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Chronic sleep deprivation differentially affects short and long-term operant memory in *Aplysia*

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ABSTRACT

The induction, formation and maintenance of memory represent dynamic processes modulated by multiple factors including the circadian clock and sleep. Chronic sleep restriction has become common in modern society due to occupational and social demands. Given the impact of cognitive impairments associated with sleep deprivation, there is a vital need for a simple animal model in which to study the interactions between chronic sleep deprivation and memory. We used the marine mollusk *Aplysia californica*, with its simple nervous system, nocturnal sleep pattern and well-characterized learning paradigms, to assess the effects of two chronic sleep restriction paradigms on short-term (STM) and long-term (LTM) associative memory. The effects of sleep deprivation on memory were evaluated using the operant learning paradigm, learning that food is inedible, in which the animal associates a specific netted seaweed with failed swallowing attempts. We found that two nights of 6 h sleep deprivation occurring during the first or last half of the night inhibited both STM and LTM. Moreover, the impairment in STM persisted for more than 24 h. A milder, prolonged sleep deprivation paradigm consisting of 3 consecutive nights of 4 h sleep deprivation also blocked STM, but had no effect on LTM. These experiments highlight differences in the sensitivity of STM and LTM to chronic sleep deprivation. Moreover, these results establish *Aplysia* as a valid model for studying the interactions between chronic sleep deprivation and associative memory paving the way for future studies delineating the mechanisms through which sleep restriction affects memory formation.

1. Introduction

Increasingly, individuals are working longer hours with approximately 19% of adults working more than 48 h per week and 7% working more than 60 h per week (Alterman, Luckhaupt, Dahlhamer, Ward, & Calvert, 2013). Consequently, chronic sleep restriction has become prevalent in modern society as a result of occupational and social demands (Akerstedt & Wright, 2009; Costa, 2015; Liu et al., 2016). Approximately 35% of American adults and an increasing number of children and adolescents report insufficient sleep at night (Centers for Disease Control & Prevention, 2011, 2012; Liu et al., 2016). In national surveys conducted in 2005 and 2010, one-third of adult workers reported an average of less than 6 h sleep per night (Centers for Disease Control & Prevention, 2011, 2012; Luckhaupt, Tak, & Calvert, 2010). Insufficient sleep and sleep disorders not only represent a public health problem in the United States and western countries, but are also arising as a significant health issue in countries across Africa and Asia (Stranges, Tigbe, Gómez-Olivé, Thorogood, & Kandala, 2012). Chronic sleep restriction results in poor performance in tasks measuring sustained attention such as the psychomotor vigilance test (Mollicone, Van Dongen, Rogers, Banks, & Dinges, 2010), working memory (Drummond, Anderson, Straus, Vogel, & Perez, 2012; Jiang et al., 2011) and long-term memory (Lo, Bennion, & Chee, 2016; Lo, Ong, Leong, Gooley, & Chee, 2016). Both mild (~3 h/day) and harsh (~7 h/day) sleep restriction for 1–2 weeks result in cumulative adverse effects on attention and cognition (Belenky et al., 2003; Dinges et al., 1997; Van Dongen, Maislin, Mullington, & Dinges, 2003).

In light of the accumulating evidence regarding the adverse effects of even short periods of sleep restriction on performance and memory, relatively little is known about the mechanisms through which restricted sleep affects performance and cognitive function. In rodent models, chronic sleep restriction paradigms involve extended sleep deprivation protocols frequently continuing for multiple weeks or months (Alzoubi, Khabour, Albawaana, Alhashimi, & Athamneh, 2016; Alzoubi, Khabour, Rashid, Damaj, & Salah, 2012; Rothman, Herdener, Frankola, Mughal, & Mattson,

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2013; Zielinski, Davis, Fadel, & Youngstedt, 2013) making it difficult to isolate the time points at which to resolve the molecular consequences of sleep deprivation. As the first step in establishing a simple model system in which the interactions between chronic sleep restriction and memory could be investigated at the molecular and cellular levels, we investigated the effects of chronic sleep deprivation on the induction of short-term (STM) and long-term (LTM) associative memory using a relatively simple invertebrate model system, *Aplysia californica*. *Aplysia* sleep almost solely during the night exhibiting decreased responsiveness to appetitive and aversive stimuli during sleep (Vorster, Krishnan, Cirelli, & Lyons, 2014). Following a single night of sleep deprivation, *Aplysia* exhibit rebound sleep demonstrating homeostatic as well as circadian regulation of sleep (Vorster et al., 2014). Recently, *Aplysia* also has been used as a model to investigate the effects of acute sleep deprivation on memory in which it was found that a single night of 9 h sleep deprivation inhibited the induction of short and long-term memory (Krishnan et al., 2016).

