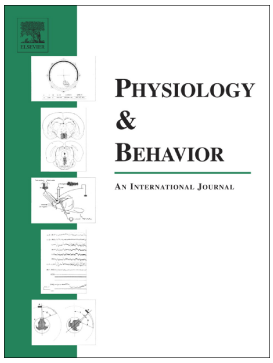
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Stress coping style does not determine social status, but influences the consequences of social subordination stress.

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Abstract:

Chronic stress exposure may have negative consequences for health. One of the most common sources of chronic stress is stress associated with social interaction. In rodents, the effects of social stress can be studied in a naturalistic way using the visual burrow system (VBS). The way an individual copes with stress, their "stress coping style", may influence the consequences of social stress. In the current study we tested the hypothesis that stress coping style may modulate social status and influence the consequences of having a lower social status.

We formed 7 VBS colonies, with 1 proactive coping male, 1 passive coping male, and 4 female rats per colony to assess whether a rat's coping style prior to colony formation could predict whether that individual is more likely to become socially dominant. The rats remained in their respective colonies for 14 days and the physiological and behavioral consequences of social stress were assessed.

Our study shows that stress coping style does not predict social status. However, stress coping style may influence the consequences of having a lower social status. Subordinate passive and proactive rats had distinctly different wound patterns; proactive rats had more wounds on the front of their bodies. Behavioral analysis confirmed that proactive subordinate rats engaged in more offensive interactions. Furthermore, subordinate rats with a proactive stress coping style had larger adrenals, and increased stress responsivity to a novel acute stressor (restraint stress) compared to passive subordinate rats or dominant rats, suggesting that the allostatic load may have been larger in this group.

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Introduction:

The body responds to environmental threats by increasing activity of the sympathetic nervous system and activating the hypothalamus-pituitary-adrenal axis. The activation of these two stress-related systems leads to physiological and biological responses that help the animal cope with environmental conditions. As defined by Selye [1, 2], stress is the non-specific response of the body to any demand for change. As such it is an adaptive, not necessarily negative response. However, in the current society the word 'stress' often has a negative connotation, due to the potentially pathological consequences of chronic exposure to stressors. In contrast to acute stress, chronic stress exposure may have negative consequences and has been associated with increased risk for several psychiatric and metabolic pathologies (reviewed in [3, 4]). Different types of environmental cues can result in a stress response, but one of the most common chronic stressors in humans and other social-living animals are stressors related to social interactions. Studying the physiological consequences of social stress in the human is complicated, due to limited ability to control their living environment, therefore animal models, such as rodents and non-human primates, are frequently employed in studies focused on social stress.

To study social stress in rodents, a laboratory model that mimics the natural environment is valuable. The natural habitat of the rat consists of underground tunnels and burrows in which rats cohabitate in mixed-sex groups [5, 6]. To allow for well-controlled studies of social behavior in a semi-naturalistic environment in the laboratory the visible burrow system (VBS) was developed by Bob and Caroline Blanchard at the University of Hawaii [7]. The VBS consists of clear Plexiglas tunnels that connect chambers with clear tops to allow for constant monitoring of the animals' behavior. Using the VBS set-up the consequences of social stress could be studied. In addition, this set-up allowed for studies looking into parameters that determine social status. The weight and size of the rat are important predictors of social dominance, with larger and heavier animals having a high chance of social dominance [8]. However, other predictors of social status have not been as clear. For example, data on the role of aggression in achieving social dominance is conflicting and depends on age, and testing circumstances [9]. There are reports showing that rats with higher levels of aggression during a 20 minute resident intruder test were more likely to achieve social dominance [10]. However, another paper reported that the attack latency, or the duration of aggression during a 10 minute resident intruder test did not predict social status [11].

The consequences of social stress and social subordination in particular, are not the same for every individual. First, within larger colonies there seem to be different levels of subordination, where some subordinates lose relatively little body weight, receive little aggression, and have (some) access to females, while other subordinates lose a large amount of body weight, receive higher levels of aggression and do not have access to the females. Within the subordinate group, a division can be made into "stress responders", those subordinates that display an increased corticosterone level during a 1-hr novel restraint stress test after VBS exposure, and "stress non-responders", those subordinates that lack an elevation of corticosterone during a novel restraint stress test [12]. The behavioral responses of the stress non-responders after VBS exposure was different in many aspects. The stress non-responders showed behaviors associated with passive defensive strategies including: increased immobility in response to handling or being held down, decreased latencies to right themselves when placed on their back, and more time spent crouching in a novel cage [11].

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In Blanchard's paper [11], it was suggested that being stress non-responsive may be associated with having a passive (or reactive) stress coping strategy.

The stress coping style describes the way an individual copes with stressors in its environment. Two distinct stress coping styles can be defined: the proactive stress coping style, and the passive (reactive) stress coping style. The proactive stress coping style is characterized by an active approach towards stressors. The proactive individual will attempt to modulate its environment to reduce or escape from the stressor. In contrast, the passive stress response is characterized by a more conservative approach towards stressors. These individuals will attempt to hide from or avoid the stressor in order to minimize harm [13]. *To characterize the stress coping style of rats the defensive burying test can be used. In this test proactive rats typically show burying behavior when exposed to an electrified prod in their home cage, whereas passive rats typically show avoidance of the prod. The results of this test have been validated under different environmental settings [14]. Typically, passive stress coping is associated with heightened HPA-Axis activity in response to stress, whereas proactive stress coping is associated with a sympathetically dominated stress response.* The stress coping style correlates with a set of behavioral constructs, for example, proactive individuals typically display higher levels of aggression, and have shorter attack latencies when an intruder enters their territory. Additionally, higher levels of proactive stress coping have been associated with reduced behavioral flexibility, and higher impulsivity levels [15]. To what extent the stress coping style influences social status in rats is currently unknown.

