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INTERSEXUAL DOMINANCE AND ITS RELATION TO STRESS AND SEXUAL COERCION

Abstract

Stress is an important factor in the etiology of depression and anxiety disorders. It is hypothesized that lasting subordination in low-ranked rats causes stress, which we try to validate. We also try to find evidence if sexual coercion is a potential stressor for female rats similar to what is known for women in our human society. This study's aim is to see if sexual coercion is occurring in rats and how this relates to the hierarchical structure in a rat colony with males and females. In rats this might be different because they are considered as animals with a much lower cognition than primates including humans. A semi-natural environment like the visible burrow system will be used to study the agonistic and sexual interactions in these animals. Consequences of living in the visible burrow system on stress and wellbeing will be studied in body and organ weight changes as well as in in hormones like corticosterone. Also changes in the structural and functional properties of brain regions involved in and affected by the behavioral and physiological response to stress will be measured. For males, the thymus was positively correlated with rank, and for females, the adrenal glands were negatively correlated with rank. No significant correlation was found between hierarchy and frequency of sexual coercion for either males or females, neither a correlation for hierarchy and frequency of successful defenses, nor between the frequency of sexual coercion and physiology of the females. Due to an abrupt ending of the research because of corona, only a small amount of data was analyzed, which makes it difficult to provide significant results, and to get a clear idea of the exact consequences of dominance hierarchies and sexual coercion in rats.

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1. Introduction

Stress that derives from social situations is a very common experience nowadays. Major causes of stress in the United States of America include job pressure and stress derived from relationships and media (Anderson, et al., 2015). The vulnerability to develop health issues due to social stress is individually different, because one's background and vulnerability play a role, but social stress in general is hypothesized promote diseases like anxiety and depression (Sontag, Graber, Brooks-Gunn, & Warren, 2008; Menard, et al., 2017; Björkqvist, 2001; Brown & Prudo, 1981). This is particularly the case when the social stressor is chronic and/or unpredictable (Koolhaas, et al., 2011). Therefore, it is legitimate to say that social stress can be seen as a risk factor in the development of stress pathology that comes with behavioral, physiological and neurobiological consequences. These consequences are frequently studied in animal models such as the social defeat in the resident-intruder paradigm where the consequences of a single or repeated loss experience, followed by social isolation in a resident-intruder set up in rats, are thought to resemble depression-like symptoms in humans (Koolhaas, et al., 1990). The resident-intruder set up is one of the existing experimental models on stress that create a dominant-subordinate relationship causing acute social stress.

A correct definition of dominance in this context, might be that of Drews (1993): 'Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterized by a consistent outcome in favor of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate. Dominance status refers to dyads while dominance rank, high or low, refers to the position in a hierarchy and, thus, depends on group composition. Dominance is a relative measure and not an absolute property of individual.'

The consequences of dominance hierarchies on quality of life in subordinate animals are severe in laboratory circumstances: 58% of subordinate male Long-Evans rats dies within 4 months; as opposed to rats in the wild that are not thought to have dominance hierarchies due to only fighting intruders (Blanchard, Blanchard, & Flannely, 1985; Barnett, 1958). It is important to realize that social stress in social colonies is only inflicted when individuals show clear agonistic behaviors towards each other. In rats particularly, male individuals exhibit aggressive behaviors to other males in order to achieve a high rank. However, not all laboratory strains of animals show aggressive behavior (de Boer, van der Vegt, & Koolhaas, 2003). In the majority of laboratory strain rats, breeding has largely eliminated aggressive propensity. Rats that still possess this aggressive behavior are for instance Long-Evans rats or feral rat strains.

Dominance hierarchies do not emerge only in laboratory settings, they also occur in many species in the wild, mostly in species that live in groups (Miller, 1995). The type of hierarchy may differ per species, some species will have a despotic group composition, which is very aggressive and has a big difference between the ranks, and some species will have an egalitarian group composition, in which animals are more equal (Kaburu & Newton-Fisher, 2015). The qualities that make an individual the leader of the group may differ amongst species. Several theories are designed to explain processes underlying the formation of dominance hierarchies. Two of these are most often used.

The first theory is called the prior attribute hypothesis. This is based on pre-existing individual differences, that are considered to have an impact on social status like body mass, age, sex, strength,

and physiological traits like sensitivity to glucocorticoids, (Hemelrijk, Wantia, & Isler, 2008; Sapolsky & Share, 1994).

The second theory is based on self-organization and is called the winner-loser effect. Winner-loser effect states that the experience an individual gains from a fight, either winning or losing, influences the outcome of a later conflict, regardless of the identity of opponents (Hsu & Wolf, 1999; Hsu, Earley, & Wolf, 2006). According to the winner-loser effect, the chance of winning another fight after losing the previous one is decreased and the individual becomes lower in ranking. Some articles state that when an individual's chance of winning a fight is significantly decreased, this individual's likeliness of initiating a fight is also altered (Schuett, 1997). This has also been studied in computational models like DomWorld, see Box A.

In multiple studies, it has been found that winner-loser effects exceed the effects that are expected by the prior attribute hypothesis (Schuett, 1997; Hemelrijk, Wantia, & Isler, 2008). Some evidence that the winner-loser effect is beneficial in nature, lies within the behavior animals show after the contest: less harm is done to losers of subsequent fights which saves time, effort and injuries (Lehner, rutte, & Taborsky, 2011).

A. DomWorld

A model has been designed in order to study the concept of the winner loser effect further; this is called DomWorld (Hemelrijk & Wantia, 2004). DomWorld is a computational model that provides an artificial world with an artificial society in which individuals interact: distance and interactions between individuals lead to a reliable ranking of the individuals. So far, this model has only been validated for species of primates, not for any species that are thought to have less cognitive abilities, even though dominance hierarchies have been studied in many of them.

1.1 Visible burrow system

The advantage of an animal model over a computational model, is the ability to obtain information about the physical condition of an individual, for example information about the endocrine system, but also to gather data about a real dominance hierarchy instead of trying to capture one in general rules. One of the primary responses to social stress is an increase in glucocorticoids due to an increased activation of the hypothalamic-pituitary-adrenocortical axis (Sapolsky, Neuroendocrinology of the stress response, 1992). The levels of such glucocorticoids can therefore provide an indication of the social status of the individual. This is, however, not flawless, because some data show elevated glucocorticoid levels in dominant males that might be struggling to maintain their high social status or that encounter many sexual interactions (Creel, 2001; Buwalda, Scholte, de Boer, Coppens, & Koolhaas, 2012). Another physical aspect that can be influenced by social ranking, is the weight of an individual: weight loss is very common among subordinate rats (Tamashiro, et al., 2007).

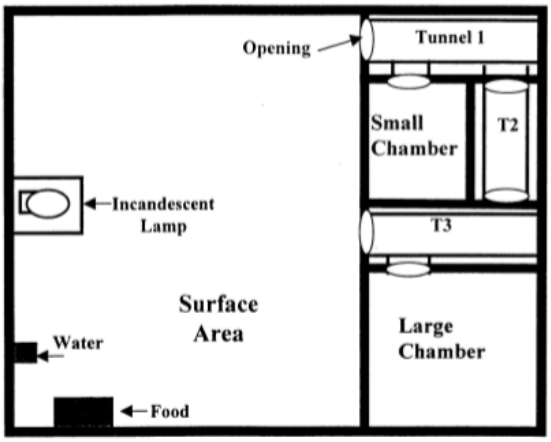


Figure 1: Schematic diagram of an example of a VBS (Blanchard, et al., 2001)

The visible burrow system (VBS) is a model in which social stress can be studied in labaratory rats that are living in a semi-natural environment (Tamashiro, et al., 2004). It consists of an open arena and burrows that are linked to nest boxes. Competition for food, water and females starts soon after the animals are placed in the VBS and a social hierarchy will be formed, making some animals subordinates and others dominant; the dominant animals can elicit stress in the subordinates via agonistic interactions, which happens mostly in the open arena. Having a higher rank will give an individual an advantage for acces to food and water, females and shelter (Korzan, Overli, & Summers, 2006). Subordinates can provide their own safe spot in the burrows by making sure the dominant animals can not get in to attack them via tunnel guarding behavior (Blanchard, et al., 2001). It has been found that the dominant-subordinate relationship can be solid enough to last six weeks even though there is daily competition (Hoshaw, Evans, Mueller, Valentino, & Lucki, 2006).

In Long-Evans rats, it has been shown that although lower ranked males can avoid interactions with dominant ones to some extension, life in a colony can have severe consequences for subordinate animals. In these rats, subordination causes changes in organs that reflect high levels of stress exposure; adrenal and spleen enlargement and reduction of the thymus (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993). Physiological consequences of subordination such as elevated corticosteroid levels and, decreased plasma testosterone levels relate to changes in the brain such as decreased activity in serotonergic neurotransmitter systems in the brain (Bernstein, Gordon, & Rose, 2003)

B. Estrus cycle

The estrus cycle in rats has an average length of four to five days, with an occasional six day cycle in some animals. Environmental factors like light, age, temperature, noise, nutrition, stress and social relationships can influence the length of the estrus cycle. Rats' estrus cycle is generally more regular when males are present.

The rat estrus cycle can be divided into four separate stages, each having its own characteristics: proestrus, estrus, metestrus and diestrus. Proestrus is the period before the female is fertile and has an average length of 14 hours, estrus is the period in which a female is willing to mate, it has a duration of 24-48 hours on average, metestrus is a short period in the absence of conception when the activity of the reproductive organs gradually subside with an average period of 6-8 hours and diestrus is a period of rest for the female with an average length of 48-72 hours (see Figure 2). (Cora, Kooistra, & Travlos, 2015)

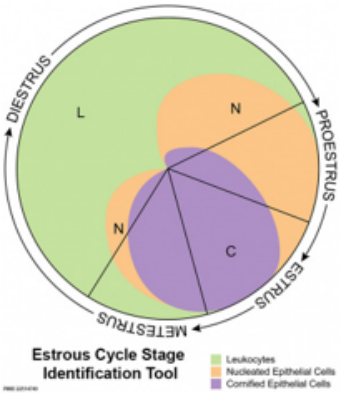


Figure 2: Different phases in the estrous cycle of the rat, length of each phase is represented in the size in the circle, types of cells are also highlighted per phase with colours

1.2 Intersexual dominance

Dominance hierarchies have been studied often in male rodents, yet hardly anything is known about the female position in rat dominance hierarchies and their physiological and behavioral reactions to this social housing in a colony together with males. It is also not known if they have agonistic

interactions with other animals, male or female, or if they win or lose agonistic interactions with conspecifics from either sex.

So far, almost all experiments focus on the behavioral and physiological effects on male rats, even when female rats are also a part of the experiment, this can be, for example when females are used to initiate competition between males (Brain, Benton, Howell, & Jones, 1980; Vidal, Buwalda, & Koolhaas, 2011a; Tamashiro, et al., 2004). This ignorance towards behavioral and physiological responses in females is most likely due to practical reasons. Their estrus cycle (see Box B) might be of influence on both physiology and behavior, and they are sometimes thought not to fight in social stress models such as the resident-intruder (Björkqvist, 2001). However, in colonies with primate species, evidence is found that females are hierarchically active members of the colony. They can be higher in ranking than some of the subordinate males, or even control the entire colony, depending on the species (Raps & White, 1991; Kaufman, 1991; Hohenbrink, et al., 2016; Meyer, Gallo, & Schultz, 1999).

DomWorld confirms the ability of female dominance to occur in certain group compositions (Hemelrijk, Wantia, & Isler, 2008). Characteristics of such group compositions that benefit female dominance are the intensity of aggression in the group, group cohesion, distribution of food, a similar diet for the sexes and sexual attraction (Hemelrijk, Wantia, & Dätwyler, 2003). Female dominance occurring in a computational model and being influenced by simple factors, suggests it could also occur in low-cognitive animals like rats.

This is an important research question in the current experiments: does female dominance occur in rat mixed-sex social colonies? Female rats display dominance-establishing behaviors; they can fight males and females, and they even perform mounting behaviors (Fang & Clemens, 1999). These types of behaviors are significantly more executed by dominant females than by subordinate female rats. The behavior was not influenced by her own estrus cycle, yet it was by male presence. However, the mounting behavior should not be seen as a sexual behavior, even though it is stimulated by a subordinate female that is in estrus, but as a social behavior to maintain one's social status within a group. This is supported by a positive correlation between female mounting and female aggressive behavior.

1.3 Sexual behavior in rats

Normally, rat sexual behavior mostly occurs during the estrus phase of the cycle. When the female is willing to mate, she displays some specific behaviors like anogenital sniffing, hopping and darting. These behaviors show the male her willingness and often the male will respond with mounts, intromissions and finally an ejaculation (Heijkoop, Huijgens, & Snoeren, 2018). When the female is not displaying such obvious behaviors, the male can still try to asses at what point of the cycle the female is by anogenital sniffing; the glands in the hind part of the female body alter in secretory activity during the estrus (Natynczuk & Macdonald, 1994). The female not being in estrus does not mean males will not try to copulate; males sometimes sexually coerce females.

According to Smuts and Smuts, sexual coercion in animals is defined as follows: 'We define sexual coercion as use by a male of force, or threat of force, that functions to increase the chances that a female will mate with him at a time when she is likely to be fertile, and to decrease the chances that she will mate with other males, at some cost to the female. The functional consequences of male sexual coercion distinguish it from other instances of male aggression against females (e.g., in the context of feeding competition) that do not appear to involve manipulation of sexual opportunities.

Our definition of sexual coercion as a subset of aggressive male behavior toward females that is delineated by their function means that sexual coercion is not a purely behavioral concept, but involves a combination of behavioral description and functional explanation. Sexual coercion cannot be identified by observing only the immediate behavior of the aggressor; it is also necessary to observe the subsequent behavior of the aggressor, the target, and even of other individuals.' (1993). This should be explained in a way that males use force or the threat of force at all times, so also when females are not in estrus, to increase the opportunities for said male when the female will be in estrus. Actions that should be considered part of sexual coercion are harassment of the female, intimidation of the female, forced copulation and infanticide (Canastar & Maxson, 2003). Whereas sexual coercion has negative consequences for female rats, it can have positive effects for males because it increases their reproductive success (Stanford, 1998).

Evidence to underline the difference between agonistic interactions which happen mostly between two males and sexual coercion, which is strictly directed towards females, comes from studies that have found a fluctuation in male aggression towards females during the estrous cycle. Males tend to be more aggressive towards females when they are moving towards the estrous phase of their cycle (Canastar & Maxson, 2003).

C. Sexual assault in humans

Over the last years this has been a growing issue in human societies. In 2014 the rape and sexual assault numbers in the USA were 1,1 per 1000 citizens, in 2018 it grew up to 2,7 per 1000 citizens and the actual numbers grew from 150.240 in 2014 to 347.090 in 2018 (Morgan & Oudekerk, 2019).

Sexual assault in humans can have severe consequences to the victim, also depending on the circumstances of the event. Factors that are of influence include the fear of sustaining injury, completion of the rape, an acquainted offender, the age of the victim, prior psychological trauma or psychiatric history, a 'freezing' response and environmental factors like restraints or captivity (Lodrick, 2007; Kilpatrick, et al., 1989; Salter, 1995; Van der Kolk, 2000; Schore, 2003; Briere & Scott, 2014; van der Kolk, 1989; Herman, 1992).

These are interesting factors, yet it is not the only striking part of sexual assault; numbers show that in the twelve months following a first rape experience, women experience three extra rape experiences on average (Tjaden & Thoennes, 2000). This might indicate some kind of predisposition for sexual assault towards some women in general and research has pointed out that women who befriend males that engage in high-risk behaviors or who frequently are at locations which are frequently visited by high-risk men, have a higher risk of rape victimization (Mustaine & Tewksbury, 2002); some research has already been done to reduce such risks (Fisher, Daigle, & Cullen, 2008).

Although rats can experience stress, it is unlikely for rats to suffer psychological trauma to the same extent as humans do as reaction to sexual assault, because they are on another level of cognition. However, it is interesting to compare facts about human sexual assault (see box C), sexual coercion in rats and the reaction of female rats to sexual harassment, since this might be a possible model to study these stress-related disorders in humans in the future. An aspect that might be particularly interesting is the impact of being able to defend oneself against the sexual assault. This could be compared to stress avoidance studies in rats; corticosterone levels differ in animals that can turn off the stressor

and animals that cannot turn off the stressor or to coping behavior during aggressive attacks (Brennan, Beck, Ross, & Servatius, 2005; Meerlo, Sgoifo, de Boer, & Koolhaas, 1999).

1.4 Consequences of social stress in rats

As mentioned above, rats can experience social stress in a mixed-sex social group. Competition for resources like food, water or a dry place to sleep are mostly the cause (Taylor & Constanzo, 1975). The consequences of social stress in rats are found in behavior, organ- and body weight, (neuro)endocrine function, and structural brain changes.

1.4.1 Behavioral changes

In studies using Long-Evans rats, dominant rats spend about 60% of the time in the open surface whereas subordinate males spend only 15% of their time in the open surface, and females spend 35% of their time in the open surface (Blanchard & Blanchard, 1990; Blanchard & Blanchard, 1989). With the water and food only accessible in the open surface, it is of higher risk for the subordinate animals to consume any, this is shown as well in both the studies of Blanchard and Blanchard; dominant animals eat and drink approximately three times as often as subordinate animals do, but they do this only 25% more often than females.

When the lights in the visible burrow systems turn off and the active phase begins, the subordinate animals take more than double the time to start moving than the dominant animals, females are in between (Blanchard & Blanchard, 1990). More interestingly, the number of transits per hour were significantly lower for subordinate animals compared to dominant animals but differed hardly from females. And lastly, the number of social contacts for subordinate animals is significantly lower than that of dominant animals.

