BEHAVIORAL AND NEUROBIOLOGICAL CORRELATES OF DOMINANCE RANKING IN MIXED-SEX COLONIES OF WILD-TYPE RATS

By A. R de Boer (S3383504)
Supervisors: prof. B. Buwalda and M. A. Puentes-Escamilla

Abstract

Being in a dominance hierarchy can cause a lot of physical and psychosocial stress. Especially subordinates experience a lot of stress as they show the most detrimental behavioral, physiological and neurological changes. Chronic stress is known to decrease dendritic complexity and spine density of the hippocampus. However, a recent study found that dominants show a similar amount of decreased dendritic complexity of the CA3 region of the hippocampus, indicating that dominants might experience a similar amount of stress. The aim of this study is to examine the effects of chronic social stress on the spine density of apical CA3 dendrites of subordinate and dominant males. To test this, 36 male and 36 female Wild Type Groningen (WTG) rats were divided into 12 colonies of 4 male and 4 female rats and placed into a visible burrow system, i.e.; a semi-natural environment for social hierarchy. Behavioral and physiological indices of stress were measured and the spine density of the dominant and subordinate males were counted. Results show that subordinates spent less time in the burrows and had a bigger bodyweight loss. There was no difference in spine density and corticosterone levels between dominants and subordinates. Time spent in the arena and body weight loss suggest that subordinates are more stressed than dominant males, however, spine count showed no difference. In conclusion, this data could indicate that subordinate males might adapt to their situation which could save them from spine loss.

Inhoudsopgave

1. Introduction

1.1 Dominance hierarchies

Living in a group is beneficial for animals as it helps protect from predation, increases access to food and increases odds to find mates. However, when competition over resources increase, conflict will arise for dominance causing a social hierarchy to be established. In this social system, dominance relationships exist between all pairs of individuals in a group which are established by performing agonistic interactions. For example, when one animal bites, chases or threatens the other, but receives no aggression back, a dominance relationship is formed (Chase et al., 2002). As resources are critical for survival and reproduction, an animal's quality of life is greatly influenced by its dominance ranking as the hierarchy produces inequalities in access to resources (Huang et al., 2011).

Different factors can influence hierarchy formation, such as prior attributes and the winner-loser effect. Prior attributes are characteristics that animals already possess before the formation of the dominance hierarchy which can influence fighting outcomes and rank positions. It can include size, distinct morphological features, fighting skill and sex (Beacham, J. L., 1988; Francis, R. C., 1988). However, many studies have found that these pre-existing factors are not as important in the determination of dominance hierarchies as external forces such as the winner-loser effect (Collias, N. E., 19743; Hemelrijk et al., 2008). This effect entails that individuals who have lost fights, have a bigger chance of losing again in the future. The opposite is true for individuals who have won fights (Dugatkin L. A., 1997).

Although females are involved in dominance hierarchies, males show more defined dominance hierarchies. For example, in captive Bonobo groups it was found that males have a steeper dominance hierarchy than females (Stevens et al., 2007). Furthermore, females possess low prior attributes causing them to be less likely to outrank a dominant male. In most species, the most dominant animal in a social hierarchy is male and males perform overall more aggressive acts than females (Reinhardt, V., 1987). For this reason, males are often the most important individuals in defining dominance hierarchies and females are mostly ignored when looking at dominance hierarchies.

Although the most dominant individual in a colony is often male, some females can become dominant over some males. Female dominance is the dominance of ranks of all females relative to those of all males in a group and different factors can affect female dominance. The winner-loser is one of them. It creates opportunities for females to become dominant over males when a male loses a fight as that causes the male to have a bigger chance of losing again in the future. That male is set on a course to keep decreasing in rank. When this happens, females have a bigger opportunity to win from the male that is losing. Afterward that females will increase in rank and because of that, female dominance increases. Next to the winner-loser effect, other factors can also influence female dominance. Female dominance can differ significantly between groups of a single species because of differences in group composition and intensity of aggression (Hemelrijk et al., 2008). Self-organization models have indicated that female dominance increases with a high intensity and frequency of aggression (Hemelrijk, C. K., 1999) Furthermore, group composition influences female dominance as a higher percentage of males causes an increase in female dominance (Hemelrijk et al., 2008).

The main way of determining dominance ranking is by looking at the direction of agonistic interactions. More dominant individuals in a group are those that begin and win more aggressive gestures to other group members and show fewer submissive behaviors. More submissive individuals do the opposite and initiate more submissive and fewer aggressive behaviors to group members. The information in these agonistic interactions can be used to calculate the dominance index, which informs about relative dominance. The 'average dominance index' (ADI) is a method to order individuals in a hierarchy by looking at the average percentage with which an individual wins in interactions with each of its group members. To calculate the ADI, the number of times that an individual has won a fight from a certain opponent is divided by the total number of fights of the pair. This dominance indices are calculated per pair of individuals. In the end, the average of all dominance indices of one individual with all its interaction partners is calculated. This value indicates the dominance value with a higher value indicating a higher dominance in the group (Hemelrijk et al., 2005). The mathematical equations described and used to calculate the ADI can be found in appendix 6.1.

1.2 The stress response

As the establishment of these dominance hierarchies mainly is defined by agonistic interactions, it might come as no surprise that living in a social hierarchy is associated with elevated levels of stress in individuals for different dominance positions. Because of the social environment and social interactions, two types of stressors can exist in a social hierarchy: physical and psychosocial stressors. The physical stressors entail the external forces that present a direct challenge to the homeostasis, including physical trauma. Psychosocial stressors are stressors that arise in a social context, including the anticipation of a challenge to the homeostasis. (Gundersen et al., 2011). The latter is mostly accompanied by a feeling of lack of control and predictability and a lack of an outlet for frustration. Animals in a dominance hierarchy are faced with competition for resources and agonistic behavior and therefore experience psychosocial and physical stressors (Sapolsky, R. M., 2005). Both activate the stress response as they trigger the release of glucocorticoids and adrenocorticotropic hormone (ACTH) (Gavrilovic, L., & Dronjak, S., 2005; Abdelall et al., 2020).

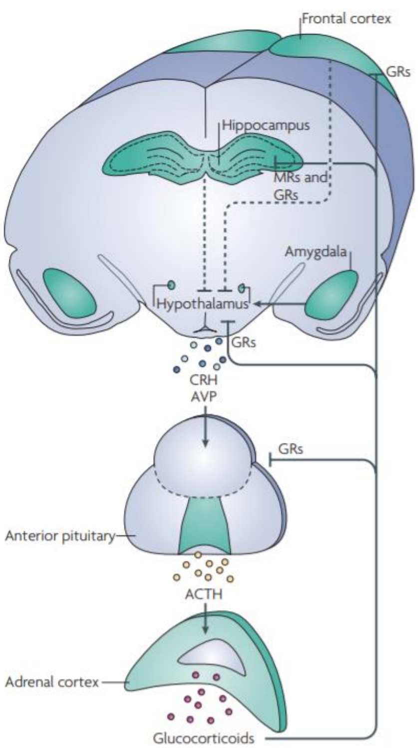


Figure 1. The hypothalamus-pituitary-adrenal (HPA) axis (Lupien et al., 2009)

However, not all ranks in a dominance hierarchy are faced with the same amount of physical and psychosocial stressors. Neither the distribution of resources nor the amount of aggression received and executed are the same for dominant and subordinate males. Subordinate males could perceive less control as they are faced with less access to resources and might get attacked by dominant males which causes them to experience a lot of psychosocial stress. Furthermore, they are also involved in fights, causing them to experience physical stress. As subordinate males deal with a lot of physical and psychosocial stress, subordination is used as a model to study the effects of stress.

Stress activates a range of processes in the body and can have beneficial but also detrimental effects on brain and body. With stress, the first reaction is the rapid activation of the sympathetic branch of the autonomous nervous system which causes symptoms that are typically associated with stress such as increased heart rate and increased blood pressure (Kemeny, M. E., 2003). After this, the slower response is the activation of the hypothalamus-pituitary-adrenal (HPA) axis depicted in Figure 1. This activation causes the hypothalamus to release corticotrophin-releasing hormone (CRH) which triggers the release of adrenocorticotrophin (ACTH) in the pituitary. ACTH in turn causes the release of glucocorticoids from the adrenal cortex. Glucocorticoids are released into the bloodstream and can pass the blood-brain barrier where it can influence different brain areas (Wolf O. T., 2003).

One of the brain areas that is affected by glucocorticoids is the amygdala. The amygdala plays a critical role in emotional learning and memory and it will respond to glucocorticoids with the formation of emotionally charged memories (Roozendaal et al., 2004). Chronic stress will cause dendritic growth of basolateral amygdala (BLA) neurons and increased branching (Vyas et al., 2002). Furthermore, spine density is not increased in BLA dendrites after chronic stress (Patel et al., 2018). The medial prefrontal cortex (mPFC) is a brain area that has connections to the amygdala and is known to influence the activity of the amygdala (Morgan and LeDoux, 1995). This area is also affected by chronic stress as decreased branch-complexity and branch-length have been observed in chronically stressed animals (Radley et al., 2004; Cook et al., 2004). When looking at spine density, there was no effect found after repeated social defeat stress (Patel et al., 2018). As chronic stress influences distinct brain areas, chronic stress has an effect on the functional output of these brain areas. The amygdala is involved in emotional memory, and stress causes increased amygdala activation which increases storage of emotionally arousing events. This is also accompanied by increased anxiety and depressive-like symptoms (Roozendaal et al., 2009).

1.3 Stress and the hippocampus

As chronic stress also affects working memory, the hippocampus is also an important area to look at. The hippocampus is a brain region that has a big role in memory formation (Squire & Zola-Morgan, 1991). In humans, the main function of the hippocampus is to support the creation of new memories. Especially memories that are tied to a specific time or place, called episodic memories, are created by the hippocampus (Knierim, J. J., 2015). Structurally, the hippocampus is composed of the dentate gyrus (DG), the proper hippocampus which is divided into subregions (CA1, CA2 and CA3), and the subiculum (Blackstad, T. W., 1956). Hippocampus-signaling starts in the entorhinal cortex which projects on to the dentate gyrus and CA3, the granule cells of the dentate gyrus signal to the CA3 pyramidal cells, the CA3 projects to the CA1 pyramidal cells which in turn projects to the subiculum. The CA1, via the subiculum, is the major output structure of the hippocampus (Amaral, D. G., 1993). Furthermore, the hippocampus also gets input from the amygdala, which helps modulate emotional memory.

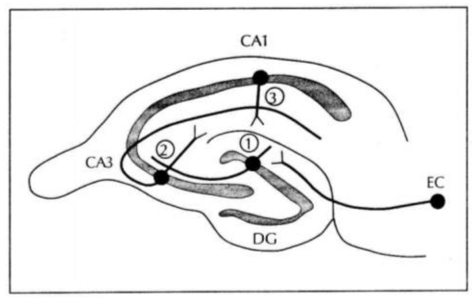


Figure 2. The classical trisynaptic pathway of the hippocampus (Amaral, D. G., 1993).

When exposed to chronic stress, the hippocampus undergoes significant morphological changes. Chronic stress reduces the hippocampal volume and changes spine morphology (Lee et al., 2009). Specifically, the CA3 region shows dendritic retraction as dendritic length and branching points decrease when corticosterone injections are administered over a longer time (Woolley et al., 1990). Furthermore, in the CA1 region a decrease in length and number of branches of the apical dendrites was found (Lambert et al., 1998). When looking at spine density, a significant loss of spines was found in apical and basal dendrites of the CA1 region (Patel et al, 2018). This decrease in the number of spines is most likely caused by the increased glucocorticoid levels as glucocorticoids can influence spine density through activation of NMDA receptors (Armanini et al., 1990). The decreased volume and complexity also has functional effects. Mainly that chronic stress has been shown to impair hippocampus-dependent learning. When faced with chronic stress, spatial recognition of rodents was impacted (McEwen & Magarinos, 1997).

1.4The visible burrow system as a chronic stress model

As explained earlier, chronic stress causes anxiety and depressive-like symptoms and is therefore used as a model to study these disorders. Adding to this, chronic stress is of frequent occurrence in humans. Therefore it is important to study its effects**.** Living in a dominance hierarchy can provide a model for chronic stress due to the physical and psychosocial stress. Especially subordinates are thought to experience a lot of stress in such a system. Because of this, chronic subordination is used as a model for chronic stress. For this, the visible burrow system (VBS) can be used.

In a VBS, animals are housed in a mixed-gender colony in a semi-natural burrow environment (Blanchard et al., 1995). It has an open arena with food and water and continuously dark burrows with nesting boxes and a light cycle of 12 hours. When the animals are put into the model, a dominance hierarchy will form in a naturalistic way through offensive and defensive behavior in dyadic agonistic encounters (Blanchard & Blanchard, 1989). The stressors that the animals in a VBS are faced with are physical stressors such as agonistic behavior and psychosocial stressors such as subordination and the anticipation of aggressive behavior. A benefit of this model is that the animals are not exposed to these stressors at specific times per day, making the social threat unpredictable. This decreases habituation as unpredictable stressors are harder to adapt and habituate to than predictable stressors **(**Blanchard et al., 1998). All animals housed in a VBS system show increase in corticosterone levels indicating that this stress is experienced by all animals (McKittrick et al., 2000).

1.5 Stress and ranks

The physical and psychosocial stressors that are experienced by animals in a social hierarchy affect both dominant and subordinate animals males. Females are mostly ignored when looking at dominance hierarchies, because they form less steep dominance hierarchies than males and are most often not the most dominant individual. However, for males there is a lot of data about ranks and stress. Some debate exists about which rank suffers the most stress in a social system. As chronic social subordination is a model for chronic stress, it is mostly thought that subordinate males suffer from the most stress. This is thought because subordinates experience a lot of psychosocial stress as they have less control over their situation and less access to resources than dominant males.

When looking at rodents studies that examine behavioral and physiological markers of stress, most results support the notion that subordinate males experience more stress than dominant males. Behaviorally, dominants show an increase in aggressive behavior and submissive rats show an increase in defensive behavior. Furthermore, subordinate males spend significantly less time on the surface than dominant males, indicating that they are avoiding the dominant male who spends the most time on the surface (Blanchard et al., 1993). Physiologically, adrenal weight is increased in both dominants and subordinates and thymus weight is decreased in dominants and highly decreased in subordinates. Basal corticosterone was moderately but significantly increased in dominants while it was highly increased in subordinates. Furthermore, looking at metabolic changes, only subordinates show a decrease in body weight. Testosterone was highly increased in dominants and decreased in subordinates (Blanchard et al., 1995). These findings show that although both dominant and subordinate rodents may be exposed to a stressful environment in social colonies like the VBS, in general, the subordinates show more stress associated changes than dominant males.

When investigating non-human primates, the amount of stress that each ranks experiences depends on the species and kind of colony. Rank is associated to the amount of access to mates, food and safe spatial locations. The amount of access to these resources that each rank has can influence the amount of stress that each rank experiences**.** Colonies where the benefits are strongly biased towards the highest-ranking males are called ‘despotic hierarchies’. ‘Egalitarian hierarchies’ are colonies where this is more equally distributed (Hemelrijk, 1998). In more despotic colonies, dominance is maintained through intense aggression against subordinates causing the subordinate animals to experience the most stress. (Cavigelli, S. A.,1999; Bercovitch & Clarke, 1995). Egalitarian societies show no difference in the amount of stress experienced by individuals between high and low ranks (Sapolsky, R. M., 2005).

Stability is another colony property that can influence the amount of stress that different ranks experience. Stability is the amount of change in rank among subjects. In a stable hierarchy there is no reversal in dominance and no change in rank (Sapolsky, R. M, 1993). Stability can influence stress levels of non-human primates. In stable colonies the dominant males show the lowest basal cortisol levels. However, in more instable situations, dominants experience the most stress. They are faced with social tension, experience a significant amount of physical and psychosocial stress as they need to fight to defend their rank and they are confronted with more social uncertainty than when they rank is secure. During these periods of instability, it was found that dominants have the highest basal cortisol concentrations (Mason & Mendoza, 1993).

As explained before, chronic social stress influences behavior, physiology and neurons in distinct brain regions. Seeing that generally subordinate males have been found to experience the most stress, it is questioned whether they also show the most neuronal remodeling. Mckittrick et al (2000) have looked at the differences in the effect of chronic stress on neuronal structure between more dominant and more subordinate rats and found some interesting results. In this experiment, a control group was used which consisted of weight- and age-matched male-female pairs which were kept in conventional cages. First, looking at the physiological measures they concluded that both the dominant and the subordinate animals suffer from stress in a VBS system as they have a similar amount of plasma corticosterone elevation. Subordinate stress was especially well reflected in weight change as they showed a dramatic weight loss of up to 20% of their original body weight, while dominants showed little to no difference. In this research, dominant males were also investigated, as they also seemed to be stressed compared to pair-housed controls (Blanchard et al., 1993, 1995). They found that although the dominants show stress in their HPA activation, this was not reflected in their behavioral and physiological deficits. However, they also took a closer look at neuronal remodeling of the dendritic trees for dominant and subordinate males. The results show that both dominant and subordinate animals had a similar decrease in number of dendritic branch points. The total length of apical dendrites was decreased in dominant animals, while no difference can be found for subordinate animals. This could indicate that dominants and subordinates are both stressed and that this stress affects dominants differently than subordinates or that perhaps another factor is underlying these results.

