The effect of photoperiod and high fat diet on the cognitive response in photoperiod-sensitive F344 rats

Samantha L. McLean[a], Haesung Yun[b], Andrew Tedder[b] and Gisela Helfer[b]*

[a]School of Pharmacy and Medical Sciences, Faculty of Life Sciences, University of Bradford, Richmond Road, Bradford, BD7 1DP, UK

[b]School of Chemistry and Bioscience, Faculty of Life Sciences, University of Bradford, Richmond Road, Bradford, BD7 1DP, UK

Running title: Seasonal rhythms in cognition

***Corresponding author:**
Gisela Helfer
School of Chemistry and Bioscience, University of Bradford
Richmond Road
Bradford
BD7 1DP, UK
E-mail: g.helfer@bradford.ac.uk

Highlights

- Diet and photoperiod have an impact on object recognition in photoperiod-sensitive F344 rats.
- Rats in long photoperiod on chow diet can perform a novel object recognition task suggesting their short-term memory remains intact.
- Our behavioral analysis suggests that high fat diet induces an impairment in memory independent of photoperiod.
- Changing rats to short photoperiod conditions impairs their ability to differentiate between normal and familiar objects independent of diet.

Abstract

In many species, seasonal changes in day length (photoperiod) have profound effects on physiology and behavior. In humans, these include cognitive function and mood. Here we investigated the effect of photoperiod and high fat diets on cognitive deficits, as measured by novel object recognition, in the photoperiod-sensitive F344 rat, which exhibits marked natural changes in growth, body weight and food intake in response to photoperiod. 32 male juvenile F344 rats were housed in either long or short photoperiod and fed either a high fat or nutrient-matched chow diet. Rats were tested in the novel object recognition test before photoperiod and diet intervention and re-tested 28 days after intervention. In both tests during the acquisition trials there was no significant difference in exploration levels of the left and right objects in the groups. Before intervention, all groups showed a significant increase in exploration of the novel object compared to the familiar object. However, following the photoperiod and diet interventions the retention trial revealed that only rats in the long photoperiod-chow group explored the novel object significantly more than the familiar object, whereas all other groups showed no significant preference. These results suggest that changing rats to short photoperiod impairs their memory regardless of diet. The cognitive performance of rats on long photoperiod-chow remained intact, whereas the high fat diet in the long photoperiod group induced a memory impairment. In conclusion, our study suggests that photoperiod and high fat diet have an impact on object recognition in photoperiod-sensitive F344 rats.

Key words: photoperiod, seasonal, rhythms, F344, cognition, high fat diet, novel object recognition

Introduction

Seasonal animals have evolved diverse seasonal variations in physiology and behavior to accommodate yearly changes in environmental and climatic conditions. These physiological and behavioral changes are initiated by changes in day length (photoperiod) and include annually occurring phenomena such as migration, hibernation, torpor and reproduction (Helfer et al., 2019). The broad importance of seasonality in physiology and biomedical research has increasingly been recognized in recent years (Stevenson et al., 2015). Seasonal animals undergo pronounced cycles of weight gain and weight loss to precisely control their energy stores as part of their natural physiology. Unlocking the basis of seasonal body weight regulation is therefore important not only to our understanding of basic physiology, but to understand the long-term impact of seasonal changes on the brain, behavior and cognition in animals, including humans (Helfer and Dumbell, 2020).

The neuroendocrine mechanisms underlying seasonal energy balance regulation and reproduction involve dynamic interactions across multiple central nervous system substrates and hormonal messengers to provide system-wide orchestration (Helfer and Stevenson, 2020). Seasonal changes in photoperiod are mediated through the nocturnal secretion of melatonin from the pineal gland which relays photoperiodic information to the pars tuberalis of the pituitary gland to regulate the release of thyroid-stimulating hormone (TSH). In short photoperiod (winter: short days and long nights), the increased duration of melatonin signal inhibits the release of TSH whereas in long photoperiod (summer: long days and short nights), the short duration of melatonin allows TSH release. The hypothalamus integrates the TSH signal by increasing the expression of deiodinase enzymes to catalyze the conversion of inactive thyroid hormone T4 to biological active thyroid hormone T3. Increased T3 regulates key downstream pathways, such as retinoic acid signaling, resulting in appropriate seasonal phenotypes (Helfer et al., 2019; Helfer and Dumbell, 2020). This process also involves the photoperiodic regulation of neuropeptides localized in discrete appetite-regulating centers of the hypothalamus to regulate food intake and body composition (Helfer and Stevenson, 2020).

