PET IN CONSCIOUS RODENTS – QUANTIFICATION OF STRESS DURING THE TRAINING PROCESS

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INTRODUCTION & METHODS

Recently several methods for performing PET studies in conscious rodents have been developed [1-3]. These methods have the potential to greatly improve the translational nature of PET studies in rodents. One of the most easily implemented methods is the training of a rat to tolerate head fixation in a restraining device. Training consists of intervals of restraint over several days. However, the stress induced by this training procedure has not been quantified in detail. Limited changes in plasma corticosterone have been reported, but this data may be confounded by sample timing and baseline levels. The aim of this study was to quantify stress associated with conscious PET training by measuring multiple physiological parameters.

Methods

➢In this initial pilot study, an implantable telemetry system (Telemetry Research) was used to remotely measure blood pressure (BP), heart rate (HR) and core temperature (CT) during 5 days of head-fixation training.

➢Transmitters were implanted in the abdominal cavity with the blood pressure sensor in the abdominal aorta. A plastic block was cemented to the skull to allow head fixation.

➢Training was started after a recovery period of at least 1 week.

➢Training consisted of a 5 min period of acclimatization in the cage containing the restraining device, followed by increasing durations of restraint in the device on subsequent training days (15, 30, 45, 60min). The head was not fixed on the first day of training.

➢Telemetry data was acquired from 5 min prior to acclimatization to 60 minutes post-training.

➢The effect of the training process on HR, BP and CT was compared across the 5 days of training.

HIGHLIGHTS

Heart rate and blood pressure were increased during acclimatization and training. Core temperature increased gradually throughout training. HR and BP generally returned to baseline within 30 min of cessation of training, but frequently a delayed peak in CT was observed.

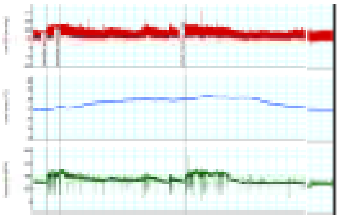

Figure 1: Example of telemetry data from 1hr training session with acclimatization and recovery periods (x axis = time, 5 min blocks). Heart rate was calculated from the BP signal. This example clearly shows the subsequent peak in core temperature after cessation of training.

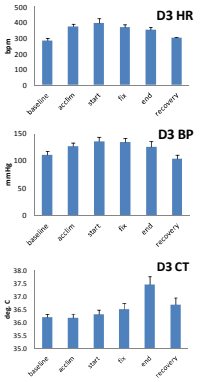

Figure 2: Example of HR, BP and CT responses to 30 min training protocol on the third training day (n=4, mean±SEM). (fix = head fixation; recovery = level after return to baseline after training)

RESULTS & DISCUSSION

➢ The restraining device to allow head fixation was closely based on that described by Itoh et al. (2009) [3]. The device allows adequate restraint and access to the head. Fixation of the head using either plastic or metal screws sufficiently limits horizontal rotation and fore/aft movement, but does not fully prevent vertical rotation. Subsequent designs will address this problem.

Figure 3: The restraining device with head fixation grid. Pair of steel bolts and a steel pin were used to fix the head in position for training. Plastic hardware will be used in PET studies.

➢ As shown in Figure 2, HR and BP were increased during the acclimatization period and at the start of restraint for training.

➢ HR and BP reduced throughout the duration of training as seen in Figure 1. After removal from the restraining device HR and BP recovered to near-baseline levels after 20-30 min.

➢ % change in HR and BP between baseline levels and acclimatization/start of training levels was used to compare the response to training over the 5 days (Figures 4 and 5). **The HR and BP responses to acclimatization and to the training protocol persisted throughout all training days** (no sig. change – 1-way repeated measures ANOVA – except BP response to start of training). During acclimatization, HR was increased by 29±4 bpm (mean±sd) on average across the 5 training days. At the start of training this value increased to 36±5 bpm. BP was increased by 14±3 mmHg during acclimatization and 21±7 mmHg at the start of training.

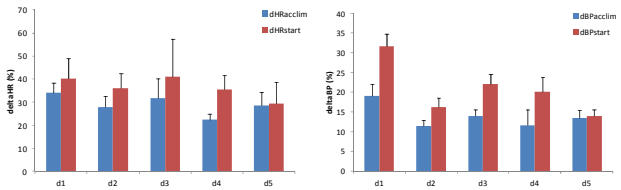

Figure 4 (left) & 5 (right): Percentage change (d, delta) in HR and BP associated with the acclimatization period (acclim) and the start of training (start) across the 5 training days (d1-d5).

➢ As shown in Figures 1 and 2, CT increased throughout training. % change in CT between baseline levels and the end of training was again used to compare the response over 5 days (Fig. 6). No significant adaption to training was found.

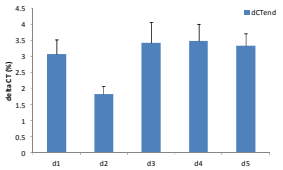

Figure 6: Percentage change in CT associated with training across the 5 training days (d1-d5). No significant change over time (rep. meas. ANOVA).

➢ In subsequent studies the effect of the training on plasma corticosterone and central glucose metabolism (using [18F]FDG) will be examined.

SUMMARY

Increased HR, BP and core temperature are indicative of a stress response. **Consistent increases in HR, BP and core temperature throughout training suggest that limited acclimatization to restraint with head fixation occurs during this 5 day training protocol.** Furthermore, the delayed effect on core temperature seen in some animals may indicate stress-induced hyperthermia. Detailed understanding of the underlying stress in PET studies using conscious animals will aid in the interpretation of such data.

REFERENCES

[1] Momosaki et al. (2004) Synapse 54:207–213. [2] Wyss et al. (2009) NeuroImage 48:339–347. [3] Itoh et al. (2009) J Nucl Med 50:749–756

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