I first met Randall Sakai in 2005 at the very beginning of my graduate studies with Anton Scheurink at the University of Groningen. During that first meeting, Randall took the time to explain the VBS and the history of the studies on social behavior to me. With that interaction, the intent to study social behavior of the passive and proactive rats in the VBS was born. Due to Randall's continuous enthusiasm and encouragement this plan never faded, and once I moved to Johns Hopkins for my post-doctoral fellowship with Kellie Tamashiro, I set up a collaborative study with Randall's lab at the University of Cincinnati so we could test the questions that Randall and I discussed during that first meeting when I was a new Ph.D. student. We used the VBS model to investigate the role of stress coping strategy on determination of social status, as well as on the consequences of being socially subordinate within the different stress coping styles.

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Methods:

Animals: 36 male Long Evans rats, and *32* female Long Evans rats (approx. 90 days of age) were obtained from Harlan (Indianapolis, IN). Rats were individually housed upon arrival in conventional shoebox cages (18 × 24.5 × 18 cm) prior to assignment to a colony. Rats were kept in a temperature and humidity controlled room with a 12/12 h light/dark cycle (lights off at 6pm). Food (Teklad sterilizable mouse/rat diet 7012; Harlan Teklad, Madison, WI) and water were available throughout the experiment. All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996).

Experimental set-up: One week after arrival, the coping style of the male rats was determined using a defensive burying test (DB1) (Day -21/-22). Two days after the first defensive burying test, the rats were tested in a second defensive burying test to confirm consistency of the coping style (DB2) (Day -19/-20). One week later, the rats were tested in an elevated plus maze test (EPM) (Day -12/-11) to assess anxiety-like behavior. *Two days after the EPM, the body composition of the rats was assessed by NMR (day -10/-9).* A week after the EPM a baseline blood sample was taken via a small nick of the tail at 10 am (Day -5). *Hereafter the male rats were matched by body weight to form 11 pairs containing 1 proactive and 1 passive coping rat, of which 7 pairs were exposed to the VBS. The remaining 4 proactive and 4 passive rats were non-stressed controls. Rats without a clear stress coping phenotype (between 10-20% burying) were excluded from the study.* The characteristics of these pairs are presented in Table 1. On Day 1, two matched males (1 passive and 1 proactive) and 4 female rats were introduced into the visible burrow system (VBS). Rats remained in their respective colonies until Day 14, on which a restraint stress test (RST) was performed with the male rats (Day 14). Hereafter the rats were individually housed until the end of the experiment. Three days after removal from the colony, the body composition of the rats was assessed by NMR (Day 16). Four days later a third defensive burying test was performed (DB3) (Day 21). This was followed by another body composition assessment on day 22. Finally, the rats were sacrificed two weeks after removal from the colony (Day 24). Throughout the experiment body weight and food intake of the rats were measured. The experimental set-up is summarized in figure 1.

Defensive burying (DB) test: All rats were tested in the defensive burying test three times. The first test was used to characterize the stress coping style of the rats. The second and third tests were used to evaluate the stability of the behavioral phenotype of the rats prior to and after exposure to the VBS colony. During the DB-test the rats were housed in a conventional shoebox cages (18 × 24.5 × 18 cm) with a hole with a diameter of 2 cm in the front of the cage. The bottom of the cage was covered with clean corncob bedding. The rats were left to habituate to the testing room for at least 10 minutes prior to testing. During the test an electric prod (length 8.5 cm, diameter 1 cm) was inserted through the hole and the latency to touch the prod was measured. Upon touching the prod the rat received a mild shock (2.5 mA). Behavior of the rat was scored using Hindsight behavioral software for 5 minutes. The time the rat spent immobile, exploring the cage, grooming, and burying the probe with bedding was measured. The percentage time spent burying the prod was used as the criterion to categorize the rats as proactive or passive coping. Rats that spent 10% or less time burying were characterized as passive coping, rats that spent 20% or more time burying were characterized as proactive coping [16]. During the second and third test the behavioral scoring commenced

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immediately when the prod was inserted in the cage and continued for 5 minutes. During this test, investigation of the probe was included as an additional behavioral parameter.

Elevated plus maze test: Prior to the start of the VBS the rats were assessed for anxiety-like behavior in the EPM. The EPM apparatus consisted of two open arms (56 × 10 cm with 0.5 cm lip) and two closed arms (56 × 10 cm with 40 cm high walls) connected by a center platform (10 x 10 cm) made of opaque grey Plexiglas (Harvard Apparatus). The arms of the plus maze were elevated 50 cm above the floor. The floor of the maze was a grey Plexiglas to accommodate the automated scoring using contrast analysis with Clever System and the maze was dimly lit *(10 lux)*. The rats were placed on the platform facing one of the open arms at the start of the test. Rats were allowed to explore the EPM for 5 minutes. The behavior of the rats was scored using an overhead camera and a computerized tracking system (TopScan, Clever Sys Inc., Reston, VA, USA) and was used to measure time spent in each arm of the EPM and total distance traveled. The maze was thoroughly cleaned between rats with 5% ammonium hydroxide solution.

Visible burrow system: The structure and procedures of the VBS have been previously described [17-23]. Briefly, the VBS is constructed of black Plexiglas and consists of a large high-walled open-field chamber, and a series of clear tubes connecting the open field to two smaller chambers. The open field is lit by a 15-W bulb on a 12:12-h light-dark cycle, while the tubes and smaller chambers are kept dark to simulate an underground burrow system. Food and water are provided ad libitum in the open field and in both of the smaller chambers. Infrared cameras are suspended above each of the VBSs to record behavior during the dark cycle. The colonies were set-up such that each colony contained 4 females and 2 weight matched males: 1 proactive coping male rat and 1 passive coping male rat. Control (CON) males were weight-matched to males in their respective VBS colony and housed with a single female in a standard conventional cage for the duration of the VBS. The behavior of the rats was scored using Hindsight software. Behavioral analysis were performed on 3 different days during the VBS; day 1, day 7 and day 14 of VBS exposure. The behavior of the rats was scored every minute for the first 15 minutes of the hour between 7 and 12 pm (dark cycle). The following behaviors were recorded: sleep, immobility, ingestive behavior, groom, explore, interact with a female, threat, bite, lateral attack, chase, freeze, defensive flight.