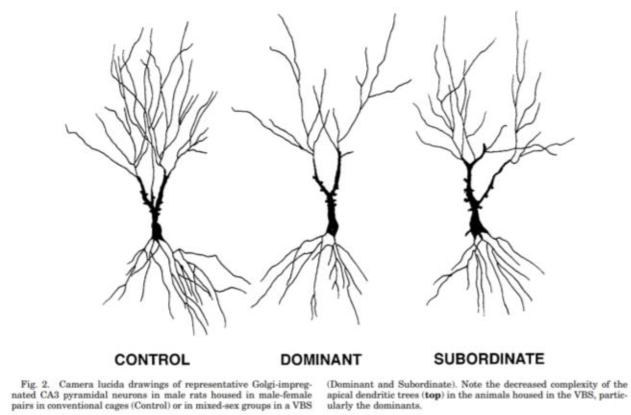


Figure 3. Neuronal remodeling in the CA3 area of the hippocampus of control, dominant and subordinate animals housed in a VBS (Mckittick et al., 2000)

1.6 Research questions

Although it is generally thought that subordinates experience more stress than dominant individuals, the findings from Mckittrick et al. (2000) found that both suffer from neuronal remodeling in the CA3 area of the hippocampus. As in other studies, chronic stress has been shown to decrease neuronal complexity in the hippocampus, it could be possible that dominants are also stressed. Therefore, the aim of this study is to explore the effects of chronic social stress on dominant and subordinate males when they are placed into a VBS system.

The main research question of this thesis is 'What is the effect of chronic social stress in a VBS system on dendritic spine density of dominant vs subordinate males'. Behavioral and physiological aspects will also be investigated, together with whether different societal qualities of colonies will also impact the amount of stress that different ranks experience. To answer the main question, smaller sub-questions are made; 'Is there a difference in body weight loss between dominant and subordinate males', 'is there a difference in corticosterone levels between dominant and subordinate males', 'is there a difference in time spent in the arena between dominant and subordinate males', 'is there a difference in spine density between dominant and subordinate males?', 'is there is correlation between stability and spine density and/or body weight change', 'is there a correlation between intensity of aggression and spine density and/or body weight' and finally 'is there a correlation between female dominance and spine density and/or bod weight'.

In this study it is hypothesized that subordinate males are more stressed than dominant males. For this reason it is thought that subordinates have a lower body weight, higher corticosterone levels and spend less time in the arena than dominant males as this has been found before in previous research. It is also expected that they have a lower spine density than dominant males in the CA1 region of the hippocampus. This is expected as higher stress levels mostly translates into lower spine density in that region. For colony properties, it is expected that a higher stability is less stressful for subordinate males than a lower stability as a higher stability is more predictable which decreases the amount of psychosocial stress. For dominant males, is thought that a lower stability is more stressful as this was found in previous research. Living in a colony with a higher intensity of aggression is thought to be more stressful for all individuals and therefore it is thought that there is no difference between dominants and subordinates. Female dominance influences male dominance as a higher female dominance causes males to be lower in rank. Because of this, it is hypothesized that stress levels of dominant males are not influenced by female dominance as females rarely outrank the most dominant male. However, for subordinates it is hypothesized that they are more stressed with a higher female dominance as this causes their rank to decrease.

2. Methods

2.1 Animals and procedures

For this experiment, 96 Wild Type Groningen (WTG) rats were used, 48 males and 48 females. This type of rat was used because they show a large variation in which aggression. This range ensures the formation of dominance hierarchies. As these rats also show a difference in intensity of aggression, they are also suitable for studying colony properties such as high and low intensity of aggression (de Boer et al, 2003).

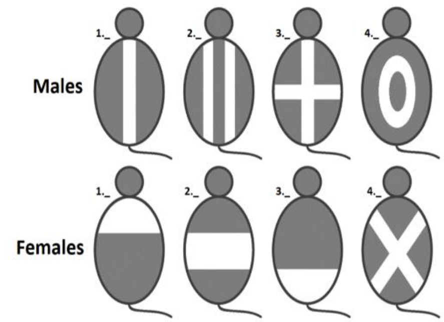


Fig 4. Fur dye patterns

The animals were first housed in same-sex house cages of 4 animals each. After this, to allow the males and females to already be exposed to sexual experiences, the animals were put into a cage in pairs of males and females for 7 days. To ensure that the females did not get any offspring during the experiment, the females were sterilized by ligating the oviducts which does not affect their estrus cycle nor their behavior. Furthermore, before to the experiment, the fur of the animals was dyed in the specific patterns of Figure 4 so they could be recognized in the visible burrow system. Nineteen hours before the experiment, all animals were housed alone in a cage before they entered the visible burrow system to collect feces for corticosterone measurements. An scheme with a timeline of the whole experiment can be found in Figure 6.

At the start of the experiment, when the animals were approximately 5 months old, the animals were put into 12 colonies of 8 animals each, 4 males and 4 females. When forming the colonies the animals were divided in such a way that all the animals were foreign to each other and did not have an existing dominance hierarchy. The groups of eight animals were put into a Visible Burrow System (VBS) for 10 days. Because there were only four VBS boxes, the animals were divided into three batches of four colonies each.

2.2 The experiment

The visible burrow system (VBS) is a big box that contains a big open space called the open arena and a system of nests and tunnels called the burrows. A scheme of the VBS is visible in Figure 5. The animals can freely move between these two areas through two openings. The open arena, there are two water bottles and food available. In the open arena has a light-dark cycle of 12:12 hours (with lights on at 17:00H) to mimic natural day and night rhythm. The burrows consist of tunnels which connect to two bigger and two smaller nesting boxes. The burrow was continuously dark because of a dark lid that did not let light through. During batch one it became apparent that not all males could go into the arena to drink water, creating an ethical issue.

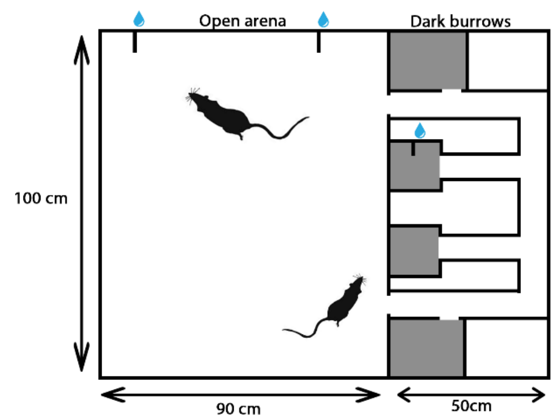


Fig 5. A scheme of the VBS with a female and male rat size as a reference for size

Figure 6. A timeline of the whole experiment.

To solve this issue, another bottle was added to the burrows to ensure that all males had proper opportunity to drink water. Standard bedding material was added to both the arena and burrows and there was an infrared camera above the VBS which recorded the VBS continuously throughout the whole 10 days of the experiment.

On days 0, 2, 5, 8 and 10 the animals were taken from the VBS and their body weight was measured to measure the bodyweight and to count the number of wounds. A scheme with an overview of all days in the VBS with their measurements can be found in appendix 6.2. After the animals were in the VBS for 10 days they were single housed for 24 hours to collect feces samples for corticosterone levels which can be found in appendix 6.3. After this the animals were sacrificed by rapid decapitation under a CO2 sedation. Brain matter was collected from the animals and the brain was cut in half. The left hemisphere was frozen with dry ice and liquid nitrogen for microbiological analyses and the right hemisphere was put into a golgi solution for structural analysis. Furthermore, the thymus, adrenal gland, testes, seminal vesicle and fat weight were measured which can be found in appendix 6.4.

2.3 Behavioral analysis

The video material collected from the VBS placement was observed by six different people using the Observer XT program by Noldus. For each observation, seven time points were chosen to be observed, 1 during the light part of the cycle and 6 during the dark part of the cycle. The chosen time points were at 05:00, 07:00, 08:00, 12:00, 12:30, 14:00, 16:00 and 18:00 (see Figure 7.). These time points were observed for days 1, 2, 5 and 10 of VBS placement. A scheme with an overview of all days in the VBS with their measurements and observations can be found in appendix 6.2. On these time points different behaviors were observed and scored to calculate the dominance hierarchy of the animals (see Table 1). Along with the behaviors, modifiers were noted to give some more information about the behavior for analysis. The modifiers were ‘opponent’, ‘fierce’, ‘mild’, ‘arena’ and ‘burrows’. For behaviors like offensive act, defensive act, patrolling, approach, retreat and status quo, the opponent with who this act is performed was noted. For the offensive and defensive acts, the intensity of aggression was noted. It was ‘fierce’ when it involved potential or actual physical damage such as the attack jump and the clinch attack and ‘mild’ if the interaction did not involve physical damage such as upright posture, chase and flee. Furthermore, for every interaction the location (either arena or burrow) was noted.

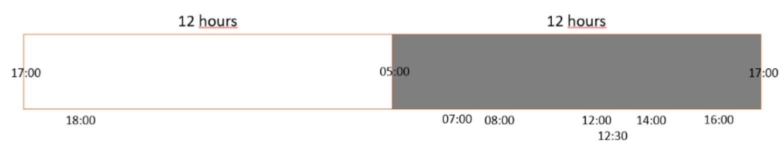


Figure 7. Scheme of the 7 observation time points

Table 1. Behaviors that were scored during VBS placement with their description.

Behavior	Description
Offensive act	An aggressive act towards an opponent including sideways lateral threats, upright defensive posture, clinch attack, pinning and chasing
Defensive	A self-protective act often in response to an aggressive act, including upright defensive posture, moving away, submissive-supine posture and fleeing

Patrolling	A behavior in the arena that blocks off access from the burrows to the arena when an opponent is in the opening of the burrows
Tunnel guarding	A behavior in the burrows that blocks off access from the arena to the burrows
Approach	When the observed rat moves towards its opponent, forcing the opponent to move backwards
Retreat	When the observed rat moves backwards in response to an approach
Status quo	When two rats in the burrows are facing each other standing still for more than 2 seconds
Drinking	Drinking water from a water bottle

2.4 Dominance ranking

The dominance ranking was calculated by using the Average Dominance Index. This uses the Average proportion of which each individual wins from every other animal to calculate a dominance score. For this, the excel extension MatrixTester version 3.0.1 was used (Hemelrijk, 2017). This program automatically calculates the dominance values which is translated into rank as the highest dominance value indicates a higher rank.

To determine the most and least dominant male, the MatrixTester analyzed the data from all the wins of agonistic interactions over all the days in the VBS. The male with the highest dominance value was determined to be the dominant male and the male with the lowest dominance value was determined to be the subordinate male. The dominance matrixes for day 10 can be found in appendix 6.5.

2.5 Structural analysis

For the structural analysis the right brain hemisphere was used. The hemispheres were stained by dropping them in a Golgi-Cox fixative and incubating them for 15 days at room temperature. After this, the hemispheres were cut in slices of 100 µm with a vibratome. The brain slices were put on a glass plate and further processed. After this the brain slices were covered with DPX Mountant and covered with a coverslip. The slides were examined under a Olympus BX53 microscope and the structural complexity of dendrites in three brain areas was measured.

Per colony, the most and least dominant males were selected and the neuronal complexity was determined by using similar methods as Patel et al. (2018) and Mckittrick et al. (2001). First, neurons that were dark and consistently colored, relatively isolated from other neurons and located in the CA1 region of the hippocampus were selected. From these neurons a primary apical dendrite that originated from the main shaft was selected, traced and segmented in 8 segments of 10 µm each starting from the beginning of the apical dendrite and continuing away from the main shaft. This was done by using software from NeuroLucida that was attached to the microscope. Per segment, spines were counted and added up for the total amount of spines per neuron. Per animal, 5-6 dendrites were analyzed. For the full protocol of the microscope see appendix 6.6.

2.6 Statistical analysis

2.6.1 Total body weight change

To determine the total body weight change over the time spent in the VBS. The body weight of the day 10 was subtracted from the body weight of day 0 per animal to get the amount of weight change. This was divided by the initial body weight to get the percentage of body weight change per animal. This data can be found in appendix 6.7.1. The data of the body weight of dominant and subordinate males and females over the full experiment can be found in appendix 6.7.2.

2.6.2 Intensity of aggression

To obtain the data of the intensity of aggression per colony, first the total sum of fierce and mild fights were calculated per animal per colony which can be found in appendix 6.8.1 After this, the proportion of fierce fights was calculated per animal per colony by dividing the amount of fierce fights by the total amount of fights per animal. The total amount of fights was calculated by adding the number of fierce fights to the number of mild fights. The average of the proportions of fierce fights of all animals in a colony was calculated per colony and this number was used as the intensity of aggression. The overview of the intensities of aggression per colony can be found in appendix 6.8.2.

2.6.3 Spine count

An overview of the spine count of the different animals can be found in appendix 6.9.1. To analyze the difference of spine count of dominant and subordinate males, the of the total amount of spines of 5 dendrites compared. To investigate the correlation between spine density and stability, intensity of aggression and female dominance, the average of the amount of spines of the 5 dendrites per male was calculated which can be found in appendix 6.9.2.

2.6.4 Stability

To calculate the stability of the hierarchy over the time spent in the VBS the dominance ranking of the first day and the last day is needed. This is calculated by using the amount of losses that each animal receives and calculating the proportion of wins of each pair in the colony. The dominance matrixes from day 1 and day 10 can be found in appendix 6.5. The dominance values have been automatically calculated with the Matrix tester version 5 and the animal with the highest average dominance value was given rank 8, the animal with the second highest ADV was given rank 7 and so on until the animal with the lowest ADV was given rank 1. Schemes with the ranks of the animals on the different days can be found in appendix 6.10.1. After this, a two-tailed non-parametric Kendall Tau correlation test was performed to determine the stability. The outcome of these tests can be found in appendix 6.10.2.

2.6.5 Female dominance

Female dominance was also determined by using the MatrixTester version 3.0.1 was used (Hemelrijk, 2017). It automatically calculates the female dominance by using the dominance matrices. The female dominance values per colony can be found in appendix 6.11.

2.6.6 Statistics

Firstly, to determine if the data is normally distributed, a normality test was performed on the data of spine count of dominants and subordinates, body weight change of dominants and subordinates, time spent in arena of dominants and subordinates, corticosterone levels of pre and post VBS situations of dominants and subordinates, fat weight, adrenal weight, testes weight, seminal vesicle weight and thymus weight. This information can be found in appendix 6.12.1. The results from these tests determine if a parametric or a nonparametric test was used in further analyses.

For the tests of body weight, spine count, time spent in arena, corticosterone levels and all the organ weight pairwise analyses were performed. If the normality test showed a normal distribution, a paired t test was performed. For the analysis of aggression over time a Jonckheere-Terpstra test was used. If the normality test showed a non-normal distribution, a non-parametric related-samples Wilcoxon signed rank test was performed. Furthermore, for some tests a two sided analysis was used and for some tests a one sided analysis was used. Which of these two analyses was used was determined by the hypothesis. When a specific direction was expected for the data, a one sided analysis was used. When this was not the case, a two sided analysis was used. In appendix 6.11.2 an overview can be seen of the tests of comparisons of means along with the outcomes. In appendix 6.11.3 an overview can be seen of the correlation tests along with the outcomes.

3. Results

Multiple tests have been performed to investigate what the physiological and neurological effects of chronic social stress are. To answer the question 'What is the effect of chronic social stress in a VBS system on dendritic spine density of dominant vs subordinate males', the spine count of dominant and subordinate males have been compared and tested for differences. To support this, body weight change from day 0 to 10, corticosterone levels of males in pre and post VBS situations, organ weights and the time spent in the arena have also been compared and tested for differences. Next to this, it was inspected whether colony properties could be correlated to higher or lower spine count and body weight loss. This included the properties stability, intensity of aggression and female dominance.

3.1 Dominants vs subordinates

A graph with the weight-changes of the highest and lowest rank of males and females over the different periods of the experiment can be seen in Figure 8. Graphs with the weights of all animals in the colony per colony together with the amount of wounds can be found in appendix 6.13. When comparing body weight of dominant to subordinate males, it is found that body weight change of dominants was significantly higher than dominants (t (11) = 2.203, p = 0.03; Figure 9). The subordinate males show a bigger percentage body weight decrease than dominants as subordinates lost about 10% of their initial body weight compared to 5% of dominant males. As there is a difference between dominants and subordinates, they will be looked at separately during the next analyses.

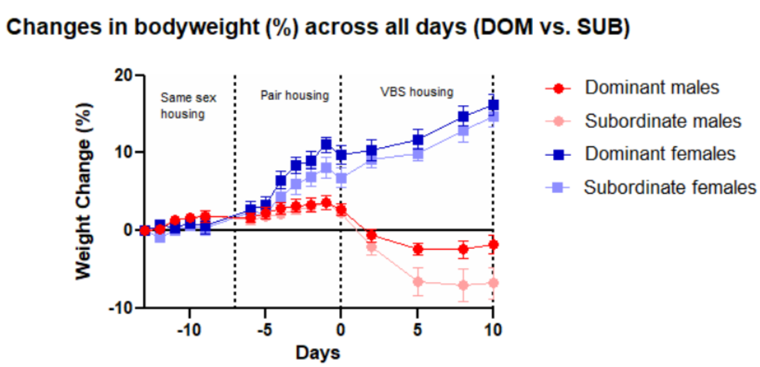


Figure 8. Body weight changes across the whole experiment of dominant males, subordinate males, dominant females and subordinate females.

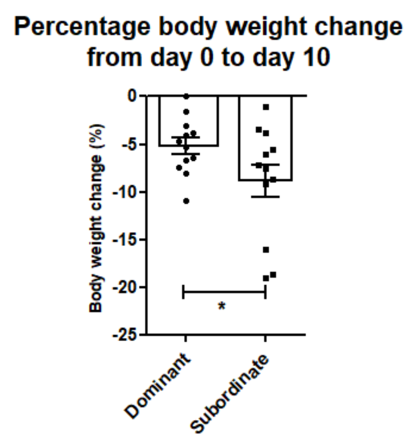


Figure 9. Percentage body weight change from day 0 to day 10 of dominant and subordinate males

Looking at time spent in the arena, a significant difference was found between the highest and lowest ranks. Dominants spent significantly more time in the arena than subordinate males (t (11) = -2.93, p = 0.002; Figure 10).

