Aggressive and nonaggressive personalities differ in oxidative status in selected lines of mice (Mus musculus)
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Published in:
Biology Letters

DOI:
10.1098/rsbl.2007.0513

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Supplementary Material

Methods

Animals and blood collection

The mice were bred and kept with their parents until weaning, which occurred at 21 days of age. Subsequently, they were housed in unisexual familiar groups in Makrolon® type II cages with sawdust as bedding material, shredded paper as nesting material and a cardboard tube as cage enrichment, and maintained under standard laboratory conditions (12:12 light/dark cycle, 22 ± 2°C). Food (AMII, AB diets, Woerden, NL) and water were provided ad libitum.

Blood was collected when mice were 38–64 days old. At this stage they were naïve concerning resident-intruder tasks. They could, however, display mild agonistic behaviour towards sibs, although real fights have been observed only in SAL animals and only at the end of adolescence, upon the establishment of hierarchies.

We avoided using very young subjects because the mechanisms regulating the body's oxidative status need some time to mature, so this study may help also to determine whether the lines differ in the time of maturation of such mechanisms. We avoided using adult animals because oxidative status may covary with age differently in the two lines. Moreover, in such housing conditions the oxidative status of adult animals could have been affected by frequent agonistic experiences.

On the sampling day, the mice were anaesthetised with a CO₂/O₂ mixture, weighed and decapitated, whereupon the trunk blood was collected on ice within 3 minutes. After 2–3 hours of storage at 4°C, the samples were centrifuged for 5 minutes at 9,000 rpm. The serum was removed and stored at −20°C.
Biochemical analyses

The d-ROMs test quantifies the levels of circulating reactive oxygen metabolites (primarily hydroperoxides, ROOH), i.e., early peroxidation products of the exposure of biological macromolecules (mainly lipids but also proteins and nucleic acids) to reactive oxygen species. The OXY-Adsorbent test (Diacron, Grosseto, Italy) uses a colorimetric determination to quantify the serum anti-oxidant capacity (OXY), i.e., the ability of the serum to cope with the oxidant action of hypochlorous acid (HOCl; oxidant of pathologic relevance in biological systems) by the whole suite of circulating anti-oxidants. Measures were run in duplicate and an average value was computed (repeatability: ROMs, $r = 0.88$, $p < 0.001$; OXY, $r = 0.84$, $p < 0.001$). For further details see Trotti et al. (2002) and Iorio (2004) and references included in the manuscript.

Statistical analyses

General linear models (GLMs) were used to evaluate which factors explained the variation in ROMs and OXY. We also ran a GLM in which the dependent variable was the ratio between ROMs and OXY ($\times 1,000$), which can be interpreted as an index of oxidative stress (OS).

ROMs and OXY were not correlated (Pearson correlation between log(ROMs + 1) and OXY, all mice: $r = 0.10$, $p = 0.61$; SAL: $r = 0.15$, $p = 0.61$; LAL: $r = 0.03$, $p = 0.93$), so a second model for oxidative stress was run with ROMs as the dependent variable and OXY as a covariate (Costantini et al. 2007).

We used the log($x + 1$) transformed ROMs and the reciprocal of OS to meet the normality assumption. Levene's test was run to check the homogeneity of variances. Main effects and two-way interactions were considered. Interactions had been removed
from the models when non significant and the analyses were repeated according to Engqvist (2005).

In all models we included as a covariate the residuals from an ordinary least squares regression of body mass on age, because body mass and age were correlated ($R^2 = 0.26$, $p < 0.01$) and this can cause problems of collinearity. The inclusion of this covariate in the model served to correct for differences that we found in the covariate and allowed us to see if the dependent variable was associated with the covariate. This second point is important because oxidative status may vary across the growth period because of the maturation of regulating mechanisms, so body mass at a given age may help reveal if the two lines differ in the time of maturation of such mechanisms or in the metabolic costs imposed by body mass.

References


