio-emotional behavior, i.e. social fear extinction. Thus, in order to evaluate the effect of mating on social fear extinction in male mice, I allowed male mice to mate for 1h before social fear extinction. Contrary to our expectations, mating *per se* did not affect social fear extinction. However, the males that ejaculated showed a facilitation to extinguish social fear. To understand if this effect was specific on social fear extinction, I first evaluated the effect of mating on cued fear extinction, which is a paradigm measuring the emotional response of the tested animal. For this purpose, male mice were allowed to mate for 1h before cued fear extinction. I could not find any effect of mating with or without ejaculation. A previous study reported that mating after the acquisition of context fear conditioning decreased freezing behavior during extinction in male rats (Bai et al., 2009). In this study, a male was paired with a female for 24h after the cued fear acquisition. It was shown that mating behavior impairs the formation of fear memory by activating the dopamine system within the hippocampus. Regarding my result in the CFC, mating occurred only before extinction and thus could not affect fear memory formation. As ejaculation seemed to not

have any effect on the emotional response in a non-social context that was assessed with CFC, I then tested the mice after mating for anxiety-like behavior, hypothesizing that if mating decreases anxiety-like behavior, mated males would be more inclined to extinguish social fear during the extinction. I found that mating induced anxiolysis in the LDB, as already shown in male rats (Waldherr and Neumann, 2007). In the marbles burying test, mating without ejaculation did not induce a decrease in burying behavior, while mating with ejaculation tends to decrease it, which is related to decreased anxiety-like behavior, even though I could not found a statistical significance. Increasing the animal numbers in each group might help to see a clearer effect. Several studies investigated the effect of mating and ejaculation on anxiety-like behavior. Fernandez-Guasti and collaborators found a clear effect of ejaculation on anxiety-like behavior in male rats using the conditioned defensive burying test (Fernández-Guasti et al., 1989; Fernández-Guasti and Saldívar, 1990; Rodríguez-Manzo et al., 1999). In their experiments, mating without ejaculation did not affect anxiety-like behavior. However, in our lab, mating alone has been shown to have an anxiolytic effect in the LDB and the elevated-plus-maze in male rats, independently of ejaculation (Waldherr and Neumann, 2007). The anxiolytic effect of mating and/or ejaculation seems to vary depending on certain conditions, i.e. laboratory conditions, the kind of test used to evaluate the behavior (passive or active behavior) or the model used. As an important component of the social fear extinction, I evaluated the social preference and motivation of male mice after mating. Negative social experiences such as social defeat induce social avoidance (Haller and Bakos, 2002; Huhman, 2006). I hypothesize than a positive social experience might promote social preference or social approach. I did not find any modification of social preference or social motivation after mating behavior. Then, mating doesn´t affect the motivation or the approach towards unknown same-sex conspecifics. The facilitation of the social fear extinction after ejaculation is then not mediated by a decrease in anxiety-like behavior or an increase social motivation.

It is also interesting to note that the mice that were conditioned in the social fear paradigm do not show fear towards the female during mating behavior. Moreover, the mating procedure does not lead to a social fear extinction. This means that during 1h of mating, the conditioned males do not extinguish the fear by contact with the female as they show social fear at the first social stimulus of the extinction. This suggests that the SFC paradigm induces

a generalized social fear, as mice are fearful of unknown male conspecific, but in a sex-specific manner as this seems not to be the case for females. In this context, it would be interesting to evaluate if male mice can be conditioned against a female which possesses inherently a higher biological valence and *vice versa*.

1.2. Neuronal activity during social fear extinction

Social fear has been investigated in our lab since the generation of the SFC paradigm in 2012 (Toth et al., 2012a). Consequently, several studies showed that OXT promotes the facilitation of the social fear extinction, more specifically when it is released into the LS (Zoicas et al., 2014; Menon et al., 2018). The measurement of OXT release using microdialysis after social fear conditioning or after extinction revealed a release within the LS only in SFC^- mice but not in SFC^+ mice during social fear extinction. However, it is important to note that, in this experiment, SFC^+ mice did not extinguish social fear, i.e. they did not have any social contact during the extinction (Zoicas et al., 2014). Thus, we cannot affirm whether the OXT release is actually impaired in SFC^+ mice or if it is the lack of social investigation that impairs OXT release in SFC^+ mice while increased social investigation triggers the release of OXT during the extinction in SFC^- mice. During social fear extinction, social fear conflicts with social motivation of the conditioned mice. Indeed, mice that have been conditioned are confronted with a social conspecific which is linked to a trauma (foot-shock), but which is also highly attractive as mice are social animals (Preston, 2017). This generates a conflict between avoidance and approach during social fear extinction. I aimed to understand better the involvement of OXT in the regulation of this process. For that, I sacrificed the mice at different time points during the social fear conditioning procedure. These were 90 min after either the first social stimulus (SFC^- and SFC^+ mice) or the last social stimulus (SFC^+ only) and I measured the expression of the protein cFos, product of the immediate-early gene *cfos* and marker of neuronal activity. I did not find any difference in cFos expression in the OXT neurons of the PVN and SON. This suggests that there is no impact of conditioning on the expression of cFos in OXT neurons at the first social fear extinction stimulus (comparison between the SFC^-/SS1 and the SFC^+/SS1). Regarding the effect of repeated social exposure, also no significant effect could be found (comparison between the SFC^+/SS1 and the SFC^+/SS6). However, two mice had to be removed from the SFC^+/SS6 group because they did not extinguish social fear. The number of mice for this group was consequently too low (n=5) and the variance too high to identify a proper

effect. By increasing the n number in each group, I expect then an increased expression of cFos in the OXT neurons of both PVN and SON after 6 social stimuli in response to social fear extinction. Two assumptions can be made to interpret the increased OXT neurons expressing cFos after 6 stimuli. This increase might happen in response to the process of social fear extinction (either as the process of social fear extinction or as a stress response), or the increase in cFos-positive OXT neurons might simply reflect the repetition of social contact. Several studies show the release of OXT and the increased activity of OXT neurons following stress (Engelmann et al., 1999; Wotjak et al., 2001; Torner et al., 2017) and social exposure (Hung et al., 2017; Resendez et al., 2020). To interpret this effect, a fourth group containing SFC^- mice exposed to 6 social stimuli should be included.

Regarding the LS, I found a decreased neuronal activation in the ventral part at the first social stimulus in SFC^+ mice compared to SFC^-. This indicates that the ventral LS exhibits reduced activation when the mice have been conditioned. Although an effect could only be confirmed in the ventral part of the LS, it does not exclude the possibility that this effect might also be present in the dorsal part, neither the possibility that the SFC^+/SS1 and SFC^+/SS6 might present a differential activation, once the group size is increased to reach the correct statistical power. The LS is known to be involved in the inhibition of fear response (Sheehan et al., 2004). In this context, high expression of cFos protein has been found in the LS after fear conditioning (Calandreau et al., 2007; Menon et al., 2018). However, after extinction, the LS presented a level of cFos protein similar to the control group (Menon et al., 2018). During the social fear extinction, the LS could participate in the inhibition of the fear expression. Intriguingly, an increased level of OXTR binding was detected within the dLS after acquisition in the SFC^+ mice. This increase reverted to baseline levels after extinction (Zoicas et al., 2014). These data showed a modulation of the OXT system within the dLS induced by social fear. However, the precise mechanisms by which OXT signaling within the LS regulates social fear extinction are still not completely understood.

Within the CeA, I found an increase of the cFos protein expression after social fear extinction in SFC^+ mice. This change in the expression of cFos in the CeA implies an involvement of the CeA during social fear extinction. Furthermore, the particular involvement of the OXT system in social fear extinction and the increase of the OXTR binding in SFC^+ mice after social fear acquisition (Zoicas et al., 2014) raises the hypothesis of a specific involvement of OXT within

the CeA during social fear extinction. This will be further discussed in section 2 of the discussion.

Altogether, I found an increased cFos expression in the PVN and SON OXTergic neurons as well as in the CeA after social fear extinction and a decreased cFos expression in the vLS before social fear extinction. These regions are then differently recruited during social fear extinction.

1.3. OXT release during mating behavior

From previous studies, OXT is known to promote social fear extinction when released into the LS (Zoicas et al., 2014; Menon et al., 2018). In order to investigate how ejaculation can affect social fear extinction, and more precisely, if OXT can be a common factor to both mating and social fear extinction, I investigated the release of OXT during mating using microdialysis in different brain regions. Based on the current knowledge of social fear mechanisms and my previous results, I aimed to target the PVN, the LS, and the CeA. It is known from the literature that OXT is released within the PVN (Waldherr and Neumann, 2007) and that OXT neurons within the PVN show an increased cFos expression level during mating (Witt and Insel, 1994). Regarding the LS, a previous student measured the release of OXT in the LS using microdialysis in male mice and found an increase of OXT release during mating. The control mice, which were co-housed with a female but did not mate, did not show any release of OXT. This means that social contact with a female alone did not drive the release of OXT. This is the first evidence of a specific release of OXT in the LS during mating. Several studies in rats and hamsters showed that the LS might facilitate male sexual behavior (Baum et al., 1982; Gogate et al., 1995; Gulia et al., 2002), however, the involvement of the OXT system within the LS during mating has not been investigated yet.

In the amygdala, I found a release of OXT in both mated and non-mated mice, indicating that the presence of the female already induces OXT release. Due to the location and the size of the microdialysis probe, the OXT release was measured within the CeA but also the dorsal MeA and the ventral BLA. As OXT release in these regions is known to occur in response to a social stimulus (Dumais et al., 2016), the results obtained are then not surprising.

I have to mention that I measured here the release of OXT during the complete process of mating. This means that I could not differentiate the mice that ejaculated from the ones that did not, due to the sampling requirements. Indeed, a 30 min-sampling ensures a minimum

amount of ECSF to obtain a reliable measurement of the OXT levels. As the ejaculation is a very brief event, I was not able to measure the release specifically during this event. Hence, due to temporal constraints, I have to say that the OXT release in the LS is specific to mating behavior, but not ejaculation. However, in men, plasma OXT increases during self-stimulation with a maximum level during orgasm and ejaculation (Ogawa et al., 1980; Carmichael et al., 1987; Murphy et al., 1987; Krüger et al., 2003). I hypothesized that the central release of OXT follows the same pattern as in the plasma, and might rise during mating behavior with a peak of release during ejaculation. Then, the specific release of OXT during mating in the LS points in the direction of the mediation of the ejaculation effect on social fear extinction by OXT and the LS.

