The Resident-Intruder model

Evaluation of the possible factors and mechanisms that control the display of aggressive behavior in rodents

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Abstract

One model that is commonly used to study aggressive behavior and its subsequent negative consequences (e.g. social stress) is the Resident-Intruder paradigm, commonly used for mice and rats. In this model, two rodents are introduced to each other in the home cage of one of the animals (the resident) and then allowed to interact for a set period of time. The aim of this thesis was to discuss the possible mechanisms that determine the display of aggressive or submissive behaviors of rodents during this social interaction. Up to date, one of the neurotransmitters that have most consistently been shown to be correlate with the display of aggressive behavior in the Resident-intruder model is serotonin. A large body of research shows a negative correlation between serotonergic activity and aggressive behavior, suggesting an inhibitory role of serotonin on the display of aggressive behavior i.e. the serotonin deficiency hypothesis. However, there are also some studies that contradict show a positive correlations between serotonergic activity and aggression. These opposing correlations can be explained by distinguishing between functional forms of aggressive behavior (which are positively correlated with serotonergic activity) and escalated/pathological forms (which are negatively correlated with serotonergic activity). A revised version of the serotonin deficiency hypothesis is proposed, which suggests that low serotonergic activity may predispose an animal towards displaying aggressive behavior but only in (1) certain regions of the rodent brain (e.g. anterior hypothalamus) and only in (2) escalated forms of aggressive behavior.

1 Introduction

In daily life, many people complain about stress, we all have those moments when nothing seems to go right. One of the physiological responses to stress is mediated by the autonomic nervous system and the fight-or-flight response, which was first described in 1915 by Walter Bradford Cannon in his book "Bodily Changes in Pain, Hunger, Fear and Rage". In his book, Cannon states that animals which are stressed, are primed to fight or flee by a general discharge of the sympathetic nervous system (Cannon, 1915). The animal can either choose to act aggressively (e.g. fight) or submissively (e.g. flee) depending on numerous external and internal factors. The theory Cannon is supported by several other studies and is still widely studied and used in research (Dhabhar, 2009; Fuller et al., 2010; Kunimatsu & Marsee, 2012; Lefèvre et al., 2012). A disruption of the normal stress response in the brain can cause disturbances in behavior, e.g. overly aggressive behavior or excessive social stress. One paradigm which is used to study the response of animals in reaction to social stress is the Resident-Intruder model. This paradigm is very frequently used to study either aggressive behavior or the influence of social stress on animals (Martinez et al., 1998; Rammal et al., 2010). Within this paradigm the aversive effects of social stress as well as aggressive behavior can be studied. Relatively little is known about the factors that determine individual variability in coping styles, which is information that we might be obtained from Resident-Intruder model studies. The goal of this thesis is therefore to elucidate what (some of) the factors are that make animals in the Resident-Intruder model either react aggressively or submissively towards the other animal in the Resident-Intruder model. Insight into the biology behind aggressive/submissive responses will shed light on the underlying neural mechanisms. In this thesis, I will first discuss the individual differences in coping style as well as the involved physiological systems of the stress response. Subsequently, I will discuss the behavior, neurology and genetics of both the aggressive and the submissive animal, first separately and later on in relation to each other.

1.1 Stressful situations: coping strategies

The Resident-Intruder paradigm has not only been used in order to study aggression and social stress, it has also proven to be a useful tool in the assessment of coping strategies. Coping strategies can be described as alternative response patterns in reaction to stress (Koolhaas et al., 2010). Generally, two categories of coping strategies are distinguished although there is still some inconsistency in the literature concerning the terminology. Different descriptions of coping strategies that are used are for example shy-bold, active-passive and proactive-reactive. In this thesis I will use the terminology of proactive versus reactive in the description of different coping strategies. Displaying aggressive behavior and adopting a proactive coping strategy is not the same thing. For instance, fleeing from another animal is seen as a submissive behavior but still is part of a proactive coping style. However, using the Resident-Intruder model it has been shown that in rodents, aggressive individuals are more likely to adopt an proactive coping strategy while nonaggressive/submissive animals are more likely to adopt a reactive coping strategy (Benus et al., 1991; Koolhaas et al., 1999). Therefore, there might be some overlap in the mechanisms that predispose an animal towards aggressive behavior/proactive coping strategy or submissive

behavior/reactive coping strategy when they are confronted with an opponent in the Resident-Intruder paradigm, which will be further discussed in chapter 3.

1.2 Hypothalamic-pituitary-adrenal axis

The Resident-Intruder model can be used to expose animals to social stress. Stressful events can either be a onetime occurrence in which case we speak of acute stress, or it can be an event - or series of events - that happens over a prolonged period of time or which occurs frequently over a prolonged period of time, in which case it is commonly referred to as chronic stress. Both acute and chronic stress are known to influence the physiology of an individual. The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the regulation of acute and chronic stress and disruptions in the HPA axis are thought to contribute to stress-related pathology and behavioral problems. Activation of the HPA axis occurs in a number of steps. In short, it starts with the activation of the paraventricular nucleus (PVN) of the hypothalamus by cortical input, after perceiving a stressful stimulus. In response, the PVN secretes corticotrophin releasing hormone (CRH) and vasopressin. These hormones stimulate the pituitary and subsequently the pituitary synthesizes and secretes adrenocorticotrophin (ACTH). ACTH acts on the adrenal glands as a result of which the adrenal glands release glucocorticoids (GCs) into the bloodstream. Glucocorticoids have a various range of physiological endpoints with distinct functions. Amongst others, glucocorticoids are known to affect cardiovascular function, immune function and inflammation, metabolism, reproduction and neurobiological systems including (emotional) memory (Sapolsky, Romero, & Munck, 2000). Through this series of events, increasing the HPA axis activity is one of multiple processes which prepares the body to respond to the stressor, i.e. cope with the situation. Accordingly, animals that were tested with the Resident-Intruder paradigm show an increase in corticosterone (a glucocorticoid) which indicates an increase in HPA axis activity (De Miguel et al., 2011). When the stressful event is resolved a negative feedback mechanism, mediated by glucocorticoid receptors in the PVN, pituitary and hippocampus, ensures the HPA activity is down-regulated and homeostasis of the system is reestablished (see figure 1) (E. R. de Kloet et al., 2005).

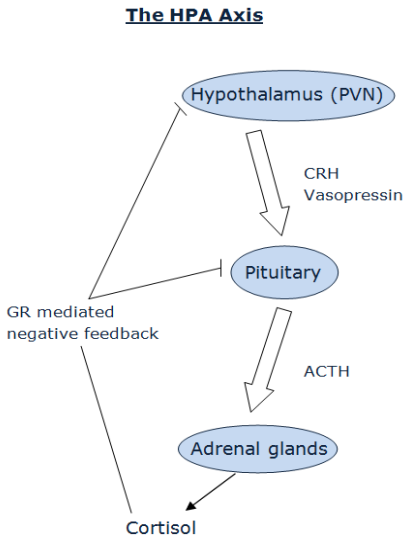

Figure 1. The hypothalamic-pituitary-adrenal (HPA) axis: negative feedback is mediated through glucocorticoid receptors (GR) under the influence of cortisol.

