Comparison of different parameters to evaluate stress responses of laboratory rats subjected to different experimental interventions

Abbreviated title: Assessment of welfare in laboratory rats

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Supporting information:

Calculation of circadian bias for a 12 hour sampling period with sampling time at midnight to detect effects of 12 hour sleep deprivation

Sleep deprivation (SD) in rats of G1 took place in the second half (12-24 h) of the 24 h time period in the activity wheels and the corresponding 12 h sampling period with sampling time at midnight solely represents the inactive (lights on) phase of the animals. This is due to the delay of fecal corticosterone metabolite (FCM) excretion of about 14 hours [1]. Due to the circadian rhythm of corticosterone secretion [2], these 12 hours samples are likely biased in comparison to the standard sampling period of 24 hours chosen in the current study and therefore have to be corrected. For this, the rhythmicity and stability of FCM excretion was determined in the 16 animals of G1 and G2 before the implantation of the electroencephalograph (EEG) electrodes one and two weeks after shifting of the animals to lights off from noon until midnight. Sampling

periods for these experiments were 12 hours; one sampling point was at noon, the other one at midnight on consecutive days to validate the stability of FCM excretion. Figure S1 shows the absolute amount of FCMs during the circadian cycle indicating that midnight samples (representing the inactive period of the animal during lights on due to the delay of corticosterone excretion via feces) show higher FCM concentrations, as expected. FCM concentrations of samples collected at midnight and noon were averaged per day and the relative difference between these mean values (mirroring a standard 24h sampling period) and the FCM concentrations detected in midnight samples were calculated (see Table S1). FCMs in the inactive phase (midnight samples) were on average 18% higher in comparison with the FCMs displaying the mean of the active and inactive phase of the animals. Consequently, the determined SD FCM values in G1 sampled at midnight to detect effects of 12 hours sleep deprivation during the course of experiments were adjusted by -18%. Whenever direct comparisons between G1 and G2 were made, the corresponding pseudo SD FCM values in G2 were equally adapted.

Sleep deprivation massively increased FCM concentration (247% in comparison to baseline values) in the current study. In comparison, the circadian rhythmicity with the detected increase of 18% plays a minor role and even if a certain bias is introduced via the calculated correction for pulsatile FCM excretion (most likely via differing amounts of feces voided during the active and inactive period, respectively), this is of minor importance. In addition, data on circadian excretion of FCMs proves to be stable two weeks after circadian shifting of the animals and a further influence of previous shifting is unlikely.

Reference List:

1. Lepschy M, Touma C, Hruby R, Palme R. Non-invasive measurement of adrenocortical activity in male and female rats. Lab Anim 2007;41(3):372-87.
2. Christiansen S, Bouzinova EV, Palme R, Wiborg O. Circadian activity of the hypothalamic-pituitary-adrenal axis is differentially affected in the rat chronic mild stress model of depression. Stress 2012;15(6):647-57.