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ORIGINAL ARTICLE

Phase shift of the circannual reproductive rhythm in European hamsters by 2 days of long photoperiod

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Abstract

OBJECTIVES AND DESIGN: In European hamsters a circannual clock drives the seasonal changes in the reproductive state. Its resetting by photoperiod is clearly phase dependent. In mid subjective winter a 1-month pulse of long photoperiod (LP) advances the onset of the reproductive phase of animals maintained in constant short photoperiod (SP) by up to 1.5 months. The present study investigated whether shorter pulses, i.e. 8, 4 or 2 days LP-pulses are still effective to phase shift the circannual rhythm.

MAIN FINDINGS: All pulses induced gonadal development after a similar time relative to the offset of the pulse and earlier than in the control group. Thus, they all shared a similar effectiveness.

CONCLUSIONS: In European hamsters a very brief LP-pulse can phase shift the reproductive rhythm but its strength is not determined by its duration at least not in the tested range.

Abbreviations:

PP - photoperiod
LP - long photoperiod
SP - short photoperiod
LD - ratio light darkness

INTRODUCTION

In natural conditions European hamsters (*Cricetus cricetus*) are reproductive in long photoperiods (LP) from spring to early summer (Vohralík 1974; Masson-Pévet *et al.* 1994). This reproductive rhythm is controlled by a circannual clock (Masson-Pévet *et al.* 1994) and synchronized by changes in photoperiod. However, in this species the phase shifting capacity of a photoperiodic stimulus depends on the endogenous season which is matched by a stimulus (Saboureau *et al.* 1999; Monecke & Wollnik 2004; Monecke *et al.* 2009). Rapid gonadal development is observed

only when the transfer from natural short photoperiod (SP) to artificial LP occurs between mid November and early March (Monecke & Wollnik 2004). Likewise, in animals in constant SP a 1-month LP-pulse in the middle of subjective winter advances the onset of the following reproductive phase by up to 1.5 months; at other times of the year the effect is less pronounced (Monecke *et al.* 2009).

Similar to circadian rhythms (Johnson 1999; Johnson *et al.* 2004) the amplitude of circannual phase shifts is not only dependent on the timing but also on the strength of the pulse. In varied carpet beetles (*Anthrenus verbasci*) the strength of a LP-pulse is determined by its duration (Miyazaki *et al.* 2007) and by the difference in photoperiod between the pulse and constant conditions of the background (Miyazaki & Numata 2009).

The aim of the present work was to test the duration model in a mammal and to determine the minimum effective duration of a photoperiodic pulse to phase shift the circannual rhythm. In European hamsters a 1-month LP-pulse during mid subjective winter induces distinct phase shifts in the reproductive rhythm (Monecke *et al.* 2009). Here we have tested the efficiency of 8, 4, and 2 days LP-pulses. Only sparse literature is available on the effect of such very brief changes in photoperiod. So far only Siberian hamsters at the age of weaning were investigated (Spears *et al.* 1990; Whaling *et al.* 1993; Finley *et al.* 1995). However this question had neither been tested in adults which already established a seasonal rhythm nor in a circannual species. In adults such brief pulses might either induce smaller phase shifts than long pulses or be too short to shift a circannual rhythm at all.

MATERIAL AND METHODS

The study was performed in accordance with the European Communities Council Directive (86/609/EEC) and French laws. We used 32 male 2 year-old European hamsters, which had been previously used as breeders. They had been maintained in LP (LD16:08) since January in the breeding facility. On September 17th they were transferred to SP (LD10:14, lights on 8:00–18:00 CET) for the experiment.

This decrease in photoperiod (PP) induced immediate gonadal atrophy in those animals which had not yet endogenously regressed testes. The animals were divided in 4 groups, A to D. Animals which had endogenously regressed gonads, were equally distributed between the groups. Their percentage per group was 37.5% at the start and ranged between 20% and 50% at the end, when animals started to die of old age. Animals were maintained individually in Macrolon type 3 cages with food and water *ad libitum*.