For corticosterone levels no significant differences were found between pre and post VBS for dominant males (t (2) = 1.978, p = 0.186) and subordinate males (t (2) = 0.970, p = 0.0.434). There is also not statistical difference between dominants and subordinates in the pre VBS situation (t (2) = -0.647, p = 0.584) nor in the post VBS situation (t (2) = 0.919, p = 0.455). These results are shown in Figure 11.

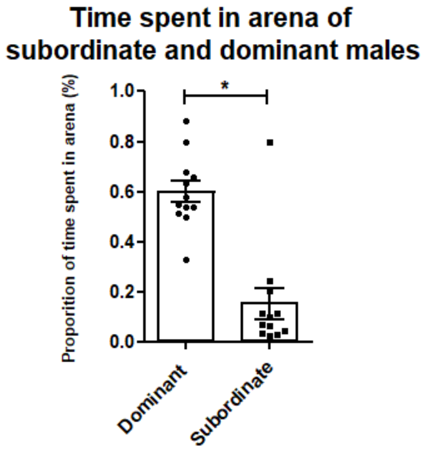


Figure 10. Proportion of time spent in arena of dominant and subordinate males. The asterix * indicates p < 0.05

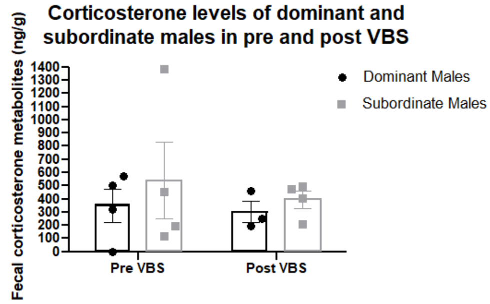


Figure 11. Corticosterone levels of dominant and subordinate males in pre and post VBS situations

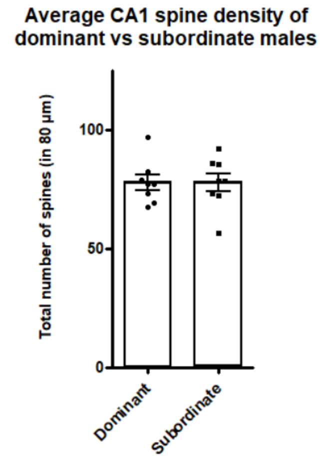


Figure 12. CA1 apical spine density of dominant and subordinate males in 80mm

The results from the pairwise comparison of spine density of dominant males and subordinate males show that there is no difference in spine count between dominant and subordinate males (t (39) = 0.232, p = 0.408; Figure 12). As there is no difference between dominant and subordinate males, for the rest of the analyses they are taken together.

3.2 Amount of fights

When looking at the amount of fights of males over time, a significant trend can be found as the amount of fights decrease over time (z (4) = -1.790, p = 0.365; Figure 13).

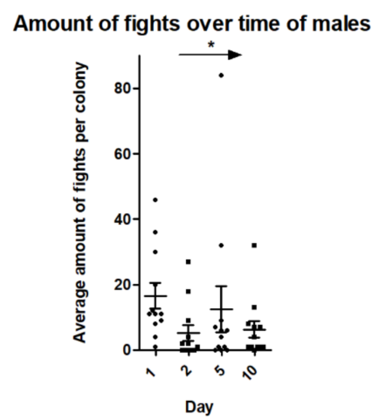


Figure 13. The amount of fights that males perform over the time spent in the VBS. The asterix indicates p < 0.05.

3.3 Stability

The correlation of fraction of body weight change and the stability of the colony was analyzed and dominant males show no correlation between stability and body weight change (r = 0.031, p = 0.89; Figure 14). For subordinates, however, there is a significant negative correlation (r = -0.431, p = 0.027; Figure 15) meaning that a higher stability or correlated with a bigger weight change.

When looking at the stability of a colony and spine count, no significant correlation can be found as the correlation coefficient (r = -0.166, p = 0.34; Figure 16).

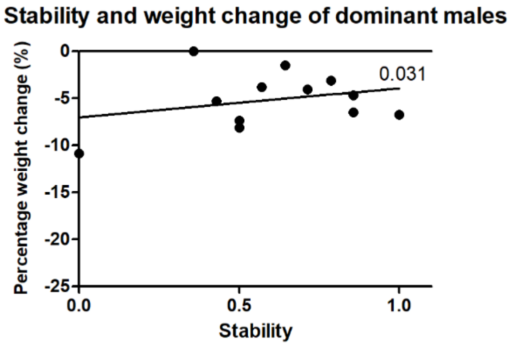


Figure 14. Stability of the colony and percentage of weight change of dominant males. The value shows the Kendall's Tau correlation coefficient.

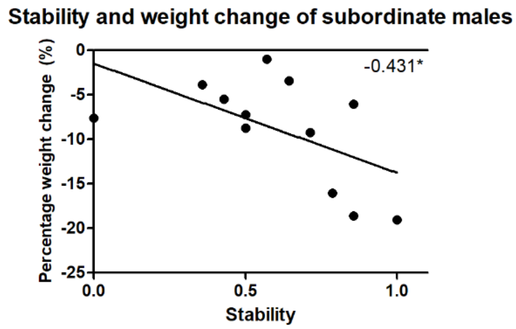


Figure 15. Stability of colonies and percentage of weight change of subordinate males. The value shows the Kendall's Tau correlation coefficient. The asterix * indicates p < 0.05)

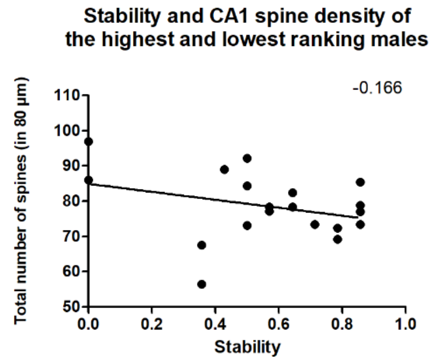


Figure 16. Stability of colonies and the total number of spines both of the highest and lowest ranking males of the colonies. The value shows the Kendall's Tau correlation coefficient. The asterix * indicates p < 0.05)

3.4 Intensity of aggression and female dominance

Female dominance is also associated with intensity of aggression (r = 0.636, P = 0.002; Figure 17). This positive correlation has a correlation value, meaning that a higher intensity of aggression is associated with a higher female dominance.

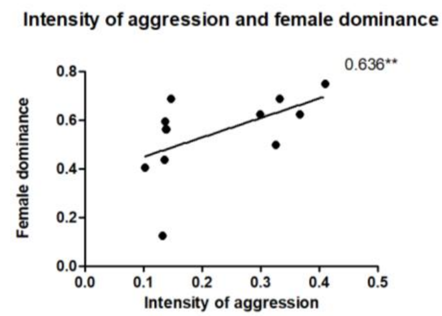


Figure 17. Intensity of aggression of colonies and female dominance of colonies. The value shows the Kendall's Tau correlation coefficient. The double asterix ** indicates p < 0.01)

3.5 Intensity of aggression

Regarding the association between bodyweight change and intensity of aggression, a correlation was found for dominant males, but not for subordinate males. For subordinate males, no significant correlation value was found of (r = 0.091, p = 0.34; Figure 18). However, for dominants there was a significant negative correlation (r = -0.515, p = 0.01; Figure 19). A higher intensity of Spine density is associated with a bigger weight loss.

When looking at intensity of aggression and spine density a significant correlation can be seen (r = 0.584, p = 0.001; Figure 20). This is a positive correlation where a higher intensity of aggression is associated with a higher spine count.

3.6 Female dominance

When looking at female dominance and body weight, subordinates show no correlation between female dominance and body weight (r = 0.047, p = 0.836; Figure 21). However, dominants do show a significant correlation (r = -0.45, p = 0.045; Figure 22). This negative correlation shows that a higher female dominance is associated with a bigger weight loss.

When examining female dominance, a correlation is found with spine count when dominant and subordinate animals are looked at separately. For dominant animals there is no correlation between spine count and female dominance (r = 0.296, p = 0.120; Figure 23). However, subordinate animals do show a correlation with a coefficient (r = 0.667, $p < 0.007$; Figure 24). This is a positive correlation meaning that a higher female dominance is associated with a higher spine count.

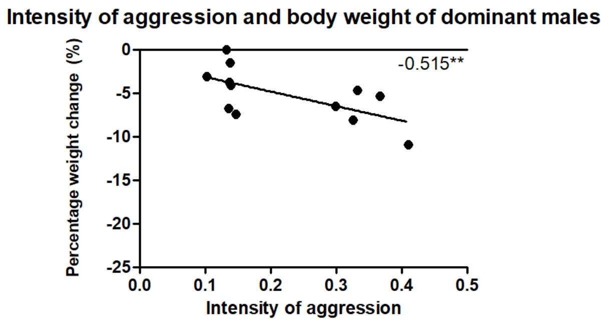


Figure 18. The intensity of aggression and the percentage of weight change of dominant males. The value shows the Kendall's Tau correlation coefficient. The double asterix ** indicates $p < 0.01$)

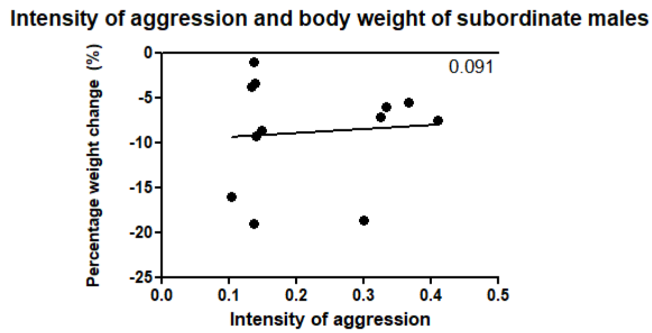


Figure 19. Intensity of aggression and the percentage of weight change of subordinate males. The value shows the Kendall's Tau correlation coefficient.

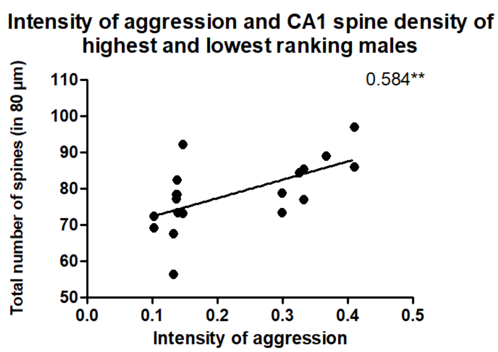


Figure 20. The intensity of aggression values of colonies correlated tot the total number of spines in 80 mm. The value shows the Kendall's Tau correlation coefficient. The double asterix ** indicates $p < 0.01$)

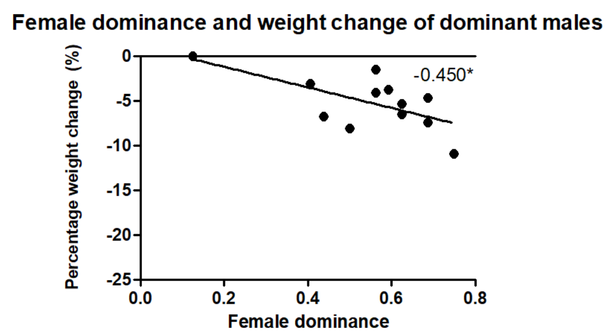


Figure 21. Female dominance of colonies correlated to the percentage of weight change of dominant males. The value shows the Kendall's Tau correlation coefficient. The asterix * indicates $p < 0.05$)

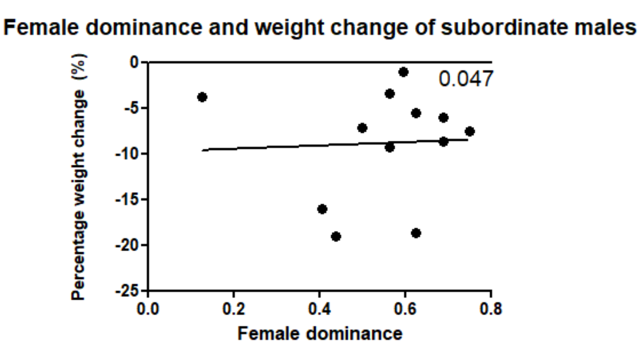


Figure 22. Female dominance of colonies correlated to the percentage of weight change of subordinate males. The value shows the Kendall's Tau correlation coefficient.

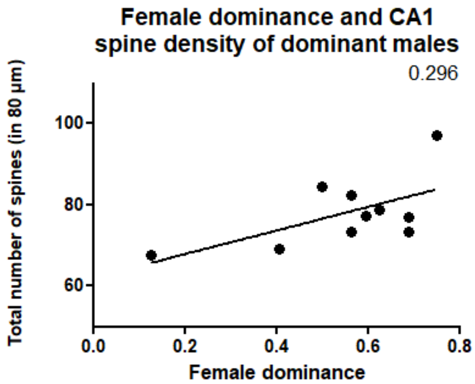


Figure 23. The correlation between female dominance and the total number of spines in 80 mm of dominant males. The value shows the Kendall's Tau correlation coefficient.

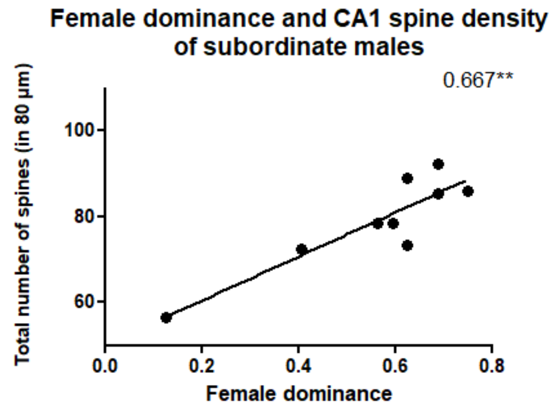


Figure 24. The correlation between female dominance and the total number of spines of subordinate males in 80 mm. The value shows the Kendall's Tau correlation coefficient. The double asterix ** indicates p < 0.01)

3.7 Organ weight

When looking at the organ weights of the animals, no difference is found between dominants and subordinates for all organs except fat weight. There is no statistical difference between dominants and subordinates for adrenal weight (t (11) = -0.872, p = 0.402; Figure 25), thymus weight (t (10) = -1.379, p = 0.198; Figure 26), seminal vesicle weight (t (11) = 1.246, p = 0.239; Figure 27) and testes weight (t (11) = 1.373, p = 0.197; Figure 28). However, for retroperitoneal fat weight, there is a difference between dominants and subordinates. Here, subordinates show a higher retroperitoneal fat weight than dominants (t (11) = -3.421, p = 0.006; Figure 29).

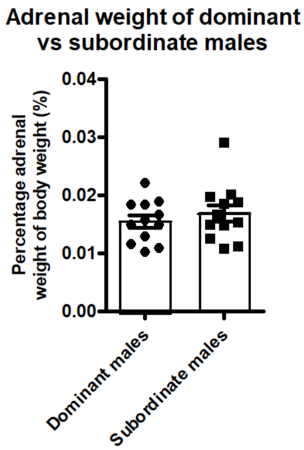


Figure 25. Percentage of adrenal weight of body weight of dominant and subordinate males.

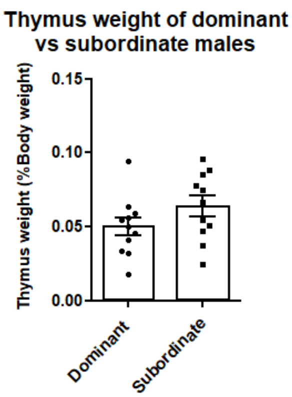


Figure 26. Percentage of thymus weight of body weight of dominant and subordinate males.

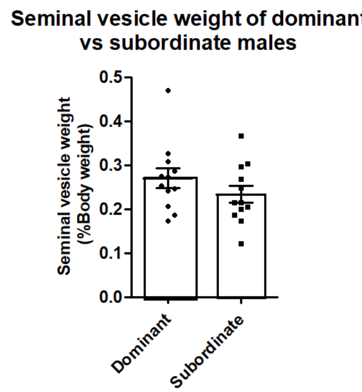


Figure 27. Percentage of seminal vesicle weight of body weight of dominant and subordinate males.

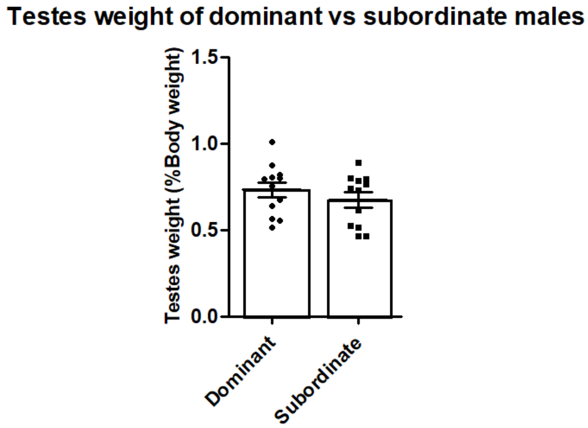


Figure 28. Percentage of testes weight of body weight of dominant and subordinate males.