Determination of social status: The social status of the rats was determined by observation of agonistic interactions between males, body weight, and wound patterns as previously described [24]. Briefly, Dominant animals are characterized by less than 5% weight loss, higher levels of offensive behavior, little wounds which are typically located on the head and shoulders of the body. In contrast, subordinate rats are characterized by more than 5% weight loss, more defensive behaviors and more wounds that are located on the back, flanks and tail.

Restraint stress test: A restraint stress test was performed to assess responsivity to a novel stressor after VBS exposure. Ten minutes prior to testing a blood sample was collected via a small nick of the tail (40 µl). Hereafter the rats were placed in a polycarbonate restraint tube with air holes and restrained for 60 minutes. After 60 minutes a second blood sample was collected and the animal was removed from the restrainer and returned to his home cage. A third blood sample was taken 60 minutes later. Blood samples were spun down and plasma was collected for determination of plasma corticosterone levels using a commercially available corticosterone radioimmunoassay kit (MP

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Biomedicals, Solon, OH, USA). Inter- and intra-assay variability for the assay was as follows: 6.5–7.1% and 4.4 –10.3%.

Body composition: To determine whole body composition the rat was placed into a Plexiglas tube, which was then inserted into an EchoMRI (Echo Medical Systems, Houston, TX) whole body composition analyzer system. This analysis provides estimates of fat mass, lean mass, and water content.

Data analysis: Data are displayed as means ± standard error of the mean (SEM). For the corticosterone response to the restraint stress test the area under the curve was calculated. For the behavioral analysis during the VBS, averages over the 3 measurements the VBS (day 1, 7, and 14) were calculated. Group differences were assessed using repeated measures ANOVA analysis or a ANOVA analysis where appropriate. The social status and stress coping style were defined as between subject factors. Specific group differences or differences at a specific time point were analyzed *with a planned comparison t-test analysis with Bonferroni correction. Significant correlations were assessed using Pearson correlation analysis. For ANOVA analysis, F and p values are displayed, for planned comparison t-tests Bonferroni corrected p-value are provided, and for correlations Pearson's r-values and p-values are given.* Differences were regarded statistically significant when $P<0.05$. All statistical analyses were performed using Statistica 7 (Systat, Tulsa, OK) software.

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Results:

Baseline characteristics: The baseline characteristics of the 7 VBS colonies are summarized in Table 1. At baseline there were no significant differences in the body weight or body composition between the experimental groups. The passive coping rats spent significantly less time burying the prod than proactive rats ($F(1,21) = 382.15$, $p<0.001$). The stress coping style did not predict social status (Chi-Square = 0.007, df =1, $p>0.9$). Out of the 7 colonies, 3 colonies had a passive coping dominant, whereas 4 colonies had a proactive dominant rat.

Body weight: *At baseline there were no significant differences in the body weight or body composition between the experimental groups.* During the period the rats were in the VBS, a repeated measurements ANOVA revealed a significant effect of social status ($F(20,190) = 29.54$ $p<0.001$) as well as an interaction between time, social status and stress coping style ($F(20,190) = 2.54$ $p<0.01$) (Fig. 2A). Planned comparison analysis revealed that subordinate rats had a lower body weight than dominant rats through the whole period in the VBS. During the first 4 days of VBS, the passive coping rats with a dominant status had a lower body weight than proactive coping rats with a dominant status ($p<0.05$). Furthermore, within the rats with a subordinate status, rats with a proactive stress coping style had a lower body weight on day 3 *($p<0.05$)* and 4 *($p<0.05$)*, whereas on day 11 their weight was higher than passive coping subordinate rats *($p<0.05$)*. The control rats not exposed to VBS had a higher body weight throughout the VBS exposure compared to all ($p<0.05$). VBS exposed rats. After the VBS exposure, the dominant rats remained heavier than subordinate rats until sacrifice ($F(22,293) = 14.36$ $p<0.01$). During this period there was however no interaction between social status and coping style.

Behavior in VBS: The behavior of the rats in the VBS was analyzed for days 1, 7, and 12. There was no significant effect of time on any of the behavior measured. Therefore we averaged the data over these three days for the further analysis (Fig. 3). As expected, there were main effects of social status on both defensive (Fig. 3A) and offensive behavior (Fig. 3B) displayed. Dominant rats showed significantly more offensive ($p=0.015$) and less defensive behavior ($p=0.013$) than subordinate rats. In addition, subordinate rats spent more time immobile than dominant rats ($p = 0.0007$) (Fig 3C). Finally, dominant rats spent more time interacting with the female rats in the colony than the subordinate rats ($p=0.049$) (Fig. 3D). More detailed analysis revealed that within the rats with a dominant status, those rats with a passive stress coping style spent more time interacting with females than proactive coping rats ($p=0.024$). Similarly, among the subordinate rats, those rats with a passive coping style interacted more with the females than those with a proactive coping style ($p=0.032$). There were no effects of coping style on time spent on defensive behavior or immobility within either dominant of subordinate rats. However, within the subordinate rats, those rats with a passive coping style spent less time on offensive behavior than those with a proactive stress coping style ($p=0.039$).

Wound patterns: Analysis of the wound patterns of the rats can inform us about aggression received and fighting strategies [25], therefore the wounds on the different sections (front, back and flank) were assessed daily. The total number of wounds sustained during the VBS was significantly lower in the dominant rats compared to subordinate rats ($F(1,10) = 26.89$ $p<0.01$) (Fig. 4). There was no social status *stress coping style interaction on the total number of wounds sustained. Analysis of the location of the wounds revealed that subordinate proactive coping rats had more wounds on head,

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neck and shoulder regions of their body compared to subordinate passive coping rats (p=0.049). In addition, there was a trend toward subordinate proactive coping rats having fewer wounds toward the back of their body (hindquarter and tail) compared to subordinate passive coping rats (p=0.069). Within the subordinates, the ratio of the wounds on the back to front was significantly higher in passive coping compared to proactive coping rats (p=0.021).