1.4. Effects of blockade of OXTR after mating prior social fear extinction

To elucidate if OXT mediates the effect of ejaculation on social fear extinction I conducted a pharmacological experiment aiming to infuse i.c.v. an OXTR-A after mating and before social fear extinction. One hypothesis is that the release of OXT during mating might facilitate a second release of OXT during social fear extinction. Indeed, this phenomenon called "self-priming" occurs in OXT neurons in the PVN (Ludwig and Leng, 2006). OXT binding to its receptor on the OXT neurons induces the accumulation of an available secretory pool at the target cell, potentiating the next release within up to 90 minutes. This process permits to prepare the system for an anticipated future trigger. Hence, a release of OXT during the mating behavior could prime the OXT neurons to potentiate for the next stimulus, the social fear extinction, which takes place 45-75 minutes after ejaculation, thus facilitating the extinction. Moreover, during mating, male mice ejaculated generally several times before to reach sexual satiety. The first ejaculation was shown to facilitate the second ejaculation in male rats by decreasing the number of intromissions needed to reach ejaculation (Rodríguez-Manzo and Fernández-Guasti, 1994). The mating protocol that I used if often too short to let the mice ejaculated a second time. Hence, if I hypothesize that the first ejaculation could prime the OXT neurons of the PVN to potentiate the next ejaculation, and as the male mice ejaculated only once during the mating protocol, then the ejaculation-induced priming of the OXT neurons should still be present during the social fear extinction. Hence, the male that ejaculated would show a potentiated release of OXT, leading to a facilitation of the extinction. However, this hypothesis needs to be proven with further experiments. I.c.v. infusion is a

reliable way to evaluate the general involvement of a system in a particular phenomenon. The infusion of the OXTR-A had to be done after mating as central OXT is involved in penile erection (Argiolas et al., 1988, 1989; Melis et al., 1988) which is essential for mating to occur. I could show that an OXTR-A infusion after mating and prior to the extinction reduced social investigation during social fear extinction in both Ej^- and Ej^+ mice. However, no difference was found between the groups SFC^+/Ej^-/Veh and SFC^+/Ej^+/Veh. It is likely that the experimental procedure abolished the effect of ejaculation on social fear extinction. This could be an effect of the surgery, or of the manipulation of the mice to perform the infusion or the infusion itself. The effect of the i.c.v. OXTR-A on social fear extinction was expected as it is known that an infusion of OXTR-A, either i.c.v. or local in the LS, impairs extinction (Zoicas et al., 2014; Menon et al., 2018). However, the fact that the mice ejaculated before did not protect them from the effect of the OXTR-A. This interpretation, of course, can be contested by the fact that the positive effect of the ejaculation is now present in the SFC^+/Ej^-/Veh and SFC^+/Ej^+/Veh groups. i.c.v. infusion of OXTR-A appears to not be ideal to investigate the effect of mating and ejaculation on social fear extinction. Hence, a local blockage of the OXTR seems to be a better option.

1.5. LS administration of OXTR-A after mating prior social fear extinction

Endogenous and applied OXT into the LS was shown to facilitate social fear extinction (Zoicas et al., 2014; Menon et al., 2018). Moreover, I showed that OXT is released during mating within the LS. As previously explained, hypothesizing that the central release of OXT follows the same time course than in the plasma, OXT would be centrally released during mating, with a peak of release during ejaculation. Considering this, I then hypothesized that OXT release within the LS might be mediating the facilitatory effect of ejaculation on social fear extinction. To confirm the involvement of the LS-OXT system activation in the facilitatory effect of ejaculation on social fear extinction, I conducted an experiment to block the OXTR during mating within the LS. This time, the local blockage of the OXTR is not expected to interfere with mating behavior and penile erection. Thus, I infused an OXTR-A directly into the LS before mating prior social fear extinction. Even though more mice need to be added to this experiment to achieve the necessary statistical power, the OXTR-A infusion seemed to not impair the mating behavior. Indeed, neither the latency and number of intromissions and the latency to ejaculate were modified following the infusion of OXTR-A. OXTRs within the LS are

then not needed to achieve mating behavior and ejaculation. In line with the i.c.v infusion, no difference was found between the groups SFC^+/Ej^-/Veh and SFC^+/Ej^+/Veh mice, which means that once again the specific effect of ejaculation on extinction was lost. The OXTR-A infusion during mating behavior did not affect the social fear extinction, neither in the Ej^- and Ej^+ mice. Thus, although OXT is released during mating in the LS, it seems to not causally influence the social fear extinction. However, more animals have to be included in this experiment to reach an acceptable statistical power. Another information that we can acquire from the above-mentioned results is that pharmacological manipulations are possibly not the most suitable approach for studying the effect of ejaculation on social fear extinction. Indeed, in both i.c.v. and local experiments, the facilitation of the extinction which was specific of the Ej^+ mice was absent. The hypothesis that the release of OXT during mating might facilitate the second release of OXT during social fear extinction could not be verified. This result reveals the importance of studying other systems in the context of social fear and its extinction. Indeed, it seems that ejaculation induces a specific effect on social fear extinction which is not strictly dependent on the OXT system within the LS. However, this does not rule out the possibility of an involvement of the OXT system within another brain region or an involvement of the LS independently of the OXT system. Even the simultaneous activation of the LS OXT and OXT in other brain regions or in the periphery may be thinkable to be responsible for the effect of ejaculation in social fear extinction.

1.6. Differential neuronal activation during sexual behavior and SFC extinction

So far, OXT release within the LS was the only overlap that I could find between mating behavior and social fear extinction. However, this did not lead to any conclusive result. One of the major issues is the differentiation of mating and ejaculation on the level of oxytocin neurotransmission, as a proper distinction during the measurement of the OXT release was problematic due to the short duration of ejaculation. Then, I aimed to identify a specific region which is specifically involved in the ejaculation and social fear extinction processes in addition to the LS, I performed an experiment which aims to measure the expression of the cFos protein, product of the immediate-early gene *cfos* during mating and ejaculation, and the level of pErk, also a marker of neuronal activation, during social fear extinction in the same animals. For that, animals were sacrificed immediately after the second social stimulus of the social fear extinction, the time point where the Ej^- and the Ej^+ mice show the greatest difference in

social investigation. The purpose is to assess which regions are recruited by both mating behavior and social fear extinction, and if there is a difference of cFos and/or pErk expressions in these regions in between males that mated with or without ejaculation. I focused on brain regions known to be involved in social fear extinction: the PVN and the SON, as sources of OXT neurons, and the LS, essential for social fear extinction as the blockage of the OXTRs in the LS inhibit social fear extinction (Zoicas et al., 2014; Menon et al., 2018). I chose to investigate the LS even though the previous results were not conclusive because I only assessed the involvement of this region in the context of the OXT system. As our knowledge about the social fear circuit is limited, I decided to also investigate some regions known to be involved in mating behavior, like the MPOA. The MPOA is the main integrative center of mating behavior, receiving afferent genitosensory information from different regions (for more details, see Introduction). This experiment is still under analysis. However, in both the LS and the MPOA a high level of cFos and pErk could be found. Moreover, it seems that cFos and pErk colocalize, suggesting that similar populations of cells might be activated by mating and social fear extinction in these brain regions. In the PVN, cFos and pErk could be observed, however, they seem to not colocalize. cFos and pErk are two different markers of cell activity, the fact that they are expressed in the same cells does not mean that one induced the expression of the other. Then, colocalization of cFos and pErk does not mean that the activation of a cell during mating induced its activation during extinction. The two processes are independent. The main focus of this experiment is to find a difference between mating with or without ejaculation. Ejaculation is a spinal reflex, which is described as the emission and expulsion of seminal fluid. This reflex can occur even following the transection of the spinal cord, indicating that the supraspinal structures are not essential for ejaculation (McKenna et al., 1991). However, even though supraspinal regions are not necessary for ejaculation, some brain regions have been identified to be particularly activated after ejaculation in male rats. Indeed, clusters of cFos-positive neurons have been found in the posterodorsal MeA, the posteromedial BNST, and the medial suprafascicular nucleus of the thalamus (Coolen et al., 1996). These brain regions might then be also investigated in the future.

1.7. Conclusion and future studies

During my PhD thesis, I investigated how mating influences social fear extinction in male mice. The principal hypothesis was a main involvement of the OXT system in this context. However,

the outcome of my work seems to point in another direction. Indeed, the blockage of the OXTR within the LS before mating did not impair social fear extinction. In the context of mating behavior, OXT might act in synergy with other neurotransmitters in the brain to affect fear and anxiety-like behavior.

One of the biggest issues is to determine if OXT is released within the LS in response to mating in a general manner, or if the central release of OXT follow the same pattern than in the plasma and significantly increases during ejaculation (Ogawa et al., 1980; Carmichael et al., 1987; Murphy et al., 1987; Krüger et al., 2003). This could be measured by collecting cerebrospinal fluid (CSF) via *cisterna magna* puncture either during mating behavior or immediately after ejaculation. Even though the spatial resolution is not optimal, the temporal resolution is better than the microdialysis experiment. After having the confirmation that ejaculation leads to an increased release of OXT in comparison to mating, the hypothesis of the "self-priming" of the OXT neurons should be explored. The second issue is the pharmacological experiments, which seem to impair the facilitation of the extinction induced by ejaculation. Hence, it seems more appropriate to use a different approach like chemogenetics which is less invasive and stressful on the day of the experiment (only an i.p. infusion). Moreover, this technique allows a long-term inhibition of the targeted neurons, which will allow the inhibition of a specific system/region during the entire mating duration. To determine which region should be inhibited, the results of the measurement of neuronal activation during mating and social fear extinction are crucial. These results would give a proper indication of which region is activated by both stimuli and might then be involved in both mating behavior and social fear extinction. A good perspective for this project would be then to investigate a possible macrocircuit involved in the regulation of social fear extinction using chemogenetics.

1.8. Outlook

The possibility that OXT interacts with other neurotransmitters to induce the facilitation of social fear extinction should not be excluded. Mating behavior is a complex behavior involving different neurotransmitters like dopamine (DA). DA is released in several brain regions like the nucleus accumbens, the MPOA, and the PVN during mating behavior (Hull et al., 1995; Sato et al., 1995; Melis et al., 2003; Succu et al., 2007). Using lesions and pharmacological studies, DA and its receptors have been shown to facilitate mating behavior, be pro-ejaculatory and participate in the sexual reward effect (Pfaus and Phillips, 1989; Markowski et

al., 1994; Dominguez and Hull, 2005; Kitrey et al., 2007; Clément et al., 2008). During mating, DA stimulates the OXT neurons in the PVN. In response, OXT is then released into the PVN and the VTA among others, stimulating DA release and OXT release in extra-hypothalamic regions (e.g. the spinal cord) to induce penile erection and promote ejaculation (Melis et al., 1989; Melis and Argiolas, 1995, 2011; Hull et al., 2004; Argiolas and Melis, 2005). Mostly known as a rewarding neurotransmitter, the involvement of DA in aversively motivated behavior was investigated first in the early 1960s (Posluns, 1962). During fear extinction, DA is released in the medial prefrontal cortex (Hugues et al., 2007; Lammel et al., 2011). In contextual fear conditioning, an increase of DA concentrations within the ECSF by blocking the dopamine transporter or a systemic administration of L-DOPA (precursor of DA) after fear extinction enhanced fear extinction retention in male mice (Abraham et al., 2012; Haaker et al., 2013). The mesocorticodopamic system contributes to the evaluation of the threat interpretation and can shift the anxiety-like behavior towards appetitive behavior when the apparent risk seems inferior to the possible rewards (e.g. food, water, mating partner). DA-based treatments were already used in clinical studies. In healthy patients, a protective effect from fear renewal was found after the administration of L-DOPA after fear extinction (Haaker et al., 2015). In PTSD-patients, the efficacy of psychotherapy was improved following the administration of MDMA (drug inducing increased level of DA) (Mithoefer et al., 2011) with robust fear reductions for up to 74 months (Mithoefer et al., 2013, 2018). A co-action of OXT and DA in fear/anxiety was shown in the shock-probe burying test. The administration of raclopride, a D2/3R antagonist, reverses the anxiolytic effect of OXT into the CeA (de la Mora et al., 2016). A co-action of OXT and DA to facilitate social fear extinction after ejaculation is then a possibility.