To study the interactions of the mechanisms involved in the regulation of body weight and growth with those involved in diet-induced obesity, we previously investigated the effect of photoperiod and high fat diet on physiology (body weight, body composition, food intake), circulating levels of hormones that regulate feeding status and hypothalamic gene expression in Fisher F344 rats (Ross et al., 2015). The study showed that photoperiod and high fat diet regulate body weight and body composition through independent pathways, with photoperiod primarily effecting growth and lean mass accretion and high fat diet effecting adipose tissue accretion. Anecdotal findings from this study suggested that photoperiod and

high fat diet had an effect on rats' behavior, insofar we noticed that animals housed in long photoperiod demonstrated increased explorative behavior and seemed generally more alert (unpublished data). It is well established that high fat diet impairs cognitive function and induces cognitive deficits in rodent models (Buie et al., 2019; Del Olmo and Ruiz-Gayo, 2018; Kanoski and Davidson, 2011; McLean et al., 2018; Noble and Kanoski, 2016). Much less common are reports on the effect of photoperiod on cognitive performance, yet seasonal environmental changes are expected to influence cognition to meet the ecological needs of animals (Buchanan et al., 2013).

The best studied examples of seasonal changes in cognitive processes come from songbirds where seasonal changes in song production and learning are accompanied by changes in the brain regions controlling song (Tramontin and Brenowitz, 2000). Interestingly, a recent study has shown that these seasonal changes are not under photoperiod control (Pozner et al., 2018). Additionally, the hippocampus undergoes seasonal changes in food-storing birds and brood parasites, but similarly this seems independent of photoperiod (Sherry and MacDougall-Shackleton, 2015). In some seasonal breeders, such as cowbirds, deer mice and voles, spatial memory is enhanced prior to the breeding season (Clayton et al., 1997; Galea et al., 1996; Galea et al., 1994). Male African stripped mice show increased spatial performance and attention during winter and it has been suggested that this might be due to greater dispersal motivation before breeding season (Maille et al., 2015). Interestingly, female stripped mice do not show the same seasonal variation (Maille et al., 2015; Maille and Schradin, 2016). These studies provide compelling evidence for seasonal patterns of cognitive performance, however, studies designed to examine photoperiod control of cognition are limited.

Interestingly, in non-seasonal animals, for example the non-photoperiodic Wistar rat, there is some evidence indicating that photoperiod has an impact on animal behavior, including depression, anxiety and stress (Barnes et al., 2017). This provides an exciting opportunity for novel treatment strategies using photoperiod that may be applicable to humans. Indeed, short photoperiod has been suggested as an approach in the management of central nervous system injuries (Subhadeep et al., 2020) and therapeutic interventions targeting photoperiodic regulated dopamine signaling could help patients suffering from seasonal affective disorders (Okimura et al., 2021).

Here we investigated the effect of photoperiod and diet on cognition in the photoperiod-sensitive F344 rat. The inbred F344 rat strain is one of the few rat strains that shows pronounced photoperiod-induced changes in its metabolic phenotype, growth and reproductive status (Tavolaro et al., 2015). In the laboratory, a simple switch in photoperiod induces large scale changes in body composition, food intake, reproduction and

