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Aggressive and non-aggressive personalities differ in oxidative status in selected lines of mice (*Mus musculus*)

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Mice selected for aggression and coping (long attack latency (LAL), reactive coping strategy; short attack latency (SAL), pro-active coping strategy) are a useful model for studying the physiological background of animal personalities. These mice also show a differential stress responsiveness, especially in terms of hypothalamic–pituitary–adrenal axis reactivity, to various challenges. Since the stress response can increase the production of reactive oxygen species, we predicted that the basic oxidative status of the lines could differ. We found that LAL showed higher serum antioxidant capacity (OXY) than SAL, while no differences emerged for reactive oxygen metabolites (ROMs) or the balance between ROMs and OXY, reflecting oxidative stress. Moreover, the lines showed inverse relationships between ROMs or OXY and body mass corrected for age. The results indicate that variation in oxidative status is heritable and linked to personality. This suggests that different animal personalities may be accompanied by differences in oxidative status, which may predict differences in longevity.

Keywords: oxidative stress; free radicals; antioxidants; personality; glucocorticoids; aggression

1. INTRODUCTION

Metabolism produces pro-oxidant compounds that damage biomolecules. To cope with pro-oxidants, the body uses antioxidants and mechanisms that are able to repair or remove damaged molecules. When the redox status, i.e. the balance between pro-oxidants and antioxidants, is shifted towards more oxidative conditions, oxidative stress (OS) arises (Finkel & Holbrook 2000). The accumulation of degenerative changes caused by OS to biomolecules may lead to pathologies, cell senescence and cell death (Beckman & Ames 1998; Finkel & Holbrook 2000).

One of the mechanisms by which organisms cope with stressful challenges is the secretion of glucocorticoid steroid hormones. Several studies show that stress response mediated by glucocorticoids may increase OS in birds or mammals (e.g. Lin *et al.* 2004; Sahin & Gümüslu 2007). Individuals show consistent and non-random differences in how they cope behaviourally and physiologically with challenges, referred to as behavioural syndromes, coping styles or personalities (Koolhaas *et al.* 1999; Sih *et al.* 2004; Groothuis & Carere 2005; Bell 2007; Wolf *et al.* 2007). One established model is provided by mouse lines selected for short and long attack latencies (SAL and LAL; Van Oortmerssen & Bakker 1981; Benus *et al.* 1991; Koolhaas *et al.* 1999; Veenema *et al.* 2003*b*). SAL mice show shorter attack latencies and higher attack counts than LAL mice. Furthermore, the lines display different coping strategies with non-social environmental challenges (Benus *et al.* 1991; Koolhaas *et al.* 1999). More importantly for the present work, the glucocorticoid response to ACTH, novelty and forced swim is significantly higher in LAL than in SAL mice (Veenema *et al.* 2004, 2005*b*). Chronic psychosocial stress induces a long-lasting increase in glucocorticoid production in LAL mice, but not in SAL mice (Veenema *et al.* 2003*a*, 2005*a*). These data show that LAL mice have a higher stress responsiveness in terms of glucocorticoid production than SAL mice (Veenema *et al.* 2004).

In line with the characterization of personality in other taxa (e.g. birds, Groothuis & Carere 2005), LAL can be described as reactive and SAL as pro-active (Koolhaas *et al.* 1999). So far, there is scant information on the relationship between personality and oxidative status. Evidence from humans shows that the activity of the glutathione peroxidase 1 gene is lower in shy than in bold individuals (Matsuzawa *et al.* 2005). There are no studies on other animals explicitly relating personality traits to oxidative status, but a few studies suggest a link. One longitudinal study found that neophobic, shy rats had increased basal corticosterone levels throughout life and a 60% higher chance of death compared with neophilic, bold individuals (Cavigelli & McClintock 2003). These rats, although not tested for aggression, may be comparable to the SAL–LAL mice, at least in terms of glucocorticoid production and hypothalamic–pituitary–adrenal axis reactivity. One potential explanation is that the higher glucocorticoid responsiveness of shy individuals could be associated with or induce OS (Behl *et al.* 1997).