However, when an individual is subjected to chronic stress, the HPA axis remains activated for a prolonged period of time which means that the stress system is in a state of imbalance. Dysfunction of the HPA axis has been associated with multiple psychiatric disorders including obsessive-compulsive disorder (Coryell et al., 1989), posttraumatic stress disorder (C. S. de Kloet et al., 2006) and depression (Pariante & Lightman, 2008). The different kinds of stress (acute and chronic) clearly have differential consequences for physiology. In addition, there are also different kinds of stressors, classified as physical stressors (e.g. illness), psychological stressors (e.g. low self esteem) and environmental stressors (e.g. pollution). Nevertheless, the majority of stressors are of a social nature (Brown & Harris, 1989) and prolonged exposure to social stress is a major risk factor for multiple diseases, including certain immune disorders (Bartolomucci, 2007; Cohen et al., 1998) and depression (Bale, 2006; E. R. de Kloet et al., 2005; Kendler et al., 2002, 2006; Schmidt et al., 2010). This notion has lead to a substantial amount of research on social stress and the underlying mechanisms. Animal models that mimic important aspects of social stress situations observed in humans have since then been developed, one of them being the Resident-Intruder model.

1.3 The Resident-Intruder model

Modeling social stress in animals is often achieved by subjecting animals to defeat or a subordinate social status. One commonly used social stress paradigm is the Resident-Intruder test. The test can be performed once, to model acute social stress, or for a prolonged period of time in order to model chronic social stress. In the Resident-Intruder test territoriality plays an important role, and as female mice and rats do not display territorial behavior towards one another unless they are pregnant or lactating (Albert et al., 1992), the majority of Resident-Intruder research is only conducted with male mice or rats. In the Resident-Intruder paradigm, one of the animals is allowed to establish a territory (the resident) in its home cage. Subsequently, another animal is placed into the residents' home cage and the two animals are allowed to interact with each other for a fixed period of time (often 5-10 minutes, see figure 2).

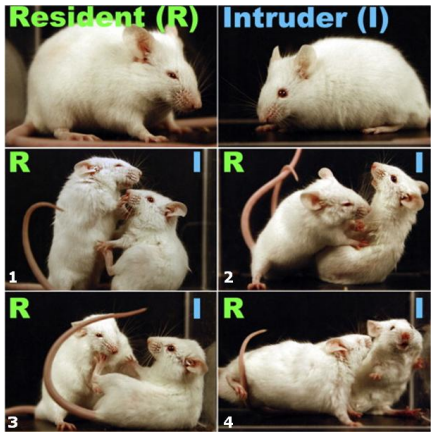

Figure 2. Behavioral responses of resident and intruder mice. The figure shows (some of) the behaviors that can be displayed by mice during the resident and intruder. For example, an offensive upright posture is shown in panel 1 by the resident mouse, while the intruder shows an upright defensive posture as it is being cornered by the resident in panel 4. Adopted from Defensor et al., 2012, and subsequently modified.

During the interaction, both animals experience a degree of social stress (indicated by an increase in corticosterone) due to being introduced to an unknown animal (De Miguel et al., 2011). When the fighting has seized and a hierarchy has been established, the dominant animal has coped with the situation by displaying aggressive behavior towards the submissive animal. The subordinate animal has not only lost the fight (which causes social stress) but also has to face the threat of being near a dominant animal for the remainder of the test (which is also stressful). So even though both animals experience a degree of social stress, the negative emotional consequences of social stress are expected to be greater for the intruder animal in comparison to the resident. Therefore the intruder animal is often used as a model for the investigation of social stress (e.g. Martinez et al., 1998; Tamashiro et al., 2005). Furthermore, the resident animal is also frequently used as model to study aggression (e.g. Mitchell & Redfern, 1997; Rammal et al., 2010). There are slight differences in the setup of the Resident-Intruder paradigm when applied to study either social stress or aggression that will be described below.

1.3.1 The Resident-Intruder model: two fields of application

The main difference between aggression and social stress studies using the Resident-Intruder paradigm lies in the selection of the animals. When the focus is on social stress, the animal that will lose the battle is the most relevant for that particular study. As a rule-of-thumb, intruder animals should be at a disadvantage because they are invading another animals' territory, which makes it most probable that the intruder animals will lose and that they are therefore most relevant for social stress research. In addition, the resident and intruder animal are chosen on the basis of specific characteristics that will ensure that the intruder will lose the battle. The favored requirements for the resident animal are described in the reviews of Bartolomucci et al., 2009 and Martinez et al., 1998 and may be summarized as follows: (1) the resident is at least 3 months old, (2) the resident is selected from a highly aggressive strain (e.g. the CD1 or NMRI mouse strain or Long Evans rat strain), (3) the resident is heavier in weight than the intruder, (4) the resident has had previous experience of victory and (5) the resident is housed differently from the intruder. The latter difference in housing is species dependent; resident rats are typically housed with a female whereas intruder rats are individually housed, resident mice are individually housed whereas intruder mice are group-housed. The specific selection of intruder animals can also increase the chance of their defeat. Preferred characteristics of the intruder animal (also described in the review of Bartolomucci et al., 2009) are: (1) the intruder is selected from a strain which is generally less aggressive than the resident strain, (2) is older than 2 months (younger rats will not elicit territorial aggression) but younger than the resident and (3) is housed differently from the intruder. Meeting as many requirements as possible when selecting intruder and resident animals for the test increases the likelihood of defeat of the intruder animal by the resident. However, meeting all requirements would be an ideal situation and this is almost never done as it also involves a lot of extra work and often it is enough to meet only a couple of the requirements to ensure defeat of the resident. The intruder animals (which are socially stressed) are of particular interest as models for human depression (Cryan & Slattery, 2007; Keeney & Hogg, 1999; Kudryavtseva et al., 1991; Malatynska & Knapp, 2005; for a review see Berton et al., 2012). By contrast, the resident animals are often used to model human aggression (for a review see Olivier & Young, 2002).

1.4 Goal

A question that underlies these studies and experimental models for social stress and aggression, which can be evoked by the same paradigm and situation, is what neurological mechanisms predispose certain animals to become submissive (and be stressed by the other animal) and others to become dominant (and act aggressive towards the other animal). As earlier mentioned, in this thesis I will discuss behavioral data, neurobiological findings and genetics studies to determine whether there are mechanisms that can be identified and that may determine individual differences in social coping strategies.

2 The Resident-Intruder model: aggression focused research

In this chapter, the brain areas, neurotransmitters, hormones and signaling molecules most commonly involved in aggressive behavior will be reported. The interpretation of these findings will be left to discuss in chapter 3. One way or the other, whenever aggression is involved in an interaction between individuals there is a high risk of an individual getting hurt. That can happen through physical aggression (e.g. fighting), but also through verbal aggression (e.g. insulting or bullying). Because of the great risks involved in the escalation of aggressive behavior, a lot of research has been done to better understand the underlying neural mechanisms of aggression. Over the past forty years many animal models have been developed to study aggression that will be discussed in the following paragraph.

