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Trait aggressiveness does not predict social dominance of rats in the Visible Burrow System

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HIGHLIGHTS

- High offensive aggression in a resident-intruder paradigm does not predict social dominance in a mixed-sex colony housing
- Average level of trait aggressiveness of male WTG subjects in a Visible Burrow System is not positively correlated with hierarchical steepness
- In WTG male rats a lower social status not necessarily coincides a maladaptive activation of stress response mechanisms and concomitant stress related behaviors
- Capacity to adopt a behavioral strategy fitting the actual hierarchical ranking position probably defines level of perceived social stress

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ABSTRACT

Hierarchical social status greatly influences health and well-being in mammals, including humans. The social rank of an individual is established during competitive encounters with conspecifics. Intuitively, therefore, social dominance and aggressiveness may seem intimately linked. Yet, whether an aggressive personality trait may pre-dispose individuals to a particular rank in a social colony setting remains largely unclear. Here we tested the hypothesis that high trait aggressiveness in Wildtype Groningen (WTG) rats, as assessed in a classic resident-intruder offensive aggression paradigm predicts social dominance in a mixed-sex colony housing using the Visible Burrow System (VBS). We also hypothesized that hierarchical steepness, as reflected in the number and intensity of the social conflicts, positively correlates with the average level of trait aggressiveness of the male subjects in the VBS.

Clear and stable hierarchical ranking was formed within a few days in VBS colonies as indicated and reflected by a rapid loss of body weight in subordinates which stabilized after 2–3 days. Social conflicts, that occurred mainly during these first few days, also resulted in bite wounds in predominantly subordinate males. Data clearly showed that trait aggressiveness does not predict dominance status. The most aggressive male in a mixed sex group of conspecifics living in a closed VBS environment does not always become the dominant male. In addition, data did not convincingly indicate that in colonies with only highly aggressive males, agonistic interactions were more intense. Number of bite wounds and body weight loss did not positively correlate with trait-aggressiveness of subordinates. In this study, rats from this wild-derived rat strain behave differently from Long-Evans laboratory rats that have been studied up till now in many experiments using the VBS. Strain dependent differences in the capacity to display appropriate social behavior fitting an adaptive strategy to a high or low social ranking position probably play an important role in the level of perceived stress in mixed sex social colonies like the VBS.

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1. Introduction

From the very start of his scientific career, Randall Sakai was intrigued by questions on the physiological regulation of mammalian behavior. More specifically, the study of the role of peripheral hormones signaling the bodily state to the brain initiating or altering ingestive

behaviors marked his line of research. This interest was sparked during his PhD research in the laboratory of Dr. Alan Epstein at the University of Pennsylvania where he studied the role of aldosterone and angiotensin on salt intake. His subsequent interest in the actions of adrenal steroid hormones on brain and behavior probably were key in joining the lab of Bruce McEwen at Rockefeller University in New York as a postdoc. There he extended his research interest to the actions of glucocorticoids on the hippocampus. This brain region was shown to be have a high density of both glucocorticoid and mineralocorticoid receptors [1] and

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therefore highly sensitive to the modulatory actions of these stress hormones as Robert Sapolsky together with McEwen previously showed [2]. During this postdoc period at Rockefeller University he initiated a collaboration with Bob and Caroline Blanchard from the University of Hawaii (the state where he grew up) and the lab of Bruce McEwen on the behavioral and biological effects of chronic social stress. The Blanchard's had cleverly developed a social housing environment to study rodent social behavior in a more naturalistic setting [3]. In this model, which they called the Visible Burrow System (VBS), male and female rats were housed together in an enclosure where an open arena with an imposed diurnal photoperiod was connected to a continuously dark burrow system consisting of tunnels and small chambers mimicking the underground burrows and nests of rat colonies in the wild. This burrow system was made of transparent Plexiglas material allowing observation of the animals in this part of the cage even though the burrows were kept in continuous dark. This collaboration resulted in a very successful research program supported by numerous grants from both the NSF and the NIH, and it was this line of research on causes and consequences of chronic stress of social subordination that brought us in contact with Randall Sakai.

The neurobiology of social behaviors in rats and mice is a central theme of our research lines that focus on individual differences in offensive aggressive behavior on the one hand and vulnerability to social stress on the other hand. In our social stress research we mainly use the resident-intruder paradigm to study the temporal dynamics of neurobiological, behavioral, and physiological consequences of winning and losing a social conflict in both adult and juvenile rats (for reviews see [4–7]). Since many stress stimuli in humans that lead to psychopathology are of social nature [8], and given the difficulty of studying the pathophysiological processes mediating between psychosocial stress and brain and behavior pathologies in humans, research using animal models with a relatively high face validity such as the consequences of social stress in experimental animal models is highly relevant.