hypothalamic gene expression within 2-4 weeks (Helfer et al., 2013; Helfer et al., 2012; Helfer et al., 2016; Ross et al., 2015). Furthermore, the F344 rat strain is one of the strains preferred in behavioral tests due to low level activation of the hypothalamic-pituitary-adrenal axis and low open field defecation (Glowa et al., 1992; Harrington, 2013; Van Der Staay and Blokland, 1996). Thus, the F344 rat is the ideal model to study photoperiod regulation of cognitive flexibility. To test photoperiod control of cognition, we used the novel object recognition (NOR) task, a spontaneous and ethologically relevant behavioral paradigm that is based on rodents' natural tendency to explore novel stimuli and environments (Ennaceur and Delacour, 1988). This is a robust, well-characterized behavioral task which is routinely used to assess cognition and natural behavior in an open field arena (Cohen and Stackman, 2015; Grayson et al., 2015; McLean et al., 2016; McLean et al., 2017). In line with previous studies, we predicted that high fat diet would decrease cognitive performance in F344 rats independent of photoperiod. Given that F344 rats breed during long photoperiod (Tavolaro et al., 2015), we hypothesized that cognitive performance would be enhanced in long photoperiod in these rats.

Methods

Ethics statement

All animal procedures were performed according to the Animals (Scientific Procedures) Act, 1986 and approved by the Animal Welfare Ethical Review Body at the University of Bradford. Animal experiments were licensed by the UK Home Office under the project license number P0D6AA50D.

Animal experiment

32 male Fischer F344/NHsd (F344) rats from barrier 208A were obtained from Envigo (Oxon, UK) at 4-5 weeks old (weight range 75-100g). Initially, rats were acclimatized for 10 days under 12h light:12h dark photoperiod in groups of four with *ad libitum* access to water and standard chow diet (2018 Teklad global 18% protein rodent diet, Envigo). Rats were then randomly assigned to four groups containing eight rats each and transferred to different photoperiod rooms (Table 1). Group sizes were selected based on power calculations performed on results from previous studies (Ross et al., 2015; Tavolaro et al., 2015). Two groups (n=8/group) were switched to a short photoperiod (SP; 8h light:16h dark) and two groups were switched to a long photoperiod (LP; 16h light:8h dark). Photoperiods were changed by intermittently shortening or lengthening the light-off time with the original light-on time remaining the same in all rooms. One group in each photoperiod room was provided *ad libitum* access to water and either a high fat diet (45% fat by kcal, TD.06415, Envigo) or a nutrient-matched chow diet (10% fat by kcal, TD.06416, Envigo) (Figure 1). In photoperiod rooms, rats were housed in pairs to avoid effects of isolation on behavior (McLean *et al.*,

2010). Rats were housed in standard rat cages (Type III rat caging, 2017cm^2 floor area, Arrowmight, Hereford, UK) with soft woodchip bedding (EC06 chips, Datesand, Manchester, UK) and a red plastic tunnel and shredded paper (Sizzle Nest, Datesand) for enrichment. Apart from photoperiod, all other environmental conditions were kept constant with a temperature of 21±1^{0}C, humidity of 50±5% and average light intensity of 150 lux. Body weight of individual rats and food intake of the cage was measured three times weekly within two hours after lights on. Food intake of the cage was measured by subtracting the remaining amount of pellets in the dispenser and cage from the pre-weighed amount provided. Food intake of the cage was then divided by two to obtain food intake values for each individual rat. Health checks were carried out twice daily and no welfare-related issues were observed.

After 28 days under experiment conditions, rats were assigned random numbers before killing to ensure blind analyses to grouping. Rats were killed by terminal anesthesia using isoflurane followed by decapitation at Zeitgeber Time 3 (=3h after lights on). Testes were dissected and paired testes weight was recorded to confirm photoperiod responses (Tavolaro et al., 2015).