2.1 Animal models of aggression

In psychopharmacology, the Resident-Intruder paradigm is by far the most commonly used rodent model to study aggression. However, not all Resident-Intruder models are comparable. There are, as mentioned earlier, many (subtle) variations that have been applied to this model. One feature that often differs between rodent Resident-Intruder paradigms is the animal which is used as resident. In mice studies, it is common to use male mice which have been isolated for a certain period of time (mostly several weeks) as a resident. This is also referred to as isolation-induced aggression. Most of these isolated male mice will consistently attack an intruder (Matsumoto et al., 2005; Sánchez et al., 1993; Valzelli, 1973). Another Resident-Intruder paradigm involves female mice or rats and is based on the maternal aggression behavior that female rodents display during pregnancy and lactation. These females act as residents and display maternal aggression towards the intruder (Olivier & Young, 2002). One more variation of the Resident-Intruder paradigm does not involve isolation and is mostly conducted with rats. In this case, the resident (a male rat) is housed with a female, a situation which induces territorial behavior (Barnett, 1975) and will promote the resident to act aggressively towards intruders. A different paradigm that also elicits aggression through social interaction like the Resident-Intruder model is the sensory contact model (SCM). The SCM was developed by Kudryavtseva (Kudryavtseva, 1991) and has many similarities with the Resident-Intruder paradigm. In the SCM, two animals are placed in a cage (neither of them is allowed to establish a territory, as in the Resident-Intruder paradigm) with between them a transparent punctured screen. Consequently, the animals are able to interact and smell, see and hear each other but not have physical contact. After a habituation period of 2 or 3 days, the screen is removed for 10 minutes each day to allow the animals to physically interact. Removing the screen generally happens once a day for up to 10-20 days. Under these conditions, the mice quickly establish a hierarchy and the behavior of the dominant animal is assessed to determine aggression in these studies. The SCM is thus similar to the Resident-Intruder paradigm in that it lets two unknown animals interact with each other, only in the SCM the animals are being kept in the same cage during the experiment and allowed to have sensory contact which is not the case in the Resident-Intruder paradigm. That pain stimuli can also lead to aggression is demonstrated in the inescapable foot shock paradigm (Dhawan et al., 1990). In this paradigm, two animals are placed in the same cage in which they receive an electrical shock to their feet which prompts aggressive behavior. Furthermore, low to moderate doses of alcohol are also known to elicit

aggression a certain subgroup of individual mice and rats (Van Erp & Miczek, 1997), whereas relatively higher doses can decrease aggressive behaviors due to sedative effects (Krsiak & Borgesová, 1973). The aggressively responding subgroup of mice and rodents is used to model alcohol-induced aggression in humans, after the alcohol treatment they are subjected to the Resident-Intruder test (as an intruder) and the aggressive behaviors are analyzed (Fish et al., 1999; Miczek et al., 1998). There are a variety of aggressive behaviors the animals can display towards each other, some of which can be used as parameters for aggressive behavior.

2.2 Parameters of aggressive behavior in rodents

In aggression focused Resident-Intruder research, the behavior of the resident is often closely monitored during the test. The most common parameters which are scored and applied as parameters of aggressive behavior are the latency until the first agonistic encounter and the frequency and duration of the agonistic encounters (Bartolomucci et al., 2009; Martinez et al., 1998; van Loo, 2001). Nevertheless, depending on the study, other aggressive behaviors can also be scored, e.g. biting, offensive upright position (see figure 1), boxing and chasing (for a full description of aggressive behaviors in mice see Miczek et al., 2001). Aggression studies are difficult to compare and interpret due to the variability between studies in the parameters that are measured. Despite these difficulties, multiple studies have used the Resident-Intruder model to investigate which brain regions are involved in the aggressive behavior of rodents.

2.3 Brain areas associated with aggression in rodents

One way to identify brain regions that are implicated in aggressive behavior is to perform lesions in specific brain areas and then observe the behavioral outcomes. This was for example described in the review of Kruk (1991), were he reviews studies in which lesions in specific brain regions of rats were performed and their subsequent behavior as residents in the Resident-Intruder paradigm was monitored. Resident rats with lesions in the lateral septum, bed nucleus of the stria terminalis, anterior hypothalamus and medial amygdala all showed a decrease in the display of aggressive behaviors towards the intruder in comparison to controls. The anterior hypothalamus has been identified as an important brain region in aggressive behavior in other subsequent studies as well. For instance, stimulation of the anterior hypothalamus either electrically (Siegel et al., 1999), hormonally (Kruk et al., 2004) or neurochemically (Ferris & Delville, 1994; Ferris et al., 1997) consistently results in a promotion of aggressive behavior. Therefore the anterior hypothalamus is more and more being referred to as the 'attack area' or 'aggression area' of the rodent brain (Halász et al., 2002; Kruk, 1991; Toth et al., 2010). In chapter 1 of the book of Weaver and Mattson (2003) it is described that forty-seven papers have reported a taming effect (which includes a decrease in aggressive behavior) caused by amygdalar lesions. However, they also describe an increase in aggression in rats that have small lesions restricted to the central nucleus. Therefore it is suggested that the medial and lateral amygdala might have different functions in the control of aggressive behavior. Aggression was also increased when lesions were placed in the orbital frontal cortex of rats, indicating that the orbital frontal cortex might exercise an inhibitory effect on the display of aggressive behavior (De Bruin et al., 1983). In addition to these lesion studies, research on c-Fos expression in the brains of aggressive and non-aggressive rodents in the Resident-Intruder paradigm has indicated the importance of the previously mentioned brain areas

as well. Several c-Fos studies indicate an increased activity in the central amygdala, anterior bed nucleus of the stria terminalis, ventrolateral hypothalamus, nucleus accumbens shell, orbital frontal cortex, periaquaductal gray and dorsal raphe of aggressive animals in comparison to non-aggressive animals (Haller et al., 2006; van der Vegt et al., 2003a; Veenema & Neumann, 2007).

2.4 Serotonin

In research on the neurobiology of aggression, multiple neurotransmitters, hormones and enzymes have been identified to contribute to aggressive behavior. However, the role of serotonin (5-hydroxytryptamine, 5-HT) which is a monoamine that serves as a neurotransmitter in the brain, has been most extensively studied (Chiavegatto et al., 2001; Chichinadze et al., 2011; Kulikov et al., 2012; Matsumoto et al., 2005; Pavlov et al., 2012; for a review see Nelson & Chiavegatto, 2001; Nelson & Trainor, 2007). An illustration of serotonin signaling can be seen in figure 3. Because of the great number of research indicating a correlation between serotonin and aggression, this thesis will also focus mainly on serotonin.