As mentioned above, our laboratory has a long history in studying physiology and behavior of rats and mice in the resident-intruder paradigm of social defeat stress. In this model experimental male animals are introduced into the territory of an experienced aggressive male conspecific. Generally, the intruder is rapidly attacked by the aggressive resident and adopts the classic defensive (avoidance/freezing) and submissive (supine) postures signaling defeat/subjugation. After this social defeat experience, ranging from only one single defeat episode [9,10] to multiple daily defeats [11–13], pronounced changes in (brain)physiology and behavior have been described that may last for weeks or even months [14]. Although principally aimed for adaptation, these changes are frequently linked to neural mechanisms underlying stress related psychopathologies like anxiety, depression and posttraumatic stress disorder (PTSD). Important in this realm is that intruder males are singly housed after the stressful confrontation [15]. To ensure the desired outcome of the social conflict, residents usually have a higher bodyweight, are familiarized with fighting and belong to a strain that express high levels of aggression. In our lab we select residential males from our so-called Wildtype Groningen (WTG) rat strain on the basis of their level of offensive aggressive behavior. These rats are descendants from male and female rat pairs caught in the wild and outbred for many generations under conventional laboratory conditions. There is a large variation in trait-like social behavior amongst these feral rats ranging from animals with very high levels of offensive aggressive behavior to animals that show no or only very little aggressiveness [16]. This individual variation allows selection of male WTG rats with high levels of offensive aggressive behavior for the purpose of defeating experimental animals as intruder males to study acute and lasting consequences of social stress [5–7,12,17]. It also allows a detailed analysis of the neurobiological basis of individual differentiation of aggressive behavior incorporating the regulation of the serotonergic neurotransmitter system and nonapeptides like vasopressin [18] and oxytocin [19] (see for further information [20]).

Chronic stress of psychosocial defeat and subjugation can also be studied by housing defeated animals next to dominant aggressive individuals in the sensory contact model as originally developed by Kudryavtseva [21] in mice and also applied in highly territorial species like the tree shrew [22]. In this experimental approach, one can refer to the stress as psychosocial stress of chronic subordination that can even be better studied in semi-natural rodent colonies.

In the VBS, as developed by the Blanchard lab, it was shown that a social hierarchy is formed rapidly when male and female rats from the Long-Evans strain [23] are housed together in groups. Male subordinates in the VBS are reliably characterized by defensive wound patterns and substantial loss of body weight indicative of social stress. Neuroendocrine changes in the hypothalamic-pituitary-adrenocortical (HPA) and hypothalamic-pituitary-gonadal (HPG) axis, like elevated baseline levels of corticosterone and suppressed testosterone plasma levels supported this interpretation. These neuroendocrine changes coincided with changes in dopaminergic [24] and serotonergic [25] neurotransmitter systems as well as altered hippocampal plasticity and structural morphology [26]. Strikingly, data showed that the subordinate individuals could be divided in two subgroups; a group in which the corticosterone stress response to restraint was heavily suppressed (called non-responsive subordinates (NRS)) and subordinates with a “normal” corticosterone response (stress responsive subordinates (SRS)). Clearly, the NRS rats showed the most profound negative impact of such chronic social subordination stress on their reproductive fitness and overall health [23].

Despite the important consequences of social rank on health and well-being, this individual differentiation in the negative consequences of occupying low social ranking positions in a group has not been explored extensively. It would be very interesting to know whether there are trait-like behavioral characteristics such as general coping style or animal personality that predict the likelihood of an animal to occupy a high or low rank in the social hierarchy that is established in the VBS. Since most animal species, including humans, live within social hierarchical organizations, questions related to causal factors contributing to the acquired ranking position are highly relevant from an evolutionary perspective. There are studies describing behavioral or physiological characteristics of individuals after they reached their hierarchical position characteristics [27,28]. Fewer studies are available, however, linking individual trait characteristics prior to placement in a social hierarchy. In a human study effects of personality and physical attractiveness were related to peer ratings of status [29]. High extraversion predicted a high social status whereas high neuroticism predicted lower status particularly in men. Physical attractiveness predicted higher status only in men.

Since social rank of an individual is mainly established during competitive encounters with conspecifics, intuitively social dominance and aggressiveness may seem intimately linked. Indeed, several older studies in birds [30], fish [31] and primates [32] have demonstrated an association between aggressive behaviors and dominance rank. However, many discrepant studies have also been reported in these same animal species [33–35]. Rank-1 mice in a so-called tube test for dominance were not more aggressive in a resident-intruder test [36] indicating that there is a distinction between aggression and dominance. Hence, although trait-aggressiveness seems likely to contribute to the expression of social dominance, there is also evidence to suggest that these traits may be dissociable.