One of the differences in behavior between dominant and subordinate males is the number of mounts performed by each male in a mixed-sex colony. Firstly, dominant males mount three times as often as subordinate males in normal visible burrow system circumstances. After the first seven hours dominant male sexual interest seems to decrease and subordinate males have higher numbers of mounting (Blanchard & Blanchard, 1989). After exposing all animals to a stressor like a cat, mounting in both dominant and subordinate animals is close to nothing; suggesting that stress has serious consequences on amount of mounting in rats.

1.4.2 Organ and endocrine changes

A study of Nguyen et al. (2007) has shown decreased body weight, elevated corticosterone and decreased testosterone levels in subordinate rats compared to dominant and control rats after fourteen days in a visible burrow system. The body weight of subordinate animals rapidly increased when rats were house individually during a recovery time after the VBS (Melhorn, et al., 2010). The subordinate animals also had significant higher levels of plasma corticosterone during the VBS period

Blanchard et al. have found symptoms of stress in subordinate rats in a mixed-sex colony of rattus norvegicus (1993). Both subordinate and dominant animals show adrenal enlargement and thymus reduction, with more severe results in subordinate animals. Subordinate animals also have decreased plasma testosterone levels and increased plasma corticosterone levels. Besides, the subordinate animals also have reduced testes weight and body weight loss. In 1994, Blanchard et al. have found severe body weight loss in subordinate animals compared to dominant animals with a smaller thymus, enlarged adrenal glands and severe higher plasma levels of corticosterone for subordinate animals.

They also found a large difference in the time spent on the open surface between dominant and subordinate animals, yet no effect of the dominance hierarchy in the size of the testes.

In 2004 a study of Tamashiro et al. has shown that significant body weight loss was found in subordinate animals, yet this difference was not as severe as found in earlier studies. No effects were found in the adrenal glands of the subordinate animals, but the thymus was significantly smaller than in dominant and control animals. Testosterone levels were lower in subordinate animals than in dominant animals, but the testes were found to be enlarged, when compared to those of dominant animals. This is very different from the study performed by Blanchard et al. in 1993, where dominant animals had higher testes weight compared to subordinates; as it is also different from the study of Blanchard et al. that was done in 1994, where no effects of dominance hierarchies were found on testes weight.

Another interesting organ that is thought to be influenced by dominance hierarchies are the preputial glands, these are major sources of pheromones in rodents (Pohorecky, et al., 2008). Pheromones can be used by animals to define territories, this is a more important aspect of dominant animals because they have more of a territory to mark, protect and defend. Glands that are producing more hormones, are found to be bigger, like it is also found in the adrenal glands (Blanchard, et al., 1994). Therefore, social ranking is expected to influence the size of preputial glands and this has been found by Pohorecky et al; the preputial glands in dominant animals are significantly enlarged compared to subdominant or subordinate animals.

1.4.3 Corticosterone

High levels of corticosterone are not always inducing suppression of the corticoid receptors: no differences were found in both hippocampus and hypothalamus in a study by Blanchard et al. (1994). Nevertheless, effects on the corticosteroid receptors are expected: corticosterone affects both mineralocorticoid and glucocorticoid receptors (Joëls, Karst, & Sarabdjitsingh, 2018) and after acute stress a rapid increase of the glucocorticoid receptor is found and a decrease in mineralocorticoid receptor sustained for a week (Yada, et al., 2007); social defeat down-regulates limbic mineralocorticoid receptors as well (Coppens, et al., 2011).

Mineralocorticoid receptors are mainly found in the hippocampus, the lateral septum and in lower levels in cortical layers and the amygdala. Its affinity to corticosterone is very high; low levels of corticosterone will lead to a constant high occupation of the mineralocorticoid receptor. This receptor is important for immediate cognitive responses to potential threats, it coordinates how individuals cope with stress in cooperation; the mineralocorticoid receptors is an important contributor in resilience (Joëls, Karst, & Sarabdjitsingh, 2018; de Kloet, et al., 2016).

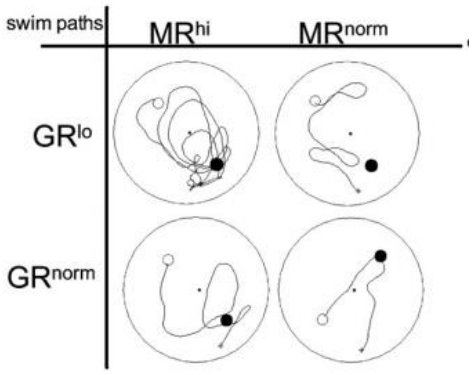


Figure 2: A representative swim path from each group, from swim 1 day 4 of reversal learning, illustrates that glucocorticoid receptor under expression and mineralocorticoid receptor over-expression mice persisted in searching in the old goal site. Black circle represents old platform location; white circle represents new platform location. (Harris, Holmes, de Kloet, Chapman, & Seckl, 2013)

Glucocorticoid receptors are present in almost all cells in the brain, but they are mostly expressed in the paraventricular nucleus of the hypothalamus and some parts of the hippocampus. The glucocorticoid receptor has a 10-fold lower affinity to corticosterone than the mineralocorticoid receptor and will be occupied mainly in stressful situations, it is important as complementary response to the immediate response and for adaptation in the long term (Joëls, Karst, & Sarabdjitsingh, 2018; Joels, 2001).

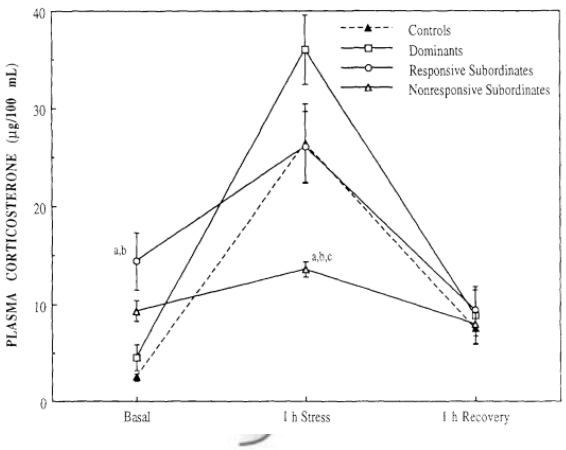


Figure 3: Plasma corticosterone levels of VBS rats before, during and after 1 h novel restraint stress. Data expressed as group mean + SEM. a = significantly different from control; b = significantly different from dominant; c = significantly different from stress

Mineralocorticoid receptors and glucocorticoid receptors affect fear, memory, reward, and other aspects of cognitive and emotional processing, and do so via cross-talk with other signaling cascades (Zalachoras, Houtman, & Meijer, 2013), but they may also affect each other and interact in specific domains of neuroendocrine and cognitive control (Harris, Holmes, de Kloet, Chapman, & Seckl, 2013). This is also shown in Figure 3, both receptors are needed to function normally to be able to learn correct new locations of goal platforms. Together, the two receptors determine the sensitivity of the brain to stress (Lucassen, et al., 2014). After acute stress, mineralocorticoid receptor is decreased and only return to control levels after seven days, which leads to thinking that they will also be decreased after 10 days of chronic stress.

1.4.4 Brain and neuro-endocrine changes

Subordination stress in rats that were not deprived for food, water or sleep, can still lead to structural brain changes: subordinate rats showed 35-50% lower levels of cell proliferation in the hippocampus (Hoshaw, Evans, Mueller, Valentino, & Lucki, 2006). Another study has shown no difference in cell proliferation in the dentate gyrus between dominant and subordinate animals after three days in a visible burrow system, but did find more new neurons in the dentate gyrus of dominant males compared to subordinate males (Kozorovitskiy & Gould, 2004).

A study of McKittrick et al. has shown dendritic atrophy in the neuronal dendrites in the CA3 region of the hippocampus of stressed, dominant animals (1995), these results were also found earlier by Watanabe, Gould and McEwen (1992). CORT levels are related to this type of atrophy in the CA3 region and were found to be higher in dominant animals after one hour of novel restraint stress than in subordinate animals (**Fout! Verwijzingsbron niet gevonden.**), which could mean that stress of maintaining a high rank could have more severe consequences in the CA3 region than being a subordinate in a dominance hierarchy. The ranking of the dominance hierarchy in this study was based on the weight changes of the animals and number of wounds and the spots on the body where they were found. Another finding of this study was enlarged adrenal glands and elevated basal corticosterone levels in subordinate animals compared to dominant and control animals.

1.4.5 Structural remodeling of dendritic neuronal structures

Neuronal dendrites are stress-sensitive, but so are other neuronal structures like spines, these dynamics are important in learning situations, which in the wild could prevent encountering dangerous

situations. Spines are sites of synaptic input with more than 90% of excitatory input ending on dendritic spines (Gray, 1959), which are important in learning and memory and can change dynamically during these processes (Kennedy, 2016). The general shape of a spine is a small head followed by a neck and then the attachment to a dendrite, but spines exist in more than one size and shape (Peters & Kaiserman-Abramhof, 1970), as can be seen in Figure 5. Yet, spines are dynamic and can be changing their shape in minutes, so it is unlikely to see the spines in exactly one of the shapes as indicated in Figure 5 (Dunaevsky, Tashiro, Majewska, Mason, & Yuste, 1999; Parnass, Tashiro, & Yuste, 2000). Spine shape is considered to be important for the functioning of the spine (Tsay & Yuste, 2004), but it is the size of the head that is correlated with synaptic strength (Schikorsky & Stevens, 1997; Matsuzaki, et al., 2001). Alterations in the shape of spines change calcium dynamics, which affects local learning rules (Majewska, Tashiro, & Yuste, 2000). Several factors are of influence on spine shape, dynamics and size, like glutamate receptors, GTPases, ephrins, adhesion molecules, sex steroids and glia (Lippman & Dunaevsky, 2005). Many of these regulating factors work via regulation of actin dynamics: the underlying source of spine morphogenesis (Fischer, Kaech, Knutti, & Matus, 1998). Dendrites arise from the actin cytoskeleton, its growth and retraction are caused by actin polymerization and depolymerization (Pollart, Blanchoin, & Mullins, 2006).

In the following sections, a few of the regulators mentioned above will be discussed more broadly.

1.4.5.1 Cofilin

Cofilin is an essential regulator of the actin cytoskeleton in many systems, which is critical in all aspects of neuronal development (Shaw & Bamburg, 2017). Some of the tasks of cofilin is working on dendritic spine structure, plasticity and volume and it is an essential factor in polymerization and depolymerization of actin, which are the backbone of dendrites, as can be seen in Figure 6 (Bamburg & Bernstein, 2016; Lei, Omotade, Myers, & Zjemg, 2016; Rust, 2015; Mizuno, 2013). The importance of cofilin is underlined in the consequences of suppressing cofilin function after sleep deprivation; spine loss, deficits in hippocampal synaptic plasticity and impairments in long-term memory that normally occur after sleep deprivation, are prevented (Havekes, et al., 2016).

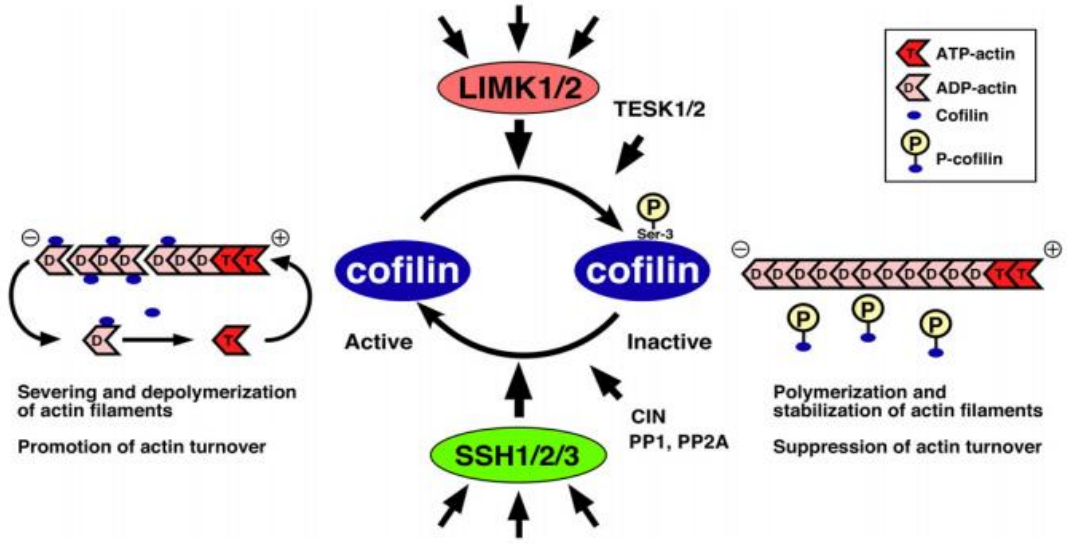


Figure 5: Control of actin filament dynamics by cofilin phospho-regulation. Cofilin preferentially binds to the ADP-bound subunits of actin filaments, stimulates severance and depolymerization of actin filaments near the pointed ends, and promotes actin filament turnover. Cofilin is inactivated by phosphorylation at Ser-3 by LIMKs and TESKs and reactivated by SSHs, CIN, and the other protein phosphatases, PP1 and PP2A. Cofilin phospho-regulation is one of the important convergence points of signaling networks that link extracellular signals to actin cytoskeletal dynamics. (Mizuno, 2013)

Cofilin can be easily inactivated by phosphorylation (pCofilin), which leads to accumulation and stabilization of actin filaments but it can be reactivated by dephosphorylation (Arber, et al., 1998). This phospho-regulation of cofilin occurs mostly via LIM-kinases and protein phosphatases and is one of the crucial events that ensures the role of cofilin in actin cytoskeletal dynamics (Mizuno, 2013; Arber, et al., 1998).

Cofilin rods are aggregates from both actin and cofilin that block intracellular trafficking and cause synaptic loss (Cichon, et al., 2012). Besides the morphological changes that are found, functional

Impairment and neurodegenerative diseases are also associated with cofilin rods. One of the intracellular trafficking pathways that is influenced by cofilin rods are glutamate receptor responses; an injection with glutamate provided a much smaller current in so called 'cofilin rod areas' than in non-rod areas (Cichon, et al., 2012); indicating a inhibition of effects of glutamate by cofilin. Another process that is altered by cofilin is long term potentiation; the learning process in which dendritic spines have critical input: alterations in AMPA receptor density and F-actin in dendritic spines are found (Michalski & Loew, 2012). F-actin is important in mechanical aspects of cell regulation like cellular shape change, cell migration and cell division (Pollard, 1976; Clarke & Spudich, 1977; Stossel, 1978). Cofilin has an apparent ability to sever filamentous (F)-actin (Hawkins, Pope, Maciver, & Weeds, 1993; Hayden, Miller, Brauweiler, & Bamburg, 1993). The alterations in F-actin are mainly because of cofilin changing the twist in the filaments; leading to another mechanism that regulates the actin cytoskeleton (McGough, Pope, W, & Weeds, 1997)

1.4.5.2 Glutamate

Another regulator in spine dynamics is glutamate. Glutamate is an excitatory neurotransmitter and its activity depends on multiple factors (Fonnum, 1984). Glutamate levels are important to maintain spines at established synapses (Matus, 2000). Stress leads to enhanced glutamate levels in hippocampus, nucleus accumbens and the medial prefrontal cortex (Moghaddam, 2002). Some effects of elevated glutamate levels are shown in a study of Wilson & Keith (1998); it is shown that a large amount of glutamate (50µM) can both enhance and inhibit dendrite length in vitro; in the short term, 50µM glutamate increases dendrite outgrowth in the hippocampus and in the long term 50µM glutamate causes dendrite retraction, resembling results were found in a study from Mattson, Dou & Kater (1988).

Glutamate has four types of receptors, NMDA, AMPA, kainate and G-protein dependent receptors (Meldrum, 2000), the main focus here will be on NMDA and AMPA. These two receptors have very distinct roles in spine motility; activation of NMDA receptors lead to spine outgrowth of dendrites as well as to activity of nascent spines, (Ziv & Smith, 1996; Maletic-Savatic, Malinow, & Svoboda, 1999; Engert & Bonhoeffer, 1999; Toni, Buchs, Nikonenko, Bron, & Muller, 1999) whereas AMPA receptors are necessary for the maintenance of yet established spines, NMDA receptor blockage however does not affect functioning of the AMPA receptors (McKinney, Capogna, Durr, & Thompson, 1999). This is supported by the finding that activation of AMPA receptors can block actin dynamics in dendritic spines in cultured hippocampal neurons (Fischer, Kaech, Wagner, Brinkhaus, & Matus, 2000).

The corticoid receptors also have their influence: mineralocorticoid receptors are found to elevate the glutamate release probability within 10 minutes in the basolateral amygdala, which lasted for several hours, in contrast to the hippocampus (Joëls, Karst, & Sarabdjitsingh, 2018). Due to the duration of the reaction of glutamate in the basolateral amygdala, an increase in corticosterone more than one hour

after the first raise, induces a reduction in glutamate release probability, leading to less spine outgrowth of dendrites .

1.5 Question and hypothesis

It is obvious that dominance hierarchies can be formed in rat species and to some extent how they affect brain and physiology; subordinance is thought to influence corticosterone levels, body weight and weight of adrenal glands, testes, thymus and preputial glands. Furthermore, subordinate animals are thought to have less spines and smaller dendrites in multiple brain areas, like the hippocampus and amygdala. It remains unclear what role females play in these dominance hierarchies, what the effects of dominance hierarchies and sexual coercion are on female brain and organs and if these dominance hierarchies can be explained by the simple rules of self-organization, like it has been done in the computational model DomWorld. When focusing on the role of females in a colony: it would be interesting if, in the far future, this could be translated to the role of women in human society in terms of sexual aggression and physical consequences of sexual aggression that have been found in women like anxiety disorders, since this has been proven to be a growing problem. Therefore, it would be interesting to find an answer to the following question:

How do intersexual dominance, sexual coercion and physiological changes in rattus norvegicus relate to each other in a visible burrow system study?