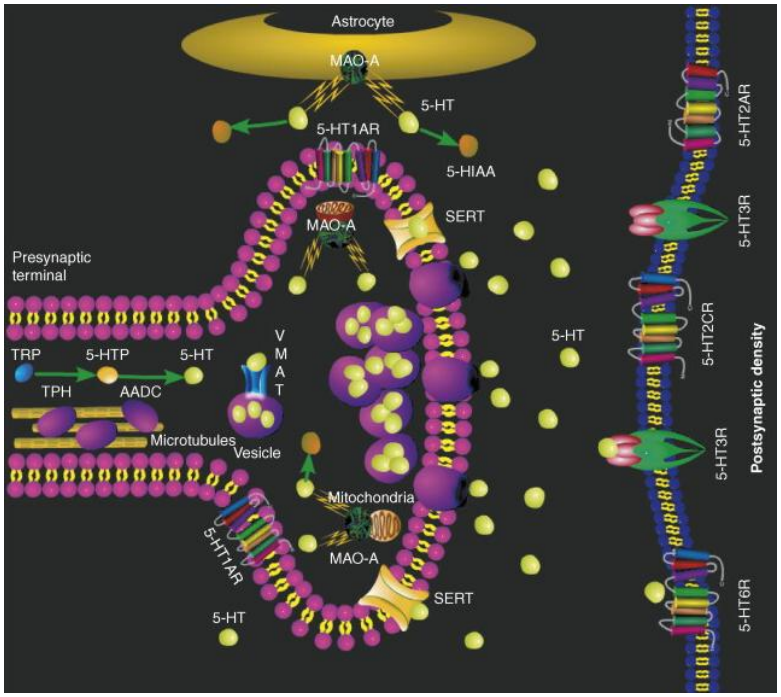

Figure 3. Idealized illustration of serotonergic signaling. Tryptophan (TRP) is converted to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH), which is subsequently turned into serotonin (5-HT) by amino acid decarboxylase (AADC). Next serotonin is packed into synaptic vesicles through the vesicular monoamine transporter (VMAT) and via these vesicles serotonin is released into the synaptic cleft. Once released, serotonin can bind to different subtypes of the serotonin receptor (not all are depicted here). Reuptake of serotonin is done by the serotonin transporter (SERT/5-HTT). Degradation of serotonin into 5-hydroxyindoleacetic acid (5-HIAA) can take place either presynaptically by mitochondrial monoamine oxidase A (MAO A) or extrasynaptically by MAO A expressed by astrocytes. Adopted from Buckholtz & Meyer-Lindenberg (2008).

One of the first to discover a correlation between serotonin and aggressive behavior was Valzelli (1973). Valzelli studied the turnover rates of serotonin in different mouse strains during the Resident-Intruder test; the animals were socially isolated prior to the testing to promote aggression. He discovered that mice which were socially isolated had a lower turnover rate of serotonin in comparison to controls. The turnover rate of serotonin is determined by the 5-hydroxyindoleacetic acid (5-HIAA)/serotonin (5-HT) ratio and is often used as an indicator of

serotonergic activity (Blanchard et al., 1993; Kudryavtseva et al., 2004; Summers et al., 2000). In addition, the mouse strains that were shown to fight most frequently also had lower serotonin turnover rates than mouse strains that did not fight so often. Lower serotonergic activity was thus correlated with increased aggression. Later on, Saudou et al. (1994) demonstrated that resident mice in which the gene of one of the serotonin receptor subtypes 5-HT_{1B} was knocked out, displayed highly aggressive behavior in comparison to wildtype resident mice. Walsh and Dinan (2001) reported in their review on selective serotonin uptake inhibitors (SSRI's) and violence the anti-aggressive effect of SSRI's on the behavior of rodents, which were also reported by Mitchell and Redfern (2005). SSRI's block the reuptake of serotonin (see figure 3), resulting in an increased concentration of serotonin in the synaptic cleft and thus enhancing serotonin signaling. Again, serotonergic activity and aggression were found to be negatively correlated. Disruption of the reuptake of serotonin can not only be achieved by the administration of SSRI's but also by knocking out the gene that codes for the serotonin transporter, which too, leads to a reduction in aggression (Holmes et al., 2002). The study of Caramaschi and colleagues (2007) also showed a correlation of low levels of brain serotonin and increased aggression. In this study six different strains of mice were obtained through three selective breeding programs for aggressive behavior, three strains were classified as highly aggressive, the other three as non-aggressive/submissive. All three of the aggressive strains showed lower serotonin levels in prefrontal cortex, hippocampus and brain stem, in comparison to the submissive strains. More recently, Mosienko et al. (2012) showed a correlation between enhanced aggression and serotonin deficiency. The researchers performed a Resident-Intruder experiment with both wildtype mice and mice lacking the tryptophan hydroxylase 2 (TPH2) gene. Tryptophan hydroxylase is one of the enzymes which are needed for the synthesis of serotonin (see figure 3). Accordingly, TPH2 knockout mice showed a significant decrease of brain serotonin levels. The Resident-Intruder test showed that the TPH2 knockout mice did not only attacked the intruder faster but also longer and more often in comparison to the resident wildtype mice.

All of the studies mentioned above, show a negative correlation between serotonergic activity and aggression, i.e. if serotonergic activity is decreased, aggression is increased. However some studies also show an opposite correlation. For instance, in the study of Van der Vegt et al. (2003b) male Wildtype Groningen rats were tested several times for aggressiveness using the Resident-Intruder test, while cerebrospinal fluid concentrations of serotonin and 5-HIAA were assessed. The results of this study show a positive correlation between aggressiveness and cerebrospinal fluid concentrations of serotonin and 5-HIAA. In addition, in the study of Kulikov et al. (2012) also a positive correlation between serotonergic activity and aggressive behavior is demonstrated in mice. In their study, Kulikov and colleagues administrated L-tryptophan (a 5-HT precursor) which increased serotonin levels and a TPH2 inhibitor which decreased serotonin levels. The administration of the L-tryptophan leads to an increase in aggressive behavior, whereas the administration of the TPH2 inhibitor leads to a decrease in aggressive behavior. These results indicate a positive correlation between serotonergic activity and aggression and thus contrast the studies mentioned before. In chapter 3 these opposing correlations will be further discussed. In summary, these studies illustrate that changes in the activity of the serotonergic system, which

may be induced by both genetic and environmental factors, alter aggressive and submissive behavior.