As mentioned above, we extensively described the neurobiological basis of individual differences in coping styles and offensive aggressive social behavior in the WTG strain. Here, we tested ranking position and behavioral and physiological responses to the VBS in WTG male rats selected for trait-like differences in aggressive behavior. We hypothesized that higher trait aggressiveness would result in an enhanced likelihood to acquire dominance in the VBS social hierarchy. We also hypothesized that hierarchical steepness in colonies, as reflected in the amount of the social conflict-induced body weight reduction and bite

wounds in subordinate animals, positively correlates with the average level of trait aggressiveness of the male subjects in the VBS.

2. Materials and methods

2.1. Animals and VBS

Experiments were performed using 4 VBS' constructed in our institute (see Fig. 1A) applying an experimental design in which rats, after screening their offensive aggressive behavior in the resident-intruder paradigm, were housed in the VBS for a period of two weeks. In this modified VBS we connected a large open arena ($82 \times 55 \times 40$ cm) with two smaller chambers ($25 \times 25 \times 30$ cm) using Plexiglas tunnels (diameter 8 cm). The burrows were covered with black plastic to keep them continuously in darkness. Food and water was offered in the open arena as well as in the burrows. In our experiments we used male and female rats from our outbred Wildtype Groningen strain (WTG). These WTG rats (*Rattus norvegicus*) were originally wild-trapped animals and outbred under conventionalized conditions (at the time of testing for 21 generations in our own laboratory) without selection for particular traits. They were housed (single-sex) in groups of 5–6 animals from weaning (28 days after birth) until the start (at age 140–150 days) of the experiments in clear Plexiglas cages ($58 \times 35 \times 20$ cm). Females were sterilized by ligating the oviduct leaving the ovaries intact and maintaining the estrous cycle. Prior to VBS housing, males were tested for their individual level of offensive aggressive behavior as residents in the resident-intruder paradigm as previously described in more detail [16]. Rats were housed in a large home cage ($82 \times 55 \times 40$ cm) together with a sterilized female conspecific for 1 week. Subsequently they were confronted on 4 subsequent days with intruder Wistar males. Intruder Wistar males were 3 months old, weighing 50–100 g less than the residential WTG males and used only for these 4 confrontations. During each of the 4 confrontations a different intruder male was placed in the resident's cage. Attack latency times were recorded on these 4 days and during the last confrontation full behavioral recordings were made for 10 min after a first clinch or, in case no aggressive attack occurred during the encounter, during 10 min after introduction of the intruder. Experimental rats were defined as non-aggressive (NA) if they failed to attack; low to medium aggressive (LA-MA) when between 1 and 50% of the time was spent on aggressive behaviors, and high-aggressive (HA) when rats spent >55% of the test time on aggressive behaviors. Control males (not screened for trait-aggressiveness) were pair-housed with a female.

Fig. 1. Schematic representation of the Visible Burrow System (VBS) with open arena ($80 \times 55 \times 50$ cm; 12 h lights on) and two smaller chambers ($25 \times 25 \times 30$ cm) connected with Plexiglas tunnels ($\varnothing$ 8 cm) which were covered with black plastic to keep this part in continuous darkness. In the VBS 3–4 male WTG rats are housed together with 2 WTG sterilized but endocrine intact (ligation of the oviduct) females.

In experiment 1, VBS' were formed with 3 males and 2 females. Males in a colony were matched for their level of offensive aggression. In three VBS' with NA males, average aggression scores were $0 \pm 0\%$; three VBS' with LA-MA rats had average aggression scores of $25.3 \pm 5.3\%$; three VBS with HA male WTG rats had an average aggression of $66.6 \pm 3.6\%$.

In experiment 2, 4 colonies were formed with each 3 males and 2 females and 8 with each 4 males and 2 females, with males differing in their level of offensive aggression. Each colony consisted of 1 low aggressive male (average aggression score $4.8 \pm 2.5\%$), 1–2 medium aggressive male(s) (average aggression score $45.3 \pm 3.6\%$) and 1 high aggressive male (average aggression score $86.5 \pm 1.4\%$).

