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DIFFERENTIAL EFFECTS OF SHORT DAY PRETREATMENT ON MELATONIN-INDUCED ADJUSTMENTS IN DJUNGARIAN HAMSTERS.

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Summary

Djungarian hamsters which did not respond physiologically to short day conditions were injected daily with melatonin. Hamsters responded to this treatment with typical body weight alterations and molt. Therefore, we concluded that the lack of short day adjustments is not based on insensitivity to melatonin in this species.

Pretreatment with short days affected the timing of melatonin-induced body weight loss and molt. Hamsters became refractory to melatonin injections earlier for both traits if pretreated with short days. Low body weight level was maintained for a shorter period of time, whereas duration of molt was not affected. These results might indicate differences in the control of melatonin-induced body weight adjustments and molt.

Djungarian hamsters exhibit seasonal adjustments including body weight loss, molt, gonadal regression and thermoregulatory improvement if transferred to short day conditions (less than 13 h of light per day; 1-3). Daylength is coded into a pineal melatonin signal through the involvement of the circadian system such that melatonin production is high only during night and duration of high melatonin level reflects nightlength (4-8). External melatonin application can induce similar physiological adjustments under long day conditions (9-11). However, these adjustments are not permanent, and spontaneous refractoriness occurs after prolonged short day exposure or melatonin administration (12-14). Recently, a short day insensitive phenotype of Djungarian hamster has been described. Due to a possible deficiency in circadian function, this phenotype lacks a proper pineal melatonin signal under short day conditions and, consequently, lacks physiological adjustments (15,16). A genetic basis for this phenotype has been proposed (17).

The lack of a proper pineal melatonin signal under short day conditions makes this nonresponsive phenotype an ideal

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experimental animal to probe for melatonin-independent direct effects of daylength on photoperiodic integration. This study addresses two questions: 1) do photoperiodically insensitive hamsters respond to external melatonin application or are they also insensitive to the melatonin signal, as shown for some populations of Peromyscus leucopus (18) and 2) if they respond to melatonin, is the timing of adjustments affected by previous short day exposure?

Methods

A total of 84 (48 females, 36 males) laboratory-bred Djungarian hamsters, originally obtained from Dr. B.D. Goldman (University of Connecticut, Storrs, CT), were used for this investigation. Hamsters were bred and raised under a long day photoperiod (lights on: 0800 h; lights off: 2400 h) and constant ambient temperature (20-22°C). Food (Wayne Lab Bloxs) and water were continuously available. Adult hamsters (older than 80 days) were singly housed and transferred to short day conditions (SD; lights on: 0800 h; lights off: 1700 h). Hamsters were then checked for body weight and fur color index (19) at weekly intervals.

After 9 weeks of SD exposure, hamsters were screened for photoresponsiveness. Hamsters were considered responsive to SD if they had started to molt (n=20). Additionally, 13 hamsters were also considered responsive, since they continuously lost body weight, a response to SD usually preceding molt. Nonresponsive hamsters did not show these SD adjustments (n=21). Eleven nonresponsive hamsters were injected daily at 12 h after lights on (2000 h) with 10 µg of melatonin dissolved in 1% Ethanol-Saline. Injections were given under dim red light. It has been shown that this timing and dosage of melatonin application induces SD adjustments under a long day photoperiod (9,10). All of the responsive and the remaining 10 nonresponsive hamsters were injected daily with vehicle only. Additional groups of nonresponsive hamsters, taken from our stock animals kept under SD, were injected daily with melatonin following 13 (n=8), 15 (n=8) and 22 (n=7) weeks or with saline following 15 (n=7) weeks of SD exposure. All groups received 26 weeks of melatonin or saline injections under a SD photoperiod.

Since one aim of this study examined the effects of SD pretreatment on melatonin-induced adjustment, only those hamsters which molted following the melatonin injections were used to determine the timing of adjustments. Body weight loss alone was not considered a physiological SD response, since the daily injections alone induced body weight loss (see Figs. 1f,g) which is not comparable to the body weight alterations seen in melatonin mediated SD responses. To compare the timing of the melatonin response with the photoperiodic response, one must consider that the endogenous melatonin signal does not reflect a novel light:dark cycle immediately, but entrains gradually. In Djungarian hamsters, it takes 3 weeks for entrainment of running wheel activity to the photoperiodic schedule used in this study (unpublished observation). Since entrainment of running wheel activity reflects the entrainment of the elevated endogenous melatonin level (20), we assumed that adjustments in pineal melatonin signal in responsive hamsters would require 3 weeks.

Therefore, data for these hamsters were corrected accordingly, such that responsive hamsters were considered as pretreated with SD for 3 weeks.

In order to visualize trends in the body weight data more clearly, a reference level was calculated independently for each experimental group. These reference points were defined as the lowest weekly mean body weight the experimental groups reached plus 5%. Characteristics measured for timing of body weight changes were: time until mean body weight dropped below this reference point, time mean body weight remained below the reference point and the time at which mean body weight exceeded the reference point again. For molt, the following parameters were calculated for each treatment group: Mean onset of molt, mean fur color index (19), time until a stable fur color index was reached, and mean onset of molt back to the summer pelt.

Data are presented as means ± SEM. Analysis of variance was performed to test for differences in continuously varying traits. If a significant treatment effect was found, comparison between means were made by t-tests. Significance of body weight loss within each treatment group was tested by Wilcoxon's test for paired differences. Pearson's correlation coefficient was calculated to test for relationships between measured characteristics and duration of pretreatment. Percent molt was compared between treatment groups by chi-square analysis.