Behavioral testing

A total of 32 rats were tested in the NOR task as described in detail previously (McLean et al., 2011). Briefly, rats were habituated to the test box for 20 min on three consecutive days. Following a 3 min habituation session on the day of testing each rat was placed in the NOR chamber (52 cm wide × 40 cm high × 52 cm long) and exposed to two identical objects for a period of 3 min. The rats were then returned to their home cage for an inter-trial interval (ITI) of 1 min; the box was cleaned with 70% ethanol, both objects removed, and one replaced with an identical familiar copy and one with a novel object. Following the ITI, rats were returned to explore the familiar and a novel object in the test box for a 3 min retention trial. The location of the novel object in the retention trial was randomly assigned for each rat using a Gellerman schedule. All experiments were video recorded (Home Guard - CCTV Home Security Kit) for subsequent behavioral analysis by an experimenter blind to the treatments. Exploration time (sec) for each object in each trial was recorded manually using two stopwatches. Locomotor activity was recorded by scoring the number of line crossings by the rat in both acquisition and retention trials (Figure 1).

Rats were then randomly assigned to one of the 4 intervention groups (as described above, Figure 1) and were re-tested after 4 weeks in the NOR task. Care was taken to use new objects for the second experiment to ensure rats still had a natural propensity to explore based on novelty.

Statistics

Body weight, food intake and energy intake data were analyzed by three-way repeated measures (RM) ANOVA (photoperiod x diet x time interaction) followed by Tukey's multiple comparisons test. Testes weight were analyzed by one-way ANOVA followed by Tukey's honestly significant difference post-hoc test for pairwise comparison. The NOR data are expressed as mean exploration time ± SD. Data passed normality (Shapiro-Wilk) and student's two-tailed paired t-test was performed to compare time spent exploring the familiar versus the novel object. The locomotor activity and total exploration levels were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant if $P < 0.05$. Data are presented as mean ± SD, n refers to the number of animals.

Results

The effect of photoperiod and high fat diet on body weight, food and energy intake and paired testes mass

A three way RM ANOVA revealed a significant interaction between photoperiod, diet and time for body weight ($F_{(12,336)}=2.276$; $P = 0.009$) comparable to our previous study (Ross et al., 2015). After 28 days of high fat feeding, body weight of rats on short photoperiod was 11% lower relative to rats on long photoperiod ($P = 0.006$; Figure 2A). The difference between chow fed rats was less pronounced with body weight of rats on short photoperiod being 7% lower compared to rats on long photoperiod ($P = 0.075$). This might be due to one rat in the long photoperiod-chow group failing to respond to photoperiod and gaining less weight than the remainder of the group, however Grubb's test did not reveal this rat as an outlier. The effects of photoperiod and high fat diet on testes size were in proportion to body weight as previously shown (Tavolaro et al., 2015). One-way ANOVA revealed a significant difference in paired testes weight ($F_{(3,28)}=5.166$, $P = 0.0057$; Figure 2B). A pairwise comparison showed that paired testes weight of short photoperiod-HF and short photoperiod-chow rats was significantly lower compared to long photoperiod-HF rats (Tukey's test, $P = 0.009$ and $P = 0.013$, respectively). Consistent with the difference in body weights, three-way RM ANOVA showed a significant interaction between photoperiod, diet and time for food intake ($F_{(3,84)}=10.31$; $P < 0.001$) and energy intake ($F_{(3,84)}=9.335$; $P < 0.001$). Food intake was highest in the long photoperiod-chow rats and these intakes were higher than the intakes of the long photoperiod-HF rats (Tukey's test, $P < 0.001$) (Figure 2C). In addition, food intake of chow diet rats was significantly higher than high fat diet rats independent of photoperiod (LD: $P < 0.0001$; SD: $P = 0.0031$). There was also a significant difference in food intake between long photoperiod-chow and short photoperiod-chow rats ($P = 0.004$). After 28 days, energy intake was higher in long photoperiod-HF than in short photoperiod chow rats ($P = 0.0002$), but all other pairwise comparisons did not reveal a significant difference (Figure 2D).