To compare the results obtained in our WTG rats with that obtained in previous studies from the Sakai lab using commercially available Long-Evans (LE) rats, we performed a third experiment. For that purpose we ordered LE rats at an age of 28 days from the same supplier (Harlan, Indianapolis, IN, U.S.A.) as used in the Sakai studies. These LE rats were group housed in our laboratory until they were 5–6 months of age and tested in the VBS. Trait aggressiveness of the LE males was not measured in the resident-intruder paradigm before placement in the VBS and they were therefore also lacking previous sexual experience. Four VBS' were formed with each 3 LE males combined with two WTG females. In another batch of 4 VBS colonies we housed 1 LE male with 2 WTG males and 2 WTG females to see if LE rats would dominate WTG rats or vice versa. Trait aggressiveness in the resident-intruder paradigm was not tested in LE rats prior to placement in the VBS.

2.2. Assessment of social status

Hierarchical status was determined by two criteria: change in body weight and number and place of bite wounds. Subordinate individuals have reliably been reported [23,37] to show a typical and rapid loss in body weight and to have bite wounds particularly in the area of the hind legs and tail. In our experiments rats were checked for bite wounds during the weighing procedure.

2.3. Neuroendocrine response to housing in the VBS and acute restraint stress

After the first housing period in the VBS, all male rats were exposed to an acute restraint stress 24 h after the start of the recovery period when the rats were solitary housed. Animals were placed for 1 h in a ventilated Plexiglas restrainer (length of 21.5 cm and inner diameter of 6.3 cm). Before, immediately and 1 h after restraint stress corticosterone was measured in tail blood and compared with plasma corticosterone levels sampled prior to housing in the VBS. All samples were collected during last 3 h of light period. Basal plasma levels of testosterone were analyzed before and 24 h after housing in the VBS. Corticosterone and testosterone levels were determined using radio immune assays obtained from respectively. Endocrine Sciences, Tarzana, USA and Diagnostic Products.

2.4. Statistical analysis

Results are presented as means $\pm$ SEM. Statistical analysis was performed using SPSS 23 software and a probability level of $p \leq 0.05$ was considered significant. Data were analyzed using one- or two-way ANOVA's and two-way repeated measures ANOVA. Linear curve fitting was performed using Microcal Origin 6.0.

3. Results

3.1. Experiment 1: VBS colonies with males matched for offensive aggressive behavior

In the majority of the 12 VBS colonies, within 2 days a clear and stable social hierarchy was formed with 1 dominant male and two

subordinates. Behavior and physiology of the female members of the VBS were not analyzed in the experiments. A rapid reduction in body weight was observed in most of the subordinate male individuals. Subordinate rats were pooled (see Fig. 2A) to compare effects of dominant ranking on body weight change with subordinate ranking. Obviously due to the selection, a significant reduction in body weight was observed in subordinate males compared to dominant males ($F(1,22) = 9.8$; $p = 0.005$). There was no interaction between time and rank on body weight changes during housing in the VBS. Body weight change in dominants was similar to that in control animals. Although subordinate rats are pooled for the presentation and analysis of the data, body weight reduction in the majority of VBS' (9 out of 12) suggested that hierarchical ranking of the two subordinate males was not always similar with one of the subordinates losing substantially more weight than the other. The hierarchical ranking that we made based upon changes in body weight was supported by the number of bite wounds that was significantly higher ($p < 0.01$) in subordinate males (Fig. 2B). Wounds in subordinate rats were all in the hind part of the body (mainly on the tail) whereas the few wounds in dominant rats were mainly frontal. Only in 1 VBS the dominant male at the end of the two-week period had multiple bite wounds. This was the only colony where hierarchical ranking order was unstable in the beginning and in which the male showing the strongest body weight reduction in the first two days

achieved dominance after 4–5 days. The bite wounds were inflicted during the first few days.

We hypothesized that the average level of trait offensive aggressiveness in the male rats in colonies would positively correlate with severity of social conflicts resulting in more bite wounds in subordinate rats and an increased body weight loss. In Fig. 3 a scatter plot shows that there was no correlation between the percentage offensive aggression in the resident-intruder paradigm prior to VBS housing and the mean change in body weight during colony housing in dominant and subordinate rats (3A). In Fig. 3B average body weight change is presented for VBS' dominant and subordinate rats with all non-aggressive (NA), low-medium aggressive (LA-MA) and high aggressive (HA) males. Body weight reduction in subordinate individuals in NA and LA-MA VBS' was not different. Only in the three VBS' with all highly aggressive males average body weight loss over the two-week housing period was slightly higher when compared with that in subordinates in NA and LA-MA colonies ($p < 0.05$). The average number of bite wounds in males within a colony, however, did not positively correlate with the average level of offensive aggression of the male inhabitants of the VBS (data not shown).