2.5 Serotonin associations

2.5.1 Steroid hormones

Several steroid hormones have been shown to influence aggression (for a review see Nelson & Trainor, 2007) but testosterone and corticosterone have been studied in most detail. It is widely known and described that castration decreases aggression (Barfield et al., 1972; Koolhaas et al., 1980), e.g. horses and dogs are often being castrated to make them more docile. In addition, transgenic male mice with a deficient estrogen receptor gene (estrogen is a derivate of testosterone), show a considerable decrease in aggressive behavior in comparison to control mice (Ogawa et al., 1998; Ogawa et al., 1997). These results suggest that testosterone facilitates aggression. However, the influence of testosterone on aggression can vary between animals with different genetic backgrounds as has been shown in a study of Simon et al. (1998). Simon and colleagues described in their review that testosterone facilitates aggression in the C57BL/6J mouse strain but not in the CF-1, CFW and CD-1 mouse strain. Therefore it is suggested that the influence of testosterone on aggression is dependent on the genetic background of the individual. The influence of testosterone and its derivate estrogen might be mediated by the serotonergic system. This is suggested as the mRNA of the 5-HT_{2A} receptor has shown to be increased by testosterone and estrogen (Sumner & Fink, 1998). In addition, the study Simon and colleagues demonstrates that the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} receptor agonists are influenced testosterone and estrogen (Simon et al., 1998). Furthermore, Singh et al. showed that the activity of neural nitric oxide synthase (nNOS) is influenced by castration and, as I later on will illustrate, nNOS has been implicated with the serotonergic system as well. Together, these studies show that testosterone and estrogen levels determine aggressive behavior and these effects appear to be mediated, at least partly, by the serotonin system.

Corticosterone is a well known hormone in the research area of animal welfare as corticosterone measurements are used as a tool to assess HPA axis activity (Sejian et al., 2011). Prolonged elevated HPA axis activity is reflected by elevated corticosterone levels (see paragraph 1.3) and corticosterone measurements and associated HPA axis activity can give an indication of the degree of acute and chronic stress an animal has been exposed to. The study of De Miguel et al. (2011) shows that in the Resident-Intruder model, both the aggressive and the submissive mice have significantly higher corticosterone levels in their serum than non-tested animals. The study also shows that submissive mice differ from aggressive mice in that they have higher corticosterone levels in their serum than the aggressive mice. These results were supported by the study of Veenema et al. (Veenema et al., 2003) in which mice were classified as either non-aggressive or aggressive using the Resident-Intruder paradigm. After this classification the animals were tested in the forced swim test, in which the corticosterone response of the non-aggressive animals was increased and prolonged in comparison to the aggressive animals. Administration of corticosterone has been shown to increase serotonergic activity in the hippocampus medial amygdala of lizards (*Anolis carolinensis*), in comparison to saline treated control animals (Summers et al., 2000). In addition, Hesen and Joëls (1996) demonstrated that in the hippocampus CA1 region of rats, the

serotonin-induced hyper polarization of neurons is very sensitive to changes in corticosterone concentration. These results suggest that among others, serotonin is involved in the effect of corticosterone on aggressive behavior.

2.5.2 Vasopressin

Another hormone which has been implicated with the regulation of aggressive behavior is Arginine Vasopressin (AVP). AVP tends to stimulate aggressive behavior, as has been shown in multiple studies. For example inhibition of AVP signaling, by using an AVP receptor antagonist, results in a decrease of aggressive behavior towards intruders in golden hamsters (Ferris & Potegal, 1988). Later on, it was also shown that aggressive behavior increases after microinjection of AVP into the anterior hypothalamus (Ferris et al., 1997). Interestingly, the group of Ferris later on demonstrated that the increase in aggressive behavior, after microinjection of AVP into the anterior hypothalamus, can be inhibited by the application of a 5-HT_{1A} receptor agonist (Ferris et al, 1999). These studies with golden hamsters identified involvement of vasopressin in aggression which again is mediated at least in part by the serotonergic system.

2.5.3 Nitric oxide

The first study which indicated a role for neuronal nitric oxide (NO) synthase (nNOS) in regulating behavior was by Nelson et al. in 1995. They revealed that male mice with a disruption of nNOS, display increased aggressive behavior in comparison to controls in the Resident-Intruder test. The notion that nNOS is involved in aggressive behavior in mice was later on supported by the study of Demas et al. (1997). Demas et al. showed that mice treated with a specific nNOS inhibitor displayed increased aggression in comparison to control mice. Soon after this research the nNOS association with aggressive behavior was also linked to the serotonergic system in the research of Chiavegatto and colleagues. They demonstrated that the increased aggression in the male mice lacking nNOS is caused by the dysfunction of the serotonin receptor 5-HT_{1A} and 5-HT_{1B} (Chiavegatto et al., 2001).

2.5.4 Monamine oxidase A

Vishnivetskaya et al. (2007) discovered that resident mice of the Tg8 mouse strain, that lack a functional monoamine oxidase A (MAO A) gene, were more aggressive towards intruders than control resident mice. Monoamine oxidase A is known to degrade serotonin (Shih et al., 1999), suggesting that MAO A-deficient mice may be expected to have higher brain serotonin levels. Indeed, the study of Cases and colleagues (1995) showed that mice lacking the MAO A gene had elevated serotonin levels. These results therefore suggest a positive correlation between serotonergic activity and aggression. However it must be kept in mind that MAO A-deficient mice tremble, are fearful and blind (Vishnivetskaya et al., 2007). Such physiological abnormalities may interfere with the interpretation of aggressive behaviors. Furthermore, it has been discovered that mice of the Tg8 mouse strain have abnormal receptors (Bou-Flores & Hilaire, 2000). The serotonergic signaling in this mouse strain might thus be altered, which makes it hard to determine the actual activity of the serotonergic system.

Box 1. The Resident-Intruder model: social stress focused research

The Resident-Intruder model can be used to socially stress animals, both acutely and chronically. Chronic social stress in humans can lead to an increased risk of depression (Bale, 2006; E. R. de Kloet et al., 2005; Kendler et al., 2002, 2006; Schmidt et al., 2010) and is therefore simulated in several animal models. One of the animal models for chronic social stress is the earlier mentioned sensory contact model, which thus can not only be used for aggression research but also for chronic social stress focused research. Another paradigm to model chronic social stress is the social disruption paradigm first described by Padgett and colleagues (Padgett et al., 1998). In this test the mice are housed in groups of unfamiliar animals and the aggressive behaviors are observed. Through these observations the animals are ranked and the most dominant animal of each group is identified. Chronic social stress is evoked by switching the dominant animals among groups multiple times (once per day, for seven days), which disrupts the social hierarchy of each group. In the colony model, female and male animals (often rats, but sometimes mice) are grouped together and allowed to establish a hierarchy (Martinez et al., 1998). The subordinate animals of this group are identified through behavioral observations and proposed as a model for chronic social stress (D. C. Blanchard et al., 1993). In the Resident-Intruder models (as well as in other models of chronic social stress), it is important to be able to recognize submissive behaviors in order to indentify the subordinate animal. Submissive behaviors that can be observed are an upright defensive posture (when cornered by the dominant, see figure 1), escape behavior, freezing and the submissive might also emit squeaking vocalizations (Bartolomucci et al., 2009). As has been shown in the studies mentioned in the previous chapter, submissive behaviors are (in contrast to aggressive behaviors) correlated with high serotonergic activity in the brain (for a review see Nelson & Chiavegatto, 2001; Nelson & Trainor, 2007; Summers & Winberg, 2006).