On the hypothesis that differential stress responsiveness is related to different oxidative profiles, we compared the serum of adolescent male SAL (generation 78) and LAL (generation 51) mice for two physiological measures: (i) the level of reactive oxygen metabolites (ROMs), which is a marker of oxidative damage and (ii) the serum antioxidant capacity (OXY; e.g. Ballerini *et al.* 2003; Costantini & Dell'Omo 2006*a*,*b*; Costantini *et al.* 2006, 2007). In this study, the mice of the two lines were not exposed to any stressor to amplify their behavioural and neuroendocrine differences; however, we expected to find different basal oxidative profiles, given the evident behavioural and physiological differences that the lines display also under non-stressful conditions.

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Table 1. Outcomes of GLMs. (Significant *p*-values are shown in italics.)

variable	source of variation	*F*	d.f.	*p*-value
ROMs	line	1.30	1,24	0.264
	body mass corrected for age	2.84	1,24	0.105
	line×body mass corrected for age	5.50	1,24	*0.028*
OXY	line	9.15	1,24	*0.006*
	body mass corrected for age	1.97	1,24	0.173
	line×body mass corrected for age	4.34	1,24	*0.048*
oxidative stress: ROMs/OXY×1000	line	2.98	1,24	0.097
	body mass corrected for age	3.49	1,24	0.074
	line×body mass corrected for age	1.07	1,24	0.312
oxidative stress: ROMs with OXY as a covariate	line	0.10	1,21	0.755
	OXY	0.00	1,21	0.948
	body mass corrected for age	0.00	1,21	0.983
	line×OXY	0.18	1,21	0.677
	line×body mass corrected for age	3.51	1,21	0.075
	OXY×body mass corrected for age	0.03	1,21	0.867

2. MATERIAL AND METHODS

Blood was collected from 38 to 64 days old male mice (*Mus musculus domesticus*; $n=14$ for each line).

ROMs and OXY were measured by the d-ROMs test and the OXY-Adsorbent test, respectively, according to previous studies (Costantini & Dell'Omo 2006*a*,*b*; Costantini *et al.* 2006).

Generalized linear models (GLMs) were performed using the STATISTICA package (v. 7.0, StatSoft, Inc., 2004, Tulsa, OK, USA).

Details of the methods are reported in the electronic supplementary material.

3. RESULTS

The lines did not differ in the levels of ROMs (table 1; figure 1). However, they showed inverse relationships between ROMs and body mass corrected for age (significant interaction term; table 1; figure 2).

The OXY was higher in LAL than in SAL mice (table 1; figure 1) and the lines showed inverse relationships between OXY and body mass corrected for age (significant interaction term; table 1; figure 2).

The ratio of OS did not differ between the lines (table 1; figure 1), nor did the relationship between this ratio and age-corrected body mass (no significant interaction term; table 1; figure 2). The difference in OS between the lines approached significance ($p=0.052$) after dropping the interaction term from the model. Similar results were found using ROMs as the dependent variable and OXY as a covariate (table 1). The line effect approached significance ($p=0.069$) after dropping all the interaction terms from the model.

SAL and LAL mice did not differ in age ($t=-1.21$, $p=0.23$; Levene's test, $p<0.001$; mean±s.e.: SAL, 49.71±2.40 days; LAL, 52.71±0.61 days). A significant difference was found for body mass ($t=2.32$, $p=0.03$; Levene's test, $p<0.001$; mean±s.e.: SAL, 18.59±0.82 g; LAL, 16.54±0.32 g) and body mass corrected for age ($t=4.00$, $p<0.001$; Levene's test, $p=0.19$; mean±s.e.: SAL, +1.32±0.55; LAL, −1.32±0.37).