In the context of this project, I showed that successful mating promotes the facilitation of social fear extinction. Negative social contact, such as the exposition to a fearful conspecific, enhances the renewal of fear after extinction (Guzmán et al., 2013; Nowak et al., 2013). However, social encounters with a positive valence such as an unconditioned conspecific or a mating partner induce a facilitation of fear extinction in rats and mice (Guzmán et al., 2014; Mikami et al., 2016; Yuan et al., 2018; Ferreira et al., 2019; Gao et al., 2020). Social support during fear extinction seems to recruit different mechanisms than a solitary extinction (Farias et al., 2019). Hence, positive relationships or social company before or during cognitive

behavioral therapy would potentiate the effect of the therapy, particularly when the patient is engaged in a healthy relationship (Tol et al., 2011; Rapee et al., 2015; Strauss et al., 2017; Hori et al., 2018; Stone et al., 2019).

2. Involvement of the OXT system into the CeA in social fear extinction

Using the SFC paradigm, our lab has previously shown that OXT within the LS can facilitate the extinction of social fear (Zoicas et al., 2014; Menon et al., 2018). However, social fear is without any doubt a complex process, likely involving many other brain areas, such as the amygdala. Therefore, in the second part of my PhD thesis, I studied the involvement of the CeA in the process of social fear extinction, focusing on the OXT system using pharmacological and chemogenetic techniques.

In the first part of my thesis, I assessed the involvement of specific brain regions in the process of social fear extinction. I found an increase of the cFos protein expression after social fear extinction in SFC^+ mice in the CeA. This resembles previous findings obtained after cued fear conditioning, in which rats that extinguished the fear presented a high level of cFos protein in the CeA, specifically in the CeL (Knapska and Maren, 2009). This change in the expression of cFos in the CeA implies an involvement of the CeA during social fear extinction. Furthermore, the particular involvement of the OXT system in social fear extinction and the increase of the OXTR binding in SFC^+ mice after social fear acquisition (Zoicas et al., 2014) raise the hypothesis of a specific involvement of OXT within the CeA during social fear extinction.

2.1. Effects of OXT infusion into the CeA on social fear

In order to assess the involvement of OXT within the CeA in social fear extinction, I infused synthetic OXT directly into the CeA before social fear extinction. As expected, I found a slight facilitation of the social fear extinction after OXT infusion into the CeA. The effects of OXT within the CeA on cued fear extinction have been investigated in several studies. Within the CeA, a microcircuit gates the fear behavior expression. The CeL part contains two subpopulations of GABAergic neurons, the $PKC\delta^-$ cells and the $PKC\delta^+$ cells, also called CeL^{ON} and CeL^{OFF} respectively. The $PKC\delta^-$ are activated when the conditioned stimulus is presented, which leads to a freezing response. The $PKC\delta^+$ or CeL^{OFF} cells, also expressing the OXTR, do not respond to the conditioned stimulus and their activation inhibits the freezing response (Ciocchi et al., 2010; Haubensak et al., 2010). $PKC\delta^+$ and $PKC\delta^-$ neurons are reciprocally

inhibiting each other. The PKCδ^{+} or CeL^{OFF} project to the CeM where they tonically inhibit the neurons projecting to output brain regions like the PAG. Hence, when a conditioned stimulus is present, PKCδ^{-} cells are activated and inhibit the PKCδ^{+} cells. In turn, these disinhibit the CeM neurons which leads to freezing. The release of OXT induces the excitation of CeA neurons in brain slices of male mice and lactating rats (Huber et al., 2005; Terenzi and Ingram, 2005; Knobloch et al., 2012). Theoretically, OXT should then excites the PKCδ^{+} neurons and, via the inhibition of the CeM neurons, inhibit the freezing response. These mechanisms have been previously investigated using pharmacological and optogenetic approaches. The activation of the OXT system within the CeA leads to an inhibition of the freezing response in classic Pavlovian fear conditioning (Viviani et al., 2011; Knobloch et al., 2012; Rickenbacher et al., 2017). In the context of SFC, I saw that the cFos expression is increased in the CeA after social fear extinction. Moreover, a previous study in our lab showed a higher OXTR binding within the CeA in SFC^{+} mice after acquisition (Zoicas et al., 2014). My result confirms then an involvement of the OXT system in social fear extinction. However, the slight facilitation of the extinction by OXT infusion into the CeA shows that the CeA is probably not the major site of action of OXT in social fear extinction.

As OXT can also bind to vasopressin receptors, the expression of vasopressin receptors in the CeM (Huber et al., 2005) raises the possibility that OXT may act through the vasopressin receptor to affect social fear extinction. A classic way to answer this question is to co-infuse an OXTR-A or a vasopressin receptor antagonist with OXT. If the effect of OXT is blocked only by the OXTR-A, then OXT acts via the OXTR. If the effect is blocked or partially blocked by the vasopressin receptor antagonist, then OXT acts at least partially via the vasopressin receptor. In the case of the CeA, the difficulties met during the first experiment (Experiment 1) show the sensitivity of this region to tissue disruptions. Thus, a double-infusion in this region could harm or disrupt the behavior. Therefore, I decided to infuse the specific agonist of the OXTR Carbetocin (Passoni et al., 2016). If the OXT effect would be mediated by the OXTR, I was then expecting a similar effect on social fear extinction using Carbetocin. I could not find any effect of Carbetocin on social fear extinction. This could mean that OXT does not act through the OXTR in the CeA to regulate social fear extinction. On top of this, *in vitro* studies showed that Carbetocin is a specific agonist of the OXTR only when the OXTR is coupled with a protein Gq (Passoni et al., 2016). If the OXTR into the CeA is Gi-coupled, then Carbetocin would have

indeed no effect. However, in my opinion, this explanation seems not plausible because if the OXTR were coupled with a Gi protein, this would mean the inhibition of the PKCδ^{+} cells by OXT. This would then theoretically lead to an increase of the freezing response, which is in opposition to the known effects of OXT within the CeA. Furthermore, OXT has been shown to activate approximately 20% of the CeL neurons (Huber et al., 2005). Carbetocin has been tested in our lab in the context of SFC. When infused into the LS before social fear extinction, Carbetocin induced a similar facilitation effect than OXT (unpublished data, Dr. Rohit Menon). In mice, this agonist was also successfully used when infused into the nucleus accumbens (Williams et al., 2020). However, a study published shortly after my experiments showed an inefficiency of Carbetocin *in vivo* (Moy et al., 2019).

2.2. Chemogenetic activation of the OXTR neurons into the CeA

The infusion of Carbetocin into the CeA did not successfully answer the question of the involvement of the OXTR in the mediation of the OXT effect. We recently obtained in the lab a new mouse line, the OXTR-Cre mice. In these mice, the expression of the enzyme Cre recombinase is driven by the OXTR promoter. Using these mice, I could directly target and manipulate the neuronal subpopulation expressing the OXTR within the CeA. In this last experiment, I infused an AAV-hSyn-DIO-hM3D(Gq)-MCherry directly into the CeA, inducing the expression of a Gq-DREADD specifically on the OXTR-positive neurons of the CeA. 3 weeks after the virus infusion, I performed the SFC and administrated before extinction CNO, the dedicated ligand of DREADD, to the mice. Theoretically, this should lead to the activation of the OXTR neurons. No effect could be found during the extinction, however, the groups need to be completed. As the effect of OXT on social fear extinction seems to be discrete, it is possible that a higher number of mice is necessary to generate a clear behavioral effect. Furthermore, even by infusing the lowest volume possible (i.e., 70nl), the virus diffused in numerous mice into the dorsal MeA. This has to be taken into consideration in future interpretations of the results.

2.1. Conclusion and future experiments

I could show a discrete facilitation of OXT on social fear extinction. However, I could not yet confirm the involvement of the OXTR in the mediation of the OXT effect. The first step would be to complete the chemogenetic experiment using Gq-DREADD as a method to specifically

activate the OXTR-positive neurons within the CeA. Moreover, a neuronal activation study would be interesting to identify the neuronal subpopulations within the CeL which are activated by OXT. In this experiment, I would infuse OXT within the CeA and sacrifice the mice 90 min later. I would then stain the CeL for the cFos protein and the PKCδ protein. I would expect an expression of the protein cFos in the $PKC\delta^{+}$ neurons.

2.2. Outlook

As previously explained, OXT is a potential new target for treating anxiety disorders in combination with cognitive behavioral therapy. In healthy and SAD patients, it was shown that OXT can dampen the amygdala response in response to fearful faces (Kirsch et al., 2005; Evans et al., 2010; Labuschagne et al., 2010). In healthy patients, OXT administrated before the cognitive behavioral therapy, increased fear expression during fear extinction but, was able to significantly decrease fear expression during the extinction retrieval (Acheson et al., 2013). The involvement of CeA-OXT signaling in social fear extinction needs to be further studied. More importantly, the circuit in which OXT acts to reduce social fear seems to be particularly relevant from the perspective of a new potential treatment for SAD.

BIBLIOGRAPHY

Abraham AD, Cunningham CL, Lattal KM (2012) Methylphenidate enhances extinction of contextual fear. Learn Mem 19:67–72.

Acheson D, Feifel D, De Wilde S, McKinney R, Lohr J, Risbrough V (2013) The effect of intranasal oxytocin treatment on conditioned fear extinction and recall in a healthy human sample. Psychopharmacology (Berl) 229:199–208.

Ackerman AE, Lange GM, Clemens LG (1997) Effects of paraventricular lesions on sex behavior and seminal emission in male rats. Physiol Behav 63:49–53.

Adolphs R, Tranel D, Damasio H, Damasio A (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature 372:669–672.

American Psychological Association (APA) (2013) Diagnostic and Statistical Manual of Mental Disorders: Depressive Disorders. American Psychiatric Publishing, Inc.

Amico JA, Mantella RC, Vollmer RR, Li X (2004) Anxiety and stress responses in female oxytocin deficient mice. J Neuroendocrinol 16:319–324.

Anderson DJ, Adolphs R (2014) A Framework for Studying Emotions Across Phylogeny.

Anthony TE, Dee N, Bernard A, Lerchner W, Heintz N, Anderson DJ (2014) Control of stress-induced persistent anxiety by an extra-amygdala septohypothalamic circuit. Cell 156:522–536.

Argiolas A, Collu M, Gessa GL, Melis MR, Serra G (1988) The oxytocin antagonist d(CH2)5Tyr(Me)-Orn8-vasotocin inhibits male copulatory behaviour in rats. Eur J Pharmacol 149:389–392.

Argiolas A, Melis MR (2004) The role of oxytocin and the paraventricular nucleus in the sexual behaviour of male mammals. Physiol Behav 83:309–317.

Argiolas A, Melis MR (2005) Central control of penile erection: Role of the paraventricular nucleus of the hypothalamus. Prog Neurobiol 76:1–21.

Argiolas A, Melis MR, Mauri A, Gessa GL (1989) Oxytocin-induced penile erection and yawning in male rats: effect of neonatal monosodium glutamate and hypophysectomy. Psychopharmacology (Berl) 97:383–387.

Arletti R, Bazzani C, Castelli M, Bertolini A (1985) Oxytocin improves male copulatory performance in rats. Horm Behav 19:14–20.

Arletti R, Benelli A, Bertolini A (1992) Oxytocin Involvement in Male and Female Sexual Behavior. Ann N Y Acad Sci 652:180–193.

Arletti R, Bertolini A (1985) Oxytocin stimulates lordosis behavior in female rats. Neuropeptides 6:247–253.

Armstrong WE, Warach S, Hatton GI, Mcneill TH (1980) Subnuclei in the rat hypothalamic paraventricular nucleus: A cytoarchitectural, horseradish peroxidase and immunocytochemical analysis. Neuroscience 5:1931–1958.

