Chronic stress shifts the phase of adrenal clock gene rhythms

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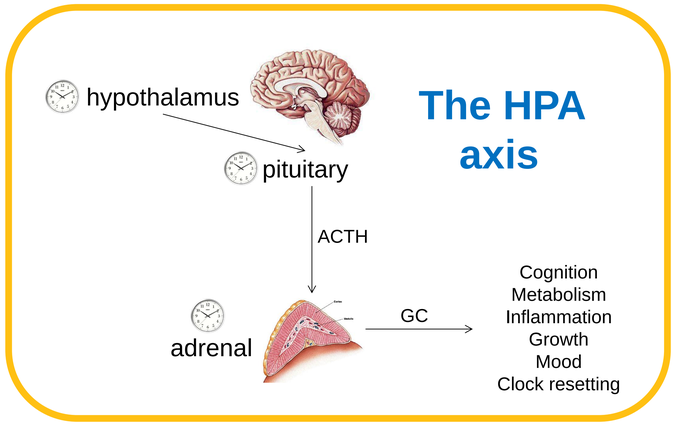
Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is characterized by a robust circadian rhythm in adrenal secretion of glucocorticoids (GC), driven in part by adrenal clock genes. The GC rhythm serves to synchronize other peripheral clocks and to maintain homeostasis[1].

Mechanisms for entrainment of the adrenal clock remain unclear. Since stress activates the HPA axis, it is possible that entrainment of the adrenal clock and, concomitantly, glucocorticoid rhythms would be susceptible to stress. The present experiment aimed to test the hypothesis that chronic stress can alter adrenal circadian rhythms.

The model of chronic subordinate stress used, consisting of daily exposure to a dominant mouse, has shown a robust metabolic phenotype in previous studies [2].

Per2::Luc mice were used to assess rhythmic expression of the clock gene Per2. Per2::Luc mice express a luciferase reporter gene driven by the Per2 promoter, so that light output measured in tissue explants reflects PER2 expression[3].



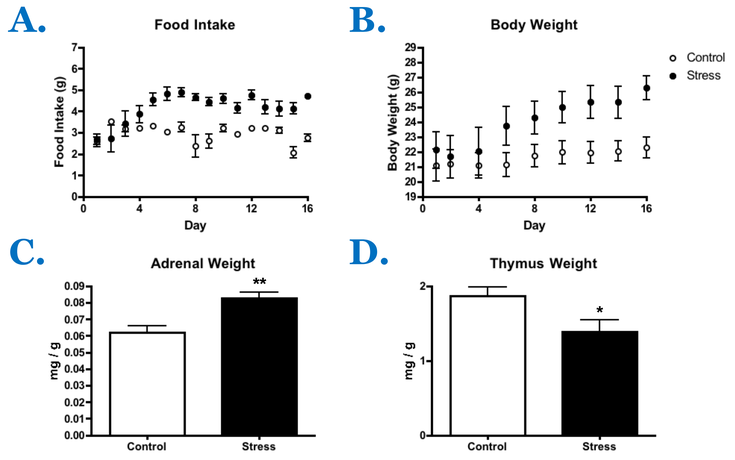
The HPA axis

Materials and Methods

- Animals: male mice aged at least 6 weeks. CD1=dominant, Per2::Luc= subordinate.
- Chronic subordinate stress: two adult male mice were housed together so that they were in constant sensory contact. Physical interaction was allowed daily for up to 10 minutes. Social hierarchy (dominant and subordinate) was established and maintained for 2 weeks, then blood and tissue were collected from subordinate mice. Control mice were housed individually for the duration of the study.
- CORT and ACTH were measured using radioimmunoassay.
- Sensitivity to ACTH was assessed *in vitro* by measuring CORT release from dispersed adrenal cells.
- Tissues from Per2::Luc mice were placed into media containing luciferin in a photomultiplier tube. Bioluminescence was sampled at ~10 min intervals for at least three days and phase and period were assessed using Lumicycle software.