Since the concept of sexual aggression is relatively new in studies in rats, it is not clear what should be expected in the visible burrow system in terms of frequency, predisposition due to ranking or effects on female health status. However, we have a number of expectations and hypotheses. It is expected that higher ranked females will be less coerced than lower ranked females, because they are expected to be able to defend themselves better, since they are also able to gain a higher rank. However, more dominant males are likely to coerce females more often to increase their own mating opportunities than males that are ranked below the females, due to lower ranked males not starting confrontations as often because of the winner-loser effect. So when overall female dominance is higher in a colony, sexual coercion is expected to occur less often. Sexual coercion is expected to induce stress in females, because it is a form of aggression. When aggression is used towards an individual, it is expected to always induce some kind of stress in the receiver, therefore sexual coercion is thought to influence brain and behavior as well as the physiological responses supporting the behavioral response..

Lower ranking in the dominance hierarchy has been found to cause stress in earlier studies, and is therefore thought to enlarge adrenal glands due to higher corticosterone production, decrease the size of preputial glands, seminal vesicle and testes, involute the thymus and decrease fat mass, both retroperitoneal and epididymal. Subordinate animals are also expected to have higher ratio of phosphorylated cofilin : cofilin in the hippocampus and higher levels of glutamate in the nervous system, which can contribute to the degeneration in dendrites and spines. Another expected consequence of stress in subordinate animals are therefore smaller dendrites and fewer spines in hippocampus and amygdala due to the stress.

2. Methods

All experiments were approved by the CCD of the Netherlands and the IvD of the university of Groningen.

2.1 Subject

For this study, 48 male and 48 female wild-type Groningen rats are used, that were originally wild-trapped and bred with at the animal facility of university of Groningen. These rats were chosen because they show a broad range of social behaviors including aggressive behaviors and individuals vary significantly in these trait-like characteristics, which make them very suitable for social dominance experiments (van der Vegt, Lieuwes, Cremers, de Boer, & Koolhaas, 2003). Wild-type Groningen rats are more resilient to social stress than the commonly used laboratory rat (Vidal, Buwalda, & Koolhaas, 2011a). The animals are approximately four months of age when they enter the visible burrow system; if they are younger, they show little to no aggression (Koolhaas, et al., 1999)

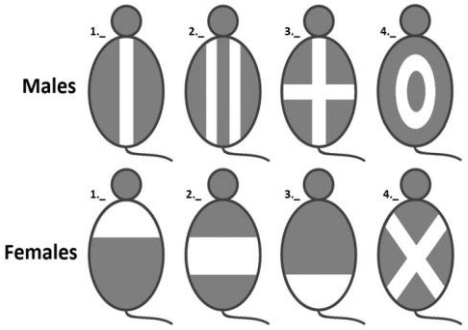


Figure 6: patterns for fur dying

2.2 Procedure

At least six weeks prior to the experiment, all females were oviduct ligated according to standard procedures: the procedure was performed while the rats were anaesthetized with isoflurane and the oviduct was cut between two ligatures, leaving the ovaries intact and maintain the estrus cycle like it was done by Buwalda et al. before (2017).

Two weeks prior to the experiment, the animals were put on a 12:12 light dark cycle, lights out at 05:00, in cages with groups of 3-4 individuals of the same sex. The cages are each enriched with a red plastic tube that the rats can play with, climb on and sit in, and multiple chewing sticks. The animals are handled every day for two weeks: they are picked up, placed on a towel on a lap and weighed.

	Males	Females
Induction box		
- Oxygen level	0,2	0,2
- Mixed air level	0,3	0,3
- Isoflurane level	5,0	5,0
Surgery tube		
- Oxygen level	0,2	0,2
- Mixed air level	0,3	0,3
- Isoflurane level	2,0	1,8

Table 1: air flow levels in induction box and tube

One week prior to the experiment, the animals were taken to the surgery room and randomly assigned to have their fur dyed in a specific pattern to be recognized in the visible burrow system (Figure 7). For this procedure, an induction box was used to anaesthetize the animals one by one for a maximum of half an hour, with air flow levels according to table 1.

After the animals were fully woken up, they were randomly assigned to be placed in a cage with a member of the opposite sex for seven days to gain sexual experience before entering the experiment.

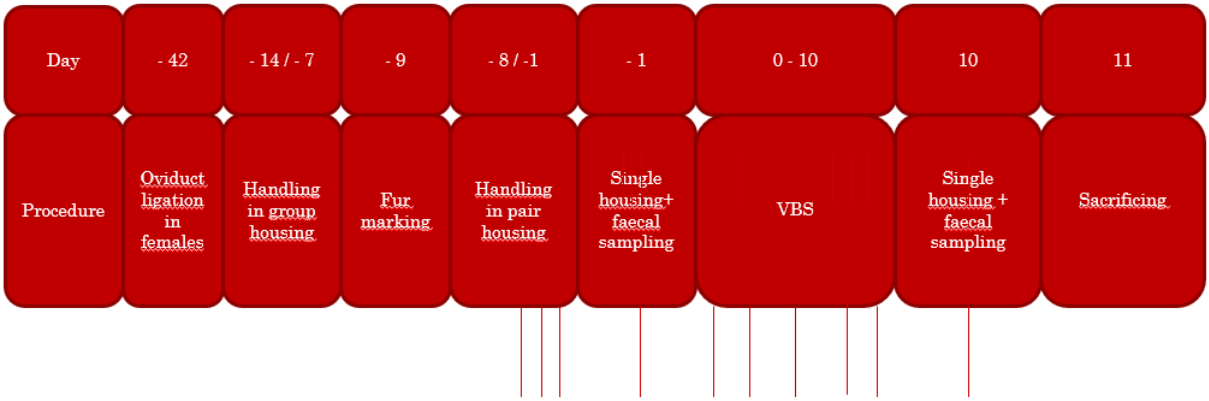


Figure 7: Timeline of the procedures, every vertical stripe represents a vaginal smear being obtained

The three days before the start of the experiment and on day 0, 2, 5, 8 and 10 of the experiment, vaginal smears were obtained from all females to determine estrus cycle, this was done by swabbing an öse twice through the vagina of the female between 09.00 and 11.00 AM at the previously mentioned days. The cells that were collected by swabbing were put on a glass slide with a drop of distilled water.

Nineteen hours prior to the experiment, near the end of the dark phase, the animals are single housed until they enter the VBS, to have an individual faeces collection which can be used for corticosterone measurements.

The animals were assigned into twelve groups of 8 individual, 4 males, 4 females, based on their weight. The groups were composed in such a way that none of the animals had met each other before entering the visible burrow system at 11:00. During the ten days the animals were in the visible burrow system, the water intake was monitored per bottle and refreshed daily and food was added on day 0, 2, 5, 8 and 10 in the arena and all nest boxes, on these days the individuals were also checked for their weight and any wounds they had gotten. After ten days, the animals were single housed in separate cages at the end of the dark phase until the next morning in order to collect individual faecal samples for corticosterone measurement again.

Sixteen hours after leaving the visible burrow system, the animals were put on a grid in a box with dry ice underneath for 30 seconds to not experience their sacrificing with a guillotine in full consciousness. Immediately after sacrificing an individual, the brain was collected and cut in halves: the left half would be instantly frozen with dry ice and liquid nitrogen, the right half would be put in a Golgi solution. Other organs of interest were taken out a few hours later and weighed.

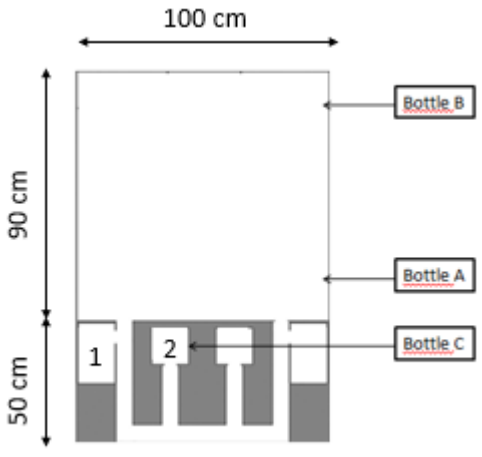


Figure 8: map of the visible burrow system, bottles are indicated, figure is from Bove et al (2018) and adjusted. Nest box 1 is 15x25x18 cm, nest box 2 is 15x15x18 cm

2.3 Apparatus

Each visible burrow system in this experiment was made of PVC and contained a large open arena, approximately 1 meter by 0.9 meter with a height of 1 meter, and burrows as shown in Figure 9, with a width and height of 7

centimeters. The burrows had a lid made out of dark Plexiglas, which is only transparent for infrared light in order for the space to be dark 24 hours per day, and no lid was placed on the open arena, so the 12:12 light-dark cycle remained with lights out at 05:00; the main light source in the arena was LED-light which was applied at all walls of the arena.

Standard bedding material was provided in the both the arena and the burrows. In both the open arena and all the nest boxes was food available ad libitum, in the arena were two bottles of water available and in one of the nest boxes was one bottle available, as indicated in Figure 9.

2.4 Data collection

2.4.1 Behavior

For the behavioral study, the animals were recorded 24 hours per day, every day with an infrared light source, a digital monochrome Basler GigE camera and Media Recorder 4 by Noldus. The videos were subsequently observed with The Observer® XT software at eight to ten timestamps of ten minutes each of day 1, 2 and 10. The timestamps were chosen based on the light-dark cycle and the activity of the animals: the main timestamps were 04:00, 05:00, 07:00, 08:00, 12:00, 14:00, 16:00, 18:00, at day 1 the time stamps 12:30 and 13:00 were added and some days we also added 06:00 and 20:00 to collect extra data of positioning of the animals in the light phase. The behaviors that are scored can be found in the behavioral catalog in the appendix (section 6.1).

2.4.2 Physiology

Corticosteroid samples can be taken in many different manners; the most common is via blood samples (Palme, 2019). About 80% of corticosterone metabolites are found in faeces and the other 20% can be found in urine (Bamberg, Palme, & Meingassner, 2001). Because blood collection from animals is impossible without restraint immobilization that influences the results and strong fluctuation of blood corticosterone levels, faecal corticosterone measurements have been introduced (Stead, Meltzer, & Palme, 2000). The collection of faeces enables monitoring of previous stressful conditions without causing any extra discomfort.

Faeces of each animal were collected before and after the visible burrow system period, they were stored at -20°C. Faecal sampling is chosen over blood sampling because plasma CORT levels are extremely elevated ten minutes after disturbing the cage, whereas faecal corticosteroid samples are much more stable (Good, Khan, & Lynch, 2003). An immunoassay was used to determine the amount of corticosterone in the faeces, as it was done previously in multiple species (Stead, Meltzer, & Palme, 2000; Rettenbacher, Möstl, Hackl, Ghareeb, & Palme, 2004; Eriksson, Royo, Lyberg, Carlsson, & Hau, 2004).

Vaginal smears were made as previously mentioned, the glass slides were air dried and stored at room temperature. Afterwards, the glass slides were stained according to the protocol in the appendix (section 6.2)

Various organs were dissected to have a general impression of the impact of the housing in the CBS and the social stress that animals perceived, depending on their dominance ranking. Adrenal glands, thymus, seminal vesicle, preputial glands, testes, retroperitoneal fat and epididymal fat were collected from all males. From all females the adrenal glands, thymus and retroperitoneal fat were collected; all

organs were precisely cleaned from other (adipose) tissues and were then weighed on an electric scale, measuring grams up to 4 decimals.

The brain was immediately divided into the two hemispheres after sacrificing each animal as mentioned before; the right half was used to study structural changes. It was put in a Golgi solution and stained according to the protocol in the appendix (section 6.3); the left half was instantly frozen with liquid nitrogen and kept at -80 °C to conserve all biochemical components until analysis.

The stained right half will be cut into sections of 100 micrometer with a vibratome. The sections will be studied under the microscope to quantify spines and dendrites. The frozen left half is used for western blotting for glutamate, cofilin, p-cofilin, corticosteroid receptors and F-actin.(Mahmood & Yang, 2012).

2.4.3 Average dominance index and further statistical analyses

The average dominance index is a linear scale that provides each individual in a colony with a score that explains their position in the dominance ranking. It is a calculation which is also used in the computational model called DomWorld. It is based on all the interactions within one group of animals and explains the dominance of each individual in this group (Hemelrijk, Wantia, & Gygax, 2005). First, the dominance index with every possible opponent is assessed, this number can then be averaged by all agonistic partners.

$$\boldsymbol{ADI} = \frac{1}{N}\sum_{j} Wij \qquad\qquad W_{ij} = \frac{X_{ij}}{X_{ij}X_{ji}}$$

Equation 1: Average dominance index. ***N*** *= number of agonistic partners,* W_{ij} *= dominance index with one specific partner,* ***j*** *= individual for who the average dominance index is calculated,* ***i*** *= individual with whom one or more fight have occurred*

The average dominance index is calculated with the number of fights won over each other individual in the colony, but it sometimes provides an equal score for multiple individuals. In this case, we chose to then calculate an average dominance index based on the number of attacks that were received by an individual, with the individual receiving the most attacks being ranked lower. When the tie is still not settled, the average dominance index is then calculated based on the number of losses each individual has, again ranking the individual the lowest who receives the most attacks.

Once the average dominance index is calculated, this data can be used to determine male and female dominance in the colony. The female dominance degree is a relative measure of female ranking over males and can be calculated with the standardized Mann-Whitney-U-Value. The value can be calculated per individual, or as a sum for all females of one colony.

$$\boldsymbol{DOM_{fem}} = \frac{\boldsymbol{M_s}}{\boldsymbol{M_t}} \qquad\qquad \boldsymbol{DOM_{femtot}} = \frac{\mathbf{1}}{\boldsymbol{N}}\sum_{\boldsymbol{F}} \frac{\boldsymbol{M_s}}{\boldsymbol{M_t}}$$

Equation 2: Female dominance degree. M_s *= number of subordinate males,* $\boldsymbol{M_t}$ ***=*** *total number of males,* ***N*** *= total number of females in the colony*

Matrix correlations are used per colony to investigate the association between agonistic behaviors and sexual behavior. Matrix analyses provided a measure to take every value in a matrix into account and calculate a correlation in comparison with all the other values in that and other matrices. All of the data in a matrix are cohesive and codependent, because if an individual loses a fight to another, the

whole group composition might change because the attitude of the loser towards all other individuals might change. Therefore, it is necessary to calculate the correlations per colony, this has been done in this study with a Kendall's Tau correlation. To combine the data and correlations of the separate colonies, a Fisher's test was performed later.

All calculations with weights were done in percentages; body weight was expressed as an alteration in percentage of the bodyweight of the starting day of the visible burrow system. To display the weight of organs for each animal in a representative way, the absolute average weight of the organs was calculated per organ, per sex; individual data was then expressed as deviating percentage of these averages.

Kendall's tau was also used to correlate changes in body weight and differences found in the weight of various organs with the dominance ranking, which was done in total per colony but also per sex per colony. Kruskal Wallis calculations were performed to see the differences between male and female organs as a consequence of dominance ranking.

Lastly, Kendall's tau was used to see if there is any correlation between the number of coercions a female has experienced and the changes in body weight and weights of organs.

3. Results

The first thing that is important in reading these results, is knowing that ranking is not done the way it is probably expected; the highest rank that can be achieved is 8 and the lowest is 1.This is because calculations are performed with number of fights won and the average dominance index displays the data as highest number being the most dominant animal. Therefore, positive correlation values are expected when talking about body weight and ranking for example. Because of the amount of data that was collected, most interesting data is visualized in this section and all individual data can be found from Appendix section 446.4 onwards.

Colony	Female dominance degree
1	0,75
2	0,625
3	0,5625
4	0,1875

Table 2: Female dominance degree for each colony. Score has a range between 0 and 1, with a higher score meaning a higher female dominance degree.

3.1 Male-female differences

Behavioral analysis has led to an average dominance index in four colonies. Dominance rank per individual per colony with ID of animal plus sex are shown in the appendix section 6.5. Females were dominant over males in three out of four colonies, as shown in Table 2. However, overall ranking was equally divided over males and females with a Chi^2 value of 8,257 (**Fout! Verwijzingsbron niet gevonden.**), also shown in Appendix section 6.6.7.

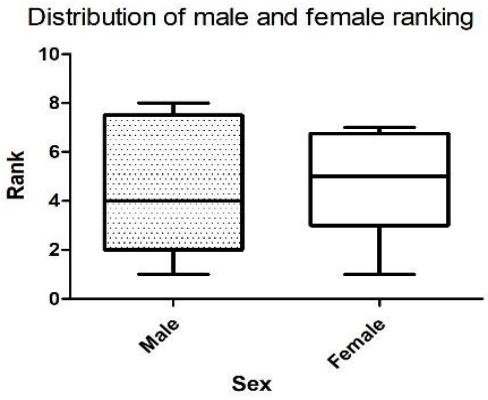


Figure 9: Distribution of male and female ranking, highest rank being 8 lowest rank being 1

Males and females reacted differently to the visible burrow system, Kruskal Wallis test pointed out a significant difference in all four colonies for body weight changes, see Figure 11 (P = 0,021 for all colonies, Chi^2 value of 30,906) and in two colonies for thymus weight (P = 0,034 and 0,043, Chi^2 value of 14,086), yet no differences were found in adrenal glands and retroperitoneal fat, this is also shown in Figure 15.