Novel object recognition before intervention

A total of three rats were excluded from the analysis as they failed to explore both objects in one or both trials of the task. There was no significant difference in time spent exploring the two identical objects during the acquisition trial in any of the treatment groups (short photoperiod-chow, $P = 0.364$; short photoperiod-HF, $P = 0.921$; long photoperiod-chow $P = 0.625$; long photoperiod-HF, $P = 0.699$; Figure 3A). In the retention trial, all four groups explored the novel object significantly more than the familiar object (short photoperiod-chow, $P = 0.016$; short photoperiod-HF, $P = 0.047$; long photoperiod-chow, $P = 0.033$; long photoperiod-HF, $P = 0.009$; Figure 3B). A one-way ANOVA revealed no significant difference in locomotor activity between the groups $F_{(3,28)} = 0.2033$, $P = 0.8931$ (Figure 3C). There was also no significant differences in total exploration time in either the acquisition ($F_{(3,28)} = 0.7881$, $P = 0.5119$) or retention ($F_{(3,28)} = 1.303$, $P = 0.2956$) trials (Table 2).

Novel object recognition following intervention

Rats were re-tested in the NOR task following four weeks of photoperiod and high fat diet intervention. A total of eight rats were excluded from the analysis as they failed to explore both objects in one or both trials of the task, therefore the final group numbers were short photoperiod-chow (n=5), short photoperiod-HF (n=6), long photoperiod-chow (n=7) and long photoperiod-HF (n=6). There was no significant difference in time spent exploring the two identical objects during the acquisition trial in any of the treatment groups (short photoperiod-chow, $P = 0.834$; short photoperiod-HF, $P = 0.883$; long photoperiod-chow, $P = 0.516$; long photoperiod-HF, $P = 0.072$; Figure 4A). In the retention trial, only the long photoperiod-chow group explored the novel object significantly more than the familiar object ($P = 0.040$; Figure 4B), whereas all other groups showed no preference (short photoperiod-chow, $P = 0.173$; short photoperiod-HF, $P = 0.397$ and long photoperiod-HF, $P = 0.493$). A one-way ANOVA revealed no significant difference in locomotor activity between the groups $F_{(3,23)} = 0.4207$, $P = 0.7402$ (Figure 4C). There was also no significant differences in total exploration time in either the acquisition ($F_{(3,23)} = 1.595$, $P = 0.222$) or retention ($F_{(3,23)} = 0.2262$, $P = 0.8.771$) trials (Table 3).

Discussion

In this study, we examined the effect of photoperiod and high fat feeding on the cognitive abilities of photoperiod-sensitive F344 rats. Our results show that short photoperiod results in cognitive impairment in the NOR test in young male F344 rats independent of diet. In contrast, rats in long photoperiod on chow diet are able to perform the object recognition task suggesting their short-term recognition memory remains intact. However, a high fat diet induces impairment in memory. This result was not unexpected, given that there is a well-established link between obesity and a decline in cognitive performance (Sellbom and

Gunstad, 2012). High fat diets have previously been shown to cause cognitive decline in animal models (Garcia-Mesa et al., 2012; McNeilly et al., 2011; Sobesky et al., 2014). There is evidence that high fat diets can also promote plaque and tangle pathology in a mouse model of Alzheimer's disease (Julien et al., 2010) suggesting a link between high fat diet and neurodegenerative diseases. Previous work has established a link between altered energy intake, insulin sensitivity and poor cognitive performance (McNeilly et al., 2012; McNeilly et al., 2011). However, the precise molecular mechanisms that underlies the observed decrease in cognitive performance remains to be established. Our results give us a first insight to understand the impact of photoperiod and high fat diet on cognition in the 'healthy' vs the 'obese' brain and might help to untangle the mechanisms involved in the regulation of body weight (related to growth) from those involved in diet-induced obesity.

As anticipated, F344 rats on long photoperiod showed no impairment in the NOR task indicating that increased cognitive performance helps them to anticipate changes in food availability during breeding season (Buchanan et al., 2013). Maintaining (or even improving) cognitive performance during long photoperiod will maximize their reproduction and survival. In contrast, cognitive performance might be impaired in short photoperiod to reduce energy-demanding physiological processes associated with the development and maintenance of the underlying enlarged and complex neural structures (Buchanan et al., 2013). Cognitive processing is energetically costly and requires neurogenesis and brain plasticity. Indeed, we recently proposed that seasonal changes in neurogenesis serve as the basis of photoperiodic control of energy balance and reproduction in seasonal animal models (Helfer et al., 2019). Interestingly, here we found that changing rats to short photoperiod conditions impairs their ability to differentiate between normal and familiar objects independent of diet. We show that this is under direct photoperiod control because a simple switch in photoperiod induces cognitive impairment in the F344 rats housed in short photoperiod.

