

Online Resource 2

Title: Development of individual differences in stress responsiveness: an overview of the factors mediating the outcome of early-life experiences

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Open field setup

In order to investigate whether *non-shared* early environmental factors could account for the development of individual differences in stress phenotype in later-life, we studied endocrine responsiveness to acute stress in animals characterized in early-life as either Low or High LG *within* the litter.

The novelty stressor consisted of 10 min exposure to a black circular open field apparatus (diameter: 90 cm, height: 40 cm) in a dimly lit room (approximately 30 lux floor level). Two animals were tested at the same time and in the same testing room using two separate open field boxes. All testing took place between 09.00 and 13.00h, and each test apparatus was cleaned thoroughly between animals using 70% ethanol. All animals were individually housed two days prior to testing (to reduce the effect of testing-order within the cage) and returned to their home cage directly after testing.

Blood sampling

Blood samples were obtained from the tail vein, by a small incision with a razorblade at three different time points: a) basal, one day before novelty exposure, b) 10 min after onset of novelty stress, i.e. directly after termination of stressor, and c) 30 min after onset of novelty stress, i.e. 20 min after termination of stressor. Blood was collected in small EDTA coated tubes (Microvette CB 300 K2E, Sarstedt, Numbrecht, Germany). Plasma was obtained by 15 min centrifugation at 13000 rpm at 4°C and subsequently stored at -20°C until further analysis.

Plasma corticosterone determination

Plasma corticosterone concentrations were determined by *in-duplo* measurement using radioimmunoassay (RIA) kits containing ¹²⁵Iodine labelled corticosterone (MP Biomedicals, Asse-Regelem, Belgium). All samples were analysed in one assay to exclude inter-assay variability.

Statistics

Statistics were performed using SPSS for Windows (version 14.0; SPSS, Chicago, IL). One-way ANOVA's were performed to compare Low LG and High LG groups. Graphs were plotted using Prism Graph Pad software.

Results

Both basal and stress-induced corticosterone levels in adolescent animals are associated with individual differences in maternal LG acquired through *non-shared* experience

during early-life. Thus, animals that received high levels of maternal LG *within* litter show significantly lower basal as well as stress-induced corticosterone levels when compared to animals that received relatively low levels of maternal LG. Similar to what is observed in adolescence; amount of *non-shared* maternal LG received in infancy also appears to have predictive value for CORT levels in adulthood. Animals that received high levels of maternal LG *within* litter show significantly lower stress-induced corticosterone levels when compared to animals that received relatively low levels of maternal LG. Due to technical issues, we are unable to show basal corticosterone levels of the animals in adulthood.

Life stage	Time point	Mean High Group	SEM High Group	Mean Low Group	SEM Low Group	F value
Adolescence	Basal	9.1 *	0.72	13.6	2.7	5.09
	10 min	161.8 *	21.1	271.7	59.5	4.93
	30 min	362.3 *	25.6	479.4	48.5	5.56
Adulthood	10 min	169.4 **	27.3	516.1	20.9	61.99
	30 min	224.5 **	32.9	456.0	36.0	14.05

Table 1. Mean corticosterone levels and SEM values of High and Low LG groups. * $p < 0.05$ compared to Low LG group; ** $p < 0.01$ compared to Low LG group.