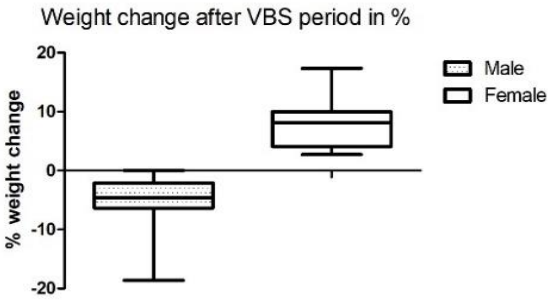


Figure 10: Weight change distribution between males and females. After Kruskal Wallis test, P values of the difference between sex per colony 0,021 for all colonies, leading to a Chi^2 value of 30.906

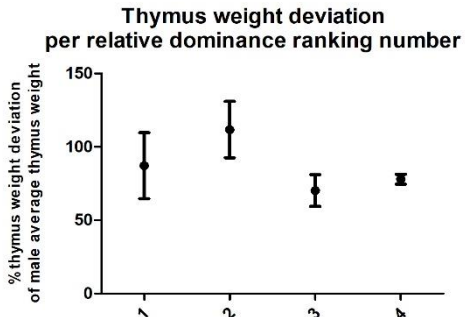
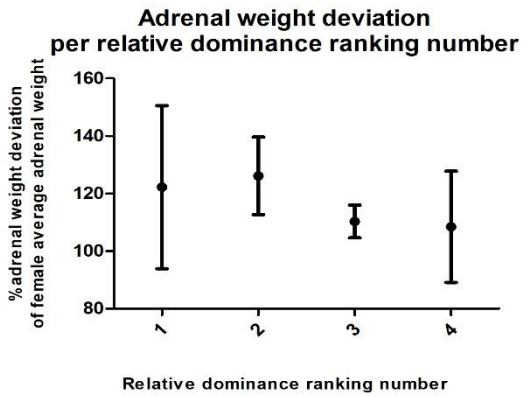


Figure 11: Deviation of individual thymus weight from average male thymus weight for relative dominance ranking number, correlation and Chi^2 values of 1, -0,333, 0,667, -0,667 and 19,067



female adrenal weight for relative dominance ranking number, correlation and Chi2 values are 0,333, -1, -0,333, -1 and 30,404

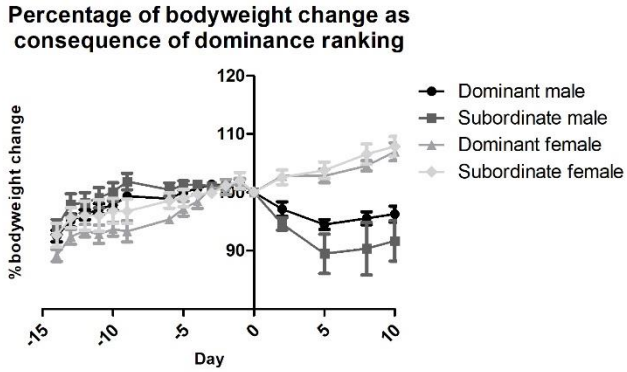


Figure 13: Average percentage of bodyweight change as consequence of dominance ranking for most dominant males, most subordinate males and females

3.2 Dominance ranking and physiology

Dominance ranking did not have a significant impact on weights of organs in combined male and female Kendall's tau correlation and a Fishers test. However, for males only, dominance ranking significantly influenced weight of thymus with correlation and Chi^2 values of 1, -0,333, 0,667, -0,667 and 19,067, critical value was again 15,507 (Figure 12). For females only, significant correlations were found for ranking and weight of adrenal glands, individual data is shown in Figure 13; correlation and Chi^2 values are 0,333, -1, -0,333, -1 and 30,404. Further graphs with relative organ weight compared per rank are shown in the Appendix (Section 6.7.3).

Body weight changes in males are very close to being significantly correlated to dominance ranking, but are not there yet, correlation values are 0, 0, 0,333 and 1,000, with a Chi^2 value of 15,214. It is shown more elaborate in Figure 14; the most dominant males on average do not lose as much weight as the most subordinate males, females are independent of rank growing during the VBS period.

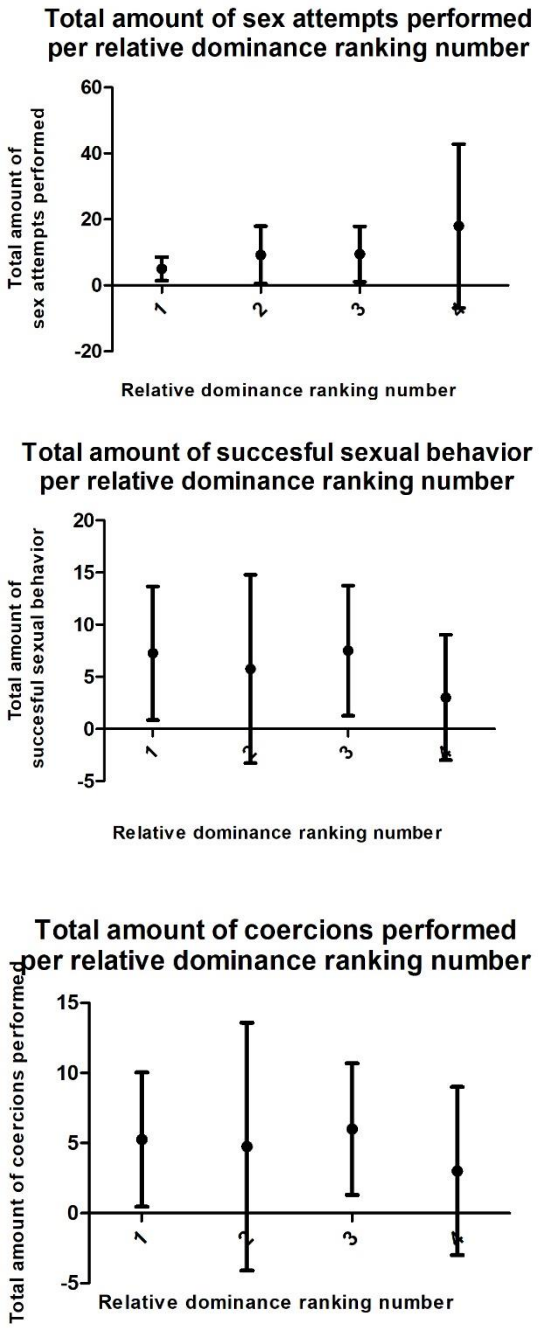


Figure 14: Total frequencies of male sexual behaviors per relative dominance ranking number.

In Figure 15, the differences in organ weight change are shown for most dominant males, most subordinate males, most dominant females and most subordinate females. For most dominant males and most subordinate males, even though dominance ranking significantly influenced thymus weight in males, this is not shown here, which is probably due to most subordinate males in all colonies not being close to having the same rank.

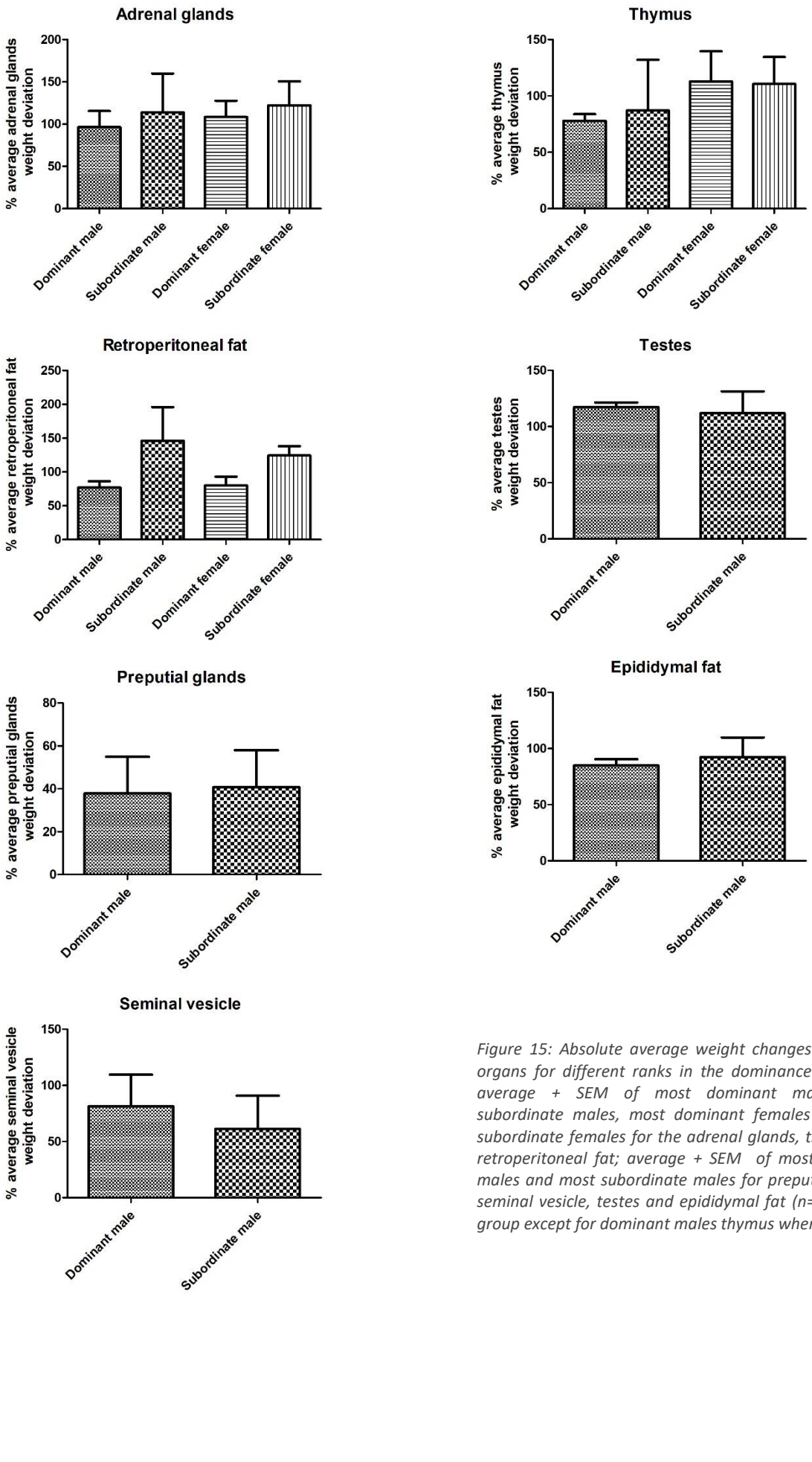


Figure 15: Absolute average weight changes of several organs for different ranks in the dominance hierarchy: average + SEM of most dominant males, most subordinate males, most dominant females and most subordinate females for the adrenal glands, thymus and retroperitoneal fat; average + SEM of most dominant males and most subordinate males for preputial glands, seminal vesicle, testes and epididymal fat (n=4 for each group except for dominant males thymus where n=3).

3.3 Dominance ranking and sexual behavior

Male ranking has a small negative correlation with the amount of sex attempts performed, correlation values are -0,286, -0,111, 0,096 and -0,387, this is not significant with a Chi^2 value of 2,271, critical value is 15,507. Male ranking does not influence the frequency of successful sexual behavior or sexual coercion; Chi^2 values o respectively 5,842 and 4,955 (also shown in Figure 7Figure 15). Female ranking also does not influence the frequency of sexual coercion, correlation values are 0,206, 0,276, -0,393 and -0,412 with a Chi^2 value of 6,611. Female ranking also does not influence the frequency nor percentage of successful defences; Chi^2 values are respectively 7,014 and 5,887.

> Sexual coercion is defined by a successful sexual attempt of the male which is not responded at with a lordosis by the female, assuming that a female in estrus will always display a lordosis.
>
> The number of successful defenses by a female is defined by the total number of sex defenses she has displayed minus the total number of coercions she has experienced.

Individual female dominance, the amount of males ranked below the specific female divided by the total amount of males in the colony, does not correlate significantly with the frequency of sexual coercion; correlation values are 0, -0,671, -0,236 and 0,775 with a Chi^2 value of 4,207, nor does it correlate with the amount of successful defenses, correlation values are 0, -0,408, 0,707 and 0,707 or

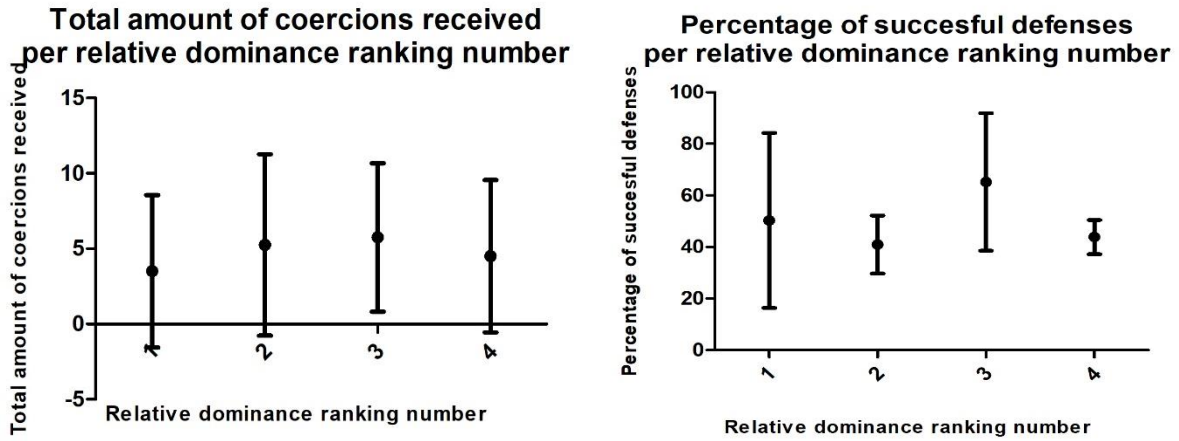


Figure 16: A: total amount of coercions received per female per relative dominance ranking number; B: percentage of defenses that was successful per female per relative dominance ranking number

with the percentage of defenses that was successful, correlation values are 0, -0,408, 0,236 and 0,236 with a Chi^2 value of 2,849. In Figure 16, a wide deviation per relative rank is also shown for total amount of coercions and successful defenses, which explains the low Chi^2 values. Female dominance degree per colony was also correlated with the total amount of coercions, successful defenses and percentage of successful defenses in that colony, which has led to correlation values of respectively 0,667, -0,667 and -0,33 with P values of 0,174, 0,174 and 0,497.

3.4 Sexual behavior and physiology

The amount of sexual coercion females experience, does not correlate significantly to any of the changes in weights of the organs. However, for the thymus all correlation values were found to be

negative, namely -0,913, -0,548, -0,333, -0,183, with a Chi^2 value of 9,909, meaning thymus weight is smaller when more coercions were received, yet not significant. For other organs correlation values were varying more and Chi^2 values were maximally 5,231. The percentage of defenses a female had made, that were successful, did also not significantly influence any weight of organs. However, the impact on retroperitoneal fat was the most striking: a positive correlation was expected because it is thought to be less stressful for females that are able to defend themselves, but correlation values of -0,183, -0.333, -1,000 and 0 were found. When total frequencies of sex defense are correlated with changes in weight of organs, correlation values for retroperitoneal fat are 0, 0, 0 and -0,6667, values for other organs can be found in appendix section 6.6.7.

4. Discussion

Due to corona, only few of the planned measurements could be performed; some of the original ideas were performed partly, some were not performed at all.

Dominance hierarchies were formed in all four colonies, this could be done mainly based on the number of wins and losses per colony, but some individuals received the same average dominance index score within a colony and these ties were settled with the number of attacks received and in one colony with the number of losses. Females were dominant over males in three out of four colonies, although there was no difference in overall ranking found between males and females. This indicates that social behaviors like dominance hierarchies in animals with low-cognitive abilities can not only be explained by older theories like the prior attribute hypothesis, leaning to the advantage of male dominance, but that more complex social models like the winner-loser effect are important.

It is clear that in all four colonies, some level of stress was experienced by the animals. Especially subordinate males showed avoidance behavior which resulted in them staying mostly in the burrows; females did not display any kind of avoidance behavior, which leads to think they experienced less stress in a way, even when considering sexual coercion; females did flee from males during a (sexual) attack, but did not stay away after the attack ended. This leads to controversy in the matter, females are clearly part of the dominance hierarchy when looking at agonistic interactions, yet they were never harmed in a way that some males were, which leads to thinking that they were not a real part of the dominance hierarchy. This could have some evolutionary origin, because wounded females are less fit to carry offspring for males, males would actually create disadvantages for themselves. Motivation in male-male fights could therefore be very different from the motivation in male-female fights; there are no disadvantages for males in harming other males. This difference in fights is also somewhat shown in subordinate males not avoiding higher-ranked females; lower-ranked males even coerce females as often as higher-ranked males.

Body weight changes are not strongly correlated to dominance ranking and are not significant when looking at all animals at once, in males only, a positive correlation is almost significant, with a Chi^2 value of 15,21422, which implies that body weight in social stress is differently affected in males and females. When looking at Figure 11, overall male body weight was negatively affected in all four colonies and overall female body weight was positively affected in all four colonies, which leads to thinking that males suffer from more stress in a visible burrow system than females do.