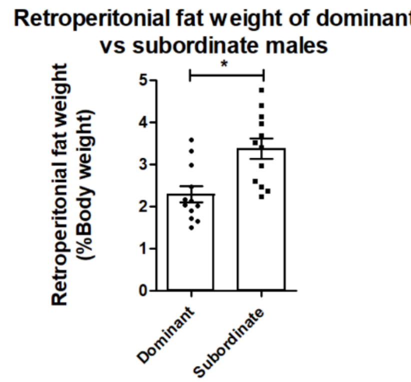


Figure 29. Percentage of fat weight of body weight of dominant and subordinate males. The asterix * indicates $p < 0.05$)

4. Discussion

In this study, it was hypothesized that subordinate males would have a lower CA1 spine density and more body weight loss than dominant males as a consequence of increased stress levels. Furthermore, it was thought that different colony properties would influence spine count and body weight. The results show that there is no difference in spine count of dominant and subordinate males, but it does show that subordinate males have a bigger body weight loss than dominant males. Furthermore, there was a difference in the amount of time spent in the arena, with dominants spending more time in the arena. For corticosterone levels, there was no difference between pre and post VBS of dominants and subordinates and between the dominants and subordinates in these two situations. Stability, intensity of aggression and female dominance also showed a few significant correlations. A high stability was associated with bigger body weight loss for subordinates. Intensity of aggression and female dominance showed similar effects as they both had a positive correlation with spine count and a negative correlation with body weight. Furthermore, only for retroperitoneal fat weight there was a significant difference, with subordinates having a higher fat weight than dominants

4.1 Difference between dominants and subordinates

Looking at the body weight change of all animals over time. It is noticeable that males start to lose body weight when they were put into the VBS system. This could indicate that males start to feel stress once they are in a social system. The body weight loss mostly happens at the beginning of the VBS period, from day 0 to about day 5. Furthermore, when comparing the weight change between the first and last day of the VBS, it becomes clear that subordinate males lose significantly more weight than dominant males throughout the experiment. As weight loss is associated with chronic stress, this could indicate that subordinates experience more stress than dominant male (Santos et al., 2000). Adding to this, when looking at time spent in the arena it further seems that subordinate males are more stressed as they spend significantly less time in the arena than dominant males suggesting that the subordinates are scared of the dominant male and avoiding him (Blanchard et al., 1990). This fear could also indicate that subordinate males are more stressed than the dominant males. Nevertheless, when looking at apical CA1 spine density, no difference was found between dominants and subordinate males. This contradicts the earlier findings as it was expected that higher stress would translate into a lower spine count for subordinates as this was also found by Patel et al. (2018). The similarity in spine count was also supported with the finding that there is no difference in corticosterone levels between dominant and subordinate males.

One explanation for this finding is that structural remodeling in the hippocampus is not entirely linked to stress as the brain is also influenced by external conditions and behavior. Although activity levels were not measured in this study, it would have been interesting to investigate this as differences in activity level could have also influenced spine count next to stress levels. When an animal is more active, higher brain derived neurotrophic factor (BDNF) levels are measured. BDNF is a neurotrophin that is vital for survival, growth and maintenance of neurons (Phillips, C., 2017). As the brain is very complex and is involved in a lot of processes, many things can influence it's plasticity next to stress. Exploring other factors that could have influenced the results would be interesting for future research.

Another explanation for the similarity of spine density is that subordinate animals adapt to their situation which prevents them from losing spines. The body weight of animals over the experiment shows that most of the weight loss happens at the beginning when the animals were put into the VBS. After day 5, their body weight stabilizes which could be a sign of adapting to the stressful situation. Buwalda et al (2017) have also studied WTG rat colonies in a VBS system. Although study was not completely comparable to the one used in this study because of a difference in amount of females and a difference in the VBS model, body weight of dominant and subordinate males was also measured throughout the experiment. The data from this study also shows that most of the body weight loss of the subordinate males takes place in the first 2 to 3 days in the VBS. Later, body weigh stabilizes and even increases. This shows that subordinate WTG rats might have the most stress at the beginning and adapt to their situation after a few days. When comparing this to studies where a difference in spine loss was found were performed with Long-Evans rats, a clear difference can be seen. When looking at body weight over time spent in a VBS in a study with Long-Evans rats, it is noticeable that body weight only starts to stabilize after around day 10 (Blanchard et al., 1993). These subordinate rats might not have the same chance of adapting to their situation in the beginning as WTG rats do which could explain the difference between the neuro-morphological findings of studies that use Long-Evans rats and this study. All in all, this provides further evidence for the hypothesis that the similarity in spine density might be caused by adaptation.

4.2 Stability

Stability is the amount of change in rank of individuals in a colony and the results from this study show that it is not correlated to spine count. When looking at stability and body weight, similarly no correlation was found for dominant animals. However, there was a significant correlation for subordinates as lower body weight was correlated to a higher stability. Weight loss is associated to stress and this finding could indicate that subordinate males experience more stress when they are in a colony with a higher stability while stability has no effect on the stress level of dominants. This is the contradictory to the hypothesis as colonies with a higher stability were thought to be more predictable which would decrease the amount of psychosocial stress that the subordinates experience. As a more predictable situation is thought to be easier to adapt to, this results also contradicts the hypothesis that subordinates adapt to their situation.

Looking at previous literature about the effect of stability of hierarchy on stress, many conflicting findings are presented. Mendonca-Furtado et al. (2014) found that cortisol levels are higher in unstable periods for dominant and subordinate males. Marson and Mendoza (1993) found that dominants have higher cortisol levels in stable hierarchies while subordinates show no correlation. However, Sapolsky (1992) showed that there were lower basal cortisol levels in stable hierarchies for dominant individuals. Sherman & Mehta (2020) stated that unstable power for dominants while stable powerlessness for subordinates is more stressful. This range in contradicting findings indicate the lack of knowledge about the effect of stability on the stress of different ranks.

One explanation for the finding of stability in this research is that subordinate males have less water accessibility which causes them to have more body weight loss. Two water bottles were placed in the arena and only one water bottle was put in the burrows in one of the nesting boxes. If one male was sitting in the nesting box with the water bottle and was blocking the entrance to prevent other males from entering that box, the other subordinate males would have limited water accessibility. Furthermore, the water bottles in the arena might be very stressful to reach as the dominant male is in the arena and subordinates are at risk of being attacked when they go to the arena. This could have caused the subordinate males to lose a lot of weight and could also help explain the correlation to stability. When a colony is very stable, there are not a lot of shifts in ranks. Because of this, the ranks of subordinates never increases. Increasing in rank might have created an opportunity to go into the arena to drink some water, however as this happens less in a more stable colony water accessibility might have been lower.

Another explanation is that stable hierarchies arise because there is a lot of stress in the beginning of the time spent in the VBS system. As said before, most of the aggression and body weight loss of males in the VBS is at the beginning of the time spent in the VBS system. One possibility is that when a colony is very stable, the amount of stress at the beginning is higher than when a colony is very unstable. It could be that the males in those stable colonies are very dominant which gives a lot of stress. Later the animals adapt to this stress, which could explain why there is no correlation to spine count.

4.3 Intensity of aggression and female dominance

A positive correlation has been found between intensity of aggression and female dominance. This has also been found by C. K. Hemelrijk, (2008) in the model "DomWorld". This finding is in accordance with the winner-loser effect which states that an individual is more likely to lose after losing and vice versa after winning. A higher intensity of aggression provides females a higher chance of rising above males in dominance rank. For this reason it is also not surprising that both intensity of aggression and female dominance have a similar correlation to body weight and CA1 spine density. They both show no correlation to subordinate body weight and a negative correlation to dominant body weight.

4.4 Intensity of aggression

As stated before intensity of aggression shows a positive correlation to spine density. Spine density increased when intensity of aggression was higher. Body weight also correlated to intensity of aggression but only for dominants as they had bigger weight loss with a higher intensity of aggression. Subordinates showed no correlation. These findings are contradictory as spine density shows that males are less stressed with a higher intensity of aggression and body weight shows that dominant males are more stressed with a higher intensity of aggression.

When looking into literature of intensity of aggression and body weight, in the study by Buwalda et al. (2017) the opposite effect was found. This study also looked at WTG rats in a VBS system. In this study, subordinates showed a big body weight decrease in colonies with high-aggressive males compared to a lower body weight decrease in colonies with non-aggressive males. Furthermore, for dominants a similar increase in body weight found in both high-aggressive and non-aggressive colonies. The results of the article of Buwalda et al. does not fit with the results from this research as the opposite was found for both dominants and subordinates. An explanation for this different result is that the colonies used in the study of Buwalda et al. were composed of males of a specific aggressiveness level possibly creating a bigger difference in intensity of aggression than in this research. The effect of intensity of aggression on subordinate body weight might not have been big enough to cause the same effects for subordinates. Furthermore, this study is not entirely comparable to the one used in this study because of a difference in amount of females and the absence of dark tunnels.

Multiple studies that looked at aggression and stress of non-human primates, found that intensity of aggression did not correspond to cortisol levels (Lynch et al., 2002; Girard-Buttoz et al., 2009). This could indicate that the difference of spine density might not be caused by stress, but by another factor. As the brain is very complex and gets a lot of input and therefore a lot of situational factors and different behaviors could influence spine count, other than stress. One example of this is BDNF levels. As already explained, BDNF levels can influence neurons as it is involved in the survival, growth and maintenance of neurons and BDNF levels can be increased with higher physical activity (Phillips, C., 2017). It could be that males in a colony with a higher intensity of aggression are more physically active, as more intense fighting involves more activity, and therefore have higher BDNF levels. This increase of BDNF could have caused the increase of spine count.

The hypothesis that males in a colony with a higher intensity of aggression are more physically active could also explain the correlation for dominant body weight loss and intensity of aggression. If a higher intensity of aggression causes dominants to be more active, the weight loss could be explained by a higher energy expenditure. The physical activity of subordinate males might be more similar between colonies of a different intensity of aggression and therefore do not have this correlation.

4.5 Female dominance

For subordinate males it was found that there was no correlation between female dominance and body weight. It was hypothesized that subordinates show a bigger weight loss with a higher female dominance as a higher female dominance causes a lower dominance rank for the subordinate males. It was hypothesized that subordinates show a bigger weight loss with a higher female dominance as a higher female dominance causes a lower dominance rank for the subordinate males. For female dominance and body weight of dominant males there was a negative correlation with body weight decreasing as female dominance increased. As the effect of female dominance on males is a subject that is not very well studied, looking into literature does not help clarify this result. One explanation for these results is that they are not caused by the actual female dominance, but are a confounding factor of the effects of intensity of aggression.

Female dominance is also linked to spine density, but only for subordinate males. That dominant males show no correlation between spine density and female dominance was expected. However, for subordinates it was thought that spine density would be negatively correlated to female dominance as a higher female dominance causes lower male dominance for subordinates which was expected to be more stressful. For subordinates the opposite effect was found where spine count increased with a higher female dominance. One explanation is that this correlation is not caused by the female dominance, but by a higher intensity of aggression. As a high intensity of aggression is correlated to a high female dominance this could also be a confounding factor.

4.6 Organ weight

When investigating the organ weight change, only retroperitoneal fat weight shows a significant difference between dominant and subordinate males. Subordinate males have a higher fat weight than dominant males. For thymus weight, adrenal weight, testes weight and seminal vesicle weight there was no significantly difference between dominant and subordinate males. The study of Mckittrick et al. (2000) showed that both dominant and subordinate males had decreased thymus weight and increased adrenal weight compared to controls which was appointed to increased stress. As this study does not have a control group, the same comparison could not be made. However, this result does hint that subordinates and dominants experience a similar amount of stress. If this amount of stress is higher than a control group should be investigated in future studies. Testes weight was studied by Tamashiro et al. (2004) and they found that there was no difference in testes weight for dominants, subordinates and for the control group. Only fat weight was higher in subordinates vs in dominants. Tamashiro et al. found the opposite, they found that both dominants and subordinates had a similar decrease in fat weight. As retroperitoneal fat is seen as visceral fat and glucocorticoids are able to cause visceral fat accumulation, the increased retroperitoneal fat weight suggests that subordinates are more stressed than dominants (Masuzaki et al., 2001). However, most organ weight results indicate that dominants and subordinates are similarly stressed. One explanation for this is that the stress in the beginning caused the retroperitoneal fat accumulation and that later the subordinate males adapt to the stress. This adaptation could prevent further differences in organ weights.

4.7 Implications and future studies

This study has helped expand on earlier research by looking deeper into the effects of chronic social stress on neurobiology and by investigating spine count specifically. It was already found that there are behavioral differences between dominants and subordinates when they are exposed to chronic social stress. These behavioral differences are thought to arise from differences in specific brain areas. Specifically, chronic stress is known to decrease working memory performance thus this research has tried to further investigate the hippocampus to help discover what the effects of chronic social stress are. By doing this, more knowledge will be gained about the effects of chronic social stress which could also be translated to humans.

As humans are also faced with social conflict and psychosocial stress, this study might give a better understanding what this does to brain and behavior.

As this study gives rise to the idea that WTG subordinate males could adapt to their situation which prevents a decrease in spine density in the CA1 region of the hippocampus. Future research could expand on this hypothesis and further investigate if this also happens in other brain areas. The medial prefrontal cortex and the basolateral amygdala could be interesting areas to examine as they are also connected to emotional memory and known to be influenced by stress. Another interesting thing to look at in future research could be the other regions of the hippocampus. This study only looked at apical CA1 dendrites, but the CA2 and CA3 are located earlier in the signaling sequence of the hippocampus and therefore might give more information about chronic social stress and neuro-morphology.

4.8 Shortcomings

As in all research, some improvements could have been made to make the results stronger. First of all, a control group should have been added. This control group should consist of animals who are exposed to the VBS, but who do not feel the chronic social stress of the experimental group. This could be accomplished by putting two rats, one male and one female, in the VBS for the same amount of time and measuring the same variables as the experimental group. These control animals would not feel the amount of social stress as the experiment animals do, as they form a dominance hierarchy very quickly. The prior attributes of males will most likely cause them to be dominant over females causing social uncertainty to be lower.

Next, for this research only the fecal corticosterone levels of batch one was analyzed. Because of this, the corticosterone levels could not be correlated to different colony properties which reduced the strength of this research. Adding to this, the most dominant and most subordinate animals were determined before all the behavioral data was collected. These preliminary males were used for the neuronal analysis, however looking at the eventual dominance ranking, there were some discrepancies with the males. Two subordinate and one dominant males that were initially selected as most and least dominant were ultimately not the most and least dominants.

4.9 Conclusion

This research investigated colony properties, and found that they are correlated to spine count and body weight loss. First, there was a correlation between stability and body weight of subordinates, which might be caused by water accessibility. Second, there was a correlation between intensity of aggression and spine count and body weight, which might be caused by activity level. Lastly, correlations found for female dominance and spine count and body weight might be confounding factors of the intensity of aggression.

Most importantly, this research tried to investigate the effects of chronic social stress on brain and behavior. Although subordinates seem more stressed when looking at their time spent in the arena and body weight loss, no difference was found between dominant and subordinate males when looking at apical CA1 spine count and corticosterone levels. This could indicate that subordinate males adapt to their situation.

5. References

Abdelall, E. S., Eagle, Z., Finseth, T., Mumani, A. A., Wang, Z., Dorneich, M. C., & Stone, R. T. (2020). The interaction between physical and psychosocial stressors. *Frontiers in behavioral neuroscience*, *14*.

Amaral, D. G. (1993). Emerging principles of intrinsic hippocampal organization. *Current opinion in neurobiology*, *3*(2), 225-229.

Armanini, M. P., Hutchins, C., Stein, B. A., & Sapolsky, R. M. (1990). Glucocorticoid endangerment of hippocampal neurons is NMDA-receptor dependent. *Brain research*, *532*(1-2), 7-12.

Beacham, J. L. (1988). The relative importance of body size and aggressive experience as determinants of dominance in pumpkinseed sunfish, Lepomis gibbosus. *Animal Behaviour*, *36*(2), 621-623.

Bercovitch, F. B., & Clarke, A. S. (1995). Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. *Physiology & behavior*, *58*(2), 215-221.

Blackstad, T. W. (1956). Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *Journal of Comparative Neurology*, *105*(3), 417-537.

Blanchard DC, Blanchard RJ. 1990. Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. Neurosci Biobehav Rev 14:455–462.

Blanchard DC, Sakai RR, McEwen B, Weiss SM, Blanchard RJ. Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behav Brain Res.* 1993 Dec 20;58(1-2):113-21. doi: 10.1016/0166-4328(93)90096-9. PMID: 8136039.

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behavioural brain research*, *58*(1-2), 113-121.

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology*, *20*(2), 117-134.

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology*, *20*(2), 117-134.

Blanchard, R. J., & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. *Journal of comparative psychology*, *103*(1), 70.

Blanchard, R. J., & Blanchard, D. C. (1990). An ethoexperimental analysis of defense, fear, and anxiety.

Blanchard, RJ, Hebert, M, Sakai, RR, McKittrick C, Henrie JA, Yudko E, McEwen BS, Blanchard DC. 1998b. Chronic social stress: changes in behavioral and hormonal indices of emotion. Aggress Behav 14:307–322.

Buwalda, B., Koolhaas, J. M., & de Boer, S. F. (2017). Trait aggressiveness does not predict social dominance of rats in the Visible Burrow System. *Physiology & behavior*, *178*, 134-143.

Cavigelli, S. A. (1999). Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, Lemur catta. *Animal behaviour*, *57*(4), 935-944.

Chase, I. D., Tovey, C., Spangler-Martin, D., & Manfredonia, M. (2002). Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proceedings of the National Academy of Sciences*, *99*(8), 5744-5749.

Collias, N. E. (1943). Statistical analysis of factors which make for success in initial encounters between hens. *The American Naturalist*, *77*(773), 519-538.

Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of neurobiology*, *60*(2), 236-248.

Dugatkin, L. A. (1997). Winner and loser effects and the structure of dominance hierarchies. *Behavioral Ecology*, *8*(6), 583-587.

Feng, X., Wu, X., Morrill, R. J., Li, Z., Li, C., Yang, S., ... & Hu, X. (2016). Social correlates of the dominance rank and long-term cortisol levels in adolescent and adult male rhesus macaques (Macaca mulatta). *Scientific reports*, *6*(1), 1-9.

Francis, R. C. (1988). On the relationship between aggression and social dominance. *Ethology*, *78*(3), 223-237.

Gavrilovic, L., & Dronjak, S. (2005). Activation of rat pituitary-adrenocortical and sympatho-adrenomedullary system in response to different stressors. *Neuroendocrinology Letters*, *26*(5), 515-520.

Girard-Buttoz, C., Heistermann, M., Krummel, S., & Engelhardt, A. (2009). Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (Macaca fascicularis). *Physiology & behavior*, *98*(1-2), 168-175.