Restraint stress test: On the last day of VBS housing the rats were exposed to a novel acute stressor, restraint stress, to assess their stress responsivity (Fig. 5). ANOVA analysis revealed a significant social status*stress coping style interaction effect ($F(4,28) = 6.41$, $p<0.05$). Planned comparison analysis revealed that within the proactive rats, subordinate rats had a higher corticosterone levels at the 60 (p=0.013) and 120 (p=0,021) minute time points compared to dominant rats (Fig 5A). In contrast, within the passive coping rats, there were no differences in corticosterone levels between subordinate, dominant and control rats (Fig. 5B).

Elevated plus maze: To assess anxiety like behavior an elevated plus maze was performed one week after VBS exposure. *Table 2 displays group differences in behavior in the elevated plus maze.* There were no differences between the groups in the total distance moved or the velocity of movement on the elevated plus maze. There was a significant effect of social status on the time spent on the closed arm ($F(1,10) = 7.67$ $p<0.05$) and the platform ($F(1,10) = 5.08$, $p<0.05$). There were no main effects of stress coping style or significant social status*coping style interaction effects. Planned comparison analysis showed that dominant rats spent less time on the closed arm than subordinate rats. For the open arm and platform dominant rats only spent significantly more time on the arm than the subordinate rats within the passive rat subgroup. No differences between dominant and subordinate rats were observed within the proactive rats.

Body composition: Three days and two weeks after VBS, the body composition of the rats was assessed using NMR. Three days after VBS, there was a significant social status effect on body weight ($F(2,18)= 14.13$, $p<0.01$), with dominant rats being heavier than subordinate rats (p=0.008), and control rats being heavier than all rats exposed to the VBS (p=0.005) (Table 3A). Control rats had a significantly higher body fat percentage than rats exposed to VBS ($F(2,18)= 12,7$, $p<0,01$). Social status in the VBS nor the coping style affected the body fat percentage of the rats.
Two weeks after VBS, there was a significant social status effect on body weight ($F(2,18)= 11.21$, $p<0.01$), with dominant rats being heavier than subordinate rats (p=0.011), and control rats being heavier than all rats exposed to the VBS (p=0.006) (Table 3B). There were no significant stress coping style or social status*coping style interaction effects on body weight. Control rats had a significantly higher body fat percentage than rats exposed to VBS ($F(2,18)= 12,4$, $p<0,01$). However, there were no social status, stress coping style, or interaction effects on the fat percentage of the rats.

Organ weights: To assess the physiological consequences of the VBS exposure we measured adrenal, thymus and spleen weights at sacrifice two weeks after VBS exposure *(Figure 6)*. There were no main effects or interaction effects on the spleen or thymus weight. There was however a group effect ($F(2,18) = 5.04$, $p<0.05$) and social status*coping style effect on the adrenal weight ($F(2,18) = 4.65$, $p<0.05$). Planned comparison analysis revealed that within the subordinate rats, rats with a proactive stress coping style had heavier adrenals than rats with passive stress coping style (p=0.021). Furthermore, control rats had smaller adrenals lower that all rats exposed to VBS (p=0.012).

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Discussion:

The first aim of this study was to investigate whether the stress coping style of a rat may predict their social status in a colony. Our data revealed that the stress coping style does not predict the social status of the rat. In the set up with two male rats, one proactive, one passive, the chance that a passive rat became dominant was similar to the chance of a proactive rat becoming dominant. A limitation of the current study was that each colony only had two males. As a result, the current study was not equipped to analyze differences in social status between multiple subordinate rats in the same colony. However, we were able to assess whether the consequences of being subordinate were different for passive and proactive rats. Therefore, the second aim of this study was to investigate whether the stress coping style may affect the consequences of having a lower social status. Within the subordinate rats, there were clear differences in offensive and defensive behavior between proactive and passive rats, with subordinate proactive rats showing more offensive behavior. The wound patterns were consistent with this; proactive subordinate rats had more wounds towards the front of their bodies whereas passive subordinate rats sustained most wounds towards the back of their bodies. These wound patterns may suggest that passive rats fled from their opponent, whereas the proactive rats engaged in the aggression. In contrast to this finding, there was no difference between the dominants in offensive behavior displayed, suggesting that the more offensive strategy in proactive subordinate rats may not have increased aggressive behavior in the dominant rat. *A limitation of our study is the low number of animals in the study. Another caveat is that all proactive subordinate rats was paired with a corresponding passive coping dominant rat due to the design of the study using just 2 male rats of opposite coping styles. It is possible that the outcomes would be different if we used 2 proactive rats or 2 passive rats, such that the coping style was the same but social status was different. This represents a future direction for these studies.*

In addition to the difference in offensive and defensive behavior, there were social status and coping style effects on the amount of time the males spent interacting with the females in the colony. Dominant males spent more time with the females than subordinate males. Further analysis showed that passive dominant rats interact more with the females than proactive dominant. In addition, the subordinate passive rats spent more time with the female than the subordinate proactive rats. Overall, independent of social status, the proactive rats spent less time with the females than passive rats. The females were ovariectomized thus these differences in time spent interacting with females could also have been due to alterations in reproductive behavior of the ovariectomized female.