McKittrick et al. (1995) and multiple studies of Blanchard et al. (1994; 1993) have shown earlier that ranking is negatively correlated with the size of adrenal glands in males. In the current study, these findings could not be replicated for males, but they were replicated for females. In males, it was found dominant males were found to have larger testes in two out of four colonies and smaller testes in the other two colonies, this displays our own observations of the first two colonies being more aggressive in general than the latter two. The two colonies that showed the most aggression have positive correlations to weight of testes and the two colonies that showed less aggression no or a negative correlation. Both subordinate and dominant animals have previously been found to have larger testes, respectively in a study of Tamashiro et al. (2004) and a study of Blanchard et al. (1993). In another study of Blanchard et al both dominant and subordinate males were found to have enlarged testes compared to control animals (1994). Ranking was shown to be positively correlated to thymus weight. This was found earlier in a study of Tamashiro et al. (2004) and studies of Blanchard et al. (1993; 1994).

Yet, as shown previously in Figure 12, this was not found to be related to relative rank or as shown in Figure 15, only for averages of most dominant and most subordinate males. If the current study would have been executed like it was planned and all animals could have been analyzed, it would have been likely to find similar results as in the before mentioned studies.

Retroperitoneal fat was far from significantly correlated when looking at rank and combined males and females, males only and females only, nor is there a difference when looking at the difference between males and females. Yet when looking only at most dominant males, most subordinate males and females, like in Figure 15, subordinate males seem to have more retroperitoneal fat compared to both dominant males and females. No differences were found in ranking related to epididymal fat, both when looking at Fisher's test for Kendall's Tau correlation values as well as only most dominant male and most subordinate male, nor was anything found on preputial glands like it was found by Pohorecky et al. (2008).

Male ranking was not found to be correlated to either the frequency of sex attempts, successful sexual behavior or coercion, neither was there any clear evidence that female ranking had any influence on the frequency of being coerced nor on how often an individual was able to successfully defend herself against coercion, which we did expect before starting of the experiment. Our findings also do not suggest any relation between frequency of sexual coercion and bodyweight, size of adrenal glands, thymus or amount of retroperitoneal fat of females; however, these findings might not be as representative as imagined.

We think it is possible that the females experienced stress already well before they went into the visible burrow system; in the pair housing period, a lot of squeaking and stressful behavior was noticed for females when entering the room daily to handle and weigh the animals, this could be due to coercions already happening at this moment. Therefore, the findings in the visible burrow system could be less strong, although these effects are not shown in body weight nor in avoidance behavior. Therefore, after the first group of four colonies, it was planned to build in a control to see whether the females in the pair housing period experienced stress already, by having another corticosterone measurement the day before pair housing. All corticosterone data, however, are not shown here due to the fact that analysis was postponed as a consequence of corona. If these analysis were to be performed, higher levels would be expected in subordinate animals (Nguyen, et al., 2007; McKittrick, Blanchard, Blanchard, McEwen, & Sakai, 1995; Blanchard, et al., 1994; Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993; Melhorn, et al., 2010)

Another reason why we might not have as strong results in organ weights as a consequence of stress as anticipated, is that we did not have control animals that did not experience any stress to compare our data with, but we used the average of the findings in stressed animals per sex; an example of a good control group would be a group of males and a group of females that stayed in the same-sex group housing situation these experimental animals were in in their first week. Another option in which the animals experience less stress but do gain sexual experience could be coupling each male with two or more females in the week before VBS. Since it is females that especially might have already been stressed before entering the visible burrow system, therefore the average for adrenal glands might have been higher than it would have been in control animals, which made our results seem smaller.

Brain analysis was also not performed due to corona, but if it did, we would have expected higher levels of corticosterone in subordinate animals compared to dominant animals, although the latter

would probably also be elevated compared to control animals. Furthermore, the amygdala in the lower-ranked animals compared to higher-ranked animals and females was thought to show more atrophy as a consequence of stress, as well as effects of stress in terms of neurodegeneration in the hippocampus (Hoshaw, Evans, Mueller, Valentino, & Lucki, 2006; Kozorovitskiy & Gould, 2004). Another expected finding was that lower ranked animals would have a higher ratio of phosphorylated cofilin : cofilin and higher levels of glutamate in both amygdala and hippocampus compared to higher ranked animals. These higher levels would lead to decreased actin polymerization, which has as consequence less dendritic branching and less spines; like is has been found before (Arber, et al., 1998; McGough, Pope, W, & Weeds, 1997; Matus, 2000; Fischer, Kaech, Wagner, Brinkhaus, & Matus, 2000).

The vaginal smears that were made prior to and during the visible burrow system, were not useful for this analysis. Because only a small amount of behavioral analysis was performed, namely days 1, 2, (3) and 10, many proestrus moments have been missed, which were hypothesized to be the moments that most coercion would occur. To correlate the proestrus values with coercion values now would be inappropriate, because we might not have scored any moment of proestrus for a certain female, so the frequency of sexual coercion that is scored could be independent of the number of times a female was in proestrus during the visible burrow system.

A last factor that was not taken into account so far, would be the effect of despotic and egalitarian hierarchies on the members of the hierarchy; when looking at the number of wounds in a colony we could clearly distinguish a difference between the four colonies: two colonies being despotic and two colonies being more egalitarian. If this could be split up in final statistical analysis, when more colonies are available to analyze, this could be a major factor to weigh in in the ranking statistics compared to body weight, organ weights, brain structures and hormone levels. When looking at the results of Kendall's tau analysis and the Fisher's test, the difference between despotic and egalitarian colonies did not affect body weight, weight of adrenal glands, retroperitoneal fat, seminal vesicle and epididymal fat , but it could have affected weight of the thymus, testes and preputial glands.

The protocol that was used was very clean and straight forward and would have brought some nice results if it would have been completed. The results that are found until now are promising in the meaning that it is very likely that results will be significant when the experiment is completed. Without these findings it is difficult to predict anything for the future, however, I think it would be interesting to keep females and sexual coercion in mind in future studies with rats in visible burrow systems; future research might point out the differences of stress reactions in rats that have been coerced compared to rats that have not been coerced, but also see if ranking of animals is somehow related to the frequency of coercion. In prospects to human health care, I think this research can be of some value to underline the physical consequences of sexual harassment that women might experience but cannot explain to their doctors.

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6. Appendix

6.1 Behavioral catalog

6.1.1 Agonistic interactions.

OFFENSIVE: ANY aggressive response performs to an opponent. This may include sideways lateral threat (rat approaches a conspecific sideways while arching its back and extending its hindlegs), upright offensive posture (rat stands on its hindlegs in reaction to the upright of the opponent), attack jump (rat jumps and then lands on conspecific's back), clinch attack (rat attempts to bite or bites effectively the body of the conspecific), pinning (pushing opponent in supine position), and/or chase (rat pursues the opponent). Offensive responses include any aggressive action from a distance (such as a chase).

DEFENSIVE: ANY self-protecting act addressed at a conspecific in response to the receipt of ANY aggressive act. This may include upright, defensive posture, move away (rat moves away from the attacker, increasing the distance to the opponent), submissive-supine posture, and flight (flee). Defensive responses can happen even in the absence of an evident offensive act from a conspecific, this means that the mere presence of a conspecific can provoke a defensive response. **(Note: Include here a modifier for "unprovoked fear", i.e. defensive responses with no apparent attack from the conspecific).**

PATROLLING: ANY behavioral response to block the exit of the burrows to the arena, intended to prevent a conspecific from entering the open arena when coming from the burrow. This includes approaching the exit when a conspecific is "guarding" and/or displaying a sideways movement against the wall in which the conspecific is located. **Patrolling occurs only in the arena and has to be scored only when a conspecific performs a "tunnel guarding (blocking)" behavior when remaining in the tunnel**.

TUNNEL GUARDING (BLOCKING): Defensive response when a rat remains inside the tunnel close to the exit of the burrows to the arena. Rat is oriented with its face to the arena, in close proximity to the gate that connects the open arena and the burrow. When there is a conspecific displaying a "patrolling" response, the number of the patroller has to be scored.

DEFINITION OF INTENSITY OF AGGRESSION

Intensity of aggression in a colony may be determined in four ways. These definitions are based on our current observations and on research literature:

1. DURATION: Based on time length of offensive response towards a conspecific, the longer the response takes, the fiercer it is.

2. NUMBER OF WOUNDS: as a measure of fierceness of a colony: The larger the number of wounds, the fiercer the colony is.

3. BEHAVIOURAL DISPLAY: Qualitatively, based on observing the offensive response repertoire of the animal, fierce aggression **usually includes a sequence of agonistic responses: sideways (lateral) threat posture, followed by attack jump, clinch attack, and chase**. We can define an offensive response as fiercer if it includes the whole sequence of actions. If we observe only a lateral threat posture or only a chase response, then we classify these as less fierce.

4. INTENSITY OF SPECIFIC AGONISTIC RESPONSES: We qualitatively categorize specific agonistic responses regarding intensity as follows:

- More intense are those actions which involve potential or actual physical damage: Attack jump, Clinch attack.

- Less intense are those actions which don't involve physical damage: Sideways lateral threat, Upright posture, Chase.

NOTE: If a dominant male skips the introductory part of aggressive behavior (no threats) it can be indicative of violent behavior (De Boer).

(Blanchard & Blanchard, 1977) (Blanchard & Blanchard, 1981)

6.1.2 Sexual responses.

ANOGENITAL SNIFFING: Sniffing the anogenital region of a conspecific of the other sex, displaying the extent to which an individual is motivated to have a sexual interaction.

From males

SEXUAL ATTEMPT: ANY attempt of a male to mate with a female, which is not successful because the female displays a defensive response (hindleg kicking, fleeing).

SUCCESFUL SEXUAL BEHAVIOR: Sexual behavior that contains all sexual male behavior, for the latter part, acceptance or cooperation is needed from a female. This consists of

- Mount: mounting on the rump of another rat from behind with pelvic thrusting
- Intromission: mounting including penile insertion
- Ejaculation: penile insertion lasts longer than at intromission and is associated with rhythmic abdominal contractions
- Postcopulatory self-grooming: self-grooming immediately after mount, intromission or ejaculation

From females

LORDOSIS: Sexual proceptive behavior displayed by a female, the female arches her back and deflects her tail to one side. Due to the setup in this experiment, it is considered a lordosis if the female sits still during the mount and no defensive behavior is seen before or after the mount.

PARACOPULATORY BEHAVIOR: Sexual proceptive behavior displayed by a female, prior to or following a mount. This includes the female approaching a male followed by a runaway, often associated with hops, darts and ear wiggling and waiting near the male that has just mounted her, to mount her again for at least three seconds.

SEX DEFENSE: ANY sexual defensive response displayed by a female towards a male when he is trying to mount her. This includes hindleg kicking, fleeing and turning around when the male is trying to mount, also biting and upright postures.

(Houwing, Heijkoop, Olivier, & Snoeren, 2018)

6.2 Gymsa staining

Benodigdheden:

- Tris buffer
- Giemsa oplossing
- Maatcilinder
- Zuigpipet
- Fles
- Bakje (object glaasjes)
- Microscoop
- Trechter
- Demiwater
- Pincet

1) **119** ml Trisbuffer in maatcilinder → in mengfles
2) **8,75** ml Giemsa met zuigpipet → in mengfles (samen met de trisbuffer maakt dit de werkoplossing)
3) Werkoplossing beetje mixen met zuigpipet
4) Objectglaasjes in bakje (schuin, laatste met rug naar elkaar toe, max 20 per bakje)
5) Werkoplossing in bakje tot ze helemaal ondergedompeld zijn
6) Timer zetten, 10 minuten wachten (12 minuten als je de werkoplossing voor de 2^{e} keer gebruikt – op fles zetten hoe vaak je mengsel gebruikt hebt, max. 2 keer)
7) Werkoplossing met trechter terug gieten in mengfles
8) Glaasjes met pincet op plaat leggen
9) Bakje omspoelen (Als de werkoplossing 2 keer gebruikt is weggooien in jerrycan onder zuurkast)
10) Achterkant van objectglaasjes onder de kraan, en afspoelen met demiwater, drogen met tissue

6.3 Modified Golgi-Cox protocol

Prepare these stock solutions separately:

Potassium dichromate solution (A) - 5%

Mercuric chloride solution (B) - 5%

Potassium chromate solution (C) - 4%

Stock solution must be prepared 24 hours before use

Mix the above solution in the following composition (5:5:4)

5 parts of A

5 parts of B

4 parts of C

If the stock is ripened you don't see a precipitate, in case you notice a precipitate, add a pinch of sodium chloride and a clear orange solution of the fixative is ready to use.

Each brain requires about 50 ml of solution

Change brains into a fresh fixative after 24hrs

Keep brains in fresh fixative for 15 days

At the end of 15 days, transfer brains into a freshly prepared solution of 6% sucrose in 0.1 M PB in the bath solution.

Collect sections on chrome alum-gelatin coated slides, add a few drops of 6% sucrose solution onto the section keeping the sections moist, then drain off excess solution further with a tissue paper (folded twice), press the sections gently with the forefinger so that the sections become completely dried an left for 5 min before starting with the color reaction process.

0.1M PB in double distilled water (pH=7.4) (1000ml)

Na_2HPO_4 14,18 g

$Na_2H_2PO_4$ 3,12 g

6.3.1 Color reaction

Double distilled water - 5 min

↓

Double distilled water - 5 min

↓

5% Na_2CO_3 in distilled water - 20 min

↓

Double distilled water - 5 min

↓

Double distilled water - 5 min

↓

70% Ethanol - 20 min

↓

100% Ethanol - 5 min

↓

100% Ethanol - 5 min

↓

100% Xylol - 1 min

↓

100% Xylol - 1 min

↓

DPX mount

Dry slides for 2-3 days before analysis

6.4 Matrices per colony

6.4.1 Colony 1

WINS	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI	Adjusted ADI
F1		4	3	0	0	0	0	0	7	0,5	0,5
F2	4		2	1	0	0	0	0	10	0,4722222	0,4722222
F3	0	1		0	0	1	0	0	5	0,2666667	0,2666667
F4	1	3	2		0	0	0	0	7	0,6875	0,6875
M1	0	0	0	0		0	0	0	5	0	0,01
M2	0	0	0	0	0		0	0	0	0	0
M3	0	0	0	0	0	0		1	1	0,125	0,125
M4	0	0	2	4	7	2	7		23	0,975	0,975

Attacks received	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI
F1		12	0	13	1	0	0	0	26	0,618506
F2	10		1	9	0	1	0	0	21	0,430125
F3	7	5		3	0	0	0	2	17	0,766667
F4	1	8	0		0	1	2	5	17	0,590336
M1	0	0	0	0		1	0	10	11	0,369697
M2	0	0	4	0	4		0	3	11	0,466667
M3	0	1	0	0	0	1		9	11	0,725
M4	0	0	0	0	1	0	1		2	0,038182

Sex attempt	F1	F2	F3	F4	
M1	20	20	7	7	54
M2	0	6	1	1	8
M3	0	0	0	0	0
M4	0	0	0	0	0

Received Sex attempt	M1	M2	M3	M4	
F1	20	0	0	0	20
F2	20	6	0	0	26
F3	7	1	0	0	8
F4	7	1	0	0	8

Succesful sexual behavior	F1	F2	F3	F4	
M1	10	0	2	0	12
M2	3	1	0	0	4
M3	0	0	0	0	0
M4	0	0	0	0	0

Received Succesful sexual behavior	M1	M2	M3	M4	
F1	10	3	0	0	13
F2	0	1	0	0	1
F3	2	0	0	0	2
F4	0	0	0	0	0

Coercion	F1	F2	F3	F4	
M1	10	0	2	0	12
M2	0	1	0	0	1
M3	0	0	0	0	0
M4	0	0	0	0	0

Received coercion	M1	M2	M3	M4	
F1	10	0	0	0	10
F2	0	1	0	0	1
F3	2	0	0	0	2
F4	0	0	0	0	0

Sex defense	M1	M2	M3	M4	
F1	27	1	0	0	28
F2	20	10	0	0	30
F3	7	2	0	0	9
F4	10	1	0	0	11

Lordosis	M1	M2	M3	M4	
F1	0	3	0	0	3
F2	0	0	0	0	0
F3	0	0	0	0	0
F4	0	0	0	0	0

Succesful defense	M1	M2	M3	M4	
F1	17	1	0	0	18
F2	20	9	0	0	29
F3	5	2	0	0	7
F4	10	1	0	0	11

Succesful defense in %	M1	M2	M3	M4
F1	100	69,565	70	100
F2	0	0	0	100
F3	0	100	0	0
F4	0	100	85,714	0

ADI	F1	F2	F3	F4
M1	2	3	7	1
M2	-3	-2	2	-4
M3	-2	-1	3	-3
M4	-4	-3	1	-5

ADI	M1	M2	M3	M4
F1	4	5	3	-2
F2	3	4	2	-3
F3	2	3	1	-4
F4	5	6	4	-1

6.4.2 Colony 2

WINS	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI	Adjusted ADI
F1		1	0	0	1	0	0	0	2	0,5	0,5
F2	0		1	2	0	0	0	0	3	0,333333	0,3333333
F3	0	2		1	0	0	0	0	3	0,583333	0,5833333
F4	2	0	1		0	0	0	0	3	0,5	0,52
M1	0	0	0	0		0	0	0	0	0	0,01
M2	1	0	0	0	8		3	1	13	1	1
M3	0	0	0	0	0	0		0	0	0	0
M4	0	1	0	0	0	0	0		1	0,5	0,51

Attacks received	F1	F2	F3	F4	M1	M2	M3	M4		AvgDI
F1				3	1	3			7	0,625
F2	1		1	2				1	5	0,566667
F3		1		4		1			6	0,690476
F4		4	3				2		9	0,52381
M1	1					7		1	9	0,46875
M2		1			1				2	0,1875
M3					2	4		1	7	0,75
M4					1	1			2	0,375

Sex attempt	F1	F2	F3	F4	
M1	0	4	3	1	8
M2	0	0	0	0	0
M3	2	0	1	4	7
M4	9	4	5	0	18

Received sex attempt	M1	M2	M3	M4	
F1	0	0	2	9	11
F2	4	0	0	4	8
F3	3	0	1	5	9
F4	1	0	4	0	5