Grønli, J., Murison, R., Fiske, E., Bjorvatn, B., Sørensen, E., Portas, C. M., & Ursin, R. (2005). Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiology & behavior*, *84*(4), 571-577.

Gundersen, C., Mahatmya, D., Garasky, S., & Lohman, B. (2011). Linking psychosocial stressors and childhood obesity. *Obesity reviews*, *12*(5), e54-e63.

Hemelrijk, C. K. (1999). Effects of cohesiveness on inter-sexual dominance relationships and spatial structure among group-living virtual entities. In *European Conference on Artificial Life* (pp. 524-534). Springer, Berlin, Heidelberg.

Hemelrijk, C. K., Wantia, J., & Gygax, L. (2005). The construction of dominance order: comparing performance of five methods using an individual-based model. *Behaviour*, 1037-1058.

Hemelrijk, C. K., Wantia, J., & Isler, K. (2008). Female dominance over males in primates: Self-organisation and sexual dimorphism. PLoS One, 3(7), e2678.

Hemelrijk, C. K., Wantia, J., & Isler, K. (2008). Female dominance over males in primates: self-organisation and sexual dimorphism. *PLoS One*, *3*(7), e2678.

Hemelrijk, C.K. (1999). An individual-oriented model on the emergence of despotic and egalitarian societies. — Proc. Roy. Soc. London B 266: 361-369.

Hemelrijk, C.K. (2000). Towards the integration of social dominance and spatial structure. — Anim. Behav. 59: 1035-1048

Huang, B., Wey, T. W., & Blumstein, D. T. (2011). Correlates and consequences of dominance in a social rodent. *Ethology*, *117*(7), 573-585.

Jameson, K. A., Appleby, M. C. & Freeman, L. C. (1999) Anim. Behav. 57, 991–998.

Kemeny, M. E. (2003). The psychobiology of stress. *Current directions in psychological science*, *12*(4), 124-129.

Knierim, J. J. (2015). The hippocampus. *Current Biology*, *25*(23), R1116-R1121.

Lambert, K. G., Buckelew, S. K., Staffiso-Sandoz, G., Gaffga, S., Carpenter, W., Fisher, J., & Kinsley, C. H. (1998). Activity-stress induces atrophy of apical dendrites of hippocampal pyramidal neurons in male rats. *Physiology & behavior*, *65*(1), 43-49.

Lee, T., Jarome, T., Li, S. J., Kim, J. J., & Helmstetter, F. J. (2009). Chronic stress selectively reduces hippocampal volume in rats: a longitudinal MRI study. *Neuroreport*, *20*(17), 1554.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews neuroscience*, *10*(6), 434-445.

Lynch, J. W., Ziegler, T. E., & Strier, K. B. (2002). Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys, Cebus apella nigritus. *Hormones and Behavior*, *41*(3), 275-287.

Mason, W. A., & Mendoza, S. P. (Eds.). (1993). *Primate social conflict*. SUNY Press.

Masuzaki, H., Paterson, J., Shinyama, H., Morton, N. M., Mullins, J. J., Seckl, J. R., & Flier, J. S. (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science*, *294*(5549), 2166-2170.

McEWEN, B. S., & Magarinos, A. M. (1997). Stress Effects on Morphology and Function of the Hippocampus a. *Annals of the New York Academy of Sciences*, *821*(1), 271-284.

McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Current opinion in neurobiology*, *5*(2), 205-216.

McGaugh, J. L. (1989). Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Annual review of neuroscience*, *12*(1), 255-287.

McKittrick, C. R., Magariños, A. M., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (2000). Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse*, *36*(2), 85-94.

Meese, G. B., & Ewbank, R. (1973). The establishment and nature of the dominance hierarchy in the domesticated pig. *Animal behaviour*, *21*(2), 326-334.

Mendonça-Furtado, O., Edaes, M., Palme, R., Rodrigues, A., Siqueira, J., & Izar, P. (2014). Does hierarchy stability influence testosterone and cortisol levels of bearded capuchin monkeys (Sapajus libidinosus) adult males? A comparison between two wild groups. *Behavioural processes*, *109*, 79-88.

Morgan, M. A., & LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral neuroscience*, *109*(4), 681.

Partanen, K., Partanen, J., Tiihonen, J., Viinamaki, H., Karjalainen, A. K., & Lehtonen, J. (2000). Quantitative MRI of the hippocampus and amygdala in severe depression. *Psychol Med*, *30*, 117125.

Patel, D., Anilkumar, S., Chattarji, S., & Buwalda, B. (2018). Repeated social stress leads to contrasting patterns of structural plasticity in the amygdala and hippocampus. *Behavioural brain research*, *347*, 314-324.

Phillips, C. (2017). Brain-derived neurotrophic factor, depression, and physical activity: making the neuroplastic connection. *Neural plasticity*, *2017*.

Preis, A., Samuni, L., Deschner, T., Crockford, C., & Wittig, R. M. (2019). Urinary cortisol, aggression, dominance and competition in wild, West African male chimpanzees. *Frontiers in Ecology and Evolution*, *7*, 107.

Price, J. (1967). The dominance hierarchy and the evolution of mental illness. *The Lancet*, *290*(7509), 243-246.

Radley, J. J., Sisti, H. M., Hao, J., Rocher, A., McCall, T., Hof, P. R., ... & Morrison, J. H. (2004). Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*, *125*(1), 1-6.

Reinhardt, V. (1987). Are male rhesus monkeys more aggressive than females?. *Primates*, *28*(1), 123-125.

Roozendaal, B., Hahn, E. L., Nathan, S. V., Dominique, J. F., & McGaugh, J. L. (2004). Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala. *Journal of neuroscience*, *24*(37), 8161-8169.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience*, *10*(6), 423-433.

Santos, J., Benjamin, M., Yang, P. C., Prior, T., & Perdue, M. H. (2000). Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *278*(6), G847-G854.

Sapolsky, R. M. (2005). c. *science*, *308*(5722), 648-652.

Sherman, G. D., & Mehta, P. H. (2020). Stress, cortisol, and social hierarchy. *Current opinion in psychology*, *33*, 227-232.

Silk, J. B. (1999). Male bonnet macaques use information about third-party rank relationships to recruit allies. *Animal Behaviour*, *58*(1), 45-51.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science*, *253*(5026), 1380-1386.

Stevens, J. M., Vervaecke, H., De Vries, H., & van Elsacker, L. (2007). Sex differences in the steepness of dominance hierarchies in captive bonobo groups. *International Journal of Primatology*, *28*(6), 1417-1430.

Tamashiro, K. L., Nguyen, M. M., & Sakai, R. R. (2005). Social stress: from rodents to primates. *Frontiers in neuroendocrinology*, *26*(1), 27-40.

Tamashiro, K. L., Nguyen, M. M., Fujikawa, T., Xu, T., Ma, L. Y., Woods, S. C., & Sakai, R. R. (2004). Metabolic and endocrine consequences of social stress in a visible burrow system. *Physiology & behavior*, *80*(5), 683-693.

Vyas, A., Mitra, R., Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience*, *22*(15), 6810-6818.

Wolf, O. T. (2003). HPA axis and memory. Best Practice & Research Clinical Endocrinology & Metabolism, 17(2), 287-299.

Woolley, C. S., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain research, 531(1-2), 225-231.

Xu, L., Anwyl, R., & Rowan, M. J. (1997). Behavioural stress facilitates the induction of long-term depression in the hippocampus. *Nature*, *387*(6632), 497-500.

6. Appendix

6.1ADI equations

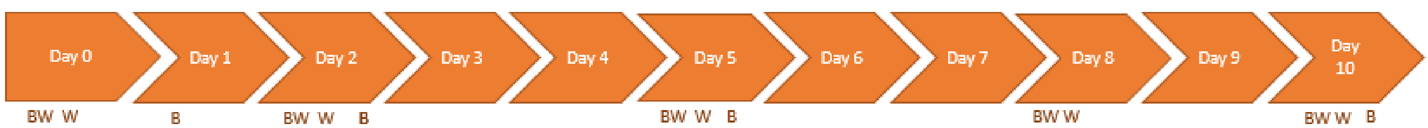
$$w_{ij} = \frac{x_{ij}}{x_{ij} + x_{ji}}$$

Dominance index equation per pair of subjects

$$1/N \sum_j w_{ij}$$

Average of all dominance indexes of a specific individual

6.2 Timeline of observations in VBS



6.3 Fecal corticosterone levels

DOMINANT	PRE VBS	POST VBS
M 2.1	317	196
M 1.4	505	459
M 1.2	569	247

SUBORDINATE	PRE VBS	POST VBS
M 1.1	195	405
M 4.1	1386	492
M 4.2	456	208

6.4 Organ weights

Dominant males

COLONY	GENDER	NUMBER	ID	ADRENAL GLANDS	THYMUS	FAT	SEMINAL VESICLE	TESTES
1	M	16	M 3.5	**0,018333**	**########**	**2,02**	**0,469744**	**0,873333**
2	M	5	M 2.1	**0,014963**	**0,0457**	**2,165848**	**0,205897**	**0,798526**
3	M	4	M 1.4	**0,015692**	**0,054615**	**1,720769**	**0,272308**	**0,805128**
4	M	2	M 1.2	**0,010284**	**0,050118**	**1,893381**	**0,286761**	**0,754137**
5	M	46	M 4.2	**0,014895**	**0,041142**	**2,038508**	**0,274336**	**0,562984**
6	M	38	M 2.2	**0,011605**	**0,094469**	**2,468612**	**0,242104**	**0,515271**
7	M	47	M 4.3	**0,012922**	**0,063219**	**1,506187**	**0,252511**	**0,555434**
8	M	39	M 2.3	**0,010875**	**0,059196**	**1,650071**	**0,326217**	**0,637376**
9	M	72	M 2.4	**0,018937**	**0,033228**	**3,582756**	**0,187028**	**0,79376**
10	M	68	M 1.4	**0,016603**	**0,018065**	**2,986983**	**0,173681**	**0,675901**
11	M	70	M 2.2	**0,022066**	**0,056221**	**2,127934**	**0,308146**	**1,009413**
12	M	66	M 1.2	**0,018329**	**0,031995**	**3,309675**	**0,245986**	**0,817053**

Subordinate males

COLONY	GENDER	NUMBER	ID	ADRENAL GLANDS	THYMUS	FAT	SEMINAL VESICLE	TESTES
1	M	6	M 2.2	**0,012506**	**0,042894**	**2,460207**	**0,248579**	**0,614987**
2	M	1	M 1.1	**0,015302**	**0,088442**	**2,592965**	**0,215327**	**0,766332**
3	M	13	M 4.1	**0,028989**	**0,024734**	**2,361968**	**0,121277**	**0,794681**
4	M	14	M 4.2	**0,011136**	**0,075056**	**3,677506**	**0,173274**	**0,801782**
5	M	34	M 1.2	**0,016706**	**0,095576**	**4,401624**	**0,214847**	**0,523529**
6	M	41	M 3.1	**0,010756**	**0,037089**	**3,509911**	**0,199733**	**0,463222**
7	M	35	M 1.3	**0,014925**	**0,085224**	**4,766468**	**0,267438**	**0,463134**
8	M	36	M 1.4	**0,019753**	**0,066181**	**2,971319**	**0,296813**	**0,728654**
9	M	65	M 1.1	**0,020087**	**0,047121**	**4,124069**	**0,186905**	**0,51474**
10	M	69	M 2.1	**0,01875**	**0,050727**	**2,2305**	**0,303432**	**0,890841**
11	M	67	M 1.3	**0,01474**	**0,054134**	**3,961688**	**0,205584**	**0,737749**
12	M	71	M 2.3	**0,018518**	**0,077459**	**3,396518**	**0,366965**	**0,785082**

6.5 Fighting matrixes

Colony 1

ADI Day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1			1						0,333333	5
F2	1			1					0,75	6
F3									0	1
F4	1	1	1					1	0,75	7
M1									0	2
M2									0	3
M3									0	4
M4			2	1	6	2			0,875	8

All days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		2	1						0,466667	5
F2	3		1	2					0,533333	6
F3		1							0,125	3
F4	3	2	3					1	0,75	7
M1									0	1
M2									0	2
M3								1	0,2	4
M4			2	1	6	4	4		0,86	8

Colony 2

Day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2									0	2
F3		2		1					1	7
F4	2								0,5	6
M1									0	3
M2	1				5		2	1	1	8
M3									0	4
M4									0	5

All days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1							0,333333	4
F2				4					0,25	3
F3		2		1					0,75	7
F4	2		1						0,5	5
M1									0	1
M2	1				14		6	3	1	8
M3									0	2
M4		1							0,5	6

Colony 3

day 1											
Rijlabels		F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1								1		0,5	5
F2				2						0,5	6
F3										0	1
F4		1	3							1	7
M1				1			4	5	3	1	8
M2										0	2
M3				1						0,333333	4
M4										0	3

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		3		1			1		0,458333	5
F2	3		2	1		1			0,591667	6
F3		1							0,083333	2
F4	2	4	1						0,822222	7
M1			1			6	5	5	1	8
M2	2								0,333333	3
M3			1						0,333333	4
M4									0	1

Colony 4

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1				1					1	7
F2									0	1
F3									0	2
F4		1							0,5	4
M1						1			0,5	5
M2							2		0,333333	3
M3			1		1	1			0,777778	6
M4			1						1	8

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1	2	2			1		0,633333	6
F2						1		1	0,266667	2
F3									0	1
F4	1	1							0,266667	3
M1		1		1		23	1	4	0,9	8
M2	2		2	3			2		0,611111	5
M3	1		1	4	1	1		2	0,666667	7
M4		2	1				1		0,5	4

Colony 5

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2	1								0,5	5
F3	1	3							1	6
F4									0	2
M1							1		1	7
M2									0	3
M3									0	4
M4						2	1		1	8

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2	1					1			0,666667	5
F3	1	5							1	6
F4									0	2
M1							1		1	7
M2									0	3
M3									0	4
M4						7	1		1	8

Colony 6

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1		2					0,5	5
F2				3					0,5	6
F3	1								1	7
F4	2								0,25	4
M1									0	1
M2					1				1	8
M3									0	2
M4									0	3

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1		2					0,5	4
F2			1	3					0,5	5
F3	1	1		1	2		1		0,9	7
F4	2								0,166667	2
M1							1		0,333333	3
M2					4		12	2	1	8
M3									0	1
M4							5		0,5	6

Colony 7

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1							0,5	3
F2									0	1
F3				1					1	7
F4		1							0,5	4
M1									0	2
M2					1		1		1	8
M3	1				1			1	0,625	6
M4							1		0,5	5

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1							0,5	3
F2									0	1
F3				1					1	8
F4		1							0,5	4
M1									0	2
M2					1		1		0,666667	6
M3	1				1			1	0,625	5
M4						2	1		0,75	7

Colony 8

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2									0	2
F3		1							1	7
F4									0	3
M1									0	4
M2								1	1	8
M3									0	5
M4									0	6

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2			3						0,75	6
F3	1	1		2					0,75	7
F4									0	2
M1									0	3
M2					2		2	4	1	8
M3									0	4
M4									0	5

Colony 9

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2									0	2
F3									0	3
F4		1							1	7
M1									0	4
M2					2				1	8
M3									0	5
M4									0	6

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1			1	1					0,75	7
F2				1					0,5	5
F3	1								0,5	6
F4		1							0,25	4
M1									0	1
M2					2				1	8
M3									0	2
M4									0	3

Colony 10

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2				2					1	7
F3				1					0,5	6
F4									0	2
M1			1						1	8
M2									0	3
M3									0	4
M4									0	5

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1		1							1	7
F2				2					0,5	5
F3				2					0,5	6
F4									0	1
M1			1			2		2	1	8
M2									0	2
M3									0	3
M4									0	4

Colony 11

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2									0	2
F3				1					0,5	7
F4		2	1						0,75	8
M1									0	3
M2									0	4
M3									0	5
M4									0	6

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2				2			1		0,5	5
F3				1					0,5	6
F4		2	1						0,5	7
M1									0	2
M2							3	1	1	8
M3		1							0,25	4
M4									0	3

Colony 12

day 1										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2									0	2
F3									0	3
F4									0	4
M1	1						3		1	8
M2									0	5
M3									0	6
M4									0	7

all days										
Rijlabels	F1	F2	F3	F4	M1	M2	M3	M4	AvgDI	rank
F1									0	1
F2			1						0,25	4
F3	2	1							0,75	5
F4		1							1	6
M1	1						3		1	7
M2									0	2
M3									0	3
M4							4		1	8

6.6 Microscope protocol

Steps

1. Turn on the computer, turn on the microscope, turn on the table
2. Open the program Neurolucida
    a. Acquire → live image
3. Make sure the lens is at x2 (white band) and not at x100 oil (white band)
4. Place the slide in between the placeholders and turn up the light
5. Move the platform up by turning the big rotary knob on the left side of the microscope
    a. Look at the screen and stop turning when the neurons are in focus
6. Move the platform with the joystick to go to the right slice/brain area
    a. Press the button on top of the joystick to move quickly
7. When arrived at the right area, search for good neurons and center it if you found a possible good neuron
    a. Make an overview picture
        i. Make sure the neuron is in focus
        ii. Select marker with number on the left side of the screen and click next to the neuron
        iii. Image → snapshot
        iv. File → save picture as → go to folder location and enter name
8. Zoom up to 10x (yellow band)
    a. Center the neuron, focus with the big rotary knob and turn up the light a bit
    b. Measure distances
        i. Make sure right lens is selected: left top edge picture of a blue 'eye', press arrow and select right lens
        ii. Measure line: trace → measure line → measure line
        iii. Press the two spots you want the distance of
    c. Measure distance of
        i. Distance to cell body
        ii. Length of dendrite
    d. Make overview picture
        i. Make sure the neuron is in focus
        ii. Select marker with number on the left side of the screen and click next to the neuron
        iii. Image → snapshot
        iv. File → save picture as → go to folder location and enter name
9. Zoom up to x20 (green)
    a. Center the neuron, focus with the big rotary knob and turn up the light a bit
10. Zoom up to x40 (light blue)
    a. Center the neuron, focus with the big rotary knob and turn up the light a bit
11. Zoom up to x60 (dark blue)
    - After x10 you could also skip the lenses x20, x40 and go straight to x60, if the neurons are centered right to save some time

    a. Make sure you have the right lens selected in the top left corner

b. Center the neuron, focus with the big rotary knob and turn up the light a bit
c. Click the "contour selection" button at this magnification to begin drawing around the cell body and tracing a line on the dendrite.
d. By clicking on the arrow in the box above the button "contour selection," you can choose different colors for the cell body and dendrite.
e. Click the "Close" button after drawing a line around the cell body. Click the "end open" button after tracing the line on the dendrite.
f. Then, on the top right, go to "tools" and click on the arrow. To separate the dendrites into segments, use the Partition contour option from the “tools” button located on the top right corner.
g. A little box will appear, allowing you to adjust the distance of your segments.