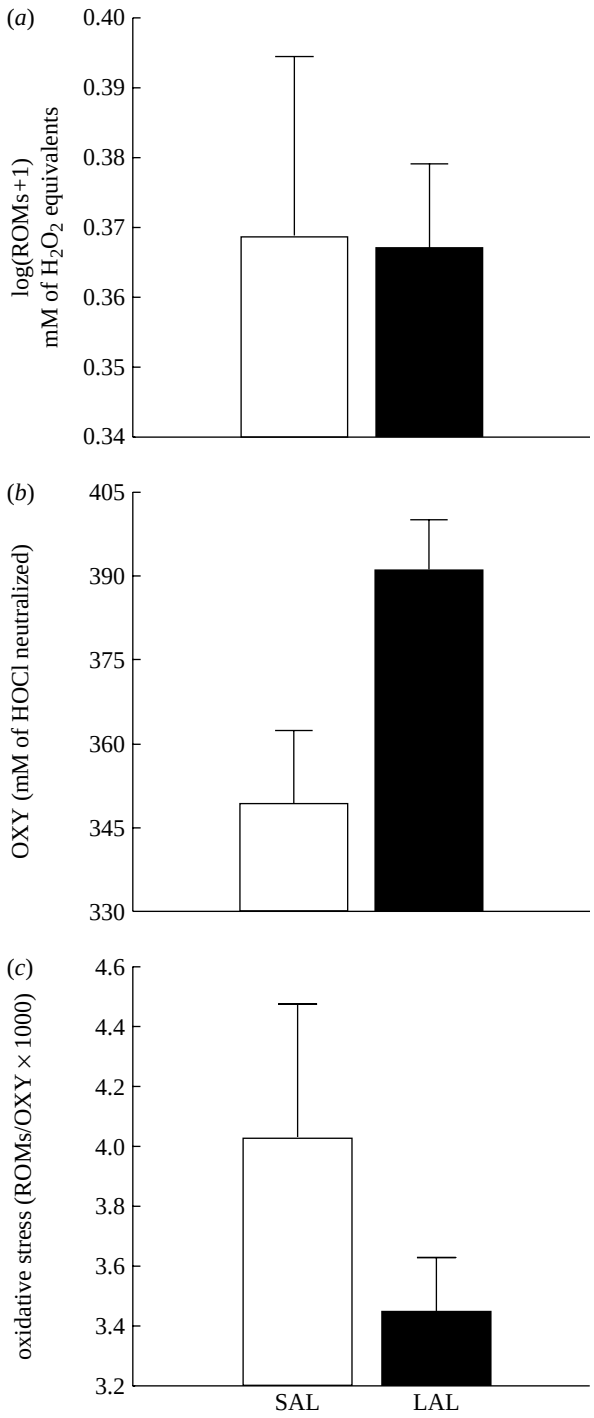

Figure 1. SAL mice show lower OXY than LAL mice. No differences emerged for ROMs or OS. Means+s.e.m. are shown. (*a*) $p=0.26$, (*b*) $p=0.006$ and (*c*) $p=0.10$.

4. DISCUSSION

We found that non-aggressive mice (LAL) showed higher OXY than aggressive mice (SAL). Moreover, the lines showed different relationships between ROMs or OXY and body mass corrected for age. This is the first evidence in non-human animals that oxidative status differs between personalities with a known genetic background.