Succesful sexual behavior	F1	F2	F3	F4	0
M1	4	9	5	1	19
M2	0	0	0	0	0
M3	5	4	2	0	11
M4	7	1	6	0	14

Received succesful sexual behavior	M1	M2	M3	M4	0
F1	4	0	5	7	16
F2	9	0	4	1	14
F3	5	0	2	6	13
F4	1	0	0	0	1

Coercion	F1	F2	F3	F4	0
M1	4	8	5	1	18
M2	0	0	0	0	0
M3	2	3	2	0	7
M4	7	0	4	0	11

Received coercion	M1	M2	M3	M4	0
F1	4	0	2	7	13
F2	8	0	3	0	11
F3	5	0	2	4	11
F4	1	0	0	0	1

Sex defense	M1	M2	M3	M4	
F1	7	0	6	16	29
F2	12	0	2	4	18
F3	11	0	4	6	21
F4	4	0	4	0	8

Lordosis	M1	M2	M3	M4	0
F1	0	0	3	0	3
F2	1	0	1	1	3
F3	0	0	0	2	2
F4	0	0	0	0	0

Succesful defense	M1	M2	M3	M4	0
F1	3	0	4	9	16
F2	4	0	0	4	8
F3	6	0	2	2	10
F4	3	0	4	0	7

Succesful defense in %	M1	M2	M3	M4
F1	42,857	0	66,667	56,25
F2	33,333	0	0	100
F3	54,545	0	50	33,333
F4	75	0	100	0

ADI	F1	F2	F3	F4
M1	-2	-1	-5	-4
M2	4	5	1	2
M3	-3	-2	-6	-5
M4	1	2	-2	-1

ADI	M1	M2	M3	M4
F1	2	-4	3	-1
F2	1	-5	2	-2
F3	5	-1	6	2
F4	4	-2	5	1

6.5.3 Colony 3

WINS	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI	Adjusted ADI
F1		2	0	1	0	0	1	0	4	0,611111	0,611111
F2	2		2	1	0	1	0	0	6	0,566667	0,566667
F3	0	0		0	0	0	0	0	0	0	0
F4	2	2	1		0	0	0	0	5	0,777778	0,777778
M1	0	0	1	0		3	4	5	13	1	1
M2	0	0	0	0	0		0	0	0	0	0,01
M3	0	1	1	0	0	0		0	2	0,5	0,5
M4	0	0	0	0	0	0	0		0	0	0,02

Attacks received	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI
F1		1	0	3	0	0	1	0	5	0,472222
F2	5		2	3	0	0	1	1	12	0,654762
F3	0	1		3	2	2	1	0	9	0,733333
F4	1	4	0		0	4	0	0	9	0,455357
M1	0	0	0	0		0	1	2	3	0,1125
M2	0	1	4	0	11		1	0	17	0,733333
M3	1	0	0	0	4	0		0	5	0,26
M4	0	0	0	0	6	0	0		6	0,375

LOSSES	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI
F1		3	0	2	0	2	0	0	7	0,566667
F2	2		1	4	0	0	0	0	7	0,383333
F3	0	2		1	1	0	1	0	5	0,916667
F4	1	1	0		0	0	0	0	2	0,177778
M1	0	0	0	0		0	0	0	0	0
M2	0	1	0	0	6		0	0	7	0,666667
M3	1	0	0	0	5	0		0	6	0,666667
M4	0	0	0	0	5	0	0		5	1

Sex attempt	F1	F2	F3	F4	
M1	20	20	7	7	54
M2	0	6	1	1	8
M3	0	0	0	0	0
M4	0	0	0	0	0

Received sex attempt	M1	M2	M3	M4	
F1	20	0	0	0	20
F2	20	6	0	0	26
F3	7	1	0	0	8
F4	7	1	0	0	8

Succesful sexual behavior	F1	F2	F3	F4	0
M1	10	0	2	0	12
M2	3	1	0	0	4
M3	0	0	0	0	0
M4	0	0	0	0	0

Received succesful sexual behavior	M1	M2	M3	M4	0
F1	10	3	0	0	13
F2	0	1	0	0	1
F3	2	0	0	0	2
F4	0	0	0	0	0

Coercion	F1	F2	F3	F4	0
M1	10	0	2	0	12
M2	0	1	0	0	1
M3	0	0	0	0	0
M4	0	0	0	0	0

Received coercion	M1	M2	M3	M4	0
F1	10	0	0	0	10
F2	0	1	0	0	1
F3	2	0	0	0	2
F4	0	0	0	0	0

Sex defense	M1	M2	M3	M4	
F1	27	1	0	0	28
F2	20	10	0	0	30
F3	7	2	0	0	9
F4	10	1	0	0	11

Lordosis	M1	M2	M3	M4	0
F1	0	3	0	0	3
F2	0	0	0	0	0
F3	0	0	0	0	0
F4	0	0	0	0	0

Succesful defense	M1	M2	M3	M4
F1	17	1	0	0
F2	20	9	0	0
F3	5	2	0	0
F4	10	1	0	0

Succesful defense in %	M1	M2	M3	M4
F1	62,963	100	0	0
F2	100	90	0	0
F3	71,429	100	0	0
F4	100	100	0	0

ADI	F1	F2	F3	F4
M1	2	3	7	1
M2	-3	-2	2	-4
M3	-2	-1	3	-3
M4	-4	-3	1	-5

ADI	M1	M2	M3	M4
F1	-2	3	2	4
F2	-3	2	1	3
F3	-7	-2	-3	-1
F4	-1	4	3	5

6.4.4 Colony 4

WINS	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI	Adjusted ADI
F1		2	2	2	0	0	1	0	7	0,8	0,8
F2	0		0	0	0	0	0	0	0	0	0,01
F3	0	0		0	0	0	0	0	0	0	0
F4	0	1	0		0	0	0	0	1	0,5	0,5
M1	0	1	0	0		4	0	3	8	1	1
M2	1	0	0	0	0		2	0	3	0,666667	0,666667
M3	0	1	0	0	0	0		1	2	0,5	0,52
M4	0	2	1	0	0	0	0		3	0,5	0,51

Attacks received	F1	F2	F3	F4	M1	M2	M3	M4	Sum	AvgDI
F1		1	2	0	0	1	1	0	5	0,366667
F2	4		1	4	3	2	1	2	17	0,728571
F3	2	2		1	0	1	2	1	9	0,805556
F4	3	1	0		0	0	2	1	7	0,573333
M1	1	0	0	0		3	0	0	4	0,357143
M2	1	1	0	0	4		3	0	9	0,31746
M3	0	0	1	1	0	3		1	6	0,277778
M4	0	2	0	0	3	1	1		7	0,5

Sex attempt	F1	F2	F3	F4	
M1	7	1	1	6	15
M2	0	0	1	4	5
M3	21	0	0	0	21
M4	6	0	2	0	8

Received sex attempt	M1	M2	M3	M4	
F1	7	0	21	6	34
F2	1	0	0	0	1
F3	1	1	0	2	4
F4	6	4	0	0	10

Succesful sexual behavior	F1	F2	F3	F4	0
M1	1	1	0	2	4
M2	0	0	0	0	0
M3	5	0	0	0	5
M4	0	0	0	0	0

Received succesful sexual behavior	M1	M2	M3	M4	0
F1	1	0	5	0	6
F2	1	0	0	0	1
F3	0	0	0	0	0
F4	2	0	0	0	2

Coercion	F1	F2	F3	F4	0
M1	1	0	0	2	3
M2	0	0	0	0	0
M3	5	0	0	0	5
M4	0	0	0	0	0

Received coercion	M1	M2	M3	M4	0
F1	1	0	5	0	6
F2	0	0	0	0	0
F3	0	0	0	0	0
F4	2	0	0	0	2

Sex defense	M1	M2	M3	M4	
F1	8	1	23	6	38
F2	2	1	0	0	3
F3	2	1	1	2	6
F4	9	3	0	0	12

Lordosis	M1	M2	M3	M4	0
F1	0	0	0	0	0
F2	1	0	0	0	1
F3	0	0	0	0	0
F4	0	0	0	0	0

Succesful defense	M1	M2	M3	M4	0
F1	7	1	18	6	32
F2	2	1	0	0	3
F3	2	1	1	2	6
F4	7	3	0	0	10

Succesful defense	M1	M2	M3	M4
F1	87,5	100	78,261	100
F2	100	100	0	0
F3	100	100	100	100
F4	77,778	100	0	0

ADI	F1	F2	F3	F4
M1	1	6	7	5
M2	-1	4	5	3
M3	-2	3	4	2
M4	-3	2	3	1

ADI	M1	M2	M3	M4
F1	-1	1	2	3
F2	-6	-4	-3	-2
F3	-7	-5	-4	-3
F4	-5	-3	-2	-1

6.5 Dominance ranking per colony

Colony 1

Rank	Sex	ID
1	M	6
2	M	3
3	M	12
4	F	28
5	F	22
6	F	18
7	F	31
8	M	16

Colony 2

Rank	Sex	ID
1	M	10
2	M	1
3	F	24
4	F	20
5	M	15
6	F	30
7	F	25
8	M	5

Colony 3

Rank	Sex	ID
1	F	27
2	M	13
3	M	8
4	M	9
5	F	23
6	F	19
7	F	29
8	M	4

Colony 4

Rank	Sex	ID
1	F	26
2	F	21
3	F	32
4	M	14
5	M	11
6	M	7
7	F	17
8	M	2

6.6 Statistics

6.6.1 Matrix correlations

Male rank*Sex attempt	KTr value	P value
Colony 1	-0,28609	0,749073
Colony 2	-0,11111	0,95912
Colony 3	0,096374	0,499725
Colony 4	-0,38695	0,894511
Chi^2 value		2,271667

Female rank*coercion	KTr value	P value
Colony 1	0,205764	0,748603
Colony 2	0,276245	0,792982
Colony 3	-0,39284	0,251027
Colony 4	-0,41153	0,246138
Chi^2 value		6,611119

Male rank*Successful sexual behavior	KTr value	P value
Colony 1	0,435645	0,247508
Colony 2	0,222222	0,372666
Colony 3	0	0,584184
Colony 4	-0,61729	1
Chi^2 value		5,841851

Female rank*successful defense	KTr value	P value
Colony 1	0,25047	0,250637
Colony 2	0,457594	0,140799
Colony 3	-0,54772	0,917001
Colony 4	-0,45759	0,926641
Chi^2 value		7,014017

Male rank*Coercion	KTr value	P value
Colony 1	-0,09637	0,665653
Colony 2	0,235926	0,333827
Colony 3	0,205764	0,377746
Colony 4	-0,61729	1
Chi^2 value		4,955305

Female rank*successful defense in %	KTr value	P value
Colony 1	0	0,668663
Colony 2	0,522774	0,125769
Colony 3	-0,32817	0,751852
Colony 4	-0,37847	0,833162
Chi^2 value		5,887056

6.6.2 Fisher's Test for Kendall's Tau correlated ranking x physiology for both males and females

Body weight	Correlation value	P value	Corrected P value
Colony 1	0,214285714	0,457901055	0,457901
Colony 2	0,285714286	0,322299596	0,3223
Colony 3	0,071428571	0,804570948	0,804571
Colony 4	-0,28571429	0,322299596	0,6777
Chi^2 value			5,039744

Adrenal	Correlation value	P value	Corrected P value
Colony 1	0,5	0,083265	0,916735
Colony 2	-0,35714	0,216021	0,216021
Colony 3	-0,42857	0,137646	0,137646
Colony 4	-,643*	0,025952	0,025952
Chi2 value			14,50776

Thymus	Correlation value	P value	Corrected P value
Colony 1	,714*	0,024271	0,024271
Colony 2	-0,28571	0,3223	0,6777
Colony 3	0,357143	0,216021	0,783979
Colony 4	-0,28571	0,3223	0,6777
Chi^2 value			9,479921

Retroperitoneal fat	Correlation value	P value	Corrected P value
Colony 1	-0,28571	0,3223	0,6777
Colony 2	0,214286	0,457901	0,542099
Colony 3	-0,35714	0,216021	0,783979
Colony 4	-0,42857	0,137646	0,862354
Chi^2 value			2,785637

6.6.3 Fisher's Test for Kendall's Tau correlated ranking x physiology for males only

Body weight	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	0	1	1
Colony 3	0,333333	0,496906	0,496906
Colony 4	1,000*	0,001	0,001
Chi^2 value			15,21422

Adrenal	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	-0,33333	0,496906	0,496906
Colony 3	-0,66667	0,174231	0,174231
Colony 4	-0,33333	0,496906	0,496906
Chi^2 value			6,292161

Thymus	Correlation value	P value	Corrected P value
Colony 1	1	0,001	0,001
Colony 2	-0,33333	0,496906	0,503094
Colony 3	0,666667	0,174231	0,174231
Colony 4	-0,66667	0,174231	0,825769
Chi^2 value			19,06709

Retroperitoneal fat	Correlation value	P value	Corrected P value
Colony 1	-0,33333	0,496906	0,503094
Colony 2	0,333333	0,496906	0,496906
Colony 3	-0,66667	0,174231	0,825769
Colony 4	-0,66667	0,174231	0,825769
Chi^2 value			3,538428

Preputial	Correlation value	P value	Corrected P value
Colony 1	0,666667	0,174231	0,174231
Colony 2	-0,33333	0,496906	0,503094
Colony 3	0,333333	0,496906	0,496906
Colony 4	-0,33333	0,496906	0,503094
Chi^2 value			7,641363

Seminal	Correlation value	P value	Corrected P value
Colony 1	0,666667	0,174231	0,174231
Colony 2	-0,66667	0,174231	0,825769
Colony 3	0,333333	0,496906	0,496906
Colony 4	0,666667	0,174231	0,825769
Chi^2 value			5,659214

Testes	Correlation value	P value	Corrected P value
Colony 1	0,666667	0,174231	0,174231
Colony 2	0	1	1
Colony 3	0,182574	0,717982	0,717982
Colony 4	-1,000*	0,001	0,999
Chi^2 value			4,159366

Epididymal fat	Correlation value	P value	Corrected P value
Colony 1	0,333333	0,496906	0,496906
Colony 2	0,333333	0,496906	0,496906
Colony 3	-0,33333	0,496906	0,503094
Colony 4	-0,66667	0,174231	0,825769
Chi^2 value			4,554256

6.6.4 Fisher's Test for Kendall's Tau correlated ranking x physiology for females only

Bodyweight	Correlation value	P value	Corrected P value
Colony 1	-0,33333	0,496906	0,503094
Colony 2	0,666667	0,174231	0,174231
Colony 3	-0,33333	0,496906	0,503094
Colony 4	-0,66667	0,174231	0,825769
Chi2 value			6,625535

Adrenal	Correlation value	P value	Corrected P value
Colony 1	0,333333	0,496906	0,503094
Colony 2	-1,000*	0,001	0,001
Colony 3	-0,33333	0,496906	0,496906
Colony 4	-1,000*	0,001	0,001
Chi2 value			30,40369

Thymus	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	0,666667	0,174231	0,174231
Colony 3	0,333333	0,496906	0,496906
Colony 4	0	1	1
Chi2 value			4,893452

Retr.fat	Correlation value	P value	Corrected P value
Colony 1	-0,66667	0,174231	0,825769
Colony 2	0,333333	0,496906	0,496906
Colony 3	-0,33333	0,496906	0,503094
Colony 4	-1,000*	0,0001	0,9999
Chi2 value			3,155747

6.6.5 Fisher's Test for Kendall's Tau correlated received coercion x physiology

Bodyweight	Correlation value	P value	Corrected P value
Colony 1	-0,18257	0,717982	0,717982
Colony 2	0,182574	0,717982	0,282018
Colony 3	1,000*	0,001	0,999
Colony 4	-0,18257	0,717982	0,717982
Chi^2 value			3,858812

Adrenal	Correlation value	P value	Corrected P value
Colony 1	0,182574	0,717982	0,717982
Colony 2	0,182574	0,717982	0,717982
Colony 3	-0,33333	0,496906	0,503094
Colony 4	-0,18257	0,717982	0,282018
Chi^2 value			5,230767

Thymus	Correlation value	P value	Corrected P value
Colony 1	-0,91287	0,070951	0,070951
Colony 2	-0,54772	0,278599	0,278599
Colony 3	-0,33333	0,496906	0,496906
Colony 4	-0,18257	0,717982	0,717982
Chi^2 value			9,908816

Retroperitoneal fat	Correlation value	P value	Corrected P value
Colony 1	0,182574	0,717982	0,282018
Colony 2	-0,18257	0,717982	0,717982
Colony 3	1,000*	0,001	0,999
Colony 4	-0,18257	0,717982	0,717982
Chi^2 value			3,858812

6.6.6 Fisher's Test for Kendall's Tau correlated successful defense in % x physiology

Body weight	Correlation value	P value	Corrected P value
Colony 1	-0,91287	0,070951	0,929049
Colony 2	0	1	1
Colony 3	-1,000*	0,001	0,999
Colony 4	0,333333	0,496906	0,496906
Chi^2 value			1,547899

Adrenal	Correlation value	P value	Corrected P value
Colony 1	-0,18257	0,717982	0,717982
Colony 2	-0,33333	0,496906	0,496906
Colony 3	0,333333	0,496906	0,503094
Colony 4	0	1	1
Chi^2 value			3,435288

Thymus	Correlation value	P value	Corrected P value
Colony 1	-0,18257	0,717982	0,282018
Colony 2	0,666667	0,174231	0,174231
Colony 3	0,333333	0,496906	0,496906
Colony 4	0,333333	0,496906	0,496906
Chi^2 value			8,823727