There were no coping style differences in body weight loss during the VBS, which may suggest that the stress coping style of dominants or subordinates does not differentially affect weight loss due to chronic social stress. Body composition was also not affected 3 days or 2 weeks after social stress. However, the subordinate proactive rats had significantly larger adrenals than passive coping rats. This may suggest that these proactive rats either had larger adrenals prior to VBS exposure, or that they experienced more stress during the VBS resulting in adrenal hypertrophy. Frequent measurements of corticosterone, and adrenalin during the VBS are needed to confirm this hypothesis. Furthermore, the proactive subordinate rats showed an elevated corticosterone response to restraint stress after VBS exposure compared to dominant and subordinate passive rats. This suggests that proactive rats may become more stress reactive due to social subordination, whereas social status has no influence on stress reactivity in passive coping rats. Since the recovery of the corticosterone levels after restraint stress took longer in subordinate proactively coping rats,

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indicated by elevated corticosterone levels at the 2 hour time point, one may hypothesize that negative feedback of the HPA-axis is impaired in these rats. Previously, it was reported that about 35 percent of subordinate rats could be identified as stress non-responders, meaning that these rats showed a increase of less than 10 microg/dl plasma corticosterone in response to 1 hour of restraint stress [11]. Although, there were no differences in aggression levels between the stress responders and stress non-responders in this study, stress non responders were showed more behavioral immobility and increased escape latencies, which may indicate a more passive stress coping strategy [11]. These data fit with our observation of lower stress response in passive subordinate rats compared to proactive subordinate rats. In our study there was only one animal that according to these criteria would be categorized as a stress non-responder, this was a passive subordinate rat, but due to the limited number of subordinate rats in this study it is hard to make conclusions about stress responder status in this study. *The observation of increased adrenal weight in proactive submissive rats compared to passive submissive rats may align well mismatch theory of disease [26] and particularly with the stress-coping (mis)match hypothesis posed by Homberg [27]. These theories propose that that stress coping responses are adaptive when they match current stress conditions, but maladaptive when they mismatch current stress conditions [27]. Translating this to our data, one may hypothesis that the environment induced by having a submissive position in the colony may not match well with the proactive stress coping rats, which may have been adapted towards having a dominant position. This notion requires further research to evaluate the stress imposed by different position of social dominance.*

The data presented here suggest that the effects of social subordination on anxiety-like behavior were not different between proactive and passive coping rats. Overall, dominant rats showed less anxiety-like behavior during an elevated plus maze test than subordinate rats. Previous studies showed that behavior in the EPM prior to VBS exposure predicted social status [21] , rats that later became dominant spent more time in the open arm of the maze compared to rats that later became subordinate. Therefore, it might be that the observed higher levels of anxiety-like behavior in subordinates in this study are not resultant of VBS exposure, but rather may be a predetermining factor for social status. A passive stress coping style is typically associated with increased levels of anxiety-like behavior [28], however, in this study no clear difference in anxiety levels between the passive and proactive stress coping style were observed in either the VBS exposed and the control rats. Although differences between our, and these previous studies may be explained by differences in the rodent strain used, future studies are needed to further elucidate the relationship between different behavioral parameters like aggression, anxiety, stress coping strategies and social dominance. *Furthermore, the current study only investigated male rats, interactions between stress coping and social status may be different, which should be addressed in future research.*

This study suggests that stress coping style does not predict social status *in male rats*, however, it may influence the consequences of experiencing social subordination stress. If we were to translate these data back to the human, one may speculate that the stress coping style of the individual may have an impact on the social stress experience of that individual. *This may occur through several pathways*, first by altering their response to being under social stress, or second by altering the social interaction itself which may then impact the stress induced by that interaction. Future research is needed to further study these relationships is both the rodent models as well as in humans to better identify individual at risk for social stress as well as understand the consequences of the social stress to these individuals.

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Legends:

Table 1: Baseline characteristics of rats in the VBS. * indicates a significant difference between passive and proactive coping rats $p<0.05$.

Table 2: Behavior during an elevated plus maze test. 'a' indicates a significant difference between subordinate and dominant rats with the same stress coping style.

Table 3: Body weight and body composition after VBS exposure. A: Body weight and body composition 3 days after VBS exposure. B: Body weight and body composition 2 weeks after VBS exposure. 'a' indicates a significant difference between subordinate and dominant rats within the same stress coping style. 'c' indicates a significant difference between control and VBS exposed rats within the same stress coping style.

Figure 1: Schematic overview of the study set-up.

Figure 2: Body weight gain during housing in visual burrow system. *CON PAS = control passive, CON PRO = control proactive, DOM PAS = dominant passive, DOM PRO = dominant proactive, SUB PAS = subordinate passive, SUB PRO = subordinate proactive. CON PAS = control passive, CON PRO = control proactive. White symbols = proactive coping rats, grey symbols = passive coping rats, circle = dominant rats, square = subordinate, triangles = control rats.* ***'****a' indicates a significant difference between subordinate proactive and all other groups, and between subordinate passive rats and all dominant rats. 'b' indicates a significant difference between dominant and subordinate rats, P<0.05.*

Figure 3: Behavior during housing in VBS. A: Time spent on defensive behavior. B: Time spent on offensive behavior. C: Time spent interacting with a female. *DOM = dominant, SUB = subordinate. Grey bars = passive coping rats, white bars = proactive coping rats. Bars with different letters are significantly different p<0.05.*

Figure 4: Bite wounds sustained during VBS Exposure. A: Bite wounds sustained on different sections of the body. B: Ratio between the numbers of bites wounds on the back vs the front of the body. *DOM = dominant, SUB = subordinate. Grey bars = passive coping rats, white bars = proactive coping rats. Bars with different letters are significantly different p<0.05.*

Figure 5: The corticosterone response curve during and after a 60 minutes restraint stress test. DOM PAS = dominant passive, DOM PRO = dominant proactive, SUB PAS = subordinate passive, SUB PRO = subordinate proactive. CON PAS = control passive, CON PRO = control proactive. **A: Corticosterone levels in proactive coping rats.** Circle = dominant rats, square = subordinate rats, triangles = control rats. **'**a' indicates a significant difference between subordinate proactive and all other groups, and between subordinate passive rats and all dominant rats. **B: Corticosterone levels in passive coping rats.** Circle = dominant rats, square = subordinate rats, triangles = control rats.