3.2. Experiment 2: VBS colonies with males that differ in offensive aggressive behavior

In this experiment we studied 4 VBS colonies in which 3 males and two females were housed and 8 with each 4 males and 2 females. Fig. 4 shows that subordinates rapidly lost a significant amount of body weight compared to dominant individuals ($F(1,42) = 17.8$; $p < 0.001$) similar to experiment 1. In this experimental approach we hypothesized that the most aggressive male in a colony would have an increased chance to become the dominant. In Fig. 5A we plotted the correlation between the percentage offensive aggression in the resident-intruder paradigm prior to the VBS housing experiment and the body weight change in dominant and subordinate males. In Fig. 5B the average percentage of trait aggression in dominants and subordinates is shown. In Fig. 5C we show in a bar graph how many of the dominant males in a colony indeed were characterized by a high trait aggressiveness and how many subordinates were non- to medium aggressive. The comparison of the average percentage of aggressive offensive behavior in Fig. 5B clearly indicates that there is no difference between dominants and subordinates as to their trait aggressiveness. Neither did the scatter plot in Fig. 5A support the hypothesized negative correlation between body weight reduction and offensive aggression in subordinates. Fig. 5C clearly shows that not only high aggressive males become dominant; there were two non-aggressive rats that dominated rats with higher aggression scores and in the subordinate group 6 rats had the highest aggression scores amongst the males in the VBS in which they were placed.

3.3. Corticosterone response to housing in the VBS and to restraint stress

In experiment 1 tail blood was sampled from rats in 8 VBS colonies before and 24 h after VBS housing to see if VBS housing induced an increased baseline level of plasma corticosterone. Immediately after this 24 h post-colony sampling, rats were placed for 60 min in a restrainer. Fig. 6 shows the corticosterone response to restraint stress and the speed of recovery by a plasma sample collected 1 h after the stress exposure. No significant differences were observed between control, dominant and subordinate males in response to restraint stress. Also no differences were observed in speed of recovery. In the Fig. 6B insert, increases in baseline levels of plasma corticosterone after colony housing are shown for dominants and subordinates and compared with control males. Housing in the VBS tended to increase baseline level of plasma corticosterone in comparison to controls. This effect did not reach, however, the level of statistical significance.

Fig. 2. A: Body weight changes in male dominant and subordinate WTG rats after housing in the VBS for a period of two weeks (indicated by the gray square). VBS colonies were formed with 3 male rats and 2 females. All males were previously screened for offensive aggressive behavior in the resident-intruder paradigm. Colonies were formed with males that had similar aggression scores: 3 colonies had all non-aggressive males, 6 had low-medium aggressive males, and 3 had all highly aggressive males. Control males were housed together with a female conspecific. B: Number of bite wounds in dominant and subordinate males in the colonies in panel A. Bite wounds were mainly in the hind part of the body (hind legs and tail).

Fig. 3. A: Relation between percentage trait aggressiveness in the resident-intruder paradigm as tested before placement in the VBS of male inhabitants and average change in body weight during two week housing period in the VBS with males matched for their aggressive behavior. There was no significant correlation between aggression levels in subordinates (open triangles), dominants (closed triangles) and body weight change. B: Average body weight change in colonies with all non-aggressive (NA), low-medium aggressive (LA-MA) and high aggressive (HA) males. Body weight drop in subordinate rats in HA colonies was larger than in NA and LA-MA colonies ($p < 0.05$).

3.4. Effect of colony housing in the VBS on plasma testosterone

In experiment 1 also plasma testosterone was analyzed before and 24 h after the colony housing in all males. Fig. 7A shows that baseline testosterone levels in dominant and subordinate males before placement in the VBS were similar for dominant males and the subordinate males that lost the least amount of body weight. The baseline levels of testosterone before colony housing were, however, significantly higher in the most subordinate individuals ($p < 0.05$). We had 4 VBS' available for the experiments in this study. In each of the three cohorts of animals these higher pre-colony baseline testosterone levels in the most subordinate rats were observed. The increase in testosterone levels during

Fig. 4. Body weight changes in male dominant and subordinate WTG rats after housing in the VBS for a period of two weeks (indicated by the gray square). VBS colonies were formed with 3 to 4 male rats and 2 females. All males were previously screened for offensive aggressive behavior in the resident-intruder paradigm. Colonies were formed with males increasing in aggression scores: each colony consisted of 1 low aggressive ($4.8 \pm 2.5\%$ aggression), 1–2 medium aggressive ($45.3 \pm 3.6\%$), and 1 high aggressive ($86.5 \pm 1.4\%$) male. Control males were housed together with a female conspecific.

VBS housing was strongest in dominant males and absent in the most subordinate individuals. There was no correlation between offensive aggression levels in the resident-intruder paradigm and the change in plasma testosterone to colony housing in dominants, and each of the subordinate animals (Fig. 7B). Pre-colony baseline levels of testosterone were also not correlated with trait aggressive behavior.