Retr. Fat	Correlation value	P value	Corrected P value
Colony 1	-0,18257	0,717982	0,282018
Colony 2	-0,33333	0,496906	0,503094
Colony 3	-1,000*	0,001	0,999
Colony 4	0	1	1
Chi^2 value			3,907523

6.6.7 Fisher's test for Kendall's Tau correlated frequency of sex defense x physiology

Body weight	Correlation value	P value	Corrected P value
Colony 1	-0,33333	0,496906	0,496906
Colony 2	0,333333	0,496906	0,503094
Colony 3	0	1	1
Colony 4	-0,33333	0,496906	0,496906
Chi^2 value			4,171375

Adrenal	Correlation value	P value	Corrected P value
Colony 1	0,333333	0,496906	0,496906
Colony 2	0	1	1
Colony 3	0	1	1
Colony 4	-0,66667	0,174231	0,825769
Chi^2 value			1,781591

Thymus	Correlation value	P value	Corrected P value
Colony 1	-0,66667	0,174231	0,174231
Colony 2	-0,33333	0,496906	0,496906
Colony 3	-0,66667	0,174231	0,174231
Colony 4	0,333333	0,496906	0,503094
Chi^2 value			9,762149

Retr.fat	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	0	1	1
Colony 3	0	1	1
Colony 4	-0,66667	0,174231	0,174231
Chi^2 value			3,494742

6.6.8 Fisher's Test for Kruskal Wallis tested sex x physiology

Bodyweight	P value
Colony 1	0,021
Colony 2	0,021
Colony3	0,021
Colony 4	0,021
Chi^2 value	30,90586

Retr. Fat	P value
Colony 1	0,773
Colony 2	0,386
Colony3	0,773
Colony 4	0,564
Chi^2 value	4,079143

Adrenal glands	P value
Colony 1	0,083
Colony 2	0,386
Colony3	1
Colony 4	0,248
Chi^2 value	9,670318

Rank	P value
Colony 1	0,248
Colony 2	0,564
Colony3	0,773
Colony 4	0,149
Chi^2 value	8,256626

Thymus	P value
Colony 1	0,034
Colony 2	0,773
Colony3	0,043
Colony 4	0,773
Chi^2 value	14,0858

6.6.9 Female dominance

Femdom*coercion	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	-0,671	0,221	0,221
Colony 3	-0,236	0,655	0,655
Colony 4	0,775	0,157	0,843
Chi2			4,207002

Femdom total *coercion	Correlation	Pvalue
Total	0,667	0,174

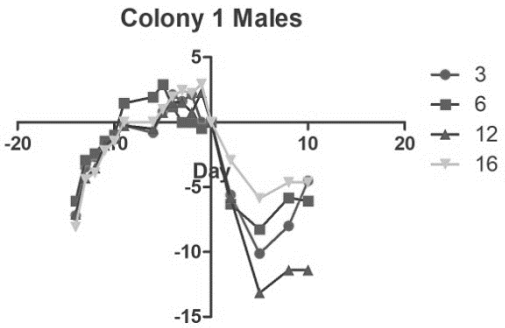
FEMDOM x succesful defense	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	-0,40825	0,438578	0,561422
Colony 3	0,707107	0,179712	0,179712
Colony 4	0,707107	0,179712	0,179712
Chi^2 value			8,020153

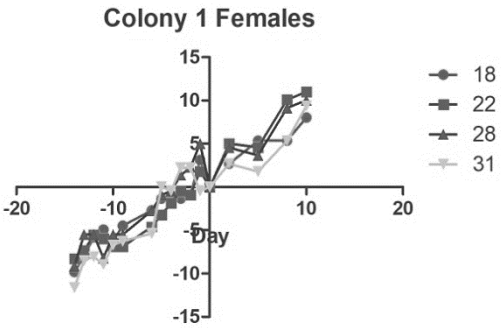
FEMDOM x succesful defense%	Correlation value	P value	Corrected P value
Colony 1	0	1	1
Colony 2	-0,40825	0,438578	0,561422
Colony 3	0,235702	0,654721	0,654721
Colony 4	0,235702	0,654721	0,654721
Chi^2 value			2,84875

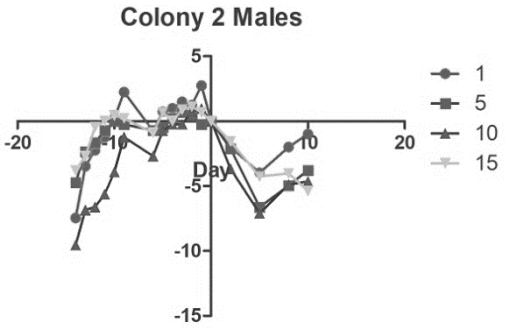
FEMDOMtot	Correlation value	P value
Defensetot	-0,66667	0,174231
Defensetot%	-0,33333	0,496906

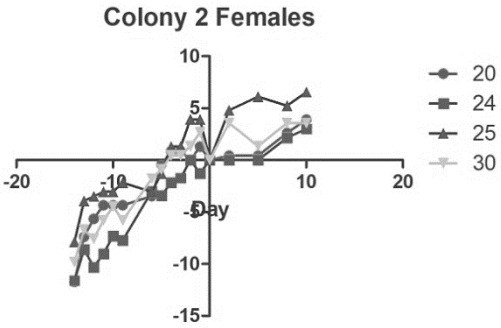
6.7 Graphs

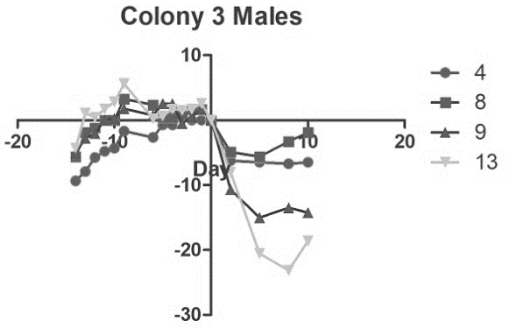
6.7.1 Weight changes per colony per ID

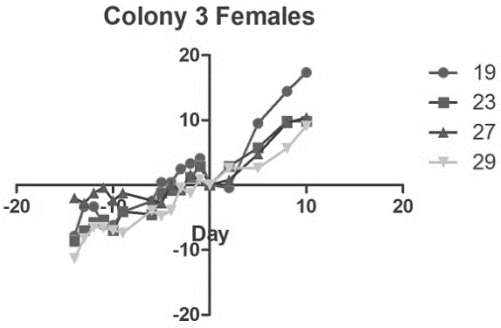


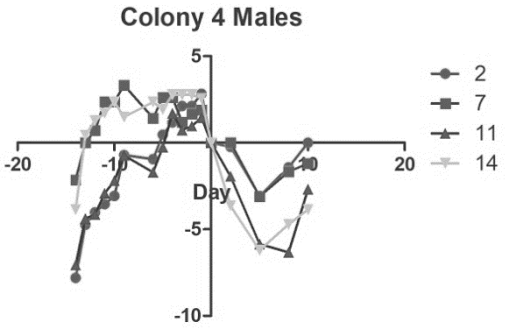


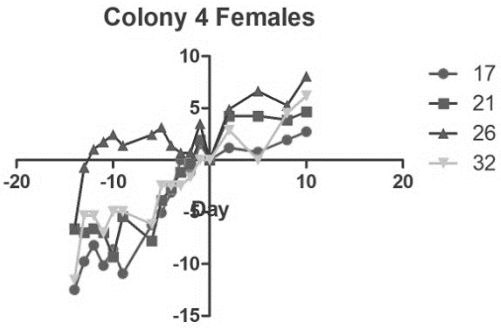


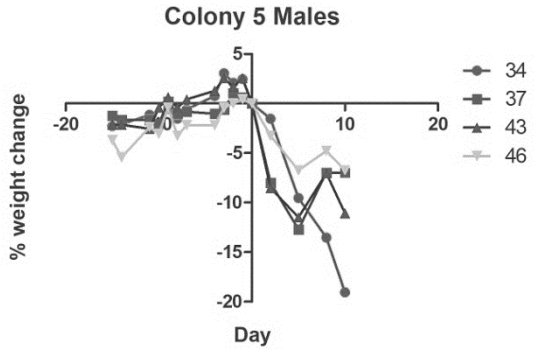


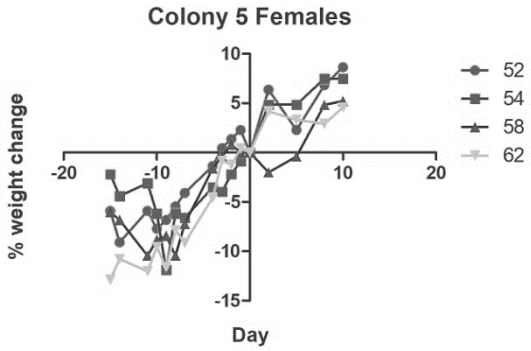


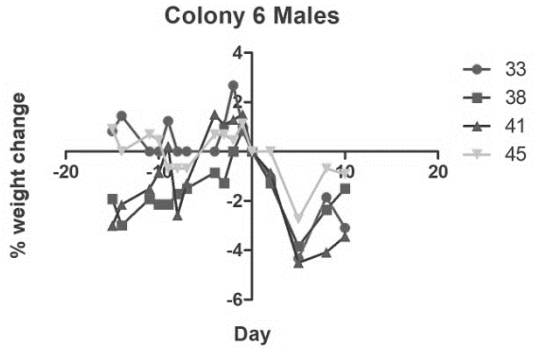




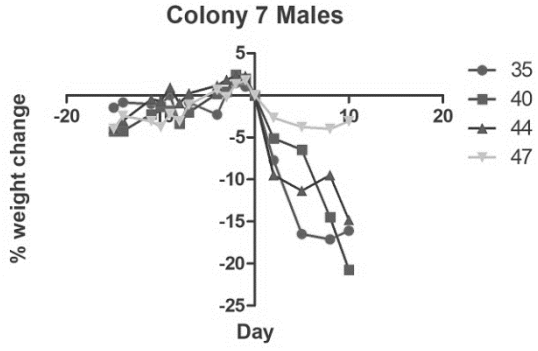



Colony 5 Males
% weight change
Day
34
37
43
46

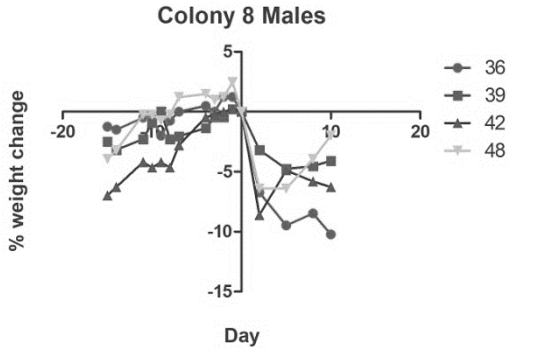

Colony 5 Females
% weight change
Day
52
54
58
62

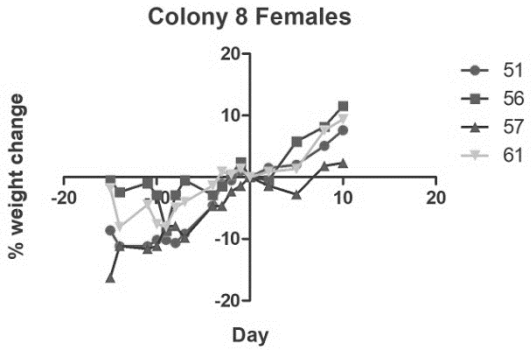

Colony 6 Males
% weight change
Day
33
38
41
45


Colony 6 Females
% weight change
Day
49
53
59
63

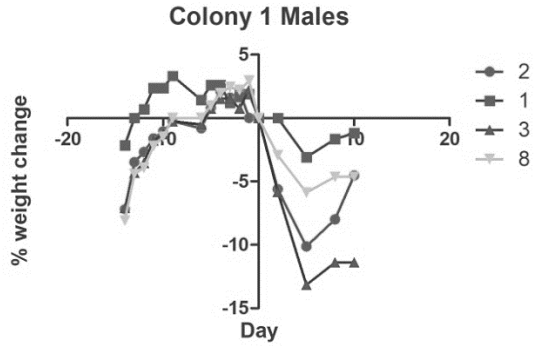

Colony 7 Males
% weight change
Day
35
40
44
47

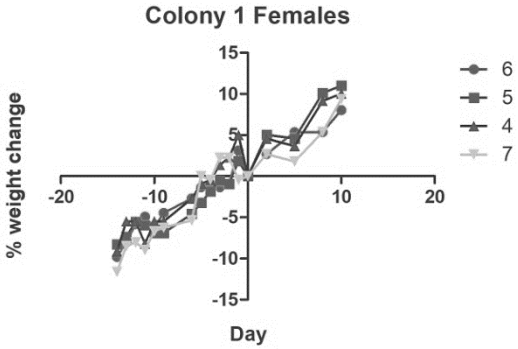

Colony 7 Females
% weight change
Day
50
55
60
64

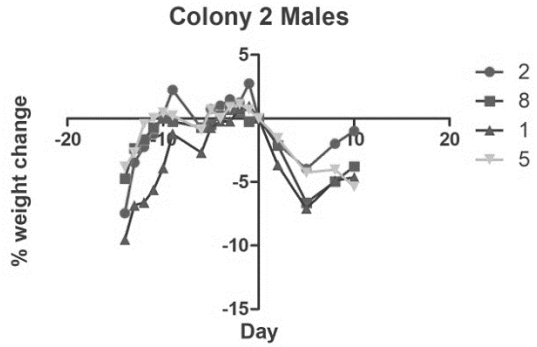

Colony 8 Males
% weight change
Day
36
39
42
48

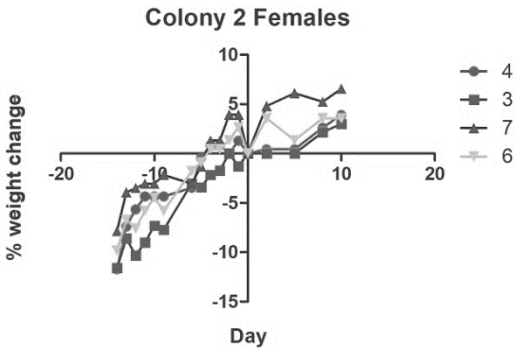

Colony 8 Females
% weight change
Day
51
56
57
61

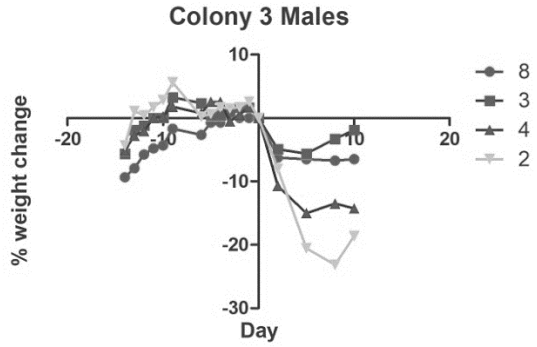
6.7.2 Weight changes per colony per rank

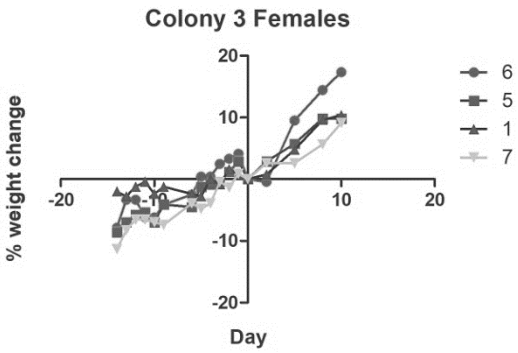


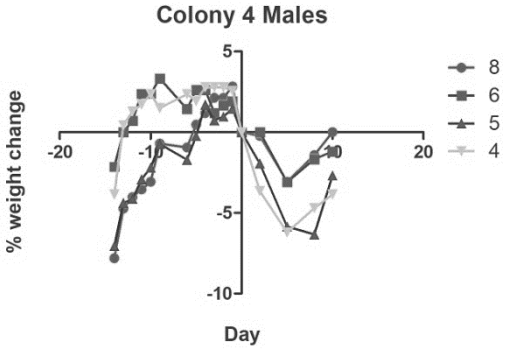


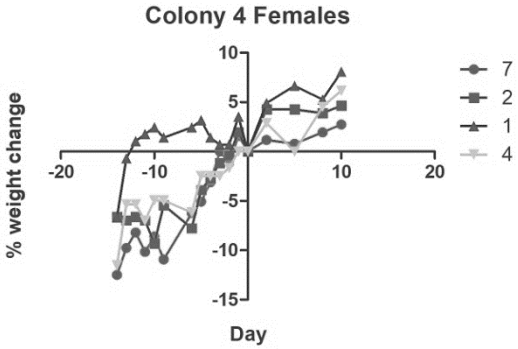




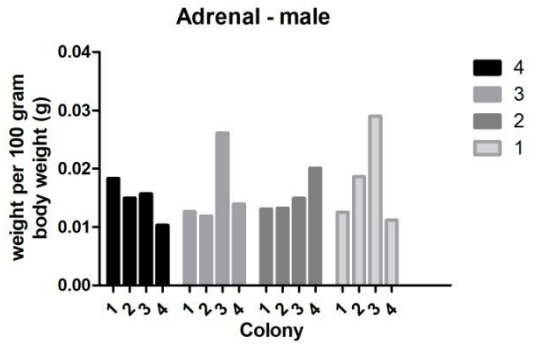


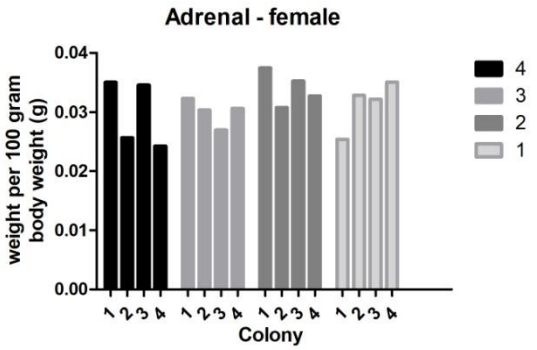


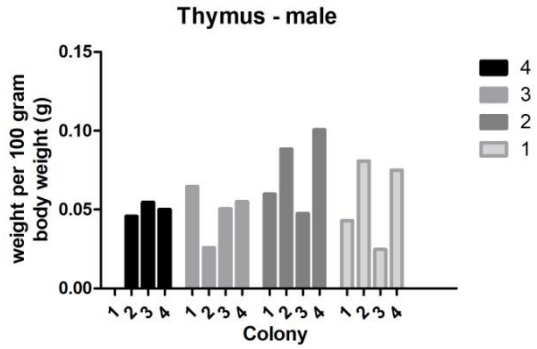


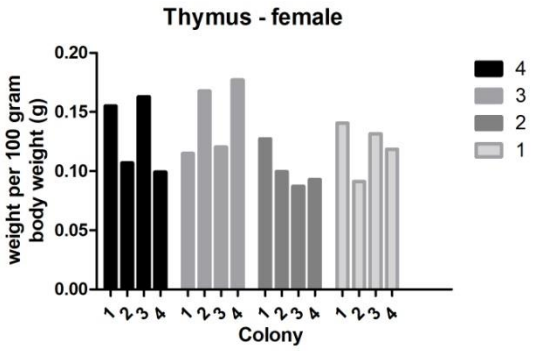


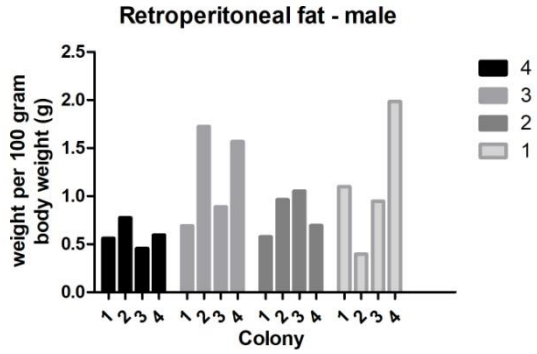
6.7.3 Relative organ weight per relative rank, males and females separated