Figure 6: Organ weights two weeks after VBS exposure. A: Spleen weight. B: Thymus Weight. C: Adrenal weights. *There were no differences among the groups in spleen or thymus weight after 2 weeks recovery from VBS stress. Adrenal glands of Passive SUB were heavier than all other groups. DOM = dominant, SUB = subordinate. Grey bars = passive coping rats, white bars = proactive coping rats. Bars with different letters are significantly different p<0.05.*

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Table 1: Baseline characteristics of rats. 'a' indicates a significant difference between passive and proactive coping rats $p<0.05$.

Colony	Body weight (g)		Time spent burying (%)		Dominant in VBS
	Passive	Proactive	Passive	Proactive	
VBS 1	435	422	0	71.8	Passive
VBS 2	425	412	0	57.1	Proactive
VBS 3	428	418	2.7	56.6	Passive
VBS 4	437	423	0.4	91.2	Proactive
VBS 5	436	435	1.7	90.6	Proactive
VBS 6	412	431	0	66.0	Passive
VBS 7	433	441	0	82.0	Proactive
VBS	**429.4 ± 3.4**	**426 ± 3.8**	**0.7 ± 0.4**	**73.6 ± 5.5[a]**	
CON 1	502	473	2.9	81.5	
CON 2	406	406	0	65.3	
CON 3	402	429	0	74.2	
CON 4	396	398	9.1	77.1	
CON	**426.5 ± 25.2**	**426.5 ± 16.8**	**3.0 ± 2.2**	**74.5 ± 3.4[a]**	

Table 2: Behavior during an elevated plus maze test. 'a' indicates a significant difference between subordinate and dominant rats with the same stress coping style.

Arm (%)	Dominant		Subordinate		Control	
	Passive	Proactive	Passive	Proactive	Passive	Proactive
Closed	66.9 ± 11.7	74.2 ± 6.6	91.5 ± 5.7[a]	83.5 ± 6.9[a]	92.1 ± 1.7	89.2 ± 1.7
Platform	15.3 ± 5.2	18.8 ± 5.9	5.5 ± 3.2[a]	8.8 ± 3.1	7.1 ± 1.8	7.3 ± 1.4
Open	17.7 ± 9.7	7 ± 2	2 ± 3[a]	7.8 ± 4	0.9 ± 0.8	3.4 ± 1.5

Table 3: Body weight and body composition after VBS exposure. A: Body weight and body composition 3 days after VBS exposure. B: Body weight and body composition 2 weeks after VBS exposure. 'a' indicates a significant difference between subordinate and dominant rats within the same stress coping style. 'c' indicates a significant difference between control and VBS exposed rats within the same stress coping style.

A	Dominant		Subordinate		Control	
	Passive	Proactive	Passive	Proactive	Passive	Proactive
Body weight (g)	459 ± 9	462 ± 6	404 ± 16[a]	419 ± 14[a]	482 ± 22[c]	485 ± 14[c]
Body fat (%)	24.8 ± 8.3	23.5 ± 6.1	21.2 ± 7.8	23.3 ± 4.3	27.1 ± 8.7[c]	27.2 ± 5.3[c]

B	Dominant		Subordinate		Control	
	Passive	Proactive	Passive	Proactive	Passive	Proactive
Body weight (g)	465 ± 6	469 ± 6	433 ± 15[a]	447 ± 13[a]	487 ± 20[c]	484 ± 13[c]
Body fat (%)	24.5 ± 2.2	23.1 ± 5.2	23.2 ± 5.8	23.9 ± 3.1	27.4 ± 8.6[c]	26.2 ± 3.4[c]

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DB = Defensive burying test
EPM = elevated plus maze test
VBS = visual burrow system exposure
RST = restraint stress test
BC= body composition measurement

Figure 1

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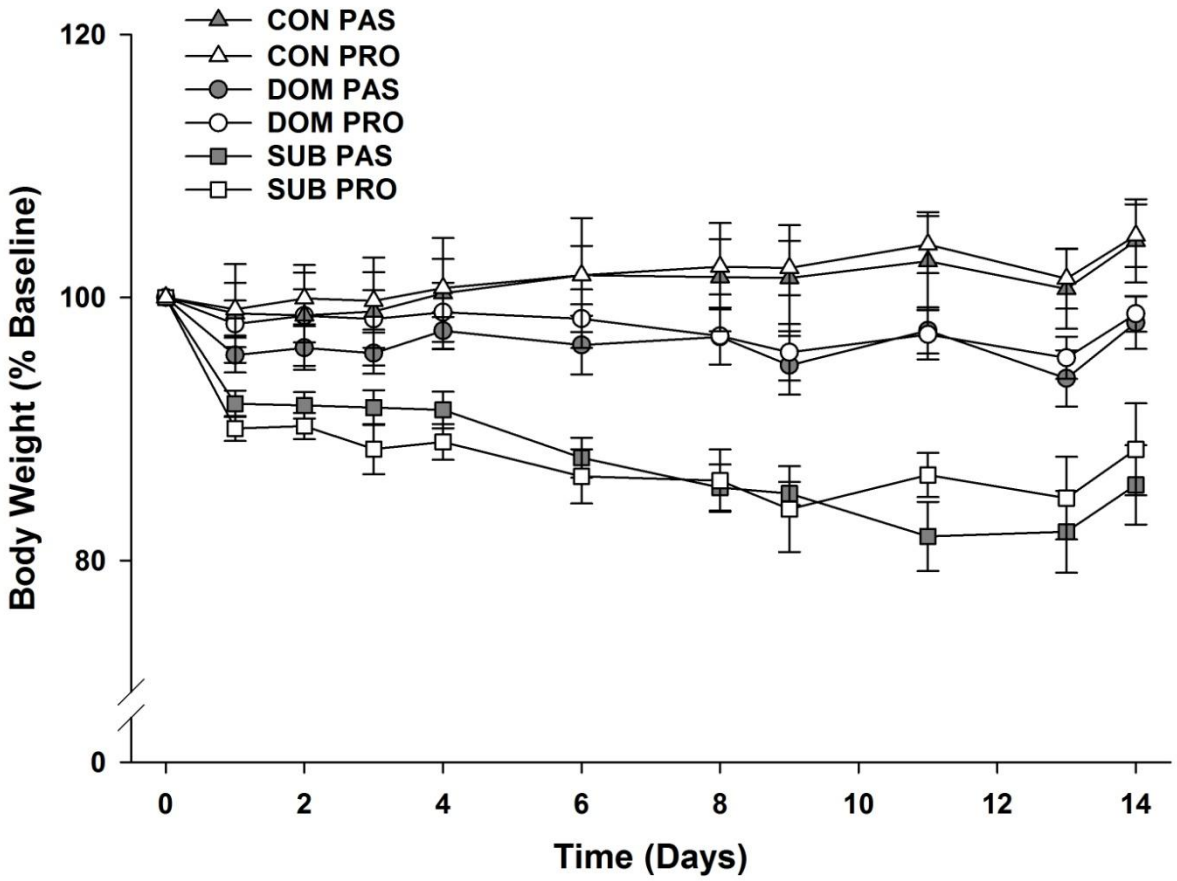