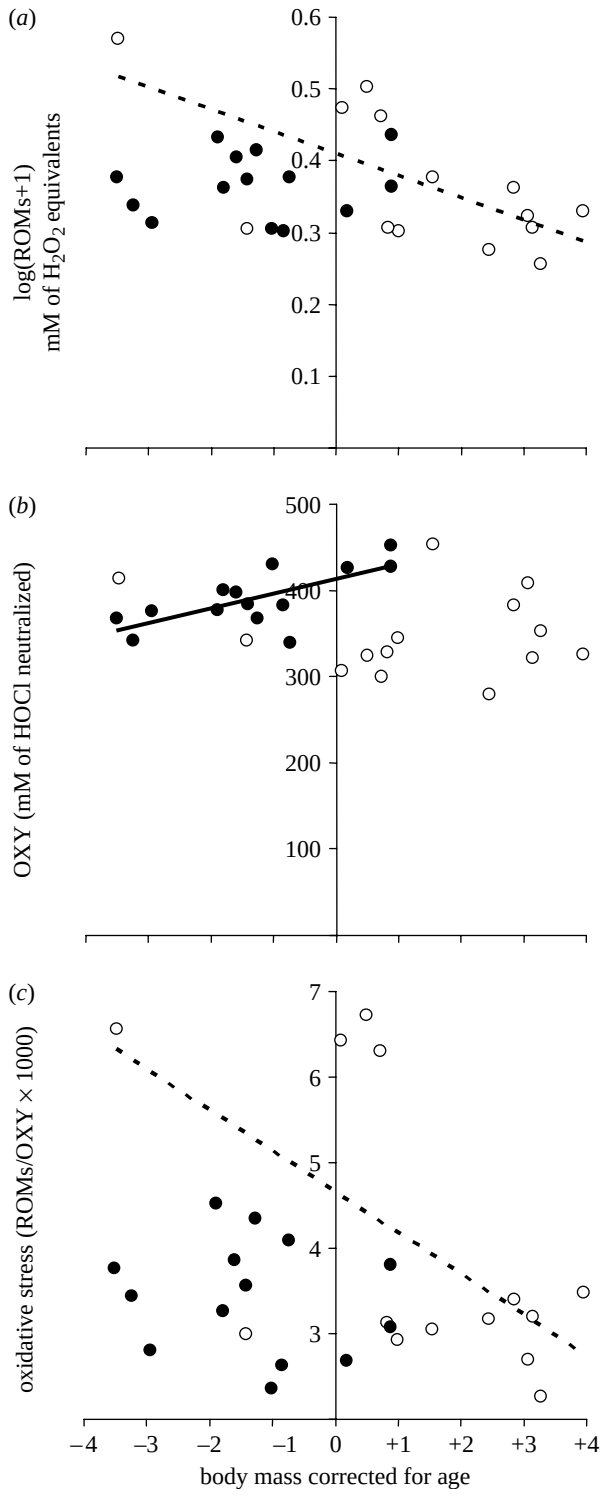

Figure 2. SAL (open circles, dashed line) and LAL (filled circles, solid line) mice show inverse relationships between body mass corrected for age and ROMs or OXY. Trend lines are shown when significant (SAL: (*a*) ROMs, $r=-0.65$, $p=0.011$; (*c*) OS, $r=-0.56$, $p=0.037$; LAL: (*b*) OXY, $r=0.69$, $p=0.006$). Body mass corrected for age is shown as residuals from a linear regression of body mass on age.

Our findings indicate that oxidative status has a genetic basis and is linked with personality and stress coping, probably having been co-selected with the behavioural phenotype. However, the effect could be indirect, as different behavioural styles could themselves induce different oxidative status profiles. This latter hypothesis is unlikely, since the subjects were adolescent and experimentally naive concerning social challenges. A genetic basis for oxidative status is known for many species (Martin *et al.* 1996), thus our finding is not surprising. However, a link with personality has been shown for humans only (Matsuzawa *et al.* 2005).

Less aggressive male mice have shorter lifespans, and it has been suggested that the age-related decline in the concentrations of catecholamines and testosterone might be responsible for their reduced longevity (Ewalds-Kwist & Selander 1996). Given the present results and the fact that OS may modulate ageing (Harman 1956; Beckman & Ames 1998), the redox status could explain the differences in lifespan that different personalities experience (Cavigelli & McClintock 2003). In our study, the less aggressive LAL showed higher OXY. This suggests that LAL may overexpress OXY to cope with higher free-radical production or to prepare the organism for the high stress levels they will experience throughout adulthood. Whether glucocorticoid production, which is higher in shy, neophobic and less aggressive individuals, mediates such differences needs to be tested experimentally, but the evidence from this and other studies and species supports this view (e.g. Lin *et al.* 2004; Sahin & Gümüslu 2007).

The patterns of covariance between both markers of OS and body mass corrected for age showed that in SAL mice, ROMs decreased with age-corrected body mass, while in LAL mice OXY increased with it. In contrast, the relationship between age-corrected body mass and the ratio between ROMs and OXY did not differ. These results suggest that the lines have different maturation times for the mechanisms that regulate redox status, or that they undergo different metabolic costs of body mass.

In conclusion, our results shed light on the personality/oxidative status nexus and call for further investigations to evaluate how the seemingly different redox statuses characterizing different personalities respond to stress challenges. A potential problem is that the selection lines are not replicated and this implies that this study does not prove causality (see Falconer & Mackay 1996, p. 318). However, studies comparing all three genetic selection lines for high and low aggression available in the world show striking similarities in behaviour, neuroendocrinology and neurochemistry (e.g. Caramaschi *et al.* 2007). Moreover, these similarities also hold for the extremes in aggressive behaviour in an unselected strain of feral rats (Koolhaas *et al.* 2007).

Future work on the two lines should compare oxidative status between basal and acute stress conditions, as well as examine its relationship to ageing. Studies should also consider the levels of oxidative damage and antioxidant capacity in other tissues to build a more comprehensive view of the individual OS of SAL–LAL lines.

Animal care and sampling procedures were performed according to the Animal Experiment Committee (DEC) of the University of Groningen.

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