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Acute psychosocial stress in mid-aged male rats causes hyperthermia, cognitive decline, and increased deep sleep power, but does not alter deep sleep duration

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ABSTRACT

Aging is associated with altered sleep architecture and worsened hippocampus-dependent cognition, highly prevalent clinical conditions that detract from quality of life for the elderly. Interestingly, exposure to psychosocial stress causes similar responses in young subjects, suggesting that age itself may act as a stressor. In prior work, we demonstrated that young animals show loss of deep sleep, deficits in cognition, and elevated body temperature after acute stress exposure, whereas aged animals are hyporesponsive on these measures. However, it is unclear if these age-altered stress responses occur in parallel over the course of aging. To address this, here we repeated the experiment in mid-aged animals. We hypothesized that mid-aged stress responses would be intermediate between those of young and aged subjects. Sixteen mid-aged (12 months) male F344 rats were implanted with EEG/EMG emitters to monitor sleep architecture and body temperature, and were trained on the Morris water maze for 3 days. On the fourth day, half of the subjects were restrained for 3 hours immediately before the water maze probe trial. Sleep architecture and body temperature were measured during the ensuing inactive period, and on the following day, endpoint measures were taken. Restrained mid-aged animals showed resistance to deep sleep loss, but demonstrated stress-induced water maze probe trial performance deficits as well as postrestraint hyperthermia. Taken in the context of prior work, these data suggest that age-related loss of sleep architecture stress sensitivity may precede both cognitive and body temperature–related stress insensitivity.

1. Introduction

There are several consequences of normal aging including cognitive decline (Barnes, 1988; Driscoll et al., 2006; Gallagher and Pelleymounter, 1988; Klempin and Kempermann, 2007; Wimmer et al., 2012), reduced deep sleep (Buechel et al., 2011; Espiritu, 2008; Kirov and Moyanova, 2002; Zepelin et al., 1972), altered circadian rhythm (Dijk et al., 2000; Monk, 2005; Pace-Schott and Spencer, 2011), and increased neuroinflammation (Gemma and Bickford, 2007; Nikodemova et al., 2007). In addition, hypothalamic-pituitary-adrenal (HPA) activity has been documented to be increased with aging (Paul et al., 2015). Loss of deep sleep and deficits in hippocampal function are also observed with stress exposure (Prenderville et al., 2015). The allostatic load hypothesis of aging (McEwen and Stellar, 1993) posits that stress exposure has a cumulative and exacerbating influence on age-related processes. Several studies have shown that stress in young rodents and humans has long-lasting consequences throughout the lifespan (Lupien et al., 2009). Furthermore, the chances of experiencing a new onset stressor, particularly a psychosocial (nonpainful) stressor such as losing a job, death of a spouse, or becoming socially isolated, increases with age (House et al., 1994) and the negative consequences of a stress exposure can be more severe in the aged population (Azuma et al., 2015; Machado et al., 2014; Prenderville et al., 2015; Stein-Behrens and Sapolsky, 1992). However, the lack of basic research on the age-related responses to new onset stressors represents an important area of investigation and has recently been referred to as part of the "stress-aging gap" (Epel and Lithgow, 2014).

To address this, in prior work (Buechel et al., 2014), we used restraint to model of acute psychosocial stress (PS) in young and aged male F344 rats. This manipulation induces sleep loss, cognitive deficit, and body temperature elevation in young animals. However, the same treatment failed to elicit these canonical responses in aged animals, despite the aged animals showing clear signs of distress during the restraint. Whether these sleep, cognitive, and

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body temperature changes in stress response occur in parallel across aging is not known, and mid-age could represent a crucial transition period in the aging stress response phenomenon.

In this study, mid-aged male (12 months) F344 rats were implanted with EEG/EMG telemetry devices to measure sleep architecture and body temperature. Effects on cognition were evaluated with the Morris water maze. Three-hour restraint was used to model new onset acute psychosocial stress in half of the animals. Middle-aged animals showed poststress cognitive deficits and hyperthermia, but did not show stress-associated deep sleep loss. In the context of earlier work on young and aged animals, these results indicate that age-related changes in sleep response to stress may be upstream of cognitive and body temperature response.

2. Materials and methods

2.1. Subjects

Sixteen middle-aged (12 months) male Fischer 344 rats were obtained from the NIA aging colony. Animals were individually housed with Enviro-dri bedding, rat tunnel, and Nylabone. They had access to food and water *ad libitum* and were acclimated to a 12-hour reverse light/dark cycle (4:30 AM lights off, 4:30 PM lights on). A timeline (Fig. 1) provides an overview of the experiment. Two additional animals were excluded due to surgical complications. Rats were randomly assigned to control and stress groups. All experiments were performed in accordance with institutional and national guidelines and regulations, and conform to our approved protocol (University of Kentucky IACUC #2008-0347).