On December 12th, after three months in SP, the animals were well in the winter state, i.e. they had regressed gonads and most of them showed hibernation bouts. According to (Monecke & Wollnik 2004) the animals were assumed to be now in the photoperiod-sensitive phase, in which they react to changes in photoperiod within 4 weeks. Groups B, C and D were thus transferred for 2, 4, or 8 days to LP (lights on 5:00–21:00, CET), while the control group A remained in SP. The increase of day length at the transfer to LP was symmetrical, the light phase being extended by 3 hours both into the early and the late night (Figure 1). This symmetrical design was chosen because European hamsters respond differently to asymmetrical changes in PP depending on whether the change occurs in the morning or evening (Monecke *et al.* 2006). Changes in PP were conducted during the light phase, so that the time of lights off changed first. The animals had constant dim red light during the dark phase.

The reproductive state was checked bi-weekly throughout the experiment except for weekly intervals during weeks 3–8 after the onset of the LP-pulse. Sexual quiescence was considered to be terminated when testes appeared for the first time outside of the abdomen. Testis size was then measured with a calliper. Animals were considered to be reproductive when the testes had a length ≥ 1.8 cm since testosterone levels then reach their maximum (Buijs *et al.* 1986; Masson-Pévet *et al.* 1994). The phase in which testes were already palpable outside the abdomen but their length did not reach 1.8 cm was defined as the transition phase.

Data were analyzed by analysis of variance (ANOVA) for repeated measures, (STATISTICA, StatSoft Inc., Tulsa, OK 74104, USA). The LSD-test was used for post-hoc comparisons.

RESULTS

In the control group A sexual quiescence was terminated on May 11 and the reproductive phase started on June 10. Though gonadal development occurred in all 3 experimental groups only 4–5 months after the pulse i.e. in April/May, its timing was significantly affected by

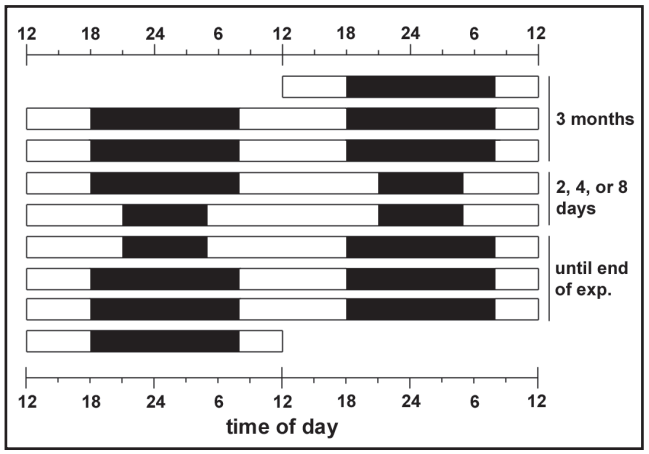

Fig. 1. Experimental schedule as a double plot showing the example of the 2 days LP-pulse. White bars indicate the light phase, black bars the dark phase.

Tab. 1. Timing of gonadal development in European hamsters whose constant SP regime was interrupted by a 2, 4 or 8 days LP-pulse.

group	LP		end of sexual quiescence				start of reproductive phase			
	start	end	date	days after start of LP	days after end of LP	SEM [d]	date	days after start of LP	days after end of LP	SEM [d]
A (Control)	-	-	11-May-08	-	-	2.8	10-Jun-08	-	-	4.3
B (2 days LP)	12-Dec-07	14-Dec-07	19-Apr-08	130	128	3.5	13-May-08	154	152	7.8
C (4 days LP)	12-Dec-07	16-Dec-07	21-Apr-08	132	128	5.6	19-May-08	159	155	7.1
D (8 days LP)	12-Dec-07	20-Dec-07	09-May-08	149	141	4.7	24-May-08	165	157	6.8