Bai HY, Cao J, Liu N, Xu L, Luo JH (2009) Sexual behavior modulates contextual fear memory

through dopamine D1/D5 receptors. Hippocampus 19:289–298.

Baldwin DS, Pallanti S, Zwanzger P (2013) Developing a European research network to address unmet needs in anxiety disorders. Neurosci Biobehav Rev 37:2312–2317.

Bale TL, Davis AM, Auger AP, Dorsa DM, McCarthy MM (2001) CNS region-specific oxytocin receptor expression: Importance in regulation of anxiety and sex behavior. J Neurosci 21:2546–2552.

Bancila M, Giuliano F, Rampin O, Mailly P, Brisorgueil MJ, Calas A, Vergé D (2002) Evidence for a direct projection from the paraventricular nucleus of the hypothalamus to putative serotoninergic neurons of the nucleus paragigantocellularis involved in the control of erection in rats. Eur J Neurosci 16:1240–1248.

Bandelow B, Michaelis S (2015) Epidemiology of anxiety disorders in the 21st century. Dialogues Clin Neurosci 17:327–335.

Barrett LF, Satpute AB (2019) Historical pitfalls and new directions in the neuroscience of emotion. Neurosci Lett 693:9–18.

Baum MJ, Everitt BJ (1992) Increased expression of c-fos in the medial preoptic area after mating in male rats: Role of afferent inputs from the medial amygdala and midbrain central tegmental field. Neuroscience 50:627–646.

Baum MJ, Kelliher KR (2009) Complementary Roles of the Main and Accessory Olfactory Systems in Mammalian Mate Recognition.

Baum MJ, Tobet SA, Starr MS, Bradshaw WG (1982) Implantation of dihydrotestosterone propionate into the lateral septum or medial amygdala facilitates copulation in castrated male rats given estradiol systemically. Horm Behav 16:208–223.

Beyer C, Contreras JL, Moralí G, Larsson K (1981) Effects of castration and sex steroid treatment on the motor copulatory pattern of the rat. Physiol Behav 27:727–730.

Blanchard RJ, Yudko EB, Rodgers RJ, Blanchard DC (1993) Defense system psychopharmacology: An ethological approach to the pharmacology of fear and anxiety. Behav Brain Res 58:155–165.

Blanco C, Bragdon LB, Schneier FR, Liebowitz MR (2013) The evidence-based pharmacotherapy of social anxiety disorder. Int J Neuropsychopharmacol 16:235–249.

Blume A, Bosch OJ, Miklos S, Torner L, Wales L, Waldherr M, Neumann ID (2008) Oxytocin reduces anxiety via ERK1/2 activation: Local effect within the rat hypothalamic paraventricular nucleus. Eur J Neurosci 27:1947–1956.

Borland JM, Walton JC, Norvelle A, Grantham KN, Aiani LM, Larkin TE, McCann KE, Albers HE (2020) Social experience and sex-dependent regulation of aggression in the lateral septum by extrasynaptic δGABAA receptors. Psychopharmacology (Berl) 237:329–344.

Bosch OJ, Meddle SL, Beiderbeck DI, Douglas AJ, Neumann ID (2005) Brain oxytocin correlates with maternal aggression: Link to anxiety. J Neurosci 25:6807–6815.

Brackett NL, Edwards DA (1984) Medial preoptic connections with the midbrain tegmentum are essential for male sexual behavior. Physiol Behav 32:79–84.

Brady J V., Nauta WJH (1953) Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. J Comp Physiol Psychol 46:339–346.

Brinton RE, Wamsley JK, Gee KW, Wan YP, Yamamura HI (1984) [3H]oxytocin binding sites in the rat brain demonstrated by quantitative light microscopic autoradiography. Eur J Pharmacol 102:365–367.

Burns-cusato M, Scordalakes EM, Rissman EF (2004) Of mice and missing data : what we know (and need to learn) about male sexual behavior. 83:217–232.

Calandreau L, Jaffard R, Desmedt A (2007) Dissociated roles for the lateral and medial septum in elemental and contextual fear conditioning. Learn Mem 14:422–429.

Calcagnoli F, Kreutzmann JC, de Boer SF, Althaus M, Koolhaas JM (2015) Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. Psychoneuroendocrinology 51:112–121.

Camacho FJ, Portillo W, Quintero-Enríquez O, Paredes RG (2009) Reward value of intromissions and morphine in male rats evaluated by conditioned place preference. Physiol Behav 98:602–607.

Campbell-Smith EJ, Holmes NM, Lingawi NW, Panayi MC, Westbrook RF (2015) Oxytocin signaling in basolateral and central amygdala nuclei differentially regulates the acquisition, expression, and extinction of context-conditioned fear in rats. Learn Mem 22:247–257.

Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ (1997) Elicitation and reduction of fear: Behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-fos. Neuroscience 78:1087–1104.

Campos AC, Fogaça M V., Aguiar DC, Guimarães FS (2013) Animal models of anxiety disorders and stress. Rev Bras Psiquiatr 35.

Caquineau C, Leng G, Guan XMM, Jiang M, Van Der Ploeg L, Douglas AJ (2006) Effects of α-melanocyte-stimulating hormone on magnocellular oxytocin neurones and their activation at intromission in male rats. J Neuroendocrinol 18:685–691.

Carmichael MS, Humbert R, Dixen J, Palmisano G, Greenleaf W, Davidson JM (1987) Plasma oxytocin increases in the human sexual response. J Clin Endocrinol Metab 64:27–31.

Ciocchi S, Herry C, Grenier F, Wolff SBE, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, Müller C, Lüthi A (2010) Encoding of conditioned fear in central amygdala inhibitory circuits. Nature 468:277–282.

Clément P, Bernabé J, Compagnie S, Alexandre L, McCallum S, Giuliano F (2013) Inhibition of ejaculation by the non-peptide oxytocin receptor antagonist GSK557296: A multi-level site of action. Br J Pharmacol 169:1477–1485.

Clément P, Peeters M, Bernabé J, Denys P, Alexandre L, Giuliano F (2008) Brain oxytocin receptors mediate ejaculation elicited by 7-hydroxy-2-(di-N-propylamino) tetralin (7-OH-DPAT) in anaesthetized rats. Br J Pharmacol 154:1150–1159.

Coolen LM, Peters HJPW, Veening JG (1996) Fos immunoreactivity in the rat brain following

consummatory elements of sexual behavior: A sex comparison. Brain Res 738:67–82.

Curran T, Morgan JI (1995) Fos: An immediate-early transcription factor in neurons. J Neurobiol 26:403–412.

Darwin C (1872) The expression of the emotions in man and animals. London: J. Murray.

de Jong TR, Menon R, Bludau A, Grund T, Biermeier V, Klampfl SM, Jurek B, Bosch OJ, Hellhammer J, Neumann ID (2015) Salivary oxytocin concentrations in response to running, sexual self-stimulation, breastfeeding and the TSST: The Regensburg Oxytocin Challenge (ROC) study. Psychoneuroendocrinology 62:381–388.

De Jongh R, Groenink L, Van Der Gugten J, Olivier B (2003) Light-enhanced and fear-potentiated startle: Temporal characteristics and effects of α-helical corticotropin-releasing hormone. Biol Psychiatry 54:1041–1048.

De Kloet ER, Rotteveel F, Voorhuis TAM, Terlou M (1985) Topography of binding sites for neurohypophyseal hormones in rat brain. Eur J Pharmacol 110:113–119.

de la Mora MP, Pérez-Carrera D, Crespo-Ramírez M, Tarakanov A, Fuxe K, Borroto-Escuela DO (2016) Signaling in dopamine D2 receptor-oxytocin receptor heterocomplexes and its relevance for the anxiolytic effects of dopamine and oxytocin interactions in the amygdala of the rat. Biochim Biophys Acta - Mol Basis Dis 1862:2075–2085.

de Vries GJ, Buijs RM (1983) The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273:307–317.

Deacon RMJ (2006) Digging and marble burying in mice: Simple methods for in vivo identification of biological impacts. Nat Protoc 1:122–124.

Degroot A, Nomikos GG (2004) Genetic deletion and pharmacological blockade of CB1 receptors modulates anxiety in the shock-probe burying test. Eur J Neurosci 20:1059–1064.

Degroot A, Treit D (2004) Anxiety is functionally segregated within the septo-hippocampal system. Brain Res 1001:60–71.

Deng K, Yang L, Xie J, Tang H, Wu GS, Luo HR (2019) Whole-brain mapping of projection from mouse lateral septal nucleus. Biol Open 8.

Dhungel S, Masaoka M, Rai D, Kondo Y, Sakuma Y (2011) Both olfactory epithelialand vomeronasal inputs are essential for activation of the medial amygdala and preoptic neurons of male rats. NSC 199:225–234.

Dodhia S, Hosanagar A, Fitzgerald DA, Labuschagne I, Wood AG, Nathan PJ, Phan KL (2014) Modulation of resting-state amygdala-frontal functional connectivity by oxytocin in generalized social anxiety disorder. Neuropsychopharmacology 39:2061–2069.

Dominguez JM, Hull EM (2005) Dopamine, the medial preoptic area, and male sexual behavior. Physiol Behav 86:356–368.

Du Vigneaud V, Ressler C, Trippett S (1953) The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin. J Biol Chem 205:949–957.

Dumais KM, Alonso AG, Bredewold R, Veenema AH (2016) Role of the oxytocin system in

amygdala subregions in the regulation of social interest in male and female rats. Neuroscience 330:138–149.

Ebner K, Bosch OJ, Krömer SA, Singewald N, Neumann ID (2005) Release of oxytocin in the rat central amygdala modulates stress-coping behavior and the release of excitatory amino acids. Neuropsychopharmacology 30:223–230.

Ebner K, Wotjak CT, Landgraf R, Engelmann M (2000) A single social defeat experience selectively stimulates the release of oxytocin, but not vasopressin, within the septal brain area of male rats. Brain Res 872:87–92.

Elands J, Beetsma A, Barberis C, de Kloet ER (1988) Topography of the oxytocin receptor system in rat brain: an autoradiographical study with a selective radioiodinated oxytocin antagonist. J Chem Neuroanat 1:293–302.

Eliava M et al. (2016) A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. Neuron 89:1291–1304.

Emery DE, Sachs BD (1976) Copulatory behavior in male rats with lesions in the bed nucleus of the stria terminalis. Physiol Behav 17:803–806.

Engelmann M, Ebner K, Landgraf R, Holsboer F, Wotjak CT (1999) Emotional stress triggers intrahypothalamic but not peripheral release of oxytocin in male rats. J Neuroendocrinol 11:867–872.

Engelmann M, Ebner K, Landgraf R, Wotjak CT (2006) Effects of Morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. Horm Behav 50:496–501.

Engelmann M, Ebner K, Wotjak CT, Landgraf R (1998) Endogenous oxytocin is involved in short-term olfactory memory in female rats. Behav Brain Res 90:89–94.

Evans S, Shergill SS, Averbeck BB (2010) Oxytocin decreases aversion to angry faces in an associative learning task. Neuropsychopharmacology 35:2502–2509.

Fadok JP, Krabbe S, Markovic M, Courtin J, Xu C, Massi L, Botta P, Bylund K, Müller C, Kovacevic A, Tovote P, Lüthi A (2017) A competitive inhibitory circuit for selection of active and passive fear responses. Nature 542:96–100.