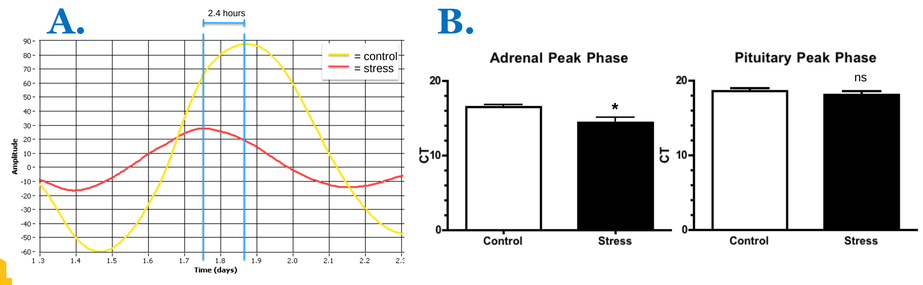
Typical posture exhibited by a subordinate mouse during free social interaction time[4].

CSS chronically activates the HPA axis



Stressed mice ate more (A) and gained weight (B) compared to controls, as observed in previous studies using the CSS model[2]. Stressed mice also experienced adrenal hypertrophy (C) and thymic involution (D), indicative of chronic stress[5]. Adrenal and thymus weights were corrected for body weight. *p<0.05, **p<0.005

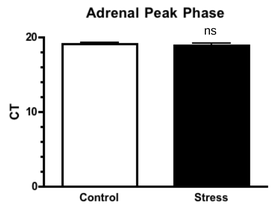
CSS shifts the phase of the adrenal Per2 rhythm



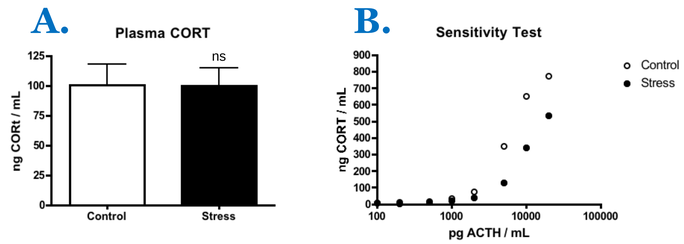
A) Circadian PER2::LUC expression in representative control and stressed mice. B) The adrenal Per2 rhythm peaked significantly earlier in stressed mice than in controls (*p<0.05). The pituitary Per2 rhythm was not affected by CSS.

Acute stress does not change the adrenal Per2 rhythm

An acute mild stress, consisting of handling the mice, did not shift the Per2 rhythm in adrenals. Per2 rhythms in pituitaries and spinal cords were also unaffected.



CSS does not change basal HPA activity



A) Basal plasma corticosterone levels and B) adrenal sensitivity to ACTH were unchanged by chronic subordinate stress. Sensitivity to ACTH was measured in pooled adrenal cells, so statistical analysis was not performed.

Discussion

- Subordinate stress chronically activates the HPA axis, as evidenced by robust adrenal hypertrophy and thymic involution in stressed mice.
- CSS appears to alter entrainment of the adrenal clock: after two weeks of stress, the circadian peak of Per2 expression was significantly shifted. In contrast, acute stress did not have an effect on entrainment of the adrenal clock.
- Despite clear effects on adrenal clock gene rhythmicity, no effects were seen on basal HPA activity.
- Timing of plasma collection may not provide sufficient resolution to observe a similar shift in CORT rhythms.
- Per2 rhythms in other tissues (pituitary and spinal cord) were unaffected by CSS. The adrenal specificity observed suggests ACTH as a possible mediator of the shift.
- These data indicate that a novel consequence of chronic activation of the HPA axis is altered entrainment of the adrenal clock gene rhythm.
- Future directions:
  - Perform CSS at different times of day to see if the clock's susceptibility to stress varies.
  - Remove adrenal glands and give back chronic CORT before beginning CSS to investigate the role of GC rhythms in the metabolic phenotype.

References

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