Once the drawing is done and the segment is set, you are ready to move on to the x100 (oil) magnification.

*Make sure a few tiny droplets of oil are placed on the specific brain slice you're investigating before turning the lens to the oil (x100) lens.

12. Zoom up to x100 (white)
*Make sure the top of the screen's magnification button is also set to 100 in the top left corner
    a. Make sure that the drawing is in the center. If not, the joystick or the arrow bars on the keyboard can be used to change it.
    b. You can score each individual spine with the markers on the left side of the screen by clicking on the right marker and next to the spines to mark them
    c. If you score each segment with a different number, you can view the total number of spines without having to count them yourself.
13. Once you are done with the spine counts, note the number of spines per segment. The distance between the cell body and the section where you began counting the spines should also be recorded.
    a. After this make an overview picture of the dendrites with the markers the same way as in step 7
14. Close off the program
    a. Delete the drawing by select all → delete
    b. Turn the big rotary knob to lower the platform
        i. Note: do not change the lens to prevent the oil from getting on the other lenses
    c. Take the object glass off the table and clean the oil from it with alcohol
    d. Turn the lens to the x10 zoom and turn down the light
    e. If you want to continue with another slice you can start again from step 4
    f. If you want to stop the observation
        i. Close the program
        ii. Turn of the light
        iii. Turn off the table
        iv. Turn off the microscope
        v. Clean the oil lens with oil paper (*Any other paper, including kimtech, should not be used since it may damage the lens)
        vi. Put a protective bag over the microscope

6.7 Body weight

6.7.1 Body weight change from day 0 to day 10

	DOMINANT MALE	WEIGHT CHANGE	SUBORDINATE MALE	WEIGHT CHANGE
C1	M16	-4,65	M6	-6,07
C2	M5	-3,78	M1	-1,00
C3	M4	-6,47	M13	-18,61
C4	M2	0,00	M14	-3,85
C5	M46	-6,74	M34	-19,05
C6	M38	-1,50	M41	-3,43
C7	M47	-3,10	M35	-16,08
C8	M39	-4,08	M36	-9,23
C9	M72	-10,88	M65	-7,60
C10	M68	-7,38	M69	-8,71
C11	M70	-5,33	M67	-5,52
C12	M66	-8,10	M71	-7,21

6.7.2 Body weight of dominant and subordinate males throughout the experiment

DOMINANT MALES

DAY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
-13	0	0	0	0	0	0	0	0	0	0	0	0
-12	0,511509	0,726392	2,34375	0,744417	-1,80587	-1,08933	0,455581	-0,69767	0,526316	1,071429	-0,66667	0,450451
-11	2,30179	1,694915	3,385417	1,240695	1,354402	0	-0,22779	0,232558	2,45614	1,428571	0,666667	1,345291
-10	3,069054	2,421308	3,90625	1,736973	0,677201	-0,21787	0	1,627907	2,982456	1,964286	1,777778	0,663717
-9	4,60358	2,179177	6,770833	4,218362	3,386004	-0,21787	0,683371	2,55814	0	-1,42857	-0,22222	-1,31868
-6	4,60358	1,694915	5,729167	3,970223	0,451467	0,217865	-0,22779	0,232558	-0,35088	0	0,444444	2,672606
-5	5,626598	1,937046	7,8125	5,459057	1,580135	0,43573	1,822323	0,465116	-0,35088	0	1,555556	0,21692
-4	6,649616	2,663438	7,8125	6,203474	1,580135	1,089325	3,644647	1,162791	0,701754	1,071429	1,333333	0,21645
-3	7,161125	3,1477	8,854167	7,19603	3,386004	0,653595	2,733485	2,093023	0,526316	1,428571	1,333333	0,431965
-2	6,905371	2,905569	8,59375	7,19603	3,837472	1,960784	4,328018	2,093023	0	0,357143	1,111111	0,430108
-1	7,672634	2,179177	8,59375	7,940447	4,288939	2,832244	4,783599	2,790698	-0,17544	1,071429	1,111111	0,856531
0	4,60358	2,421308	8,59375	4,962779	3,837472	1,960784	2,961276	2,55814	0	1,607143	0	-0,42463
2	1,534527	0,242131	1,822917	4,71464	0,451467	0,653595	0,22779	-0,69767	-5,08772	-2,67857	-4,22222	-4,26439
5	-1,53453	-4,35835	1,5625	1,736973	-3,16027	-1,96078	-0,91116	-2,32558	-7,36842	-4,10714	-4,88889	-1,78174
8	-0,25575	-2,66344	1,302083	3,473945	-1,12867	-0,43573	-1,13895	-2,09302	-11,2281	-6,07143	-5,55556	-3,40136
10	-0,25575	-1,45279	1,5625	4,962779	-3,16027	0,43573	-0,22779	-1,62791	-10,8772	-5,89286	-5,33333	1,173709

DOMINANT FEMALES

DAY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
-13	0	0	0	0	0	0	0	0	0	0	0	0
-12	0,487805	0,454545	1,895735	1,731602	-0,8547	-2,89855	4,020101	6,111111	0	-1,96078	-1,35747	0,4830918
-11	-0,4878	0,909091	1,895735	-0,4329	-4,70086	1,449275	1,507538	5,555555	-0,96154	-0,78431	-2,26244	3,381643
-10	1,95122	0,909091	1,421801	1,298701	-2,99145	-1,44928	2,01005	6,111111	0,961538	0	-1,80996	3,381643
-9	2,439024	1,818182	0,947867	-1,2987	-2,5641	-1,44928	2,01005	10	0	-2,35294	-0,90498	-1,449275
-6	3,414634	0,909091	4,739336	3,896104	-4,70086	1,932367	3,517588	10	0,480769	0,392157	0	7,246377
-5	9,268292	2,727273	3,791469	5,194805	-1,28205	2,415459	2,512563	7,777778	-1,44231	-0,39216	0,904977	8,212561
-4	8,780488	5,454545	4,739336	7,359307	4,700855	7,246377	5,527638	13,88889	1,442308	0,784314	5,882353	12,56039
-3	11,70732	5,454545	8,530806	10,82251	5,982906	8,212561	9,547739	13,88889	4,807693	0,784314	9,049774	12,07729
-2	11,70732	8,181818	7,582938	9,523809	7,264957	9,661836	10,05025	16,66667	5,288462	1,960784	6,78733	14,00966
-1	8,780488	8,181818	9,952606	12,55411	6,410256	10,14493	14,07035	17,77778	9,134615	7,058824	13,12217	15,45894
0	9,268292	4,090909	9,00474	10,82251	6,410256	8,695652	11,05528	19,44444	8,653846	5,098039	13,12217	12,07729
2	12,19512	9,090909	11,84834	12,12121	4,273504	14,00966	14,57286	17,77778	6,25	1,176471	9,502262	12,56039
5	11,21951	10,45455	11,84834	11,68831	5,982906	19,32367	15,57789	16,11111	9,615385	3,529412	14,02715	12,07729
8	15,12195	9,545455	15,16588	12,98701	11,53846	21,25604	16,58291	21,66667	12,01923	7,843137	16,28959	17,3913
10	19,5122	10,90909	18,95735	13,85281	11,96581	25,12077	14,57286	22,22222	14,42308	8,235294	16,74208	18,35749

SUBORDINATE MALES

DAY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
-13	0	0	0	0	0	0	0	0	0	0	0	0
-12	0,5	1,28866	-0,6424	0,852879	0,194932	0,884956	0,635593	-0,25253	-0,20243	1,082251	-0,42194	1,605505
-11	1,5	2,319588	0,642398	1,279318	1,169591	1,548673	1,694915	0,757576	1,214575	0,649351	1,265823	3,211009
-10	2	3,865979	1,713062	1,918977	-0,19493	2,212389	0,423729	0,757576	1,417004	1,515152	1,054852	3,899083
-9	4,5	5,927835	4,496788	1,066098	1,754386	3,318584	1,483051	-0,75758	0,202429	-0,4329	0,210971	2,522936
-6	5	2,835052	-0,85653	1,918977	0,779727	1,548673	-1,90678	0,505051	0,202429	2,813853	-0,21097	3,899083
-5	6	4,381444	-0,42827	1,492537	1,754386	1,769912	0,635593	1,262626	-0,40486	2,380952	0,421941	3,440367
-4	4,25	4,639175	0,642398	2,345416	3,118908	4,646018	-0,84746	1,767677	-0,60729	2,597403	0	3,440367
-3	3	5,154639	0,428266	2,345416	5,45809	4,20354	1,694915	1,262626	-0,80972	4,329004	1,054852	4,587156
-2	3	4,896907	0,642398	2,345416	4,483431	4,424779	2,966102	2,525253	0,202429	3,896104	1,687764	5,045872
-1	2,5	6,443299	1,498929	2,132196	4,873294	4,646018	2,542373	2,525253	2,024292	5,627706	3,797468	5,275229
0	3	3,608248	-1,07066	-0,42644	2,339181	3,097345	1,483051	1,262626	1,214575	4,329004	3,164557	5,045872
2	-3,5	1,546392	-8,99358	-4,05117	0,779727	2,212389	-6,35593	-5,55556	-0,60729	-1,7316	-1,68776	2,752294
5	-5,5	-0,51546	-21,4133	-6,60981	-7,40741	-1,54867	-15,2542	-8,33333	-7,08502	-1,51515	-3,37553	0
8	-3	1,546392	-23,9829	-5,11727	-11,501	-1,1062	-15,8898	-7,32323	-7,69231	-4,76191	-3,16456	-2,06422
10	-3,25	2,57732	-19,4861	-4,26439	-17,154	-0,44248	-14,8305	-8,08081	-6,47773	-4,76191	-2,53165	-2,52294

SUBORDINATE FEMALES

DAY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
-13	0	0	0	0	0	0	0	0	0	0	0	0
-12	0	-1,87793	1,646091	1,766784	-3,38164	2,072539	-1,86916	-2,77778	-1,31004	-1,8018	-1,97044	-0,45045
-11	-2,89855	-0,46948	2,469136	2,473498	0	1,554404	0,934579	-2,77778	0	-1,8018	-2,46305	3,153153
-10	0	1,408451	0,411523	3,180212	-1,93237	3,108808	-1,86916	-1,66667	3,056769	-2,7027	0,492611	4,504505
-9	0	0,938967	1,646091	2,120141	-0,96618	5,699482	-4,6729	-1,66667	2,620087	-3,6036	-0,49261	2,252252
-6	2,898551	6,103286	0,411523	3,180212	0,483092	6,735751	-3,27103	-2,22222	5,676856	1,801802	4,433497	3,153153
-5	4,830918	5,633803	0	3,886926	1,932367	3,626943	-3,73832	-0,55556	3,49345	1,351351	4,433497	3,153153
-4	5,31401	7,042253	2,880658	2,120141	4,830918	11,9171	-3,27103	4,444445	5,240175	0,450451	5,91133	5,855856
-3	7,246377	7,511737	2,057613	1,413428	6,763285	15,02591	-2,33645	8,333333	10,48035	2,252252	8,866995	4,954955
-2	8,212561	9,389671	4,526749	1,413428	7,729469	15,02591	-0,46729	8,888889	8,733624	4,504505	8,866995	6,306306
-1	11,11111	7,981221	3,703704	4,240283	8,695652	17,09845	-0,46729	10,55556	8,296944	5,855856	13,30049	7,657658
0	5,797101	9,389671	2,880658	0,706714	6,280193	15,02591	1,869159	9,444445	11,35371	2,702703	10,34483	6,306306
2	10,62802	9,389671	3,703704	5,65371	13,04348	16,06218	7,476635	11,11111	13,10044	5,855856	7,389163	6,756757
5	9,661836	9,389671	7,81893	7,420495	8,695652	15,54404	6,542056	11,66667	13,53712	8,108109	13,30049	6,756757
8	15,45894	11,73709	12,7572	6,007067	13,52657	23,31606	10,74766	15	9,606987	9,009009	19,21182	8,108109
10	16,42512	12,67606	13,58025	8,833922	15,45894	25,3886	12,14953	17,77778	12,66376	12,61261	20,68966	9,009009

6.8 Intensity of aggression

6.8.1 proportion of fierce fights per animal per colony

COLONY 1 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	30	3	33	0,090909
F2	34	5	39	0,128205
F3	23	4	27	0,148148
F4	35	5	40	0,125
M1	6	13	19	0,684211
M2	16	7	23	0,304348
M3	4	7	11	0,636364
M4	14	17	31	0,548387

COLONY 2 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	7	2	9	0,222222
F2	11	0	11	0
F3	6	1	7	0,142857
F4	17	0	17	0
M1	10	5	15	0,333333
M2	12	8	20	0,4
M3	12	0	12	0
M4	9	0	9	0

COLONY 3 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	12	4	16	0,25
F2	16	4	20	0,2
F3	14	2	16	0,125
F4	18	0	18	0
M1	15	11	26	0,423077
M2	20	1	21	0,047619
M3	1	10	11	0,909091
M4	5	4	9	0,444444

COLONY 4 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	19	4	23	0,173913
F2	24	4	28	0,142857
F3	13	3	16	0,1875
F4	28	6	34	0,176471
M1	37	2	39	0,051282
M2	42	9	51	0,176471
M3	22	4	26	0,153846
M4	24	0	24	0

COLONY 5 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	16	1	17	0,058824
F2	28	0	28	0
F3	14	1	15	0,066667
F4	17	0	17	0
M1	0	0	0	0
M2	7	3	10	0,3
M3	2	1	3	0,333333
M4	4	2	6	0,333333

COLONY 6 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	7	2	9	0,222222
F2	9	2	11	0,181818
F3	9	1	10	0,1
F4	13	2	15	0,133333
M1	8	2	10	0,2
M2	11	1	12	0,083333
M3	15	1	16	0,0625
M4	7	1	8	0,125

COLONY 7 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	11	0	11	0
F2	14	0	14	0
F3	5	1	6	0,166667
F4	7	1	8	0,125
M1	2	0	2	0
M2	2	1	3	0,333333
M3	4	1	5	0,2
M4	2	0	2	0

COLONY 8 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	10	1	11	0,090909
F2	14	3	17	0,176471
F3	15	8	23	0,347826
F4	3	3	6	0,5
M1	1	0	1	0
M2	11	0	11	0
M3	2	0	2	0
M4	9	0	9	0

COLONY 9 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	17	8	25	0,32
F2	15	5	20	0,25
F3	7	6	13	0,461538
F4	9	3	12	0,25
M1	0	4	4	1
M2	0	5	5	1
M3	0	0	0	0
M4	0	0	0	0

COLONY 10 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	17	6	23	0,26087
F2	16	6	22	0,272727
F3	10	1	11	0,090909
F4	9	4	13	0,307692
M1	3	1	4	0,25
M2	2	0	2	0
M3	1	0	1	0
M4	2	1	3	0,333333

COLONY 11 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	11	2	13	0,153846
F2	26	24	50	0,48
F3	19	14	33	0,424242
F4	20	21	41	0,512195
M1	0	0	0	0
M2	14	8	22	0,363636
M3	5	5	10	0,5
M4	1	1	2	0,5

COLONY 12 ID	SUM OF MILD ATTACK	SUM OF FIERCE ATTACK	TOTAL	PORPORTION OF FIERCE FIGHTS PER ANIMAL
F1	7	4	11	0,363636
F2	7	1	8	0,125
F3	17	8	25	0,32
F4	11	6	17	0,352941
M1	5	10	15	0,666667
M2	9	4	13	0,307692
M3	25	7	32	0,21875
M4	6	2	8	0,25

6.8.2 Average proportion of fierce fights per colony

COLONY	INTENSITY AGGRESSION
COLONY 1	0,333196
COLONY 2	0,137302
COLONY 3	0,299904
COLONY 4	0,132792
COLONY 5	0,13652
COLONY 6	0,138526
COLONY 7	0,103125
COLONY 8	0,139401
COLONY 9	0,410192
COLONY 10	0,147775
COLONY 11	0,36674
COLONY 12	0,325586

6.9 Spine count of dominant and subordinate males

6.9.1 Total spine count of 5 to 6 dendrites of dominant and subordinate males

Dominant males

Animal ID	M16	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	80-90	7	10	13	8	9	8	6	7	**68**	77
Colony	**1**	2	90	6	11	8	13	12	8	5	7	**70**	
Scorer ID	**D**	3	60-70	3	10	6	9	11	11	11	3	**64**	
		4	100-110	13	14	14	14	14	9	14	12	**104**	
		5	50-60	8	4	10	13	12	13	9	10	**79**	

Animal ID	M5	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	50-60	12	11	11	9	12	11	8	8	**82**	77.2
Colony	**2**	2	100	13	8	10	11	9	7	11	4	**73**	
Scorer ID	**D**	3	60	6	12	8	9	10	8	11	13	**77**	
		4	80-90	13	13	8	13	11	10	8	7	**83**	
		5	50-60	8	9	10	7	9	11	7	10	**71**	