Fadok JP, Markovic M, Tovote P, Lüthi A (2018) New perspectives on central amygdala function. Curr Opin Neurobiol 49:141–147.

Farias CP, Furini CRG, Nachtigall EG, Behling JAK, De Assis Brasil ES, Bühler L, Izquierdo I, De Carvalho Myskiw J (2019) Extinction learning with social support depends on protein synthesis in prefrontal cortex but not hippocampus. Proc Natl Acad Sci U S A 116:1765–1769.

Fernández-Guasti A, Roldán-Roldán G, Saldívar A (1989) Reduction in anxiety after ejaculation in the rat. Behav Brain Res 32:23–29.

Fernández-Guasti A, Saldívar A (1990) Participation of the GABA-benzodiazepine System in the Inhibition of Defensive Burying Produced by Ejaculation. Behav Pharmacol 1.

Ferreira FF, Rodrigues FS, Schmidt SD, Cavalcante LES, Zinn CG, Farias CP, Furini CRG, Myskiw JC, Izquierdo I (2019) Social support favors extinction and impairs acquisition of both short- and long-term contextual fear conditioning memory. Neurosci Lett 712.

Ferretti V, Maltese F, Contarini G, Nigro M, Bonavia A, Huang H, Gigliucci V, Morelli G, Scheggia D, Managò F, Castellani G, Lefevre A, Cancedda L, Chini B, Grinevich V, Papaleo F (2019) Oxytocin signaling in the central amygdala modulates emotion discrimination in mice. Curr Biol 29:1938-1953.e6.

Filippi S, Morelli A, Vignozzi L, Vannelli GB, Marini M, Ferruzzi P, Mancina R, Crescioli C, Mondaini N, Forti G, Ledda F, Maggi M (2005) Oxytocin mediates the estrogen-dependent contractile activity of endothelin-1 in human and rabbit epididymis. Endocrinology 146:3506–3517.

Filippi S, Vannelli GB, Granchi S, Luconi M, Crescioli C, Mancina R, Natali A, Brocchi S, Vignozzi L, Bencini E, Noci I, Ledda F, Forti G, Maggi M (2002) Identification, localization and functional activity of oxytocin receptors in epididymis. In: Molecular and Cellular Endocrinology, pp 89–100. Mol Cell Endocrinol.

Gao F, Huang J, Guan YF, Huang G Bin, Li WJ, He XY, Qiu ZC, Zhang YL, Zhao ST, Li J, Xuan A, Sun XD (2020) Social company by a receptive mating partner facilitates fear extinction. Front Neurosci 14.

Gerendai I, Tóth IE, Kocsis K, Boldogkoi Z, Rusvai M, Halász B (2001) Identification of CNS neurons involved in the innervation of the epididymis: A viral transneuronal tracing study. Auton Neurosci Basic Clin 92:1–10.

Gil M, Bhatt R, Picotte KB, Hull EM (2011) Oxytocin in the medial preoptic area facilitates male sexual behavior in the rat. Horm Behav 59:435–443.

Gil M, Bhatt R, Picotte KB, Hull EM (2013) Sexual experience increases oxytocin receptor gene expression and protein in the medial preoptic area of the male rat. Psychoneuroendocrinology 38:1688–1697.

Gogate MG, Brid S V., Wingkar KC, Kantak NM (1995) Septal regulation of male sexual behavior in rats. Physiol Behav 57:1205–1207.

Goldin PR, Ziv M, Jazaieri H, Weeks J, Heimberg RG, Gross JJ (2014) Impact of cognitive-behavioral therapy for social anxiety disorder on the neural bases of emotional reactivity to and regulation of social evaluation. Behav Res Ther 62:97–106.

Gorka SM, Fitzgerald DA, Labuschagne I, Hosanagar A, Wood AG, Nathan PJ, Phan KL (2015) Oxytocin modulation of amygdala functional connectivity to fearful faces in generalized social anxiety disorder. Neuropsychopharmacology 40:278–286.

Gould TD, Dao DT, Kovacsics CE (2009) The Open Field Test. In, pp 1–20. Humana Press, Totowa, NJ.

Griebel G, Belzung C, Perrault G, Sanger DJ (2000) Differences in anxiety-related behaviors and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl) 148:164–170.

Grinevich V, Neumann ID (2020) Brain oxytocin: how puzzle stones from animal studies

translate into psychiatry. Mol Psychiatry:1 Available at: http://www.ncbi.nlm.nih.gov/pubmed/32514104 [Accessed June 18, 2020].

Gulia KK, Kumar VM, Mallick HN (2002) Role of the lateral septal noradrenergic system in the elaboration of male sexual behavior in rats. Pharmacol Biochem Behav 72:817–823.

Gupta J, Russell RJ, Wayman CP, Hurley D, Jackson VM (2008) Oxytocin-induced contractions within rat and rabbit ejaculatory tissues are mediated by vasopressin V 1A receptors and not oxytocin receptors. Br J Pharmacol 155:118–126.

Guzmán YF, Tronson NC, Jovasevic V, Sato K, Guedea AL, Mizukami H, Nishimori K, Radulovic J (2013) Fear-enhancing effects of septal oxytocin receptors. Nat Neurosci 16:1185–1187.

Guzmán YF, Tronson NC, Sato K, Mesic I, Guedea AL, Nishimori K, Radulovic J (2014) Role of oxytocin receptors in modulation of fear by social memory. Psychopharmacology (Berl) 231:2097–2105.

Haaker J, Gaburro S, Sah A, Gartmann N, Lonsdorf TB, Meier K, Singewald N, Pape HC, Morellini F, Kalisch R (2013) Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. Proc Natl Acad Sci U S A 110.

Haaker J, Lonsdorf TB, Kalisch R (2015) Effects of post-extinction l-DOPA administration on the spontaneous recovery and reinstatement of fear in a human fMRI study. Eur Neuropsychopharmacol 25:1544–1555.

Haller J, Bakos N (2002) Stress-induced social avoidance: A new model of stress-induced anxiety? Physiol Behav 77:327–332.

Han RT, Kim YB, Park EH, Kim JY, Ryu C, Kim HY, Lee JH, Pahk K, Shanyu C, Kim H, Back SK, Kim HJ, Kim YI, Na HS (2018) Long-Term Isolation Elicits Depression and Anxiety-Related Behaviors by Reducing Oxytocin-Induced GABAergic Transmission in Central Amygdala. Front Mol Neurosci 11.

Hascoët M, Bourin M (2009) The Mouse Light–Dark Box Test. In, pp 197–223. Humana Press, Totowa, NJ.

Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, Biag J, Dong HW, Deisseroth K, Callaway EM, Fanselow MS, Lüthi A, Anderson DJ (2010) Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature 468:270–276.

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003) Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 54:1389–1398.

Henry B, Vale W, Markou A (2006) The effect of lateral septum corticotropin-releasing factor receptor 2 activation on anxiety is modulated by stress. J Neurosci 26:9142–9152.

Hoge EA, Lawson EA, Metcalf CA, Keshaviah A, Zak PJ, Pollack MH, Simon NM (2012) Plasma oxytocin immunoreactive products and response to trust in patients with social anxiety disorder. Depress Anxiety 29:924–930.

Hoge EA, Pollack MH, Kaufman RE, Zak PJ, Simon NM (2008) Oxytocin levels in social anxiety disorder. CNS Neurosci Ther 14:165–170.

Holmes EA et al. (2020) Multidisciplinary research priorities for the COVID-19 pandemic: a call for action for mental health science. The Lancet Psychiatry 7:547.

Hori A, Morita T, Yoshida I, Tsubokura M (2018) Enhancement of PTSD treatment through social support in Idobata-Nagaya community housing after Fukushima's triple disaster. BMJ Case Rep 2018.

Huber D, Veinante P, Stoop R (2005) Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science 308:245–248.

Hughes AM, Everitt BJ, Lightman SL, Todd K (1987) Oxytocin in the central nervous system and sexual behaviour in male rats. Brain Res 414:133–137.

Hugues S, Garcia R, Léna I (2007) Time course of extracellular catecholamine and glutamate levels in the rat medial prefrontal cortex during and after extinction of conditioned fear. Synapse 61:933–937.

Huhman KL (2006) Social conflict models: Can they inform us about human psychopathology? Horm Behav 50:640–646.

Hull EM, Dominguez JM (2007) Sexual behavior in male rodents. Horm Behav 52:45–55.

Hull EM, Du J, Lorrain DS, Matuszewich L (1995) Extracellular dopamine in the medial preoptic area: Implications for sexual motivation and hormonal control of copulation. J Neurosci 15:7465–7471.

Hull EM, Muschamp JW, Sato S (2004) Dopamine and serotonin : influences on male sexual behavior. 83:291–307.

Hung LW, Neuner S, Polepalli JS, Beier KT, Wright M, Walsh JJ, Lewis EM, Luo L, Deisseroth K, Dölen G, Malenka RC (2017) Gating of social reward by oxytocin in the ventral tegmental area. Science (80-) 357:1406–1411.

Hurtazo HA, Paredes RG, Ågmo A (2008) Inactivation of the medial preoptic area/anterior hypothalamus by lidocaine reduces male sexual behavior and sexual incentive motivation in male rats. Neuroscience 152:331–337.

Jirikowski GF, Caldwell JD, Häussler HU, Pedersen CA (1991) Mating alters topography and content of oxytocin immunoreactivity in male mouse brain. Cell Tissue Res 266:399–403.

Johnson Z V., Walum H, Jamal YA, Xiao Y, Keebaugh AC, Inoue K, Young LJ (2016) Central oxytocin receptors mediate mating-induced partner preferences and enhance correlated activation across forebrain nuclei in male prairie voles. Horm Behav 79:8–17.

Jolkkonen E, Pitkänen A (1998) Intrinsic connections of the rat amygdaloid complex: projections originating in the central nucleus. J Comp Neurol 395.

Kelliher KR, Liu YC, Baum MJ, Sachs BD (1999) Neuronal Fos activation in olfactory bulb and forebrain of male rats having erections in the presence of inaccessible estrous females. Neuroscience 92:1025–1033.

Kendrick KM, Keverne EB, Chapman C, Baldwin BA (1988) Microdialysis measurement of oxytocin, aspartate, γ-aminobutyric acid and glutamate release from the olfactory bulb of the sheep during vaginocervical stimulation. Brain Res 442:171–174.

Kendrick KM, Keverne EB, Hinton MR, Goode JA (1992) Oxytocin, amino acid and monoamine release in the region of the medial preoptic area and bed nucleus of the stria terminalis of the sheep during parturition and suckling. Brain Res 569:199–209.

Kessler RC, Demler O, Frank RG, Olfson M, Pincus HA, Walters EE, Wang P, Wells KB, Zaslavsky AM (2005) Prevalence and treatment of mental disorders, 1990 to 2003. N Engl J Med 352:2515–2523.

Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci 25:11489–11493.

Kitrey ND, Clément P, Bernabé J, Alexandre L, Giuliano F (2007) Microinjection of the preferential dopamine receptor D3 agonist 7-hydroxy-N,N-di-n-propylaminotetralin hydrobromide into the hypothalamic medial preoptic area induced ejaculation in anesthetized rats. Neuroscience 149:636–641.

Knapska E, Maren S (2009) Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. Learn Mem 16:486–493.

Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, Osten P, Schwarz MK, Seeburg PH, Stoop R, Grinevich V (2012) Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73:553–566.

Kondo Y (1992) Lesions of the medial amygdala produce severe impairment of copulatory behavior in sexually inexperienced male rats. 51:939–943.