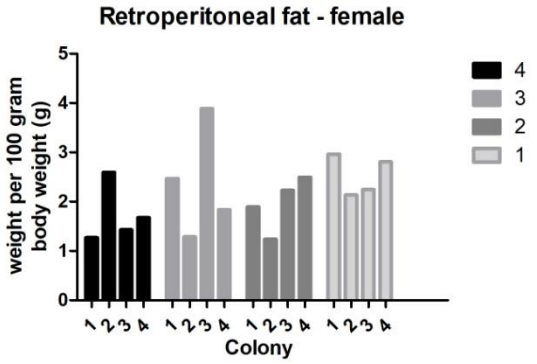


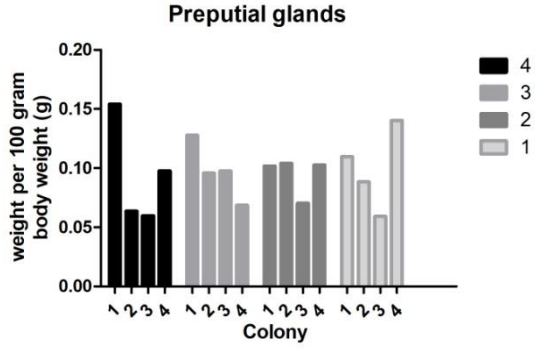


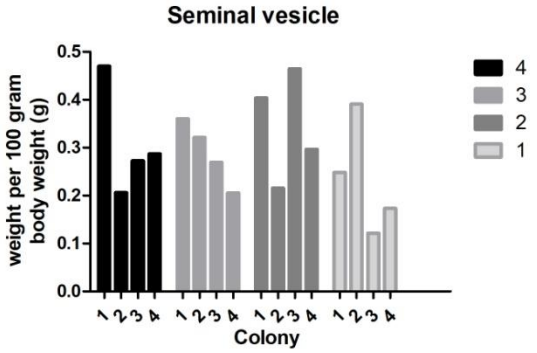


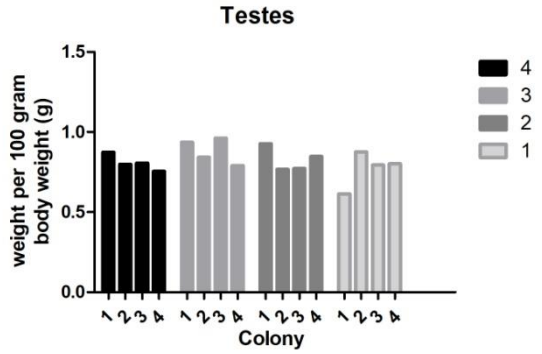


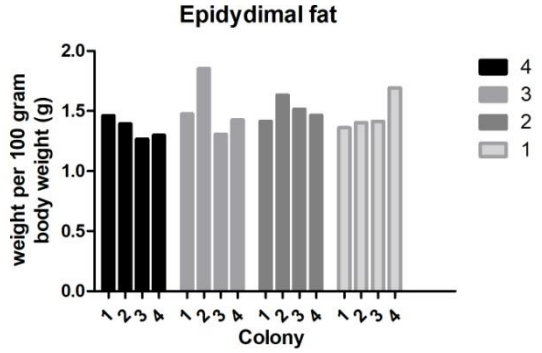




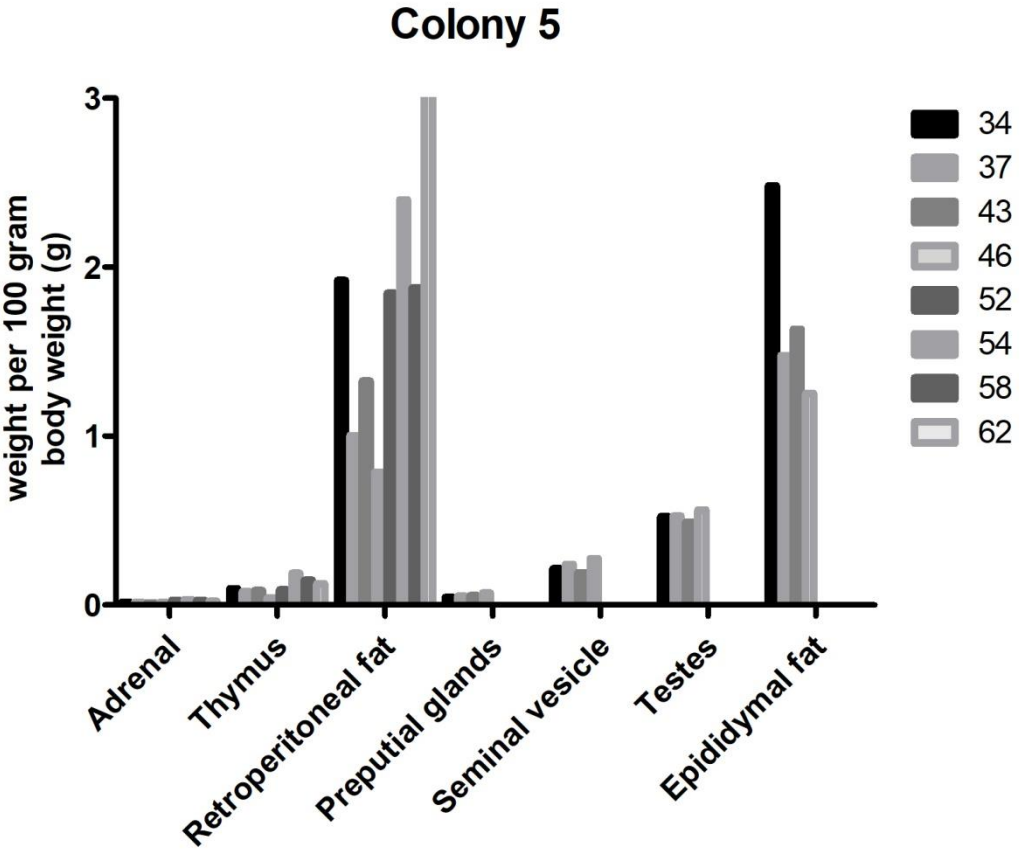


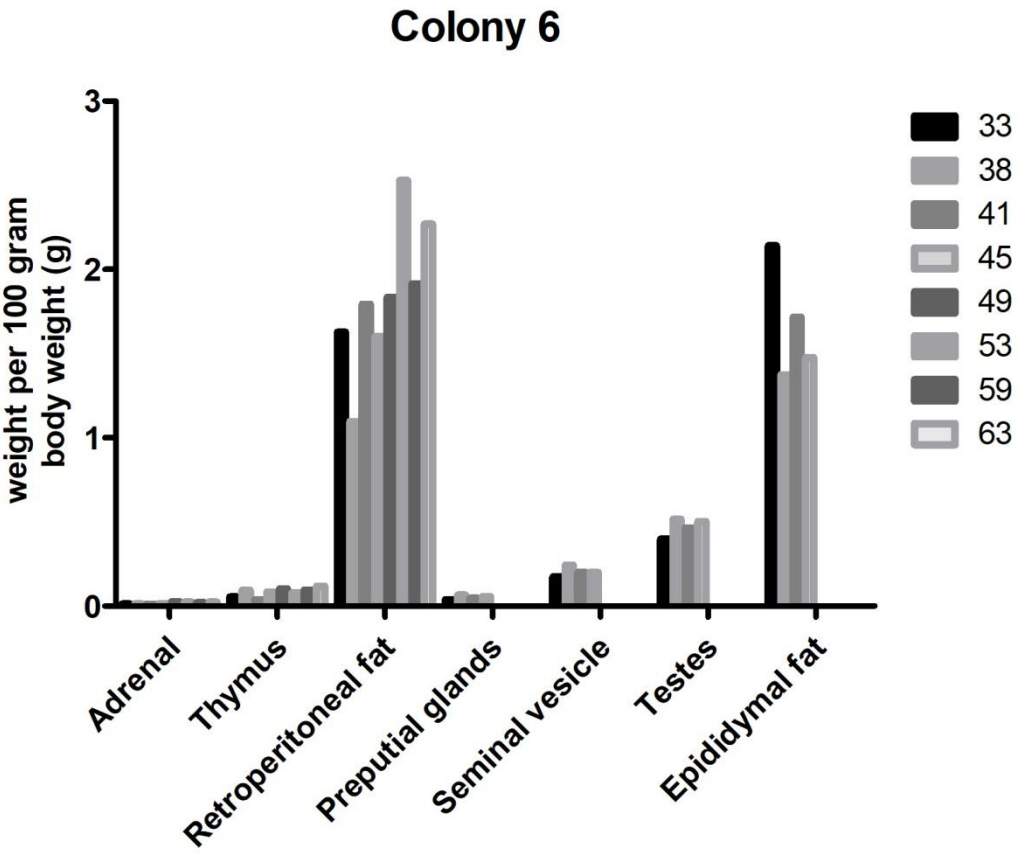


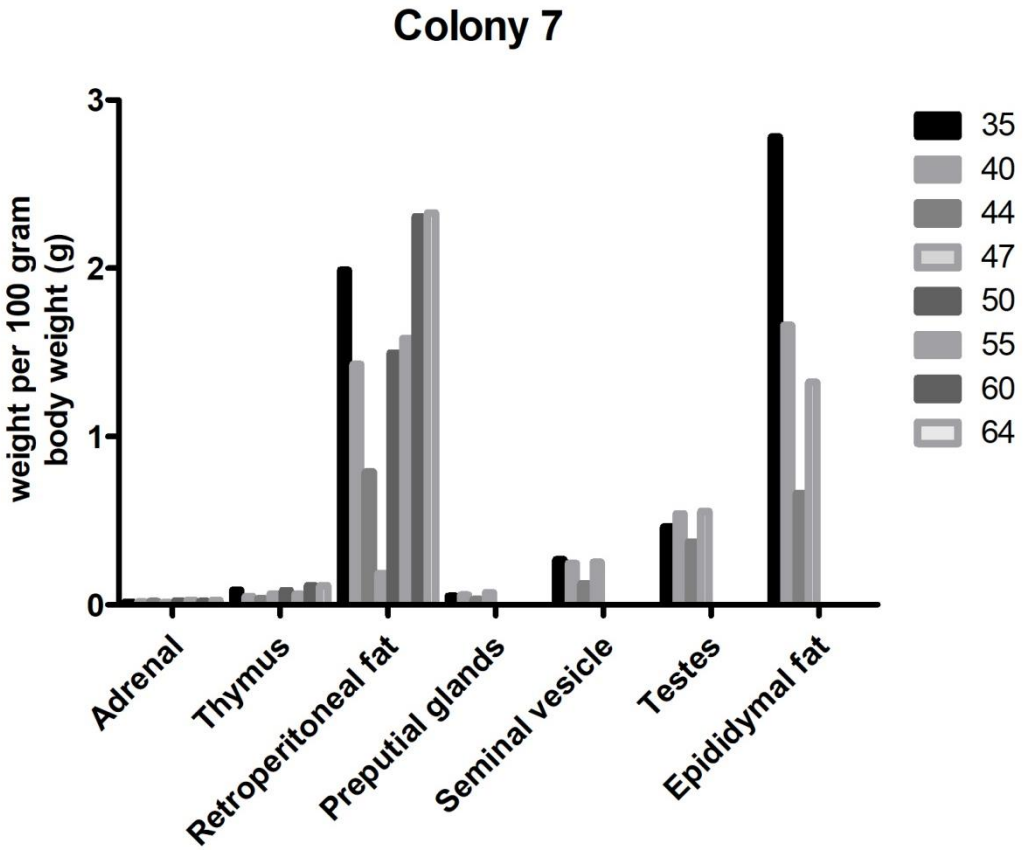

Testes
weight per 100 gram body weight (g)
1.5
1.0
0.5
0.0
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
Colony
4
3
2
1

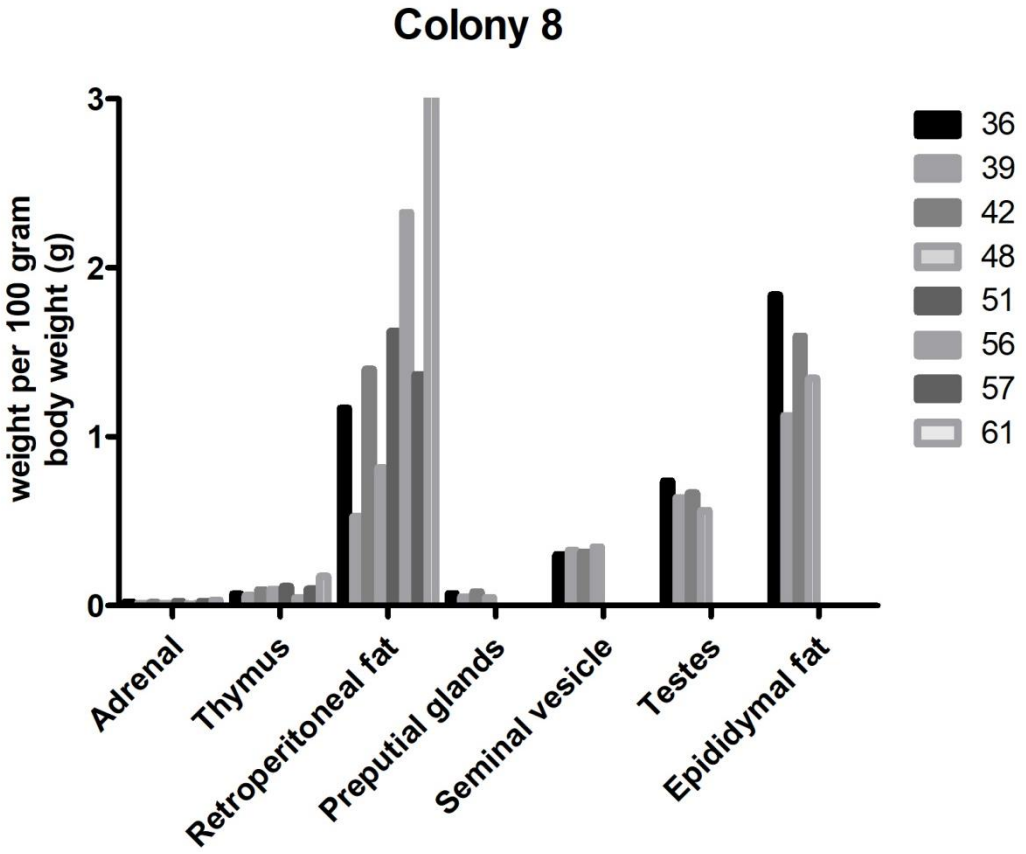

Epidydimal fat
weight per 100 gram body weight (g)
2.0
1.5
1.0
0.5
0.0
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
Colony
4
3
2
1

6.7.4 Relative organ weight per colony (colonies 5-8)






Colony 7
weight per 100 gram body weight (g)
3
2
1
0
Adrenal
Thymus
Retroperitoneal fat
Preputial glands
Seminal vesicle
Testes
Epididymal fat
35
40
44
47
50
55
60
64


Colony 8
weight per 100 gram body weight (g)
3
2
1
0
Adrenal
Thymus
Retroperitoneal fat
Preputial glands
Seminal vesicle
Testes
Epididymal fat
36
39
42
48
51
56
57
61