3 Aggression, submission and coping strategies

As has been described in the previous chapters, the behavior of a rodent towards another (unfamiliar) individual can be submissive or aggressive and sometimes these behaviors are seen as an indication of a certain coping strategy. The previously discussed studies using the Resident-Intruder model have shown that serotonergic functioning plays a critical role in the display of such behaviors (for a review see Nelson & Chiavegatto, 2001; Nelson & Trainor, 2007; Summers & Winberg, 2006). In this chapter I focus more on the underlying mechanisms that drive an animal to either react aggressively or submissively but first I will explain more about the relationship between aggressive and submissive behaviors and their implications in coping strategies.

3.1 Coping strategies

Coping strategies have been introduced earlier in the introduction and it has been mentioned that a tendency to aggressive behavior and a tendency to a proactive coping style are not the same. However, relative high levels of aggressive behavior in the Resident-Intruder model are considered to be an expression of a more general predisposition to adopt a proactive coping strategy (Koolhaas et al., 2010). This is supported by several tests for coping strategies, that aggressive animals generally adopt a proactive coping strategy, whereas non-aggressive animals generally adopt a reactive coping style (see table 1).

Table 1. Overview of the general outcomes of high and low aggressive rodents in tests for proactivity. Adopted and subsequently modified, from the review of Koolhaas et al. (2010).

Measure	High-aggressive	Low-aggressive
Tests for proactivity		
Attack latency of an intruder (Resident-Intruder model)	Short	Long
Defensive burying of a shock-prod	High	Low
Active shock-avoidance (shuttle box)	High	Low
Fleeing from dominant aggressor as an intruder (Resident-Intruder model)	High	Low
Nest-building (mice)	High	Low
Swimming/struggling in the forced swim test	High	Low

Therefore, some overlap in the underlying neural mechanisms that predispose an animal towards aggressive behavior or a proactive coping strategy is expected. Indeed, there are several studies that report a major role of serotonin in coping strategies (De Miguel et al., 2011; Koolhaas et al., 2007; Veenema & Neumann, 2007) as well, as has been found in aggression research. Furthermore, it is also hypothesized that changes in the serotonergic system are causally related to coping strategies (Koolhaas et al., 2007). The majority of research done on the neurobiology of coping strategies in rodents has focused on neurobiological findings on the aggressiveness of rodents in the Resident-Intruder paradigm (Koolhaas et al., 2010) and is similar to the studies earlier discussed in paragraph 2.4. Therefore, in the description of possible hypothesis for the display of aggressive or submissive behavior below, I will focus mainly on the studies discussed in paragraph 2.4. Keep in mind however, that the neural mechanisms discussed in the following

paragraph that predispose an animal towards aggressive behavior thus also indicate a tendency towards a proactive coping strategy.

3.2 Aggression or submission: hypotheses on the underlying mechanisms

That changes in serotonin activity can influence the level of aggression or submission in the Resident-Intruder paradigm has been shown in many studies (see paragraph 2.4 and box 1), but the precise role of serotonin in the expression of these behaviors is still up for debate. Hypotheses on the role of serotonin in the expression of aggressive and submissive behaviors are discussed here.

3.2.1 Brain region specificity of the influence of serotonin levels on aggression

Nelson and colleagues (2001) state in their review that although many other molecules are implicated in the display of aggressive behavior they all influence the behavior indirectly by affecting the serotonergic system. For that reason, serotonin is the primary molecular determinant of aggression and it is suggested that the low serotonin activity could be causally related to aggressive behavior. Indeed most studies on aggression and serotonin reviewed in this thesis report a negative correlation between the two (Caramaschi et al., 2007; Chiavegatto et al., 2001; Ferris et al., 1999; Mosienko et al., 2012; Saudou et al., 1994; Valzelli, 1973 for a review see Nelson & Chiavegatto, 2001 or Pavlov et al., 2012;). This suggests that if serotonin is causally related to aggressive behavior, high serotonin activity would inhibit aggression while low serotonin activity would promote aggression. This is sometimes referred to as the "serotonin deficiency hypothesis" (De Boer & Koolhaas, 2005). However, there are some conflicting results found on serotonin-aggression correlations (as also is mentioned in paragraph 2.4 and 2.5). For instance, it is known that in the Resident-Intruder paradigm both animals experience stress (De Miguel et al., 2011) and that stress elevates serotonin activity (see Box 1). So how could elevated serotonin levels inhibit aggression, while these levels are increased due to displaying the aggressive behavior itself? Accordingly, it is more likely that regional (in contrast to global) changes in serotonin activity are influencing aggressive behavior. This notion is supported by the research of Summers and Winberg (2006). They acknowledge the importance of serotonin in aggressive behavior, but also question the causality of the changes in behavior. They hypothesized that it is not necessarily the global change in serotonin activity that brings about the changes in aggressive/submissive behavior but more region specific serotonin changes within the brain that influence whether the animal reacts aggressively or submissively. In addition, they emphasize that aggressive behavior is regulated by multiple systems, not only the serotonin system but possibly the dopamine, GABA and glutamate system as well which view is supported in a later review of Nelson and Trainor (2007) and earlier research of Miczek et al. (2002). The hypothesis that regional rather than global changes in serotonin activity affect aggression is based on their study with lizards (*Anolis carolinensis*). In this study the aggressiveness of the animals was determined by several behavioral tests, which in earlier research had proven to be reliable indicators of aggressiveness (Korzan et al., 2004). This allowed the researchers to determine aggressiveness without a social interaction between animals. After these tests the animals were euthanized and the levels of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were analyzed. The ratio of 5-HIAA/serotonin is an approximation of serotonergic turnover and activity. The results demonstrate that serotonin

turnover/activity is heightened in aggressive/dominant animals but only in specific brain regions. The septum, nucleus accumbens, striatum, medial amygdala, locus ceruleus, raphe nuclei and certain regions of the hypothalamus (anterior hypothalamus, lateral hypothalamus, paraventricular nucleus, ventromedial hypothalamus) of aggressive/dominant animals show low serotonin activity in comparison to less aggressive/subordinate animals, whereas in the CA_3 region of the hippocampus, the lateral amygdala, substantia nigra, ventral tegmental area and the preoptic area of the hypothalamus show no significant difference. These results indicate that the correlation of serotonin and aggression is indeed brain region specific, at least in lizards. As the organization of the serotonergic system is surprisingly stable across vertebrate and mammalian species (Descarries, Riad, & Parent, 2010; Jacobs & Azmitia, 1992), it is possible that this brain region specificity of the influence of serotonin levels on aggressive and submissive behavior also applies to other vertebrate species e.g. rats and mice.