Kondo Y, Arai Y (1995) Functional association between the medial amygdala and the medial preoptic area in regulation of mating behavior in the male rat. Physiol Behav 57:69–73.

Kondo Y, Shinoda A, Yamanouchi K, Arai Y (1990) Role of septum and preoptic area in regulating masculine and feminine sexual behavior in male rats. Horm Behav 24:421–434.

Krüger THC, Haake P, Chereath D, Knapp W, Janssen OE, Exton MS, Schedlowski M, Hartmann U (2003) Specificity of the neuroendocrine response to orgasm during sexual arousal in men. J Endocrinol 177:57–64.

Kunkhyen T, Mccarthy EA, Korzan WJ, Doctor D, Han X, Michael J, Cherry JA (2017) Optogenetic activation of accessory olfactory bulb input to the forebrain differentially modulates investigation of opposite versus same-sex urinary chemosignals and stimulates mating in male mice. 4:1–16.

Labuschagne I, Phan KL, Wood A, Angstadt M, Chua P, Heinrichs M, Stout JC, Nathan PJ (2010) Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. Neuropsychopharmacology 35:2403–2413.

Lahoud N, Maroun M (2013) Oxytocinergic manipulations in corticolimbic circuit differentially affect fear acquisition and extinction. Psychoneuroendocrinology 38:2184–2195.

Lammel S, Ion DI, Roeper J, Malenka RC (2011) Projection-Specific Modulation of Dopamine Neuron Synapses by Aversive and Rewarding Stimuli. Neuron 70:855–862.

Lamontagne SJ, Olmstead MC, Menard JL (2016) The lateral septum and anterior hypothalamus act in tandem to regulate burying in the shock-probe test but not open-arm avoidance in the elevated plus-maze. Behav Brain Res 314:16–20.

Landgraf R, Hacker R, Buhl H (1982) Plasma vasopressin and oxytocin in response to exercise and during a day-night cycle in man. Endokrinologie 79:281–291.

LeDoux JE (2000) Emotion Circuits in the Brain. Annu Rev Neurosci 23:155–184.

Lehman MN, Winans SS, Powers JB (1980) Medial nucleus of the amygdala mediates chemosensory control of male hamster sexual behavior. Science (80-) 210:557–560.

Lévy F, Kendrick KM, Goode JA, Guevara-Guzman R, Keverne EB (1995) Oxytocin and vasopressin release in the olfactory bulb of parturient ewes: changes with maternal experience and effects on acetylcholine, γ-aminobutyric acid, glutamate and noradrenaline release. Brain Res 669:197–206.

Liu ZW, Jiang N, Tao X, Wang XP, Liu XM, Xiao SY (2020) Assessment of sexual behavior of male mice. J Vis Exp 2020.

Louvart H, Maccari S, Ducrocq F, Thomas P, Darnaudéry M (2005) Long-term behavioural alterations in female rats after a single intense footshock followed by situational reminders. Psychoneuroendocrinology 30:316–324.

Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. Nat Rev Neurosci 7:126–136.

Luiten PGM, ter Horst GJ, Karst H, Steffens AB (1985) The course of paraventricular hypothalamic efferents to autonomic structures in medulla and spinal cord. Brain Res 329:374–378.

Lukas M, Toth I, Veenema AH, Neumann ID (2013a) Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. Psychoneuroendocrinology 38:916–926.

Lukas M, Toth I, Veenema AH, Neumann ID (2013b) Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. Psychoneuroendocrinology 38:916–926.

Mantella RC, Vollmer RR, Li X, Amico JA (2003) Female oxytocin-deficient mice display enhanced anxiety-related behavior. In: Endocrinology, pp 2291–2296. Endocrinology.

Marazziti D, Abelli M, Baroni S, Carpita B, Ramacciotti CE, Dell'Osso L (2015) Neurobiological correlates of social anxiety disorder: An update. CNS Spectr 20:100–111.

Markowski VP, Eaton RC, Lumley LA, Moses J, Hull EM (1994) A D1 agonist in the MPOA facilitates copulation in male rats. Pharmacol Biochem Behav 47:483–486.

Marson L, Mckenna KE (1996) CNS Cell Groups Involved in the Control of the Ischiocavernosus and Bulbospongiosus Muscles: A Transneuronal Tracing Study Using Pseudorabies Virus. J Comp Neurol 374.

Marson L, Platt KB, McKenna KE (1993) Central nervous system innervation of the penis as revealed by the transneuronal transport of pseudorabies virus. Neuroscience 55:263–280.

Martínez-Mota L, López-Rubalcava C, Rodríguez-Manzo G (2005) Ejaculation induces long-lasting behavioural changes in male rats in the forced swimming test: Evidence for an increased sensitivity to the antidepressant desipramine. Brain Res Bull 65:323–329.

Mayhew TM (1992) A review of recent advances in stereology for quantifying neural structure. J Neurocytol 21:313–328.

McKenna KE, Chung SK, McVary KT (1991) A model for the study of sexual function in anesthetized male and female rats. Am J Physiol - Regul Integr Comp Physiol 261.

Melis MR, Argiolas A (1995) Dopamine and sexual behavior. Neurosci Biobehav Rev 19:19–38.

Melis MR, Argiolas A (2011) Central control of penile erection: A re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. Neurosci Biobehav Rev 35:939–955.

Melis MR, Argiolas A, Gessa GL (1989) Apomorphine increases plasma oxytocin concentration in male rats. Neurosci Lett 98:351–355.

Melis MR, Argiolas A, Stancampino R, Gessa GL (1988) Oxytocin-induced penile erection and yawning: Structure-activity relationship studies. Pharmacol Res Commun 20:1117–1118.

Melis MR, Succu S, Mascia MS, Cortis L, Argiolas A (2003) Extra-cellular dopamine increases in the paraventricular nucleus of male rats during sexual activity. Eur J Neurosci 17:1266–1272.

Menard J, Treit D (1996) Lateral and medial septal lesions reduce anxiety in the plus-maze and probe-burying tests. Physiol Behav 60:845–853.

Menon R, Grund T, Zoicas I, Althammer F, Fiedler D, Biermeier V, Bosch O., Hiraoka Y, Nishimori K, Eliava M, Grinevich V, Neumann ID (2018) Oxytocin signalling in the lateral septum prevents social fear during lactation. Curr Biol 28:1–13.

Mikami K, Kiyokawa Y, Takeuchi Y, Mori Y (2016) Social buffering enhances extinction of conditioned fear responses in male rats. Physiol Behav 163:123–128.

Miller SM, Piasecki CC, Lonstein JS (2011) Use of the light-dark box to compare the anxiety-related behavior of virgin and postpartum female rats. Pharmacol Biochem Behav 100:130–137.

Mithoefer MC, Mithoefer AT, Feduccia AA, Jerome L, Wagner M, Wymer J, Holland J, Hamilton S, Yazar-Klosinski B, Emerson A, Doblin R (2018) 3,4-methylenedioxymethamphetamine (MDMA)-assisted psychotherapy for post-traumatic stress disorder in military veterans, firefighters, and police officers: a randomised, double-blind, dose-response, phase 2 clinical trial. The Lancet Psychiatry 5:486–497.

Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Doblin R (2011) The safety and efficacy of ±3,4-methylenedioxymethamphetamine- assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: The first randomized controlled pilot study. J Psychopharmacol 25:439–452.

Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Martin SF, Yazar-Klosinski B, Michel Y, Brewerton TD, Doblin R (2013) Durability of improvement in post-traumatic stress disorder symptoms and absence of harmful effects or drug dependency after 3,4-methylenedioxymethamphetamine-assisted psychotherapy: A prospective long-term follow-up study. J Psychopharmacol 27:28–39.

Mongeau R, Miller GA, Chiang E, Anderson DJ (2003) Neural correlates of competing fear behaviors evoked by an innately aversive stimulus. J Neurosci 23:3855–3868.

Moy SS, Teng BL, Nikolova VD, Riddick N V., Simpson CD, Van Deusen A, Janzen WP, Sassano MF, Pedersen CA, Jarstfer MB (2019) Prosocial effects of an oxytocin metabolite, but not synthetic oxytocin receptor agonists, in a mouse model of autism. Neuropharmacology 144:301–311.

Murphy MR, Seckl JR, Burton S, Checkley SA, Lightman SL (1987) Changes in oxytocin and vasopressin secretion during sexual activity in men. J Clin Endocrinol Metab 65:738–741.

Neumann I, Russell JA, Landgraf R (1993) Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: A microdialysis study. Neuroscience 53:65–75.

Neumann ID (2007) Stimuli and consequences of dendritic release of oxytocin within the brain. In: Biochemical Society Transactions, pp 1252–1257. Biochem Soc Trans.

Neumann ID, Krömer SA, Toschi N, Ebner K (2000) Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. Regul Pept 96:31–38.

Neumann ID, Landgraf R (1989) Septal and hippocampal release of oxytocin, but not vasopressin, in the conscious lactating rat during suckling. J Neuroendocrinol 1:305–308.

Normandin JJ, Murphy AZ (2011) Serotonergic lesions of the periaqueductal gray, a primary source of serotonin to the nucleus paragigantocellularis, facilitate sexual behavior in male rats. Pharmacol Biochem Behav 98:369–375.

Nowak A, Werka T, Knapska E (2013) Social modulation in extinction of aversive memories. Behav Brain Res 238:200–205.

Nyuyki KD, Waldherr M, Baeuml S, Neumann ID (2011) Yes, I am ready now: Differential effects of paced versus unpaced mating on anxiety and central oxytocin release in female rats. PLoS One 6.

Ogawa S, Kudo S, Kitsunai Y, Fukuchi S (1980) Increase in oxytocin secretion at ejaculation in male. Clin Endocrinol (Oxf) 13:95–97.

Oh KS, Kim EJ, Ha JW, Woo HY, Kwon MJ, Shin DW, Shin YC, Lim SW (2018) The relationship between plasma oxytocin levels and social anxiety symptoms. Psychiatry Investig 15:1079–1086.

Paredes RG (2009) Evaluating the neurobiology of sexual reward. ILAR J 50:15–27.

Parfitt GM, Nguyen R, Bang JY, Aqrabawi AJ, Tran MM, Seo DK, Richards BA, Kim JC (2017) Bidirectional Control of Anxiety-Related Behaviors in Mice: Role of Inputs Arising from the Ventral Hippocampus to the Lateral Septum and Medial Prefrontal Cortex.

Neuropsychopharmacology 42:1715–1728.

Passoni I, Leonzino M, Gigliucci V, Chini B, Busnelli M (2016) Carbetocin is a Functional Selective Gq Agonist That Does Not Promote Oxytocin Receptor Recycling After Inducing β-Arrestin-Independent Internalisation. J Neuroendocrinol 28.

Pattij T, De Jong TR, Uitterdijk A, Waldinger MD, Veening JG, Cools AR, Van Der Graaf PH, Olivier B (2005) Individual differences in male rat ejaculatory behaviour: Searching for models to study ejaculation disorders. Eur J Neurosci 22:724–734.

Petrovic P, Kalisch R, Singer T, Dolan RJ (2008) Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. J Neurosci 28:6607–6615.

Pfaus JG, Kippin TE, Centeno S (2001) Conditioning and sexual behavior: A review. In: Hormones and Behavior, pp 291–321. Academic Press Inc.

Pfaus JG, Phillips AG (1989) Differential effects of dopamine receptor antagonists on the sexual behavior of male rats. Psychopharmacology (Berl) 98:363–368.