In line with our previous studies, juvenile F344 rats housed in long photoperiod increase their body weight compared to rats housed in short photoperiod (Ross et al., 2011; Ross et al., 2015; Tavolaro et al., 2015) and are susceptible to mild obesity after four weeks of high fat feeding (Ross et al., 2015). Similarly, the results of food intake are consistent with our previous reports and extend them to show that young F344 rats, irrespective of single or pair housing, have higher food intake in long photoperiod relative to short photoperiod. While there is evidence that food intake is higher in single housed rats, relative to socially housed rats, body weight is not effected by housing conditions (Schipper et al., 2018). Given that photoperiodic response is predominantly assessed by changes in body weight which is accompanied by changes in food intake, our results suggest that in future photoperiod

studies pair housing can be utilized to avoid emotional stress in the study animals (McLean et al., 2010).

A limitation of the current study is the low number of animals used in the NOR task. Previous studies from our laboratory suggest robust and reproducible effects in NOR when using 8 to 10 rats (McLean et al., 2011; McLean et al., 2016; McLean et al., 2017). In this study we started with 8 rats per group but due to the application of our exclusion criteria final group numbers ranged between 5 and 8. Nevertheless, statistically significant differences were observed between the groups.

While the present study does not address molecular mechanisms underlying the effect of photoperiod on cognition, recent studies have implicated that mechanisms may involve insulin, leptin, BDNF, inflammatory pathways and blood brain barrier dysfunction (Cordner and Tamashiro, 2015). Considering that cognitive decline after high fat feeding is a pathophysiological response, it may be expected that different mechanisms are in place to regulate cognition in the 'healthy' seasonal brain. Interestingly, photoperiod-regulated changes in inflammatory signals have been proposed to be involved in the photoperiodic response in seasonal mammals (Helfer et al., 2019). Microarray analysis of F344 rat hypothalami identified a range of inflammatory chemokines and cytokines in response to photoperiod (Helfer et al., 2016; Ross et al., 2011). NF-kB, a major transcription factor regulating the innate and adaptive immune response, has been investigated in more detail and was found to be upregulated in the hypothalamus of F344 rats housed in short photoperiod and regulated by TSH (Stoney et al., 2017). This is noteworthy, because it has been suggested that the immune system regulates cognitive function in seasonal animals (Buchanan et al., 2013). In support, short photoperiod Siberian hamster injected with lipopolysaccharide exhibit diminished fever response with decreased locomotor inactivity (Fonken et al., 2012). However, it is currently unknown whether this effect is mediated by direct communication or a trade-off between the nervous and immune systems (Buchanan et al., 2013). In future work, it will be interesting to investigate whether photoperiodic regulation of the immune system and inflammatory pathways mediates cognitive function.

In conclusion, our study demonstrates for the first time an association between short photoperiod and cognitive impairment and provides the basis for mechanistic studies into how photoperiod effects cognition in the healthy brain. Recent evidence indicates that photoperiod has an impact on animal behavior even in non-photoperiodic Wistar rats (Barnes et al., 2017), thus a better understanding of how photoperiod effects cognition might lead to novel preventive and therapeutic strategies in humans.

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Tables

Table 1: Baseline data for each experimental group prior to testing. All rats were healthy and test naïve. SP, short photoperiod; LP, long photoperiod; HF, high fat diet; n=8/group.

Treatment Group	Weight mean (g)	Weight SD	Weight median (g)
SP Chow	126.8	10.3	126.4
SP High fat	132.6	6.6	132.2
LP Chow	127.2	10.0	130.6
LP High fat	132.4	9.1	130.4

Table 2: Total exploration times in the novel object recognition test before random assignment to intervention. Data shown are the mean total exploration time ± SD. SP, short photoperiod; LP, long photoperiod; HF, high fat diet; n=7-8/group.