3.2.2 Brain region specificity of 5-HT receptor subtypes

Still, this reasoning does not account for the aggression reducing effect of 5-HT_{1A} and 5-HT_{1B} while they are known to exert negative feedback on the serotonergic system (Claeysen et al., 2010). The 5-HT_{1A} and 5-HT_{1B} receptors can be found not only postsynaptically, but also presynaptically where they exert negative feedback on the serotonergic neuron itself. Therefore 5-HT_{1A} and 5-HT_{1B} receptors agonists can have two effects on serotonergic signaling, postsynaptically they copy the effect of increased serotonin signaling but presynaptically they bind to the so-called 5-HT_{1A} and 5-HT_{1B} autoreceptors which causes a reduction in serotonin signaling (Claeysen et al., 2010). Therefore, to be able to interpret the role of serotonin in aggression it is necessary to do more research with 5-HT_{1A} and 5-HT_{1B} receptor agonists and antagonists of which the major site of action (pre- or postsynaptic) is known. De Boer and Koolhaas (2005) accomplished this by using a novel compound called S-15535, which is a selective agonist for the presynaptic 5-HT_{1A} autoreceptor and a partial antagonist for the postsynaptic 5-HT_{1A}. Both the agonistic effect on the presynaptic 5-HT_{1A} autoreceptor and antagonistic effect on the postsynaptic 5-HT_{1A} reduce serotonergic signaling. The animals treated with S-15535 showed a reduction in aggressive behavior in the Resident-Intruder test in comparison to non-treated controls. Accordingly, these results question the serotonin deficiency hypothesis in that they shows that the reduction of serotonergic signaling (mediated by the 5-HT_{1A} receptor through administration of S-15535) is correlated with a decrease in aggression. However, these results do not necessarily oppose the serotonin hypothesis if the brain region specificity of serotonergic activation is taken into account. It is described in the book chapter of Quadros et al. (2010) that microinjections of 5-HT_{1A} (as well as other receptor subtype) agonists have different effects on aggressive behavior dependent on the brain area of injection. This notion not only strengthens the brain region specific serotonin deficiency hypothesis of aggression. It also suggests that subcutaneous injection of S-15535 and subsequent reduction of global serotonin brain levels and aggressive behavior (as has been demonstrated in the research of De Boer and Koolhaas (2005)) does not say anything about the brain region specific effects of S-15535 on serotonergic activity and therefore the discovery that S-15535 reduces aggressive behavior is still in line with the brain region specific serotonin deficiency hypothesis of aggression.

3.2.3 Differential role of serotonin in functional and escalated aggressive behavior

However, this hypothesis does not offer an explanation for the positive correlation of aggressiveness and serotonergic activity found in some studies (as mentioned in paragraph 2.4, Kulikov et al., 2012; van der Vegt et al., 2003b). A possible explanation for the contrasting correlations between aggression and serotonergic activity is presented in the research of De Boer and Koolhaas (2005). In addition to their research on the compound S-15535 De Boer and Koolhaas also describe in their report that they did a critical assessment of the data on serotonergic activity in aggressive humans. During this evaluation they noticed that the negative correlation between serotonergic activity and aggression in humans seems to be only observed in individuals who display escalated and pathological forms of aggression and not in humans who display functional forms of aggressive behavior. Therefore they suggest that: "the involvement of serotonin in functional aggressive behavior is distinctly different from its role in violence defined as the pathological form of aggression" (De Boer & Koolhaas, 2005). Indeed, the aggression reducing properties of 5-HT_{1A} agonists have been shown to be particularly potent in animals that display escalated aggressive behavior (De Boer et al., 2000; Miczek et al., 1998; Moechars et al., 1998; Stork et al., 1999). Furthermore, the hypothesis is also supported by a subsequent review of the group of Miczek as well as a review by Nelson and Trainor on the role of serotonin in aggressive behavior (Miczek et al., 2007; Nelson & Trainor, 2007). Additionally, De Boer and Koolhaas suggest that a short rapid release of serotonin during the display of aggressive behavior controls both forms of aggression, where the animals displaying the pathological form of aggression can be characterized, prior to the aggressive act, by lower basal levels of serotonergic activity (De Boer & Koolhaas, 2005). The hypothesis that serotonergic activity may only inhibit escalated aggressive behavior, may possibly explain the contrasting results found earlier in paragraph 2.4 on the correlation between aggression and serotonergic activity. In table 2 an overview is seen of the discussed studies in paragraph 2.4.

Table 2. An overview of the discussed studies in paragraph 2.4 including the correlation between serotonergic activity and the kind of animal in which the behavior was observed.

Study	Correlation between serotonergic activity and aggressive behavior?	Aggressive behavior studied in
(Valzelli, 1973)	Negative	Prolonged socially isolated mice
(Saudou et al., 1994)	Negative	Knockout mice
(Holmes et al., 2002)	Negative	Knockout mice
(Caramaschi et al., 2007)	Negative	Mouse strains particularly selected for aggressive behavior
(Mosienko et al., 2012)	Negative	Knockout mice
(Van der Vegt et al., 2003b)	Positive	Wildtype rats
(Kulikov et al., 2012)	Positive	Conventionally used mouse strains (CC57BR and C57BL/6J)

The table shows that all studies which have demonstrated a negative correlation between serotonergic activity and aggressive behavior are in animals that have been manipulated in some way. It could be possible that the manipulation (i.e. knocking out of genes, prolonged social isolation, and selection of aggressive behavior) caused the animals to display escalated forms of aggressive behavior. In case of the studies where a positive correlation between serotonergic activity and aggression was found, no manipulations were done. Therefore it could be possible that in these studies functional forms of aggressive behavior were observed. Although the description of the aggressive behaviors in these studies is not detailed enough to assess if these animals really display escalated forms of aggressive behavior. Still, the hypothesis of de Boer and Koolhaas (2005) on the differential role of serotonin in different forms of aggression, i.e. inhibitory in escalated aggressive behavior and stimulating in functional aggression, offers a possible explanation for the contrasting correlations found in the studies discussed in this thesis.

4 Discussion

In this thesis I have evaluated multiple studies in order to gain more knowledge on the factors that may predispose a rodent in the Resident-Intruder paradigm to either act aggressively or submissively. I found that as a rule-of-thumb the resident animal is more likely to display aggressive behavior. In addition, I have found a great number of studies indicating a major role of serotonin in the neural mechanisms that predispose an animal to display escalated forms of aggressive behavior. Some thoughts on the design of the Resident-Intruder model and the serotonin deficiency hypothesis are given below finishing with my conclusions and recommendations for future research.

4.1 The Resident-Intruder paradigm: some considerations

4.1.1 The control group

The selection of a control group in the Resident-Intruder model is an important choice. In pharmacological and knockout studies the choice for a control group is often evident. The researchers want to test the influence of pharmaca or the knockout of genes on the aggressive or submissive behavior of rats or mice. Therefore the test group exists of mice or rats which have been given the drug in question or have been genetically manipulated to create the knockout. The control group are given saline or are not genetically manipulated (e.g. Caramaschi et al., 2007; de Boer & Koolhaas, 2005). However, in some social stress research the manipulation is not present during the test itself, as for example when rodents are chronically stressed by using the Resident-Intruder paradigm and are subsequently compared to non-stressed animals in their reaction to pharmaca (e.g. in Lumley et al., 2000). In these situations, the selection of a control group requires more attention. There are different kinds of control animals that are generally used in such a situation for example individually housed animals or animals that are also placed in a new environment but with an nonaggressive unknown conspecific (for a review see Martinez et al., 1998). In the last case, both the control and test animal undergo the experience of being placed in a novel environment and social interaction. This is, in my view, the best control group as it allows the researchers to study the effect of social stress without the confounding effects of a novel environment or the social interaction itself.

