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Multiple effects of circadian dysfunction induced by photoperiod shifts: Alterations in context memory and food metabolism in the same subjects

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HIGHLIGHTS

- Circadian disruption (CD) impairs conditioned place preference learning.
- CD shifts diet preference from complex (starch) to simple (dextrose) carbohydrate.
- CD elevates fasting blood glucose levels.
- CD increases percentage of weight gain following food restriction.

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ABSTRACT

Humans exposed to shiftwork conditions have been reported to have increased susceptibility to various health problems including various forms of dementia, cancer, heart disease, and metabolic disorders related to obesity. The present experiments assessed the effects of circadian disruption on learning and memory function and various food related processes including diet consumption rates, food metabolism, and changes in body weight. These experiments utilized a novel variant of the conditioned place preference task (CPP) that is normally used to assess Pavlovian associative learning and memory processes produced via repeated context-reward pairings. For the present experiments, the standard CPP paradigm was modified in that both contexts were paired with food, but the dietary constituents of the food were different. In particular, we were interested in whether rats could differentiate between two types of carbohydrates, simple (dextrose) and complex (starch). Consumption rates for each type of carbohydrate were measured throughout training. A test of context preference without the food present was also conducted. At the end of behavioral testing, a fasting glucose test and a glucose challenge test were administered. Chronic photoperiod shifting resulted in impaired context learning and memory processes thought to be mediated by a neural circuit centered on the hippocampus. The results also showed that preferences for the different carbohydrate diets were altered in rats experiencing photoperiod shifting in that they maintained an initial preference for the simple carbohydrate throughout training. Lastly, photoperiod shifting resulted in changes in fasting blood glucose levels and elicited weight gain. These results show that chronic photoperiod shifting, which likely resulted in circadian dysfunction, impairs multiple functions of the brain and/or body in the same individual.

1. Introduction

Temporal organization of physiology and behavior in mammals is provided by circadian rhythms that are generated in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (for reviews, see [1,2]). The primary experimental evidence that supports the view that the SCN is the site of rhythm generation comes from: (1) ablation studies that demonstrate the absolute requirement of the SCN for the overt expression of rhythmicity [3,4]; (2) explantation studies that demonstrate the autonomous generation of circadian rhythms in the SCN in vitro [5–7]; and (3) transplantation studies in which overt rhythms are expressed in recipient hosts following grafting of SCN tissue [8–11].

In the natural environment, exogenous cues such as sunrise have consistent rhythms and these cues provide information that the brain uses to entrain the master clock. These temporally predictable cues in the environment are called *zeitgebers* and they are used by the organism to set the clock [1]. In artificial environments, cues can be manipulated to disrupt clock function and result in SCN arrhythmicity.

Manipulating zeitgebers (e.g., introducing an artificial sunrise in the middle of the night) provides a method for disturbing circadian function

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that does not rely on altering neural regions directly. Non-invasive disruption of circadian rhythms is typically accomplished by altering light schedules because the SCN is heavily reliant on retinal input [12]. In rats, phase-shifts are typically induced by adjustment of the light schedule [13] and this technique has been used to study the functional impacts of shiftwork and jetlag [14,15]. The effects of phase-shifting within the SCN are well documented [16–18] and include altered gene expression, desynchronization between the core and shell regions, and resetting of the clock to the adjusted time.

Alterations or disruptions of biological circadian rhythms commonly occur in people exposed to shiftwork. Although many workers experience shiftwork schedules of some type, the resultant long-term health effects might be more serious than originally thought [19]. These effects include dementia, obesity, cancer, poor cardiovascular health, hypertension, immune dysfunction, gastrointestinal disorders, disrupted hormonal balance and infertility [20–24]. This broad array of suspected effects of circadian dysfunction as demonstrated in shift workers is consistent with the idea that the suprachiasmatic nucleus, the master clock, mediates important synchronizing effects with a variety of peripheral effector sites that have oscillatory properties. Importantly, desynchronized signals from the SCN can result in serious health effects associated with the dysfunction of these regions. For example, there is a tight coupling of circadian signals from the SCN to heart, lung, liver and pancreatic clock genes that regulate the disparate functions of these internal organs across the light/dark cycle [25].

Although the importance of the human shift worker studies should not be underestimated, there are interpretive issues associated with these kinds of reports because they are not controlled experiments. Making causal links between circadian dysfunction and health based on these studies is difficult because of many confounds associated with the populations sampled from. These confounds include but are not limited to self-selection in or out of shift-work jobs as well as lifestyle issues (diet, exercise, etc.). For these reasons, it is important to complete animal studies in which these population sampling confounds are not present. Of particular interest for the present study is to provide evidence for the idea that photoperiod shifting and associated circadian dysfunction would produce effects on the brain and the body in the same subjects. For the present study, we were interested in assessing how circadian disruption affects cognitive functions linked to the hippocampus and metabolic functions associated with food consumption.