3.5. VBS colonies with LE male rats and mixed colonies with WTG and LE males

The 4 VBS' with three LE males and two WTG females showed a striking difference with the colonies formed with WTG males. Fig. 8A shows the body weight changes in LE rats. In WTG colonies subordinate rats dropped body weight in the first few days and subsequently a gradual increase in body weight gain appeared as indicated by the absence of a statistical interaction between strain and time in experiment 1 and 2. In colonies with only LE rats, however, body weight in subordinate animals continuously dropped during housing in the colony whereas bodyweight in dominants was stable (interaction between rank and time $F(1,10) = 5.1; p = 0.04$). This is consistent with the behavioral observations of the colonies that agonistic and highly aggressive social interactions did not decrease after formation of a stable social hierarchy. Also the general appearance of subordinate LE rats in the VBS was indicative of poor well-being with many bite wounds of some subordinates. After 8 days we stopped the colony housing in the VBS with only LE males for that reason.

In the experiment where we aimed to study ranking positions of VBS colonies with both LE and WTG males, body weight reduction was also particularly severe in the LE males. In three out of four colonies, WTG males became the dominant rats. In these VBS' the subordinate LE rats lost substantially more body weight than the WTG subordinates. In one VBS the LE male became the dominant male. In this VBS the subordinate male WTG rats also lost more weight than WTG subordinates in colonies with WTG dominants. Main reason for this was that the number of agonistic interactions initiated by the dominant LE rats did not drop substantially after settlement of the social hierarchy. Also these housing experiments were stopped after one week.

Fig. 5. A: Relation between percentage trait aggressiveness as tested before placement in the VBS in the resident-intruder paradigm of male inhabitants and average change in body weight during two week housing period in the VBS with males with differential levels of trait-aggressiveness. There was no significant correlation between aggression levels in subordinates (open triangles), dominants (closed triangles) and body weight change. B: Average level of trait aggressiveness was similar in dominant and subordinate rats. C: Percentage of rats that were highly, medium and non-aggressive in dominant and subordinate rats. Number of animals is indicated in each bar as well as average trait-aggressiveness ($\pm$ sem).

Fig. 6. A: Plasma corticosterone response on day 15 in single housed male WTG rats (24 h after housing in the VBS) to restraint stress for 60 min in rats that were either dominant (closed triangles) or subordinate (open triangles). Responses in colony housed rats were compared with control males housed with a female conspecific. There were no differences in corticosterone responses. B: Increases in plasma corticosterone levels 24 h after housing in VBS (dominants and subordinates) or with a female partner (controls) compared to pre-colony corticosterone values. There were no significant differences between dominant and subordinate rats.

Fig. 7. A: Plasma testosterone levels in dominant and subordinate rats before and after colony housing. Subordinates were ranked based upon their decrease in body weight. Subordinates that lost the most weight were pooled as subordinate 2, the other subordinates lost less weight and were pooled as subordinate 1. Plasma testosterone levels prior to housing in the VBS were higher in the most subordinate males (asterisk, $p < 0.05$). In dominant (hash, $P < 0.001$) and least subordinate males ($p < 0.01$) plasma testosterone was significantly increased after VBS housing while this was not the case in the most subordinate rats. B: Increases in plasma testosterone levels following VBS housing did not correlate with trait aggressiveness in dominant (closed triangles) or subordinate (open triangles) rats.

4. Discussion

Dominance hierarchies are integral aspects of animals living in social groups. Hierarchical social status is a key regulator of health and well-being in mammals. The driving force behind high rank formation is the ability to maximize control over access to resources like food, water, mates and shelter [38]. High rank generally translates into a better physical condition and higher survival rate while low social ranking is usually associated with increased levels of stress both in animals and humans [23,27,39]. Negative consequences for health and well-being are particularly strong for individuals of low rank in a society with a steep or unstable hierarchy including many overt agonistic interactions [39]. Recently it was shown in baboons that subordination does not always result in activation of stress response mechanisms and

concomitant stress related behaviors and that behavior of the dominant male plays an important role in this [40]. Individual differences in personality may also play an important role in attaining a high social status. A specific personality variable involved in hierarchical ranking may be aggressive behavior. Offensive aggressiveness is known to vary enormously between individuals of virtually all animal species including humans. In humans, for example, neuroticism and agreeableness are particular personality dimensions that are associated with aggression [41]. Because the social rank of an individual is established during competitive encounters with conspecifics, aggressiveness and social dominance may seem intuitively linked.