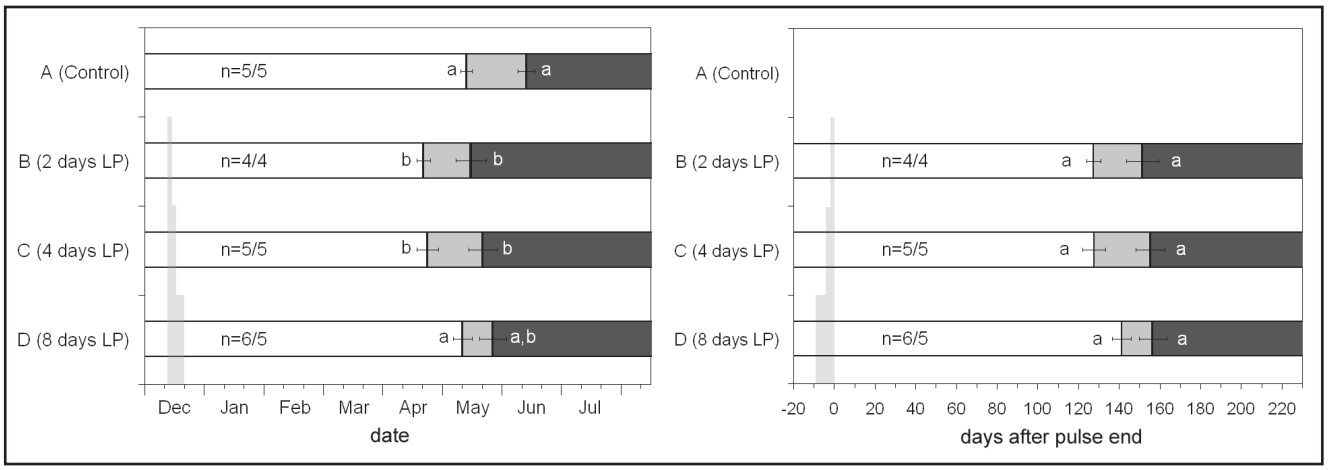

Fig. 2. Timing (mean ± SEM) of gonadal development after a brief LP-pulse of 2, 4 or 8 days in constant SP. Grey shaded areas on the left side of each graph show the time each group spent in LP. Bars indicate the reproductive state. *White*: sexual quiescence, *black*: fully developed testes, *grey*: transition. The number of surviving animals (*n*) at the end of sexual quiescence and at the beginning of the reproductive phase, respectively, is indicated for each group. The timing of changes in the reproductive state marked by different letters of the same colour differs significantly from each other ($p<0.05$, ANOVA for repeated measures, LSD-test). Data are plotted on a date scale (*left panel*), thus relative to the onset of the pulse and in days after the offset of the pulse (*right panel*).

the LP pulse ($F_{(6,28)}=2.99$, $p<0.05$; Figure 2 left panel). In groups B (2 days LP) and C (4 days LP) both the end of sexual quiescence ($p<0.01$) and the start of the reproductive phase ($p<0.05$) were significantly advanced compared with the control group A. In group D (8 days LP) however, neither the mean date of the end of sexual quiescence nor the mean date of the start of the reproductive phase differed significantly from group A ($p=0.67$ and $p=0.57$, respectively). However, the mean date of the end of sexual quiescence differed significantly between group D and groups B and C ($p<0.05$).

Group B showed the maximum advance compared with the control group A. The end of sexual quiescence and the start of the reproductive phase were advanced by 22 days and 28 days, respectively. Between experimental groups there was a tendency that gonadal development occurred the later, the longer the LP-pulse had lasted. However, the differences in gonadal development diminished when its timing was related to the end of the LP-pulse (Table 1, Figure 2 right panel). The ANOVA then revealed no significant group effect on the time between pulse offset and changes in the reproductive state ($F_{(4,20)}=1.12$, $p=0.38$).