The idea is that circadian dysfunction would, in parallel and independently, alter these brain and body functions precisely because of the circadian systems' wide range of influence over multiple effector sites and related functional mechanisms.

1.1. Hippocampal memory dysfunction and altered circadian rhythms

There are also links between episodic memory impairments and circadian dysfunction in the literature in human and non-human animals [26–29,38]. For example, our animal studies have provided a direct link between circadian dysfunction and hippocampal learning and memory functions. These studies assessed the effects of acute or chronic photoperiod shifting on the acquisition and retention of place learning in the water task [30,31].

For the acute experiments, rats experienced three hour phase-advances for 6 days while they were simultaneously trained on the standard spatial version of the water task. Following behavioral testing, the rats were placed on a 12/12 light/dark cycle to re-entrain their circadian rhythms and given retention tests one week and three weeks later. The results showed that although the photoperiod-shifted rats displayed normal acquisition of the place learning task, they had severe retention deficits. This pattern of data, combined with other evidence lead to the conclusion that memory consolidation processes in the hippocampus were compromised in the rats with circadian dysfunction induced during training.

For the chronic experiments, rats experienced four cycles of the three hour phase-advances for 6 days, used in the acute experiments, with the addition of a partial re-entrainment phase in-between shifting cycles. At the end of the chronic shifting procedure, rats were trained on a variant of the place version of the water task. The chronic photoperiod-shifted rats showed a severe impairment in acquisition and retention of place information in the water task. Taken together, these studies showed that acute photoperiod shifting impairs hippocampal memory consolidation processes [30] and chronic shifting results in severe hippocampal learning and memory impairments [31].

1.2. Metabolic disturbances and circadian dysfunction

Shiftwork and associated circadian dysfunction have also been implicated in various metabolic disturbances [32,33] that may have serious health implications. These changes consist of alterations in: hormonal responses to night eating [34]; body mass index (BMI) in night–day shift workers [35]; food and nutrient intake [36]; obesity incidence in shift workers [37,38]; and diabetes incidence rates [39–42].

Of specific interest for the present study is the possibility that circadian dysfunction associated with shiftwork alters diet preferences and overall food intake as well as causing changes in glucose metabolism mechanisms. Carbohydrates are a heterogeneous class of macronutrients which compose the bulk of an organism's daily food ingestion [43]. They hold crucial value in an organism's diet because they provide a source of energy found in many different foods [44]. In addition to providing nourishment, carbohydrates are also an important fuel for the nervous system. Under normal circumstances, carbohydrates are the only nutrient to fuel the brain [45]. The recommendations of daily carbohydrate intake have been formulated on the basis of the brain's reliance on these nutrients [43]. To adequately fuel the brain and body, the Dietary Guidelines for Canadians recommends 55% of a diet to be comprised of carbohydrates [46]. Furthermore, these guidelines encourage this amount to consist of carbohydrates from a variety of different sources while limiting sugar intake [46]. High sugar diets are linked to obesity, oral health issues, heart disease and Type 2 diabetes [44]. However, certain carbohydrates can provide health benefits. Fiber-rich foods like complex carbohydrates promote healthy body weight and lower the risk for obesity [43]. These foods also lower the risk of type 2 diabetes, cardiovascular disease and gastrointestinal disorders [43].

We were interested in the possibility that photoperiod-shifting and associated circadian dysfunction would result in an alteration in eating habits towards unhealthier carbohydrate consumption.

1.3. Goals of current research

The goal of the present experiments is to assess the effects of photoperiod shifting on learning and memory functions and various food related processes including diet consumption rates, glucose metabolism, and changes in body weight. These experiments will utilize a novel variant of the conditioned place preference task (CPP) that is normally used to assess Pavlovian associative learning and memory processes produced via repeated context-reward pairings [47–49]. In the present experiments, the standard CPP paradigm was modified so that both contexts would be paired with food, but the dietary constituents of the food would be different. In particular, we were interested in whether rats could differentiate between two types of carbohydrates, simple and complex. The simple carbohydrate was dextrose and the complex carbohydrate was starch. The predicted outcomes for these experiments were threefold: 1) chronic photoperiod shifting would result in hippocampal-based learning and memory impairments similar to those found in previous work using different behavioral paradigms; 2) consumption rates for these different carbohydrate diets would be altered in rats experiencing chronic photoperiod shifting like that