4.1.2 Variation in the behavior of non-test animals

The behavioral observation in the Resident-Intruder paradigm often focuses on either the resident (aggression focused research) or on the intruder (social stress focused research). However, the behavior performed by these test animals strongly depends on the behavior performed by the opponent. Therefore I would advise to standardize the non-test animal as much as possible. This can be achieved by making sure the non-test animal meets as many of the characteristics (mentioned before in paragraph 1.4.2). In addition, it can be suggested to use a naïve opponent each time the test is done. Animals learn from social interaction and this can cause changes in behavior in subsequent interactions. In order to prevent this, researchers can choose to use a naïve opponent for their test animal. However, consideration is needed as it will increase the number of animals that is needed for the experiment. In studies where changes over time are

critical it is in any case better to use naïve opponents, when changes over time are less critical it might be better to use the same opponent for every test round.

4.2 The serotonin deficiency hypothesis

4.2.1 Rodents

In my view the serotonin deficiency hypothesis of aggression (i.e. that serotonin inhibits aggression) is too generally stated. Brain region specificity of the negative correlation between serotonin levels and aggressive behaviors has been demonstrated already in lizards (Summers & Winberg, 2006) and is likely to also exist in rodents. In addition, the research reviewed in this thesis as well as the human research reviewed by de Boer and Koolhaas (2005) indicate that the inhibitory role of serotonin may only apply to escalated or pathological forms of aggressive behavior. Therefore, I suggest a more detailed version of the serotonin deficiency hypothesis i.e. the serotonin deficiency hypothesis for aggression only applies to (1) certain brain regions within the rodent brain (e.g. the anterior hypothalamus) and (2) escalated forms of aggressive behavior. Some thoughts on the possible cause of the low serotonergic activity found in some brain regions of highly aggressive animals in comparison to non-aggressive animals are given below.

4.2.2 Differential sensitivity of the 5-HT (auto)receptors in aggressive and non-aggressive mice

After all the studies showing a correlation between low basal levels of serotonergic activity and the display of escalated forms of aggression, the question arises what could be the cause of these low basal levels of serotonergic activity. One hypothesis is a difference in negative feedback on the serotonergic system between animals displaying escalated forms of aggression and non-aggressive animals. Negative feedback on the serotonergic system is, at least partially, regulated by the inhibitory actions of 5-HT autoreceptors (e.g. the 5-HT_{1A} and 5-HT_{1B} autoreceptor)(Claeysen et al., 2010). In the study of Caramaschi et al. (2007) the difference in sensitivity of the 5-HT_{1A} and 5-HT_{1A} receptors between aggressive and non-aggressive mouse strains was investigated. In this experiment six different mouse strains were used, that were obtained through three different breeding programs selecting for aggressive and non-aggressive animals (as earlier mentioned in paragraph 2.4). Animals of all strains were operated to implant a biotelemetry transmitter which enabled the researcher to record body temperatures. After the operation, animals were tested in the Resident-Intruder paradigm which confirmed the aggressive and non-aggressive phenotypes of a vast majority of animals of each particular strain. The next step was to give the animals an injection with either vehicle, S-15535 (a selective agonist of the presynaptic 5-HT_{1A} autoreceptor and antagonist of the postsynaptic 5-HT_{1A} receptor) or CP-94253 (a selective 5-HT_{1B} receptor agonist). The injection with vehicle induced hyperthermia in all mice. Attenuation of this injection-induced hyperthermia by the compounds S-15535 or CP-94253 was used as a measure of 5-HT_{1A} (auto)receptor sensitivity as has been done before in rats (De Boer et al., 2000). The results show that the majority of the aggressive mouse strains had higher 5-HT_{1A} and 5-HT_{1B} (auto)receptor sensitivity in comparison to the non-aggressive mouse strains. Previous research on the sensitivity of the 5-HT_{1A} receptor show similar results in both rats and mice, i.e. increased sensitivity in aggressive animals in comparison to non-aggressive animals (Van der Vegt et al., 2001). The higher 5-HT_{1A} and 5-HT_{1B} (auto)receptor sensitivity in highly aggressive animals in combination

with the knowledge that in aggressive mice the forebrain 5-HT_{1A} receptor expression is enhanced in comparison to non-aggressive mice (Korte et al., 1996), suggests that the negative feedback system in these mice may be more sensitive than in non-aggressive animals. This could be one of the factors contributing to the lower basal levels of serotonergic activity seen in animals displaying escalated forms of aggressive behavior.

4.3 Conclusion and recommendations

The main question of this thesis was which factors determined if a rodent in the Resident-Intruder test is going to act aggressively or submissively towards the other animal in the test. One factor that has great influence on this is the role of the animal in the Resident-Intruder paradigm. Due to the territoriality of rodents, resident animals are much more likely to display aggressive behavior than intruder animals. Research on coping strategies has shown that mice or rats who generally adopt a more proactive coping strategy are likely to display aggressive behavior during the Resident-Intruder paradigm. Furthermore, numerous studies have shown that animals with low levels of activity of the serotonergic system will behave more aggressively in the Resident-Intruder paradigm in comparison to animals with high levels of serotonergic activity. In addition, this correlation was also shown in other models of aggression. However, this correlation between low levels of serotonergic activity and aggressive behavior seem to be brain region dependent, at least in lizards. It would be interesting to see if the same region specificity of this correlation exists in rodents. However, current research showed that serotonin may have an opposite role in functional and escalated forms of aggressive behavior, i.e. that low serotonergic activity is only correlated with escalated forms of aggression. Based on the information reviewed in this thesis, a more specified serotonin deficiency hypothesis is suggested which implies that low levels of serotonergic activity in certain brain areas may predispose an animal to display escalated/pathological forms of aggression in the Resident-Intruder paradigm.

Acknowledgements

Writing a thesis demands a lot of self-discipline and concentration, which are two things that I have had problems with over the last year due to personal issues. Fortunately for me, I have had Heidi Lesscher as my supervisor for both my minor research project and thesis. Thanks to her support and catching enthusiasm, I got motivated again in times when I most needed it. Exceptional quick and constructive reviewing of the thesis drafts I send her offered me the chance to finalize my thesis in time and for this I am very grateful. In addition, I want to thank Iris van Gogh with whom I have had many brainstorm sessions which helped me a great deal in structuring my thesis. Furthermore, I would like to thank my mother Wil van Dijk who has always been there for me in the completion of my master.

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