Animal ID	M4	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	90-100	12	12	15	11	11	11	16	12	**100**	78.8
Colony	**3**	2	60-70	10	11	10	9	9	11	8	8	**76**	
Scorer ID	**D**	3	50-60	7	5	13	13	14	9	9	10	**80**	
		4	80-90	7	10	8	6	7	9	9	5	**61**	
		5	50-60	10	10	10	11	11	7	10	8	**77**	

Animal ID	M2	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	45	8	6	9	13	11	10	8	10	75	67.6
Colony	**4**	2	30	5	9	8	7	8	7	7	7	58	
Scorer ID	**AM**	3	25	3	7	5	8	7	9	5	6	75	
		4	20	5	1	6	6	9	4	6	7	64	
		5	5	2	8	11	9	8	9	8	6	66	
		6	10	0	0	7	6	7	6	8	3	47	

Animal ID	M38	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**2**	1	5	5	9	11	10	13	5	9	6	**68**	82.4
Colony	**6**	2	10	1	6	10	17	14	12	16	14	**90**	
Scorer ID	**AM**	3	35	4	7	5	10	14	8	10	13	**106**	
		4	20	2	8	9	10	9	9	5	4	**76**	
		5	10	3	5	7	10	12	6	8	11	**72**	
		6	10	6	12	9	12	9	7	11	8	**84**	

Animal ID	M47	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	2	1	60	6	9	11	6	9	7	6	6	**60**	69.2
Colony	7	2	50-60	5	6	9	9	4	7	9	5	**54**	
Scorer ID	D	3	60	5	8	11	11	11	11	13	9	**79**	
		4	70-80	11	12	11	13	11	10	8	11	**87**	
		5	50	8	8	7	4	7	11	9	12	**66**	

Animal ID	M39	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**2**	1	30	6	4	9	10	8	11	8	8	**64**	73.4
Colony	**8**	2	50	9	11	14	14	12	11	8	8	**87**	
Scorer ID	**AM**	3	20	11	9	9	10	12	11	9	10	**101**	
		4	5	0	3	6	10	5	6	5	4	**44**	
		5	30	1	5	2	9	8	8	3	5	**71**	
		6	15	3	7	6	9	6	5	3	8	**62**	

Animal ID	M72	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	70-80	15	12	12	10	12	12	11	10	**94**	97
Colony	**9**	2	50-60	9	9	17	7	11	11	8	11	**83**	
Scorer ID	**D**	3	90-100	18	18	17	18	12	15	15	14	**127**	
		4	50-60	13	13	10	8	12	11	9	11	**87**	
		5	70-80	13	12	11	13	10	11	12	12	**94**	

Animal ID	M68	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	90-100	8	10	9	7	9	9	17	11	**80**	73.2
Colony	**10**	2	70-80	7	6	11	7	12	6	9	10	**68**	
Scorer ID	**D**	3	50	6	4	11	7	11	9	10	8	**66**	
		4	80-90	12	8	11	10	11	12	7	7	**78**	
		5	90-100	12	13	10	8	10	7	7	7	**74**	
		6											

Animal ID	M66	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	15	2	4	7	7	9	11	10	11	61	84.4
Colony	**12**	2	35	10	13	10	8	14	14	13	9	91	
Scorer ID	**AM**	3	20	9	9	11	15	12	11	6	11	104	
		4	10	5	9	10	12	12	8	8	7	81	
		5	10	3	8	9	11	11	7	15	11	85	
		6	15	7	9	11	8	11	11	11	10	93	

Subordinate males

Animal ID	M6	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	50	8	12	13	17	10	11	10	10	**91**	85.4
Colony	**1**	2	30	3	6	12	13	12	8	12	10	**76**	
Scorer ID	**D**	3	100	11	12	14	11	13	11	13	10	**95**	
		4	100	11	11	12	11	12	8	10	9	**84**	
		5	90	11	10	10	11	11	10	9	9	**81**	

Animal ID	M1	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	60-70	8	9	14	8	10	10	7	10	**76**	78.4
Colony	**2**	2	60-70	13	8	7	12	10	11	11	9	**81**	
Scorer ID	**D**	3	90-100	12	11	7	9	8	8	8	13	**76**	
		4	90-100	11	13	10	9	11	12	10	7	**83**	
		5	50-60	7	13	11	6	12	10	7	10	**76**	

Animal ID	M13	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	50	9	7	15	9	11	12	11	11	**85**	73.4
Colony	**3**	2	80-90	9	9	4	5	12	11	10	9	**69**	
Scorer ID	**D**	3	40	7	9	6	11	10	7	9	6	**65**	
		4	120	12	7	10	10	11	9	12	9	**80**	
		5	50	5	11	11	7	8	7	9	10	**68**	

Animal ID	M14	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**1**	1	15	1	5	4	8	6	7	6	7	**44**	56.4
Colony	**4**	2	25	2	6	9	8	10	4	11	8	**58**	
Scorer ID	**AM**	3	25	2	4	7	11	11	4	5	9	**78**	
		4	5	1	3	3	7	11	5	8	5	**48**	
		5	10	2	4	6	6	5	7	8	6	**54**	
		6	50	7	15	8	11	10	11	8	8	**128**	

Animal ID	M41	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**2**	1	25	6	7	8	12	8	7	9	12	69	78.4
Colony	**6**	2	20	9	7	9	3	5	2	3	4	42	
Scorer ID	**AM**	3	45	3	9	11	8	8	5	5	3	97	
		4	15	5	4	12	11	16	9	10	13	95	
		5	10	3	5	11	12	8	15	15	10	89	
		6	20	2	5	8	12	8	8	8	10	81	

Animal ID	M35	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**2**	1	50-60	4	4	7	10	10	5	8	6	**54**	72.4
Colony	**7**	2	100-110	14	9	7	11	11	10	9	9	**80**	
Scorer ID	**D**	3	80-90	9	10	11	10	11	14	10	11	**86**	
		4	50	6	8	7	8	11	11	8	11	**70**	
		5	50	8	6	8	7	10	13	9	11	**72**	

Animal ID	M65	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	60-70	9	12	18	12	12	10	13	9	**95**	86
Colony	**9**	2	60-70	8	5	8	12	11	8	11	12	**75**	
Scorer ID	**D**	3	40-50	8	11	13	10	13	10	9	13	**87**	
		4	60	9	11	14	9	10	8	7	12	**80**	
		5	60-70	11	12	12	11	9	13	12	13	**93**	

Animal ID	M69	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	60-70	11	12	14	10	10	12	13	18	**100**	92.2
Colony	**10**	2	50	12	10	11	12	12	13	4	14	**88**	
Scorer ID	**D**	3	60-70	11	10	11	8	7	9	7	10	**73**	
		4	90-100	15	13	11	13	12	12	13	10	**99**	
		5	90-100	15	16	13	13	10	9	14	11	**101**	

Animal ID	M67	neuron	Distance to celbody (µm)	10 µm	20 µm	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	Total spines/neuron	Average of total spine count of 5 neurons
Batch	**3**	1	45	5	7	10	11	13	14	11	12	83	89
Colony	**11**	2	25	4	4	9	13	12	6	10	12	70	
Scorer ID	**AM**	3	40	11	5	8	4	13	8	9	10	108	
		4	10	6	7	10	9	9	11	11	9	82	
		5	30	5	6	9	10	13	9	12	8	102	
		6	35	4	6	12	14	12	8	10	9	110	

6.9.2 Average of total spine count of 5 dendrites per male of dominant and subordinate males

	DOMINANT	AVERAGE SPINE COUNT	SUBORDINATE	AVERAGE SPINE COUNT
C1	M16	77	M6	85.4
C2	M5	77.2	M1	78.4
C3	M4	78.8	M13	73.4
C4	M2	67.6	M14	56.4
C5	M46	-	M34	-
C6	M38	82.4	M41	78.4
C7	M47	69.2	M35	72.4
C8	M39	73.4	M36	-
C9	M72	97	M65	86
C10	M68	73.2	M69	92.2
C11	M70	-	M67	89
C12	M66	84.4	M71	-

6.10 Stability data of the colonies

6.10.1 ranks of animals at days 0 and 10

COLONY 1

ANIMAL	rank day 1	rank day 10
F1	5	5
F2	6	6
F3	1	3
F4	7	7
M1	2	1
M2	3	2
M3	4	4
M4	8	8

COLONY 2

ANIMAL	rank day 1	rank day 10
F1	1	4
F2	2	3
F3	7	7
F4	6	5
M1	3	1
M2	8	8
M3	4	2
M4	5	6

COLONY 3

ANIMAL	rank day 1	rank day 10
F1	5	5
F2	6	6
F3	1	2
F4	7	7
M1	8	8
M2	2	3
M3	4	4
M4	3	1

COLONY 4

ANIMAL	rank day 1	rank day 10
F1	7	6
F2	1	2
F3	2	1
F4	4	3
M1	5	8
M2	3	5
M3	6	7
M4	8	4

COLONY 5

ANIMAL	rank day 1	rank day 10
F1	1	1
F2	5	5
F3	6	6
F4	2	2
M1	7	7
M2	3	3
M3	4	4
M4	8	8

COLONY 6

ANIMAL	rank day 1	rank day 10
F1	5	4
F2	6	5
F3	7	7
F4	4	2
M1	1	3
M2	8	8
M3	2	1
M4	3	6

COLONY 7

ANIMAL	rank day 1	rank day 10
F1	3	3
F2	1	1
F3	7	8
F4	4	4
M1	2	2
M2	8	6
M3	6	5
M4	5	7

COLONY 8

ANIMAL	rank day 1	rank day 10
F1	1	1
F2	2	6
F3	7	7
F4	3	2
M1	4	3
M2	8	8
M3	5	4
M4	6	5

COLONY 9

ANIMAL	rank day 1	rank day 10
F1	1	7
F2	2	5
F3	3	6
F4	7	4
M1	4	1
M2	8	8
M3	5	2
M4	6	3

COLONY 10

ANIMAL	rank day 1	rank day 10
F1	1	7
F2	7	5
F3	6	6
F4	2	1
M1	8	8
M2	3	2
M3	4	3
M4	5	4

COLONY 11

ANIMAL	rank day 1	rank day 10
F1	1	1
F2	2	5
F3	7	6
F4	8	7
M1	3	2
M2	4	8
M3	5	4
M4	6	3

COLONY 12

ANIMAL	rank day 1	rank day 10
F1	1	1
F2	2	4
F3	3	5
F4	4	6
M1	8	7
M2	5	2
M3	6	3
M4	7	8

6.10.2 stability values per colony

COLONY	CORRELATION VALUE	P VALUE
1	0,857	0,003
2	0,571	0,048
3	0,857	0,003
4	0,357	0,216
5	1	0,005
6	0,643	0,026
7	0,786	0,006
8	0,714	0,013
9	0	1
10	0,5	0,083
11	0,429	0,138
12	0,5	0,083

6.11 Female dominance values

FEMDOM OVERVIEW	
COLONY	**Female dominance**
COLONY 1	0,6875
COLONY 2	0,59375
COLONY 3	0,625
COLONY 4	0,125
COLONY 5	0,4375
COLONY 6	0,5625
COLONY 7	0,40625
COLONY 8	0,5625
COLONY 9	0,75
COLONY 10	0,6875
COLONY 11	0,625
COLONY 12	0,5

6.12 Statistics

6.12.1 Normality tests and outcomes

Test of normality	**P value**
Spine density dominant males	**0.012***
Spine density subordinate males	0.08
Body weight dominant males	1.00
Body weight subordinate males	0.116
Time spent in arena dominant males	0.687
Time spent in arena subordinate males	**0.000***
Adrenal weight dominant males	0.831
Adrenal weight subordinate males	0.174
Thymus weight dominant males	0.645
Thymus weight subordinate males	0.805
Fat weight dominant males	0.126
Fat weight subordinate males	0.580
Testes weight dominant males	0.665
Testes weight subordinate males	0.119
Seminal vesicle weight dominant males	0.094
Seminal vesicle weight subordinate males	0.921
Corticosterone dominant male pre VBS	0.471
Corticosterone dominant male post VBS	0.351
Corticosterone subordinate male pre VBS	0.401
Corticosterone subordinate male post VBS	0.580

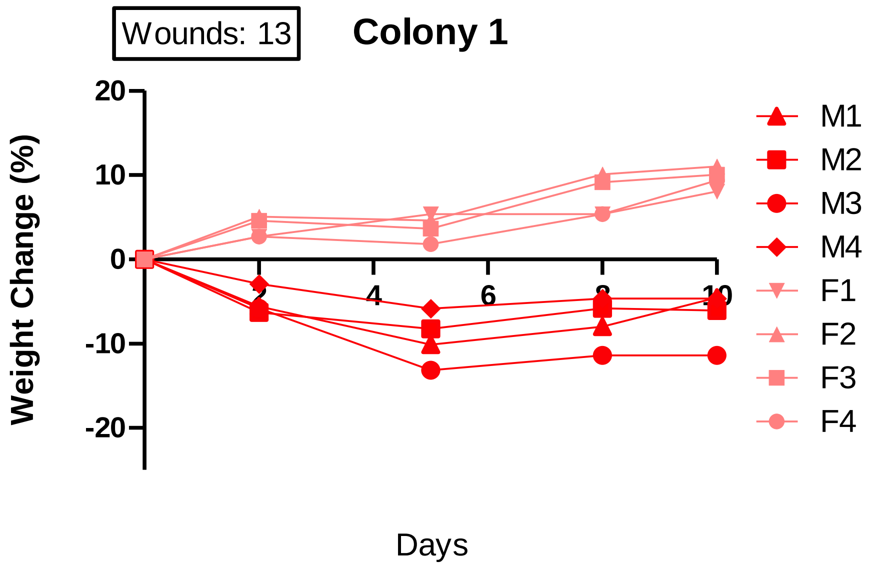
6.12.2 Tests of comparisons of means and outcomes

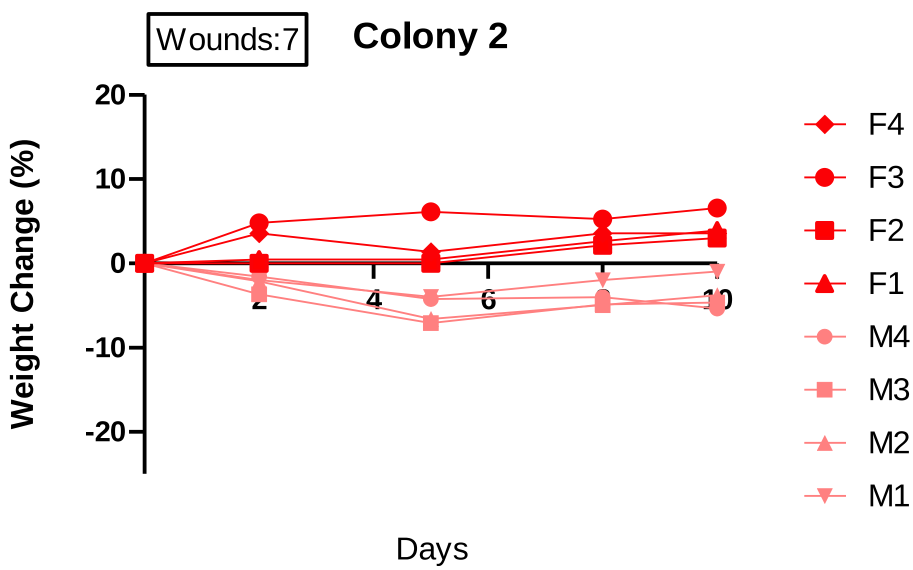
	Test	P value
Spine density subordinate vs dominant males	Non parametric two sided related-samples Wilcoxon signed rank test	0.816
Body weight subordinate vs dominant males	One sided paired-samples T-test	**0.025***
Time spent in arena subordinate vs dominant males	Non parametric one sided related-samples Wilcoxon signed rank test	**0.0015***
Corticosterone levels dominant males pre vs post VBS	Two sided paired-samples T-test	0.186
Corticosterone levels subordinate males pre vs post VBS	Two sided paired-samples T-test	0.434
Corticosterone levels pre VBS of subordinate and dominant males	Two sided paired-samples T-test	0.584
Corticosterone levels pre VBS of subordinate and dominant males	Two sided paired-samples T-test	0.455
Adrenal weight dominant vs subordinate males	Two sided paired-samples T-test	0.402
Thymus weight dominant vs subordinate males	Two sided paired-samples T-test	0.198
Fat weight dominant vs subordinate males	One sided paired-samples T-test	**0.003***
Seminal vesicle weight dominant vs subordinate males	Two sided paired-samples T-test	0.239
Testes weight dominant vs subordinate males	Two sided paired-samples T-test	0.197
Amount of fights of males over time	One sided Jonckheere-Terpstra test	0,0365