	Total Exploration Time (sec)	
Treatment Group	**Acquisition**	**Retention**
SP Chow	17.1 ± 7.0	17.4 ± 7.2
SP High fat	22.4 ± 9.5	19.1 ± 11.7
LP Chow	22.1 ± 6.2	13.4 ± 6.9
LP High fat	20.1 ± 6.0	11.9 ± 5.4

Table 3: Total exploration times in the novel object recognition test following 4 weeks of intervention. Data shown are the mean total exploration time ± SD. SP, short photoperiod; LP, long photoperiod; HF, high fat diet; n=5-7/group.

	Total Exploration Time (sec)	
Treatment Group	**Acquisition**	**Retention**
SP Chow	16.0 ± 5.7	13.6 ± 9.6
SP High fat	9.5 ± 4.2	12.3 ± 6.2
LP Chow	14.6 ± 4.1	11.9 ± 10.4
LP High fat	13.7 ± 7.0	15.8 ± 10.4

Figures and legends

Figure 1: Schematic study design and timeline. Rats were acclimatized in 12:12h photoperiod on chow diet. Before photoperiod and diet intervention, rats were tested in the novel recognition test. Rats were then randomly assigned to four groups. Two groups were switched to short photoperiod and two groups to long photoperiod. One group in each photoperiod was provided *ad libitum* with either a high fat diet or nutrient-matched chow diet. Rats in photoperiod and diet groups were re-tested after 28 days of intervention. SP, short photoperiod; LP, long photoperiod; HF, high fat diet.

Figure 2: Effect of photoperiod and high fat diet on body weight, testes weight, food intake and energy intake. (A) Body weight was significantly lower in short photoperiod-HF compared to long photoperiod-HF **(B)** Paired testes weight of male F344 rats after 4 weeks exposure to photoperiod and high fat diet. Data were analysed by One-way ANOVA, Significance is shown compared to the long photoperiod-chow group. *$P < 0.05$; **$P < 0.01$ **(C)** Food intake in grams was highest in the long photoperiod-chow group and **(D)** energy intakes in Kcal was highest in the long photoperiod-HF group. For (A), (C) and (D), data were analysed by three-way RM ANOVA followed by Tukey's multiple comparisons test. Data are presented as mean ± SD (n=8/group). SP, short photoperiod; LP, long photoperiod; HF, high fat diet.

Figure 3: Novel object recognition before intervention. Performance in the novel object recognition (NOR) task before groups were exposed to short/long photoperiod chow/high fat interventions. Data are expressed as the mean ± SD. (n=7-8 per group). **(A)** Mean exploration time of identical objects in the acquisition phase. Data were analyzed by Student's paired t-test, no significant difference observed between left and right object. **(B)** Mean exploration time of a familiar object and a novel object in the retention trial following. Data were analyzed by Student's paired t-test. *$P < 0.05$, **$P < 0.01$; Significant difference between time spent exploring the familiar and novel object. **(C)** Mean total number of line crossings in the acquisition and retention trials. One-way ANOVA revealed no significant difference in locomotor activity between the groups. SP, short photoperiod; LP, long photoperiod; HF, high fat diet

Figure 4: Effect of photoperiod and high fat diet on novel object recognition. The effect of short photoperiod/long photoperiod chow/HF intervention on the novel object recognition task. Data are expressed as the mean ± SD. (n=5-7 per group). **(A)** Mean exploration time of identical objects in the acquisition phase. Data were analyzed by Student's paired t-test, no significant difference observed between left and right object. **(B)** Mean exploration time of a familiar object and a novel object in the retention trial following. Data were analyzed by

Student's paired t-test. *$P < 0.05$; Significant difference between time spent exploring the familiar and novel object. **(C)** Mean total number of line crossings in the acquisition and retention trials. One-way ANOVA revealed no significant difference in locomotor activity between the groups. SP, short photoperiod; LP, long photoperiod; HF, high fat diet