Previously, we have shown in several studies that individual differences in offensive aggressiveness as measured in the resident-intruder paradigm in males from a feral rat strain, the Wildtype Groningen

Fig. 8. A: Colonies were formed with three Long-Evans (LE) male rats co-housed with 2 sterilized WTG females. Subordinate males (open triangles) continuously dropped in body weight after start of the VBS housing. Dominant LE (closed triangles) rats did not lose body weight. VBS housing was stopped after 8–10 days because it caused too much discomfort in subordinate males. B: Colonies were formed with two WTG males, 1 LE male and 2 WTG females. In three out of four VBS' a WTG male achieved dominance (closed triangles). In these colonies WTG subordinate rats (open triangles) lost body weight which stabilized after 6 days. Subordinate LE rats (open diamonds) kept losing body weight. In the VBS where an LE achieved dominance, the WTG subordinates (open hexagon) also kept losing weight.

(WTG) rat [7], are consistent over time and context and can be considered as important indicators of distinct coping styles or animal personality (see for review [20]). In this study we aimed to link these trait-like differences in aggressive behavior in WTG rats with ranking position achieved and hierarchical steepness of rat colonies in the VBS. First we had to study, however, if a clear social hierarchy would be formed in mixed-sex colonies of our WTG rats. We expected to rank our male individuals in the VBS based upon their change in body weight and the pattern and number of bite wounds. As shown previously in rat VBS studies of Bob and Caroline Blanchard [42] and Kellie Tamashiro [37, 43], studies in which Randall Sakai played a pivotal role, the reduction in body weight is typical for the subordinate males in the VBS and correlates with bite wound characteristics. All these studies were performed in Long-Evans rats, a laboratory rat strain that is commonly used in studies focusing on causes and consequences of social aggressive behavior. Our studies support their findings, showing that hierarchical ranking in male rats from the WTG strain is reflected in body weight changes that corresponds with the number and location of bite wounds. As shown in our studies social conflicts between WTG males in the VBS were causing numerous bite wounds in subordinate individuals. Therefore social status in the VBS was primarily based upon the amount of body weight loss. This does hold a certain risk of misinterpreting the social status in a VBS and actually a full behavioral analysis of all animals in the colony incorporating for example social avoidance behavior would be a better way to score social status of all male individuals in the colony. In the vast majority of the VBS colonies a substantial differentiation occurred between the two-three subordinates with one subordinate losing a significantly higher amount of body weight than the others and receiving more bite wounds. This seems to indicate that also within subordinates a ranking hierarchy occurs with one animal being the most subordinate and lowest in rank. Usually within 1–2 days hierarchical ranking was clear and stable amongst the WTG males. Particularly in this starting phase agonistic social interactions occurred with a relative high frequency that decreased after the formation of a stable hierarchy.

We hypothesized that rats with high trait aggressiveness as measured in a pre-colony resident-intruder paradigm would be more likely to reach a dominant ranking position in the social hierarchy. Although males with a high trait aggressiveness seemingly have a higher chance to get the highest social status in a colony; in half of the 12 colonies a male with high trait aggressiveness (6 out of 12: 50%) is the dominant male. In the other half of the VBS, however, either a medium aggressive (4 out of 22: 18%) or a low-aggressive animal (2 out of 10: 20%) achieved dominance. Neither was there a correlation between level of offensive aggression and changes in body weight during housing in the VBS nor were dominant rats characterized by previously defined high aggression scores. Apparently, rats that were not aggressive at all in the resident-intruder paradigm were able to dominate rats that showed to be very aggressive in the resident-intruder-test. This is a surprising finding. In male lizards a fast response to various social stimuli including attack latency predicted social dominance [38]. This dominance was observed in paired interactions and not in a complex social structure with multiple male and female inhabitants as in the present study. In our study also attack latency was not predictive of social status.

We also expected that social agonistic behavior would be fiercer in VBS colonies in which all male rats had high levels of aggression in comparison with colonies where the average offensive aggression level in a resident-intruder paradigm was very low. This potentially could yield colonies with relatively high stress levels in all individuals, particularly in the subordinates, due to a high number of fierce social combats. Since subordination induces body weight reduction we assumed to see an increased drop in body weight in subordinate males in colonies with a high average level of aggression. A comparison between mean body weight loss in colonies with high aggression scores (HA) and non-aggressive (NA) to low/medium aggression (LA-MA) scores

showed the strongest body weight reduction in the most aggressive colonies suggesting that our expectations were justified. However, we failed to observe a clear and strong correlation between offensive aggression and VBS induced body weight loss present in subordinate males. Furthermore, the number of bite wounds was low in colonies with only non-aggressive males but there was no difference in number of bite wounds between colonies with low, medium or high trait aggressiveness levels. A better analysis of social interactions in the VBS is needed to draw firm conclusions on the relation between personality characteristics and steepness of the social hierarchy.