DISCUSSION

The experiment has shown that even a brief exposure to LP for only 2 days results in significant phase shifts of a circannual reproductive cycle. However, the response in all 3 pulsed groups was remarkably late, i.e. only 4–5 months after the pulse.

Such a very late response can be explained in 2 ways: Either the pulse did not match one of the 2 photoperiod-sensitive phases in which European hamsters respond to changes in photoperiod within 4 weeks (Saboureau *et al.* 1999; Monecke & Wollnik 2004). Or, considering the circannual phase response curve (Monecke *et al.* 2009), the timing of the pulse did not allow larger phase shifts. In addition, the strength of the stimulus, i.e. the pulse duration (Miyazaki *et al.* 2007) or the difference in photoperiod with respect to the constant background conditions (Miyazaki & Numata 2009), might had an influence as well.

The results allow some insights in the entrainment process of the circannual rhythm, even if group differences are only small. In contrast to findings in *A. verbasci* (Miyazaki *et al.* 2007) in the present study it

was the shortest pulse which induced the largest phase shift (Figure 2a). Nevertheless, it seems to be extremely unlikely that the amplitude of the phase shift should be inversely proportional to the duration of the pulse. Plotting the data relative to the offset of the pulse (Table 1; Figure 2b) revealed that the time span between pulse and gonadal development was similar indicating a similar efficiency for all pulses. Consequently, the duration of the pulse had no effect on its strength.

Moreover, the comparison of Figure 2a and Fig. 2b showed that it is not the onset but the offset of the pulse which determines the timing of gonadal development. This fits well with what is known from the synchronisation of circannual rhythms in vertebrates. Data from rainbow trouts (*Oncorhynchus mykiss*) (Randall *et al.* 1998) and European hamsters (Monecke *et al.* 2009) strongly suggest that a photoperiodic pulse does not act as one single entrainment cue (pulse PP versus constant PP) but as two: a change in photoperiod at the beginning and one at its end. In European hamsters it is the decrease in photoperiod which is the dominant resetting signal (Monecke *et al.* 2009). Since the animals of the present study experienced a LP-pulse, the decrease in photoperiod is at the end of the pulse, which occurred at different dates for the groups. Consequently, the dates of gonadal development differed between groups.

Our results indicate that the duration of a LP-pulse do not determine its strength at least when the pulse lasts up to 8 days and most likely even up to 1 month, since in a former study the maximally induced phase shifts after a 1 month LP-pulse were in a similar range (up to 1.5 months (Monecke *et al.* 2009), present study up to 28 days).

Longer pulses have never been tested in European hamsters but one-step transitions from natural PP to constant LP (Monecke & Wollnik 2004) which induced phase advances of up to 3 months. This seems to be contradictory to the former conclusion that the duration of a pulse does not affect its strength since a one-step transition might be interpreted as an extremely long (never-ending) LP-pulse. However, the effects of LP-pulses are not to be confused with the ones of one-step transitions to LP. In the latter the animals experience only an increase in photoperiod, while a LP pulse subjects them to an increase followed by a decrease. Thus, at a one step transition to LP the induced phase shift is caused by the increase in photoperiod while it is due to the decrease in photoperiod after an LP pulse, since the decrease in photoperiod is a stronger resetting cue for European hamsters than the increase (Monecke *et al.* 2009).

In summary, the critical duration of a LP-pulse necessary to induce a phase shift during subjective winter in European hamsters is only 2 days or less, indicating a surprisingly high sensitivity to changes in photoperiod at a time when in natural conditions animals hibernate in dark burrows (Waßmer & Wollnik 1997). In the present study the efficiency of a LP-pulse was not increased by exceeding this duration. Thus, in this species the strength of a LP pulse seems to be essentially determined by other factors than its duration, for example by the photoperiod difference between the pulse and the constant background conditions as in *A. verbasci* (Miyazaki & Numata 2009).

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