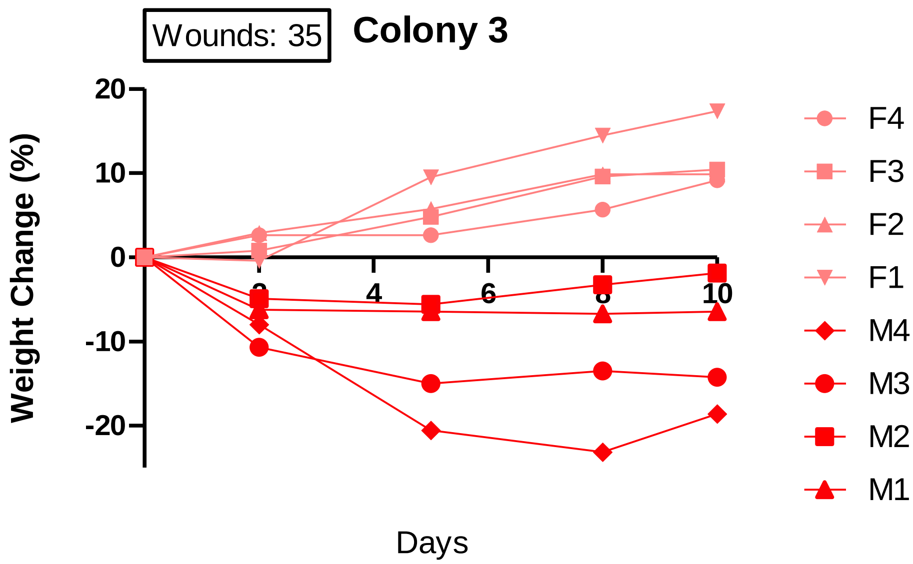
6.12.3 Correlation tests and outcomes

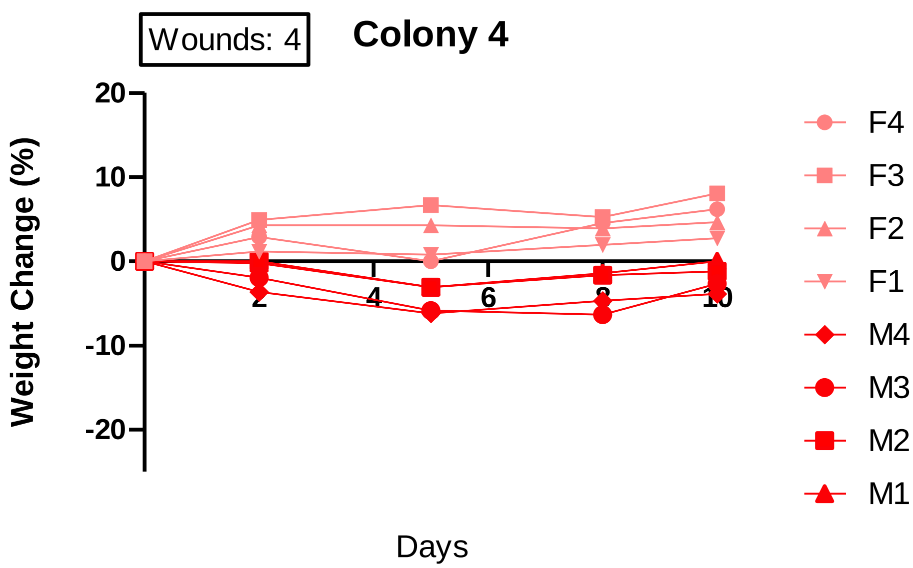
	One or two tailed	Correlation value	P value
Stability and Body weight of dominant males	One tailed	0.031	0.445
Stability and body weight of subordinate males	One tailed	-0.431	**0.027**
Intensity of aggression and body weight of dominant males	One tailed	-0.515	**0.01**
Intensity of aggression and body weight of subordinate males	One tailed	0.091	0.34
Female dominance and body weight of dominant males	Two tailed	-0.45	0.045
Female dominance and body weight of subordinate males	Two tailed	0.047	0.836
Female dominance and intensity of aggression	One tailed	0.636	**0.002**
Intensity of aggression and spine density	One tailed	0.584	**0.00032**
Stability and spine density	One tailed	-0.166	0.17
Female dominance and spine density	Two tailed	0.296	0.241
Stability and intensity of aggression	Two tailed	-0.246	0.27

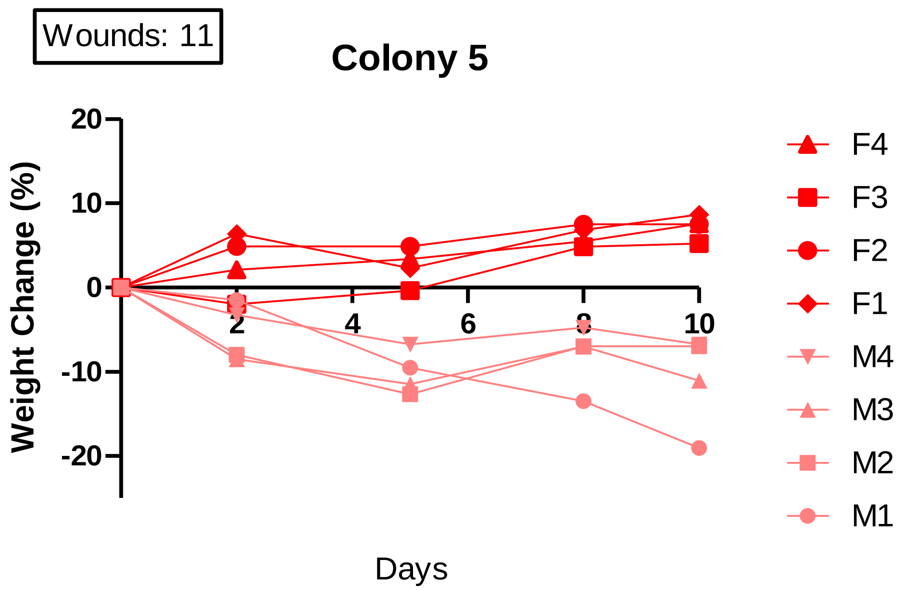
6.13 Body weight of dominant and subordinate males over time spent in the VBS plus body wounds

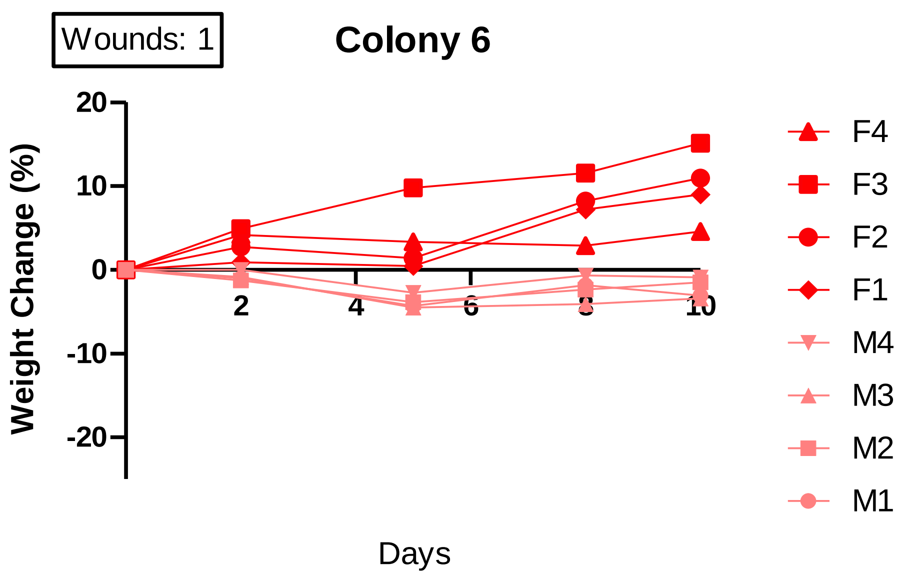


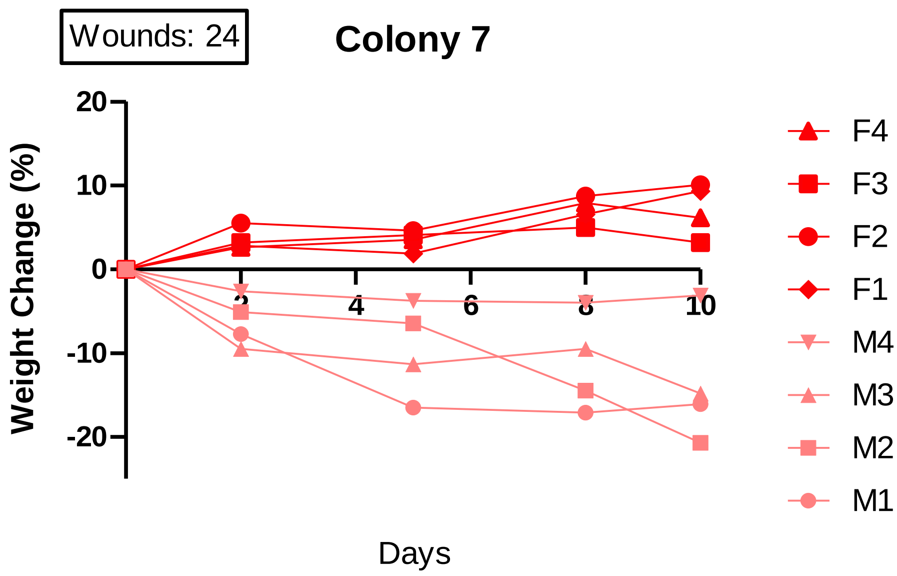


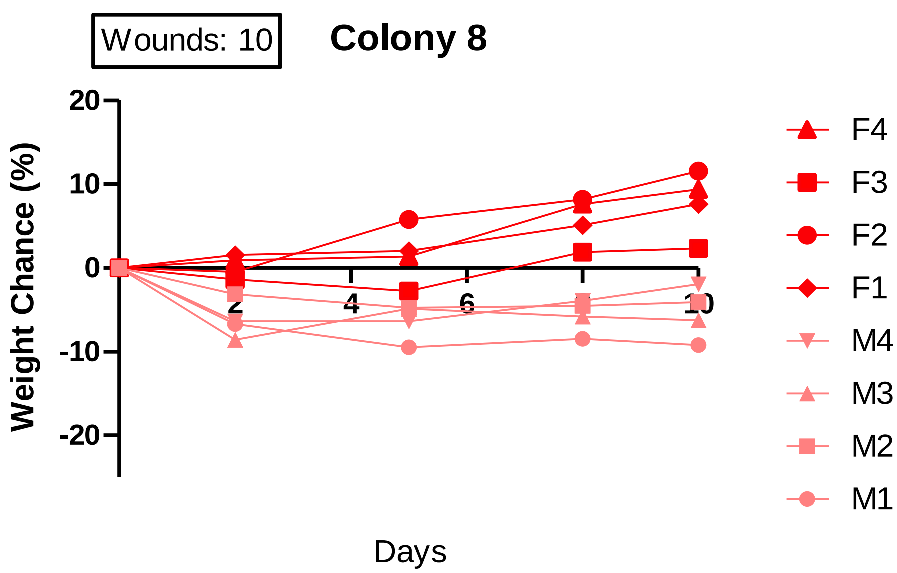

Wounds: 35
Colony 3
Weight Change (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1

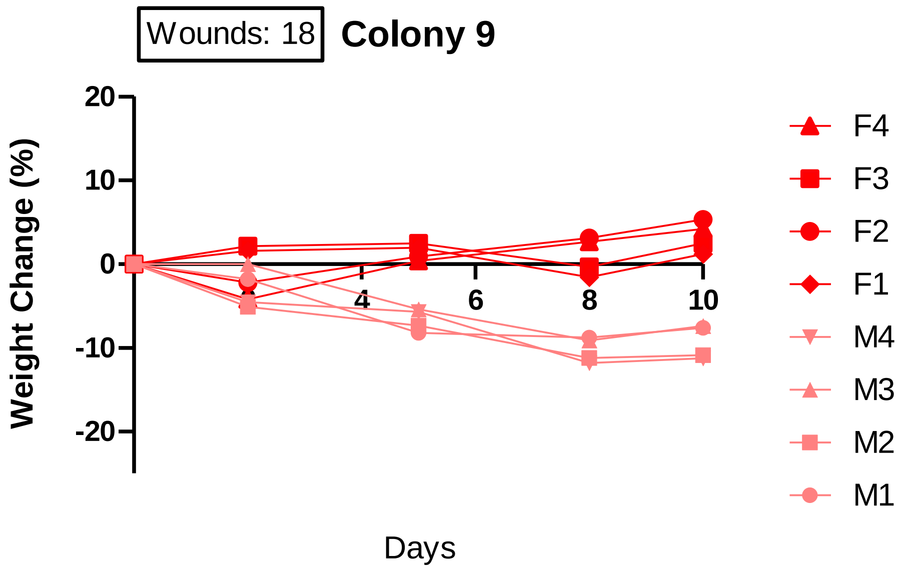

Wounds: 4
Colony 4
Weight Change (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1

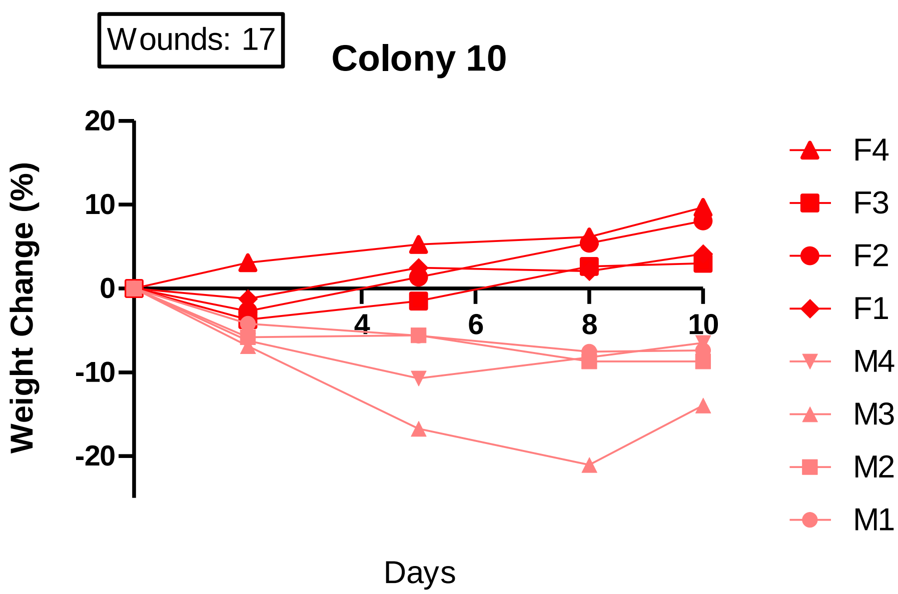

Wounds: 11
Colony 5
Weight Change (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1

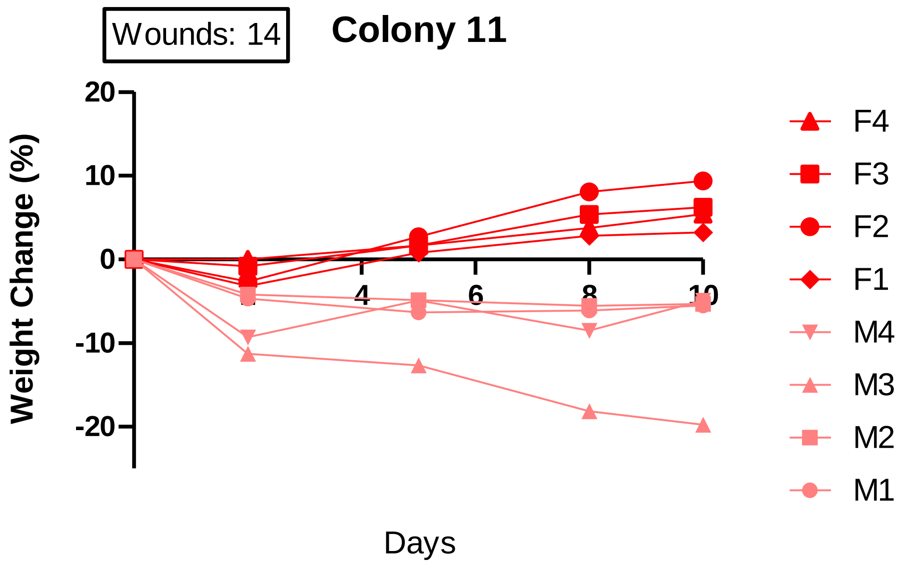

Wounds: 1
Colony 6
Weight Change (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1

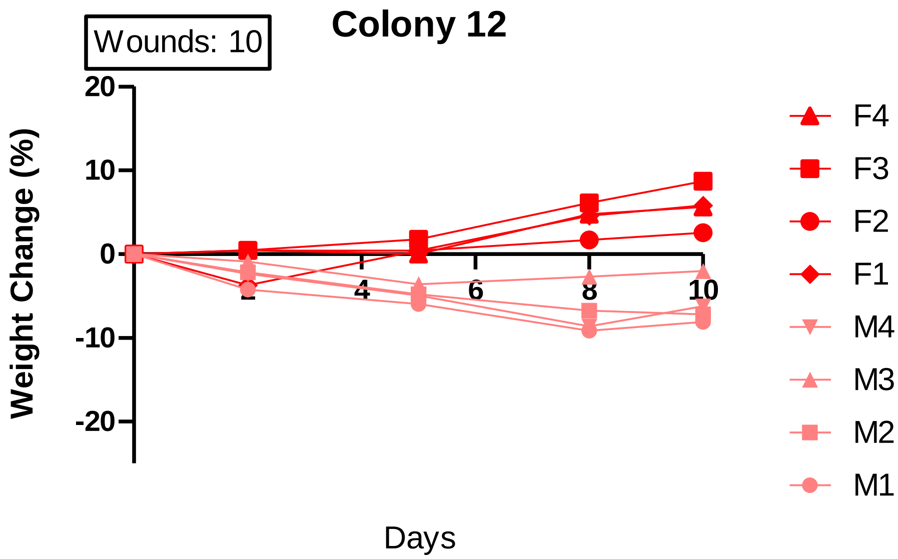

Wounds: 24
Colony 7
Weight Change (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1


Wounds: 10
Colony 8
Weight Chance (%)
20
10
0
-10
-20
2
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1


Wounds: 18
Colony 9
Weight Change (%)
20
10
0
-10
-20
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1


Wounds: 17
Colony 10
Weight Change (%)
20
10
0
-10
-20
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1


Wounds: 14
Colony 11
Weight Change (%)
20
10
0
-10
-20
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1


Wounds: 10
Colony 12
Weight Change (%)
20
10
0
-10
-20
4
6
8
10
Days
F4
F3
F2
F1
M4
M3
M2
M1