2.2. Surgery

All subjects were implanted according to standard procedures with wireless EEG/EMG emitters (Data Sciences International-TL11M2-F40-EET) as in prior work (Buechel et al., 2011, 2014). Before surgery, EEG wires were cut to length and a sterile 1/8″ stainless steel screw was soldered to the end of each lead. To begin surgery, animals were anesthetized with isoflurane and placed in a stereotaxic frame. A 2-inch incision was made to expose the skull and spinotrapezius muscles. The emitter was placed under the skin between the left scapulae and the left ileum along the flank. The exposed dorsal region of skull was cleaned with 3% peroxide and the skull surface dried with sterile cotton swabs soaked in 70% ethanol. For EEG electrodes, a 0.7-mm hole was drilled 1 mm from either side of the sagittal suture line and 1–2 mm anterior to the lambda suture line. Screws were inserted into the holes and positioned so that the flat screw tip rested on the dura. Screw heads were covered with dental cement and left to dry. EMG electrodes were inserted through the trapezius muscle with a 21 gauge needle, perpendicular to the muscle fibers. The free wire end was capped with insulation and both sides of the incision were tied off with surgical thread to prevent fluid infiltration. The incision was then closed with 6–8 mattress stitches.

2.3. Sleep data acquisition and analysis

Sleep data was acquired according to established protocols in prior work (Buechel et al., 2014). Animals were housed individually, and cages were positioned at least 18″ apart to avoid interference during radiotelemetry data acquisition. EEG, EMG, and temperature data were recorded continuously with DSI's Data Art acquisition software and binned in 10 seconds epochs. For these nocturnal rodents, the first 4 hours of their resting period (light) and the first 4 hours of their active period (dark) were evaluated for sleep architecture on the day before the start of water maze training (baseline), and after the stress/probe trial paradigm. Architecture was scored using NeuroScore's (v. 2.1.0 Data Sciences International) analysis console in 30-second increments while being viewed in 2–5 minute windows. EEG waves were stratified into "low amplitude" (≤50% of maximum) and "high amplitude" (>50% of maximum) tiers, and underwent fast Fourier transforms for each of 5 frequency ranges: Δ (0.5–4 Hz), Θ (4–8 Hz), A (8–12 Hz), Σ (12–16 Hz), and B (16–24 Hz). EMG waves were stratified into 3 tiers: "basal" ≤33% (seen during REM), "intermediate" (between 33% and 66%), and "high" (>66%). Stages based on EEG/EMG signaling were established as follows: Wake—intermediate or high EMG ± locomotor activity, EEG variable; Light Sleep—low-amplitude EEG, intermediate EMG, and no locomotion; REM (paradoxical) Sleep—high-frequency EEG, "basal" EMG and no locomotor activity; Deep Sleep—high-amplitude EEG activity enriched in delta band frequency, basal to light EMG activity, no locomotor activity. Prior assigned sleep stages informed subsequent assignments. Ambiguous epochs, as well as those containing artifacts, were not scored and accounted for <5% of scored time.

2.4. Water maze

The water maze task was performed as in previous studies (Buechel et al., 2011, 2014). A 190-cm diameter circular, black painted pool was centered (250 cm/side) in a cubicle of floor to

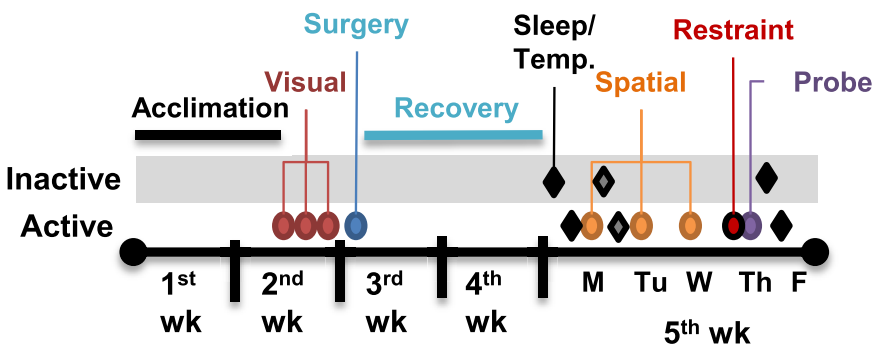

Fig. 1. Timeline. Animals spent a week of acclimating to a reverse light-dark cycle (12:12 lights on at 4:30 PM). During the second week, all animals participated in 3 days of visual cue (3 trials/d, 12 PM–3 PM). At the beginning of the third week, animals were surgically implanted with wireless telemetry devices and were allowed to recover. Baseline sleep and body temperature data were collected before spatial cue training (inactive period: 4 hours—4:30 PM–8:30 PM on Sunday, and active period: 4 hours—4:30 AM–8:30 AM on Monday). Spatial cue training began during the fifth week and lasted 3 days (Monday–Wednesday, 12 PM–3 PM). Body temperature data (gray symbols) also were collected for the first 4 hours of the inactive and active periods after the first spatial cue water maze training session on Monday. On the fourth day (Thursday), half of the animals were restrained for 3 hours (12 PM–3 PM) and all animals participated in the probe trial after the restraint period (3:15–3:45 PM) and postrestraint inactive and active period sleep and body temperature were recorded. On Friday, animals were transported from the vivarium to the lab for blood collection and tissue harvesting (9 AM–11 AM).