Using the operant learning paradigm, learning that food is inedible (LFI), in which the animal associates a specific netted seaweed with the inability to swallow the seaweed (Susswein, Schwarz, & Feldman, 1986), we compared the effects of two different patterns of repeated sleep deprivation (6 h/day for 2 consecutive nights or 4 h/day for 3 consecutive nights) on STM and LTM. We found that two consecutive nights of 6 h/day sleep deprivation during either the first half (ZT 12-ZT 18) or last half (ZT 18-ZT 24) of the night blocked the induction of both STM and LTM. Moreover, the impairment in STM following two nights of restricted sleep persisted for 24 h. In contrast, there were no persistent effects of repeated sleep deprivation on LTM. As the effects of chronic sleep restriction on cognitive impairments is proportional to the degree of restricted sleep in humans (Belenky et al., 2003; Van Dongen et al., 2003), we also investigated the effects of a milder form of chronic sleep restriction, in which the animals were sleep deprived for 4 h/night for 3 consecutive nights, on short and long-term LFI memory. We found that three nights of mild sleep restriction inhibited STM, although no adverse effects were observed on the induction of 24 h LTM. Thus, short-term memory appears to be more sensitive to the detrimental effects of chronic sleep restriction. These behavioral studies established *Aplysia* as a suitable model system for investigating the interactions between repeated sleep restriction and memory formation.

2. Materials and methods

2.1. Animal maintenance

Wild-caught *Aplysia californica* (100–200 g; South Coast Bio-Marine, San Pedro, CA) were maintained within individual boxes in chilled 110 gallon tanks containing artificial seawater (Instant Ocean) at 15 °C on a 12 h light/12 h dark (LD) cycle. Animals were fed romaine lettuce every two – three days with feeding times varied across the day.

2.2. Sleep deprivation

Sleep deprivation was performed as previously described (Vorster et al., 2014) with slight modifications. Animals were transferred in the dark to individual, chilled and aerated plastic open containers (25 cm × 30 cm) filled with artificial seawater and varying substrates (large smooth stones, small pebbles mixed with coral sand, aquarium filter, terrarium liner). Animals were sleep deprived through context changes every 30 min via transfer to a different container and tactile stimulation. Each animal was observed once per minute to assess mobility and the animal was handled if the animal remained immobile with a resting body posture (Vorster et al., 2014) for 3 consecutive observations. Typically, animals were handled 2–5 times per half hour to achieve sleep deprivation. Sleep deprivation procedures were performed in the dark under dim red light.

2.3. Behavioral training and testing

All animals, including experimental, control and naïve animals, were fed to satiation with laver seaweed as in previous studies of non-associative and associative memory in *Aplysia* (Fernandez, Lyons, Levenson, Khabour, & Eskin, 2003; Lyons, Rawashdeh, Katzoff, Susswein, & Eskin, 2005; Michel, Gardner, Green, Organ, & Lyons, 2013; Michel, Green, Gardner, Organ, & Lyons, 2012) and then removed from appetitive stimuli for 6 days prior to LFI training or testing. All training was performed at Zeitgeber Time 1 (ZT 1) with ZT 0 defined as the time of lights on and ZT 12 referencing lights off. LFI training was performed as previously described using a single 25 min training protocol (Michel et al., 2012, 2013). During LFI training, animals were presented with laver seaweed that provides strong chemosensory cues in a tulle-mesh bag that could not be swallowed. Animals responded with head-waving, orienting toward the seaweed and biting responses. Although all animals were trained using a 25 min training protocol, small individual variation in the duration of training occurs between animals due to the time necessary to gently extract the seaweed bag from the mouth during cycles of protraction and retraction of the radula. Testing occurred using similar procedures as training, either 30 min later for short-term memory (STM) or 24 h later for long-term memory (LTM) and proceeded until 3 min elapsed without the animal taking the netted seaweed into the mouth after egestion. Two parameters were measured during testing: total response time and the cumulative time the netted seaweed was retained in the mouth. Response times were measured individually for each animal using digital waterproof stopwatches (VWR). Memory is represented by a decrease in response times when trained animals are compared to naïve animals. Naïve animals were tested for behavioral responses either at the time of training (similar Zeitgeber time and time elapsed since feeding to satiation) for control and sleep-deprived animals or at the time of LTM testing. As no significant differences were observed in the responses between the two groups of naïve animals, the data was pooled for each set of experiments. For all behavior experiments including sleep deprivation, LFI training and LFI testing, a single experiment involved multiple individuals so that an experimenter at any one step was frequently blind to the history of the animal or subsequent experimental plans for the animal.

2.4. Statistical analysis

Statistical analysis of the data was performed using one-way ANOVA with Bonferroni's post hoc analysis for comparisons between groups. P values less than 0.05 were considered significant.

3. Results

3.1. Repeated 6 h sleep deprivation inhibits long-term memory

In humans, chronic sleep restriction leads to cumulative cognitive impairments (Belenky et al., 2003; Van Dongen et al., 2003). To determine whether chronic sleep restriction affected memory in *Aplysia*, we investigated the effect of two nights of 6 h sleep deprivation on the induction of long-term memory. *Aplysia* sleep