Pierrehumbert B, Torrisi R, Laufer D, Halfon O, Ansermet F, Beck Popovic M (2010) Oxytocin response to an experimental psychosocial challenge in adults exposed to traumatic experiences during childhood or adolescence. Neuroscience 166:168–177.

Pliota P, Böhm V, Grössl F, Griessner J, Kraitsy K, Kaczanowska J, Pasieka M, Lendl T (2018) Europe PMC Funders Group Stress peptides sensitize fear circuitry to promote passive coping. :1–25.

Poling A, Cleary J, Monaghan M (1981) Burying by rats in response to aversive and nonaversive stimuli. J Exp Anal Behav 35:31–44.

Posluns D (1962) An analysis of chlorpromazine-induced suppression of the avoidance response. Psychopharmacologia 3:361–373.

Potegal M, Blau A, Glusman M (1981) Effects of anteroventral septal lesions on intraspecific aggression in male hamsters. Physiol Behav 26:407–412.

Preston SD (2017) The rewarding nature of social contact: New research in mice elucidates neural mechanisms underlying social interactions. Science (80-) 357:1353–1354.

Quintana GR, Desbiens S, Marceau S, Kalantari N, Bowden J, Pfaus JG (2019) Conditioned partner preference in male and female rats for a somatosensory cue. Behav Neurosci 133:188–197.

Radulovic J, Rühmann A, Liepold T, Spiess J (1999) Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: Differential roles of CRF receptors 1 and 2. J Neurosci 19:5016–5025.

Rapee RM, Peters L, Carpenter L, Gaston JE (2015) The Yin and Yang of support from significant others: Influence of general social support and partner support of avoidance in the context of treatment for social anxiety disorder. Behav Res Ther 69:40–47.

Rassovsky Y, Harwood A, Zagoory-Sharon O, Feldman R (2019) Martial arts increase oxytocin production. Sci Rep 9.

Resendez SL, Namboodiri VMK, Otis JM, Eckman LEH, Rodriguez-Romaguera J, Ung RL, Basiri

ML, Kosyk O, Rossi MA, Dichter GS, Stuber GD (2020) Social stimuli induce activation of oxytocin neurons within the paraventricular nucleus of the hypothalamus to promote social behavior in male mice. J Neurosci 40:2282–2295.

Rickenbacher E, Perry RE, Sullivan RM, Moita MA (2017) Freezing suppression by oxytocin in central amygdala allows alternate defensive behaviours and mother-pup interactions. Elife 6.

Risold PY, Swanson LW (1997) Chemoarchitecture of the rat lateral septal nucleus. Brain Res Rev 24:91–113.

Rodríguez-Manzo G, Fernández-Guasti A (1994) Reversal of sexual exhaustion by serotonergic and noradrenergic agents. Behav Brain Res 62:127–134.

Rodríguez-Manzo G, López-Rubalcava C, Fernández-Guasti A (1999) Anxiolytic-like effect of ejaculation under various sexual behavior conditions in the male rat. Physiol Behav 67:651–657.

Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P, Zandi MS, Lewis G, David AS (2020) Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. The Lancet Psychiatry 7:611.

Sabihi S, Dong SM, Maurer SD, Post C, Leuner B (2017) Oxytocin in the medial prefrontal cortex attenuates anxiety: Anatomical and receptor specificity and mechanism of action. Neuropharmacology 125:1–12.

Sabihi S, Durosko NE, Dong SM, Leuner B (2014) Oxytocin in the prelimbic medial prefrontal cortex reduces anxiety-like behavior in female and male rats. Psychoneuroendocrinology 45:31–42.

Sachs BD, Bitran D (1990) Spinal block reveals roles for brain and spinal cord in the mediation of reflexive penile erections in rats. Brain Res 528:99–108.

Sachs BD, Garinello LD (1980) Hypothetical spinal pacemaker regulating penile reflexes in rats: Evidence from transection of spinal cord and dorsal penile nerves. J Comp Physiol Psychol 94:530–535.

Saldívar A, Ríos C, Fernández-Guasti A (1991) Differential role of serotonin and noradrenaline on anxiety reduction after ejaculation in the rat. Pharmacol Biochem Behav 38:807–812.

Sato Y, Wada H, Horita H, Suzuki N, Shibuya A, Adachi H, Kato R, Tsukamoto T, Kumamoto Y (1995) Dopamine release in the medial preoptic area during male copulatory behavior in rats. Brain Res 692:66–70.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675.

Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: Implications for neuropsychiatry. Brain Res Rev 46:71–117.

Simerly RB, Swanson LW (1988) Projections of the medial preoptic nucleus: A Phaseolus vulgaris leucoagglutinin anterograde tract-tracing study in the rat. J Comp Neurol 270:209–242.

Singewald N, Salchner P, Sharp T (2003) Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. 3223.

Sluyter F, Korte SM, Bohus B, Van Oortmerssen GA (1996) Behavioral stress response of genetically selected aggressive and nonaggressive wild house mice in the shock-probe/defensive burying test. In: Pharmacology Biochemistry and Behavior, pp 113–116. Elsevier Inc.

Sofroniew M V. (1983) Morphology of vasopressin and oxytocin neurones and their central and vascular projections. Prog Brain Res 60:101–114.

Stangier U (2016) New developments in cognitive-behavioral therapy for social anxiety disorder. Curr Psychiatry Rep 18:1–8.

Stone LB, Mennies RJ, Waller JM, Ladouceur CD, Forbes EE, Ryan ND, Dahl RE, Silk JS (2019) Help me Feel Better! Ecological Momentary Assessment of Anxious Youths' Emotion Regulation with Parents and Peers. J Abnorm Child Psychol 47:313–324.

Strauss B, Koranyi S, Altmann U, Nolte T, Beutel ME, Wiltink J, Herpertz S, Hiller W, Hoyer J, Joraschky P, Nolting B, Stangier U, Willutzki U, Salzer S, Leibing E, Leichsenring F, Kirchmann H (2017) Partner-related attachment as a moderator of outcome in patients with social anxiety disorder-A comparison between short-term cognitive-behavioral and psychodynamic therapy. Psychotherapy 54:339–350.

Substance Abuse and Mental Health Services Administration (2016) DSM-5 Changes. Substance Abuse and Mental Health Services Administration (US).

Succu S, Sanna F, Melis T, Boi A, Argiolas A, Melis MR (2007) Stimulation of dopamine receptors in the paraventricular nucleus of the hypothalamus of male rats induces penile erection and increases extra-cellular dopamine in the nucleus accumbens: Involvement of central oxytocin. Neuropharmacology 52:1034–1043.

Swanson LW, Kuypers HGJM (1980) The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J Comp Neurol 194:555–570.

Swanson LW, Sawchenko PE (1983) Hypothalamic Integration: Organization of the Paraventricular and Supraoptic Nuclei. Annu Rev Neurosci 6:269–324.

Swanson LW, Sawchenko PE, Wiegand SJ, Price JL (1980) Separate neurons in the paraventricular nucleus project to the median eminence and to the medulla or spinal cord. Brain Res 198:190–195.

Tenk CM, Wilson H, Zhang Q, Pitchers KK, Coolen LM (2009) Sexual reward in male rats: Effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions. Horm Behav 55:93–97.

Terenzi MG, Ingram CD (2005) Oxytocin-induced excitation of neurones in the rat central and medial amygdaloid nuclei. Neuroscience 134:345–354.

Thomas E, DeWolfe M, Sancar F, Todi N, Yadin E (2012) Electrophysiological analysis of the interaction between the lateral septum and the central nucleus of the amygdala.

Neurosci Lett 524:79–83.

Tol WA, Barbui C, Galappatti A, Silove D, Betancourt TS, Souza R, Golaz A, Van Ommeren M (2011) Mental health and psychosocial support in humanitarian settings: Linking practice and research. Lancet 378:1581–1591.

Torner L, Plotsky PM, Neumann ID, de Jong TR (2017) Forced swimming-induced oxytocin release into blood and brain: Effects of adrenalectomy and corticosterone treatment. Psychoneuroendocrinology 77:165–174.

Toth I, Neumann ID, Slattery DA (2012a) Social fear conditioning: A novel and specific animal model to study social anxiety disorder. Neuropsychopharmacology 37:1433–1443.

Toth I, Neumann ID, Slattery DA (2012b) Central administration of oxytocin receptor ligands affects cued fear extinction in rats and mice in a timepoint-dependent manner. Psychopharmacology (Berl) 223:149–158.

Trainor BC, Crean KK, Fry WHD, Sweeney C (2010) Activation of extracellular signal-regulated kinases in social behavior circuits during resident-intruder aggression tests. Neuroscience 165:325–336.

Treit D (1990) A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. Pharmacol Biochem Behav 36:203–205.

Treit D, Pesold C (1990) Septal lesions inhibit fear reactions in two animal models of anxiolytic drug action. Physiol Behav 47:365–371.

Treit D, Pinel JPJ, Fibiger HC (1981) Conditioned defensive burying: A new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 15:619–626.

Tribollet E, Barberis C, Jard S, Dubois-Dauphin M, Dreifuss JJ (1988) Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. Brain Res 442:105–118.

Ückert S, Becker AJ, Ness BO, Stief CG, Scheller F, Knapp WH, Jonas U (2003) Oxytocin plasma levels in the systemic and cavernous blood of healthy males during different penile conditions. World J Urol 20:323–326.

Valcourt RJ, Sachs BD (1979) Penile reflexes and copulatory behavior in male rats following lesions in the bed nucleus of the stria terminalis. Brain Res Bull 4:131–133.

Veening JG, Böcker KBE, Verdouw PM, Olivier B, De Jongh R, Groenink L (2009) Activation of the septohippocampal system differentiates anxiety from fear in startle paradigms. Neuroscience 163:1046–1060.

Veening JG, Coolen LM (2014) Neural mechanisms of sexual behavior in the male rat: Emphasis on ejaculation-related circuits. Pharmacol Biochem Behav 121:170–183.

Viviani D, Charlet A, Van Den Burg E, Robinet C, Hurni N, Abatis M, Magara F, Stoop R (2011) Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. Science 333:104–107.

Wagner CK, Clemens LG (1991) Projections of the paraventricular nucleus of the hypothalamus to the sexually dimorphic lumbosacral region of the spinal cord. Brain Res 539:254–262.

Wagner CK, Clemens LG (1993) Neurophysin-containing pathway from the paraventricular nucleus of the hypothalamus to a sexually dimorphic motor nucleus in lumbar spinal cord. J Comp Neurol 336:106–116.

Waldherr M, Neumann ID (2007) Centrally released oxytocin mediates mating-induced anxiolysis in male rats. Proc Natl Acad Sci U S A 104:16681–16684.

Walf AA, Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2:322–328.

Williams JR, Insel TR, Harbaugh CR, Carter CS (1994) Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). J Neuroendocrinol 6:247–250.

Williams T, Hattingh CJ, Kariuki CM, Tromp SA, van Balkom AJ, Ipser JC, Stein DJ (2017) Pharmacotherapy for social anxiety disorder (SAnD). Cochrane Database Syst Rev 2017 Available at: https://pubmed.ncbi.nlm.nih.gov/29048739/ [Accessed July 14, 2020].

Williams A V., Duque-Wilckens N, Ramos-Maciel S, Campi KL, Bhela SK, Xu CK, Jackson K, Chini B, Pesavento PA, Trainor BC (2020) Social approach and social vigilance are differentially regulated by oxytocin receptors in the nucleus accumbens. Neuropsychopharmacology.