The neuroendocrine stress response to VBS housing and restraint as reflected in the plasma corticosterone levels before and 24 h after colony housing was not different between subordinate and dominant WTG rats. We also did not observe a group of non-responder subordinates as previously published using the Long-Evans strain [23,37]. Strain differences probably play an important role in behavioral, physiological and neurobiological consequences of colony housing. A complicating factor in comparing the neuroendocrine responses to colony housing and a physical stressor like restraint in our experiments and those in previously published experiment using LE rats is that timing of exposure to the stressor was different. In studies with LE rats the neuroendocrine response to restraint including baseline plasma levels reflecting responses to VBS housing were measured in rats that were still housed in the VBS (1 h after removal) on day 13 [23,28,37]. In our studies we measured plasma levels 1 day later. It is not unlikely that the effects of VBS housing on plasma corticosterone secretion were already gone one day after colony housing. It is also possible that chronic activation of the HPA-axis to housing in the VBS was lower in subordinate WTG males than in Long-Evans rats. In our experiments we had the impression that subordination is not necessarily a stressor. After initial conflicts during initiation of the social hierarchy all individuals seemed to adapt to their ranking position and the number of agonistic interactions reduced and bite incidents were relatively rare as soon as the hierarchy was formed. This was clearly different in colonies formed with LE rats. The LE rats we imported from the U.S. behaved considerably different in the VBS than our WTG rats. Agonistic interactions continued during the entire housing period and subordinate males continuously dropped body weight and bite incidents were high throughout the housing period. Even when housed together with WTG males, LE subordinates kept losing weight and overt conflicts continued to occur even after clear settlement of the hierarchy. In the single colony where a LE male achieved dominance, both WTG subordinate rats heavily dropped in body weight and were bitten regularly in conflicts during the entire VBS housing period. Strain dependent differences in the capacity to behaviorally adapt to dominant or subordinate ranking position seem to underlie the chronic stress that particularly LE male rats experience during VBS housing. LE rats were not tested prior to the VBS housing for trait aggressiveness as residential males in the resident-intruder paradigm. Therefore, they were also lacking the sexual experience the WTG males had prior to placement in the VBS. We do not know if and how this different experience may have affected the behavior of the LE rats in the VBS.

A remarkable finding was that subordinate rats that lost the highest amount of body weight (likely the most subordinate animal, which we defined as “subordinate 2” in Fig. 7) were characterized by the highest pre-colony plasma testosterone levels. Since this consistently occurred in the three cohorts of colonies we studied, this seems to be a typical phenomenon. The absence of a testosterone increase in these animals following VBS housing was also striking. Tamashiro et al. [37] reported a strong reduction in plasma testosterone levels in subordinate males, especially in the ones that were housed in a female biased colony. In another study it was shown that already at day 4 of colony housing testosterone levels are significantly reduced in subordinates [44] and it was suggested that this may be causal in the reduction of body weight.

In conclusion, the experiments seem to indicate that WTG rats are capable to successfully adapt to social structures like the VBS. Although

body weights significantly drop initially in subordinate individuals, stabilization occurs as soon as a clear social hierarchy is established. Neuroendocrine (re)activity 24 h after the VBS colony housing supports an interpretation that in WTG rats there is no evidence that part of the males are exposed to chronic social stress of subordination as previously shown in rats from the Long-Evans strain. Strain dependent differences in the capacity to display appropriate social behavior as either dominant or subordinate male probably play an important role in the level of perceived stress in mixed sex social colonies like the VBS.

In future experiments we will explore in more detail the neurobiological mechanisms of hierarchical ranking in behaviorally characterized rats focusing on individual differences in coping strategy or animal personality aimed to understand health consequences of differential ranking positions. In these studies state-of-the-art techniques will allow individual recording of physiological parameters like blood pressure, heart rate and temperature using telemetry measuring each individual in the VBS simultaneously. Also techniques will be applied to study social behavior of each animal and monitor individual food intake and body weight changes via fully automated weighing systems. Many clinical trials indicate that the predictive power of most single niche tests for neuropsychological disorders in animal is disappointingly low. Therefore, the scientific approach of Randall Sakai studying animals in semi-natural environments as the VBS, is highly relevant to get a better understanding of brain systems involved in adaptive and maladaptive capacity to function as an individual in complex social structures. In this approach behavioral consequences of pharmacological interventions used for multiple targets in various disease models will hopefully more accurately show undesired side effects reflected in the impact of drug treatment on the capacity to function in a social hierarchy. Furthermore, it will demonstrate if there are individual differences in the response to drug treatment based upon differences in ranking position or personality.

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