Figure 2

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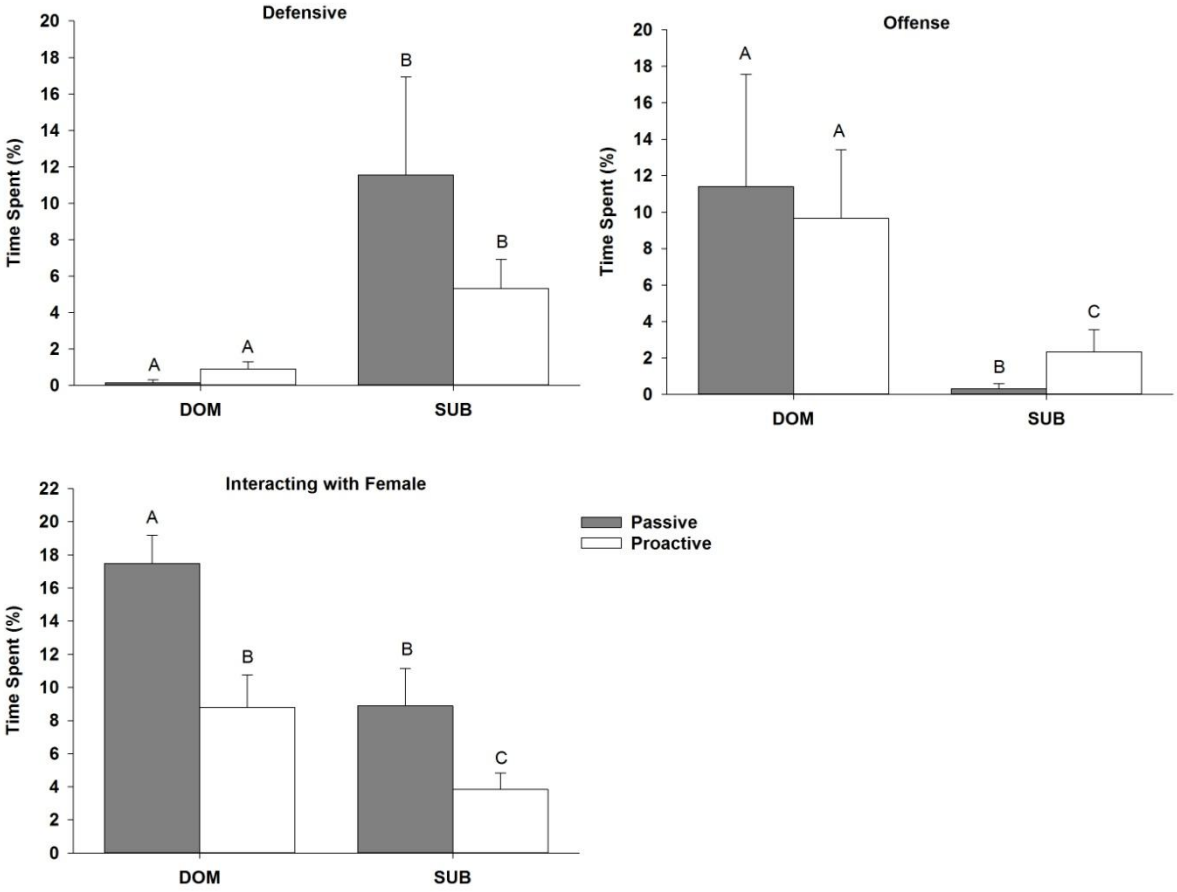


Figure 3

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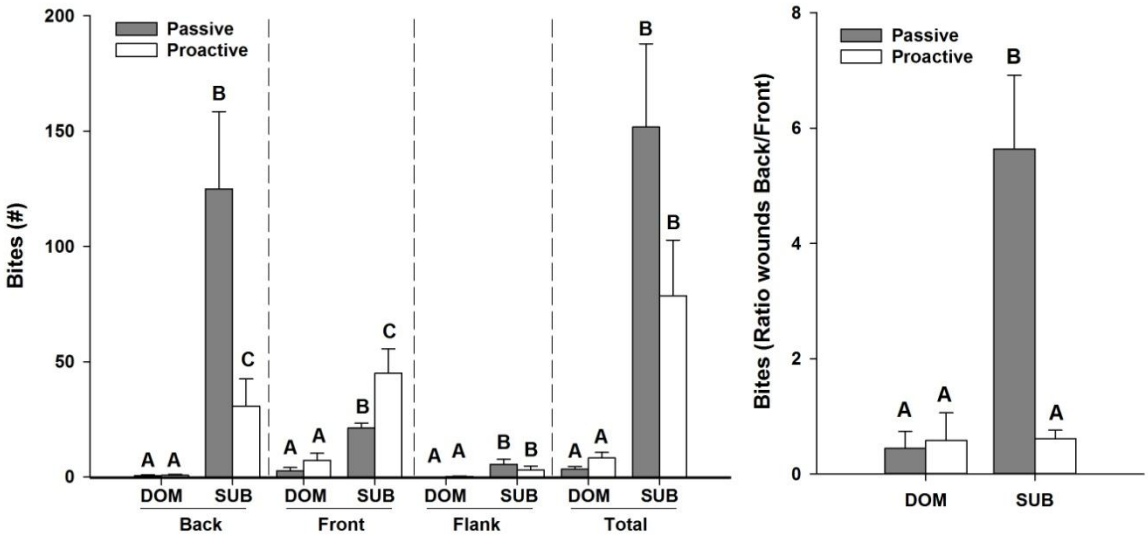