Windle RJ, Shanks N, Lightman SL, Ingram CD (1997) Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138:2829–2834.

Witt DM, Insel TR (1994) Increased Fos Expression in Oxytocin Neurons Following Masculine Sexual Behavior. J Neuroendocrinol 6:13–18.

Wittchen HU, Jacobi F (2005) Size and burden of mental disorders in Europe - A critical review and appraisal of 27 studies. Eur Neuropsychopharmacol 15:357–376.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-Carulla L, Simon R, Steinhausen HC (2011) The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol 21:655–679.

Wotjak CT, Ganster J, Kohl G, Holsboer F, Landgraf R, Engelmann M (1998) Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: New insights into the secretory capacities of peptidergic neurons. Neuroscience 85:1209–1222.

Wotjak CT, Naruo T, Muraoka S, Simchen R, Landgraf R, Engelmann M (2001) Forced swimming stimulates the expression of vasopressin and oxytocin in magnocellular neurons of the rat hypothalamic paraventricular nucleus. Eur J Neurosci 13:2273–2281.

Yells DP, Hendricks SE, Prendergast MA (1992) Lesions of the nucleus paragigantocellularis: effects on mating behavior in male rats. Brain Res 596:73–79.

Yuan J, Yan M, Xu Y, Chen W, Wang X (2018) Social company disrupts fear memory renewal: Evidence from two rodent studies. Front Neurosci 12 Available at: https://pubmed.ncbi.nlm.nih.gov/30174582/ [Accessed June 24, 2020].

Zoicas I, Slattery DA, Neumann ID (2014) Brain oxytocin in social fear conditioning and its extinction: involvement of the lateral septum. Neuropsychopharmacology 39:3027–3035.

LIST OF ABBREVIATIONS

BLA	Basolateral nucleus of the amygdala
CeA	Central nucleus of the amygdala
CeM	Medial part of the central nucleus of the amygdala
CeL	Lateral part of the central nucleus of the amygdala
CFC	Cued fear extinction
CNO	Clozapine N-oxide dihydrochloride
CS	Conditioned stimulus
DA	Dopamine
DREADD	Designer receptor exclusively activated by a designer drug
Ej^-	Mated mice without ejaculation
Ej^+	Mated mice with ejaculation
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
LDB	Light/Dark box
LS	Lateral septum
dLS	dorsal part, lateral septum
vLS	ventral part, lateral septum
MeA	Medial nucleus of the amygdala
MPOA	Medial preoptic area
MS	Medial septum
NM	Non-mated mice

nPGi	nucleus paragigantocellularis
OXT	Oxytocin
OXTR	Oxytocin receptor
OXTR-A	Oxytocin receptor antagonist
PAG	Periaqueductal grey
SAD	Social anxiety disorder
SFC	Social fear conditioning
SFC^-	Unconditioned mice
SFC^+	Conditioned mice
SNT	Social novelty test
SPT	Social preference test
SPF	Suprafascicular nnucleus of the thalamus
US	Unconditioned stimulus

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

First of all, I would like to thank Prof. Dr. Inga Neumann for giving me the opportunity to do my PhD thesis in her lab, for all the support and for the resources that she provided all along these 3 and a half years. I want then to thank the DFG GRK 2174 “Neurobiology of emotion dysfunctions” for financing my PhD. Naturally, I would like to also thank Prof. Dr. Christian Wetzel as my second supervisor and PD. Dr. Carsten Wotjak as my mentor during my PhD thesis for their advice.

Next, I would like to thanks Prof. Dr. Oliver Bosch for his help to take care of all the animal regulations and well-being. Thank you to Dr. Rohit Menon for his help, support and advice throughout this thesis. I would also like to thank all my students who helped with experiments and analysis. Acknowledgements also go to Prof. Dr. Maurice Manning for the OXTR-A and to Prof. Dr. Harold Gainer for the OXT-neurophysin antibody. Many thanks to Rodrigue Maloumby for his help with the surgeries and to be always in such good mood. You eased a lot my experiments! I would also like to thank Andrea Havasi and Martina Fuchs for their technical help. Thank you to Tanja Janoschek and Eva Hofmann for the administrative help. I would now like to thank all my co-workers from the Neumann lab: Julia, Vini, Marianella, Anna B, Rohit, Anna S and Kathi; and from the GRK: Carl, Tobi, Magdalena, Meli. I would also like to thanks the GRK students from the Klinikum: Celia, Kerstin, Felix, Lisa and Viola. Thank you for the nice time during all the summer parties, Christmas parties, GRK weekend retreats, etc.

Maintenant vient les remerciements non-professionnels. Merci à mes amis, ceux qui ont été et sont toujours présents, pour me supporter et me changer les idées quand j´en ai eu besoin. Merci, Pschi pour toutes ces pauses café et running time. Merci Vini, toujours présent pour picoler et commérer. Merci the « Party Hard » people pour ces soirées mémorables. Merci à eux et à la communauté française pour m´avoir fait me sentir chez moi à Regensburg. Merci aussi à mes anciens camarades de Master que j´ai toujours plaisir de retrouver. Merci Laurie, pour ton accueil chaleureux chaque fois que je suis passé par Dijon.

Pour finir, merci à mes parents et mon frère pour m´avoir soutenu à distance, quitte à me proposer d´imprimer ma thèse de France (hein, Maman). Merci Cousine, nos échappées m´ont agréablement dépaysées. Et pour finir, merci, merci Michael pour ton soutien durant la thèse, ta patiente et tes conseils. Je m´excuse pour le stress infligé mais je saurais me faire

pardonner, promis.

(Je m´excuse Rodrigue si une ou plusieurs personnes t´ont forcé à traduire cette section).

CURRICULUM VITAE

Cindy GROSSMANN
Brunnleite 8
93 047 Regensburg - Germany
cindy.grossmann@ur.de
☎ +49 (0)172 72 85818 (M)

Citizenship: French
Date of birth: 10/10/1993

SCIENTIFIC EXPERIENCE

March 2017 - now: PhD at the Department of Behavioral and Molecular Neurobiology, Universität Regensburg, Regensburg, Deutschland.

Supervisors: Prof. Dr I.D. Neumann

Research subject: Role of oxytocin on social fear in male mice

- Behavioral tests: social and cued fear conditioning, marble burying test, light-dark box
- Stereotaxic surgery for guide cannula implantation and pharmacology
- Virus microinfusion
- Microdialysis
- Fluorescent immunohistochemistry
- Confocal microscopy

January 2015 - July 2016 (7 months): Internship at the Research Centre on Animal Cognition (CRCA), CNRS, Toulouse, France.

Supervisors: Drs BP. Guiard and C. Rampon

Research subject: Impact of hippocampal insulin resistance on the development of Alzheimer's disease in a mouse model

- Metabolism assessment: Glucose tolerance test, fasting glycemia, insulinemia (ELISA test)
- Western-blotting
- Behavioral tests: learning and memory (Object location, Novel object recognition) and depression/anxiety (Open-field, Tail-suspension test, Elevated-plus maze test, Splash test)
- Immunohistochemistry**:** evaluation of adult hippocampal neurogenesis from cell proliferation to neuronal maturation (BrdU, DCX, NeuN, GFAP …) in light and fluorescent microscopy

July 2015 - August 2015 (2 months): Internship at the Department of Anatomy and Neurosciences, University College of Cork, Cork, Ireland.

Supervisors: Dr Y. Nolan and Prof. J. Cryan

Research subject: Assessment of neurogenesis in the hippocampus of adult Nr2e1 (frc/frc) mice

- Quantification of DCX positive-cells
- Learning cell culture techniques (HEK cells, Hippocampal neural progenitor cells)
- Learning PCR techniques

November 2014 - January 2015 (2 months): Internship at the Research Centre on Animal Cognition (CRCA), CNRS, Toulouse, France.

Supervisor: Dr BP. Guiard

Research subject: Role of Cx43-mediated astrocytic network on the modulation of the serotoninergic system

- Electrophysiology in the dorsal raphe nucleus: serotoninergic firing rate in anesthetized mice
- 8-OHDPAT-induced hypothermia
- Behavioral tests: learning and memory (object recognition) and depression/anxiety (Novelty suppressed feeding, Tail suspension test, Open-field, Porsolt's test, Elevated-Plus Maze)

June 2014 - July 2014 (2 months): Internship at the Research Centre on Animal Cognition (CRCA), CNRS, Toulouse, France.

Supervisors: Drs BP. Guiard and C. Rampon

Research subject: Impact of diabetes on adult hippocampal neurogenesis in mice

- Immunohistochemistry: evaluation of adult hippocampal neurogenesis from cell proliferation to neuronal maturation and gliogenesis (BrdU, DCX, NeuN, GFAP …) in light and fluorescent microscopy

EDUCATION AND DEGREES

2012 - 2014: Undergraduate studies (BSc) in Cellular Biology and Physiology, University Paul Sabatier, Toulouse, France

2014 - 2016: Master's degree (MSc) in Neuroscience, Cognition and Behaviour, University Paul Sabatier, Toulouse, France

March 2017 - now: PhD at the Department of Behavioral and Molecular Neurobiology, Universität Regensburg, Regensburg, Deutschland.

Supervisors: Prof. Dr I.D. Neumann

Research subject: Role of oxytocin on social fear in male mice

SKILLS

FELASA B

Language:

- French: Native language
- English: certified European level B2 (CLES 2)
- German: level B2

CONFERENCES

- ISPNE – Poster presentation , 07.09.17 – 09.09.17 (Zurich, Switzerland)
- The 2nd Munich Winter Stress Conference on Stress – Poster presentation, 16.03.19 – 20.03.19 (Garmisch-Partenkirchen, Germany)
- FAPESP-BAYLAT Workshop – Talk and Poster presentation, 01.05.19 – 03.05.19 (Munich, Germany)
- German-Israeli Minerva School – Talk, 18.06.19 – 20.06.19 (Regensburg, Germany)
- 48th Meeting of the European Brain and Behaviour Society – Poster presentation, 21.09.19 -24.09.19
- IBNS 2020 – Poster presentation, 03.08.2020 – 07.08.2020 (Online)

PUBLICATIONS

Zemdegs, J., Rainer, Q., **Grossmann, C. P**., Rousseau-Ralliard, D., Grynberg, A., Ribeiro, E., & Guiard, B. P. (2018). Anxiolytic- and Antidepressant-Like Effects of Fish Oil-Enriched Diet in Brain-Derived Neurotrophic Factor Deficient Mice. *Frontiers in Neuroscience*, *12*, 974.

Masis-Calvo, M., Schmidtner, A. K., de Moura Oliveira, V. E., **Grossmann, C. P**., de Jong, T. R., & Neumann, I. D. (2018). Animal models of social stress: the dark side of social interactions. *Stress*, *21*(5), 417–432.

REFERENCES

Prof. Inga Neumann: inga.neumann@biologie.uni-regensburg.de, Department of Behavioral and Molecular Neurosciences; Universität Regensburg (Regensburg, Germany)

Dr Bruno GUIARD : bruno.guiard@univ-tlse3.fr, MCU, CRCA-UMR CNRS-5169 (Toulouse, France)

Dr Claire RAMPON: claire.rampon@univ-tlse3.fr, DR2 CNRS, CRCA-UMR CNRS-5169 (Toulouse, France)

Prof. John CRYAN: J.Cryan@ucc.ie, Prof. & Chair, Dept Anatomy & Neuroscience; PI, APC Microbiome Institute (Cork, Ireland)