Figure 4

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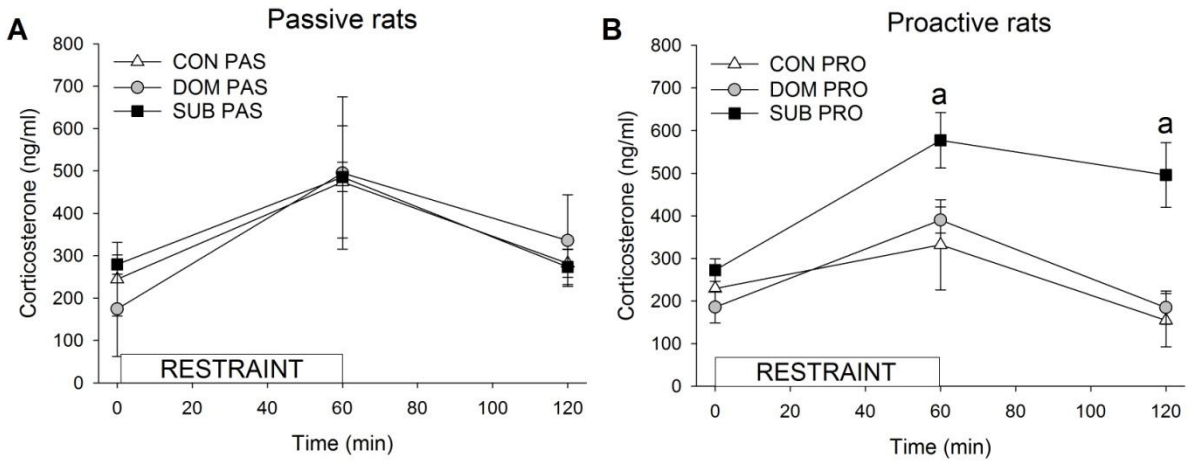


Figure 5

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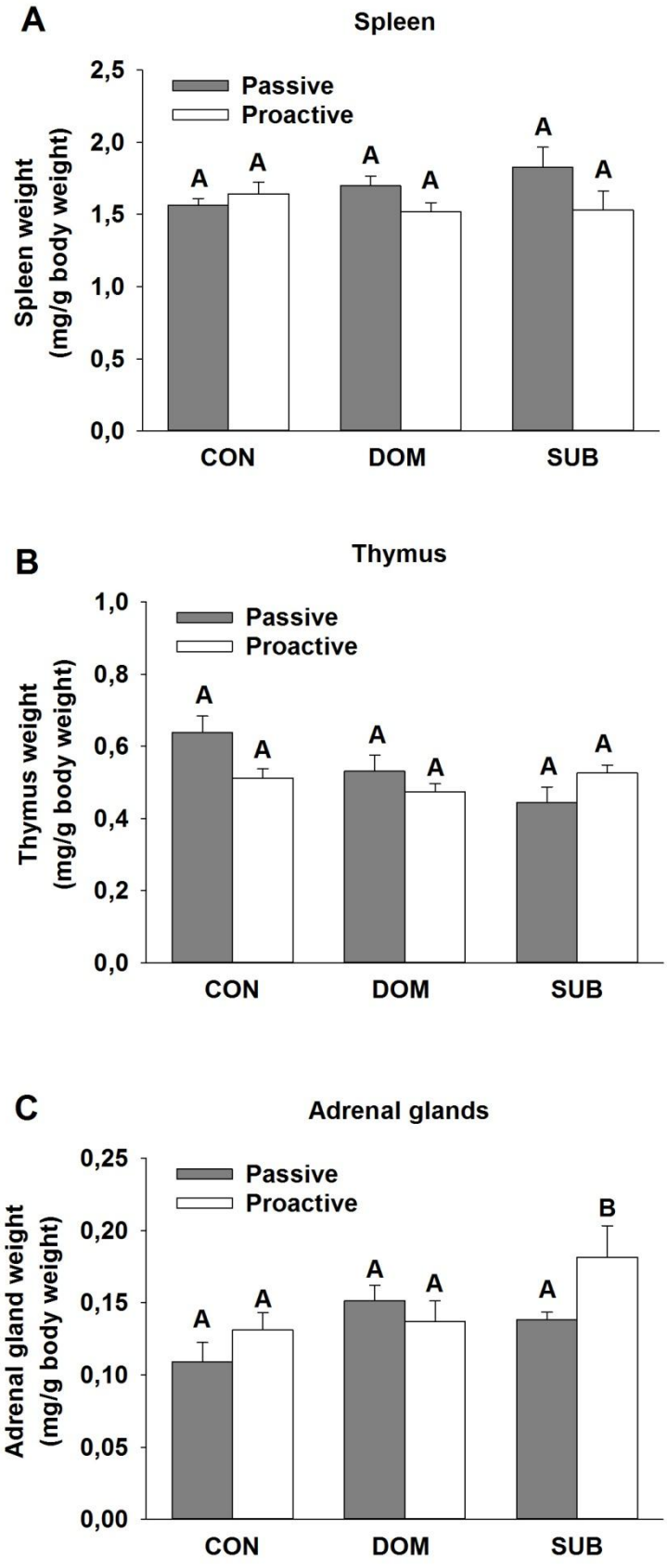


Figure 6

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Highlights:

1: The stress coping style does not predict the social status of the rat.

2: The consequences of being subordinate are different for passive and proactive rats.

3: Larger adrenals in subordinate proactive rats suggest that they may experienced more stress during social housing.

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