Results

Melatonin injections elicited a molt to the winter pelt in 76% of the hamsters (Table 1). Additionally, these hamsters exhibited the typical changes in body weight (Table 1). They lost body weight, maintained a low body weight plateau, and finally gained weight again (Figs. 1a-e). The remaining melatonin-injected hamsters (n=2

Table 1.
Characteristics of melatonin or SD induced changes in body weight and molt (means±SEM).

	SD responsive	Melatonin wk 9	wk 13	wk 15	wk 22
body weight:					
n	33	9	6	6	5
initial (g)	39.3±1.1	41.9±1.8	40.1±2.7	43.8±1.9	42.7±2.0
lowest (g)	35.0±1.2#	34.3±2.3#	33.5±3.8	33.1±3.9#	37.1±3.1
low from (wk)	7	9	7	8	9
until (wk)	25	20	16	17	15
molt:					
n	27	9	6	6	5
onset (wk)	7.6±0.5	7.8±0.9	6.0±0.9	6.2±1.0	5.8±1.3
fur index	3.6±0.1	3.0±0.3	3.3±0.2	2.7±0.2	2.8±0.2
from (wk)	14.2±0.6	12.0±0.6	11.3±1.3*	7.8±1.1*	8.8±1.6*
until (wk)	24.8±0.4	22.6±0.9*	18.1±0.8*	21.3±1.8*	17.8±1.6*

* $p<0.02$ versus SD responsive; # $p<0.05$ versus initial state

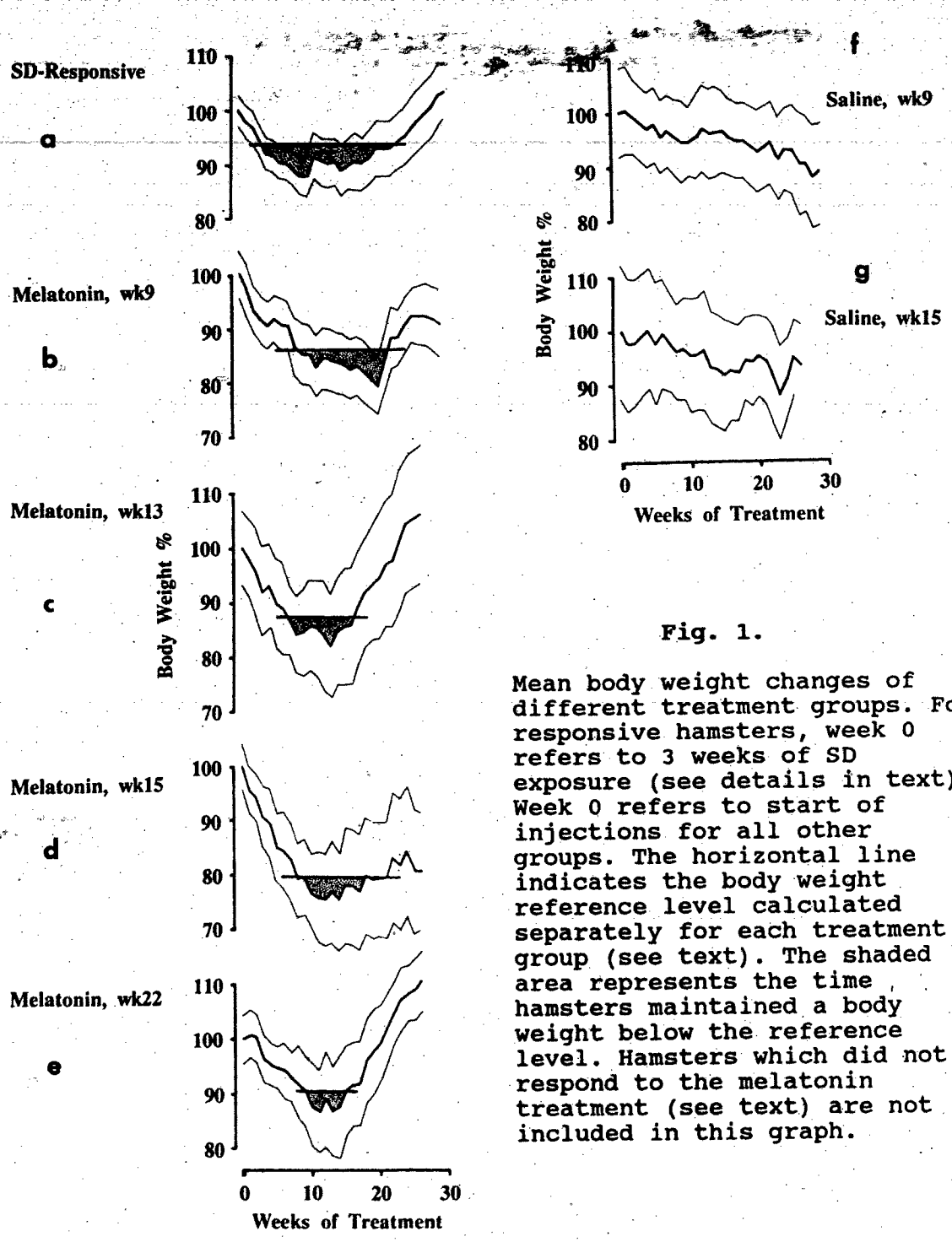

Fig. 1.

Mean body weight changes of different treatment groups. For responsive hamsters, week 0 refers to 3 weeks of SD exposure (see details in text). Week 0 refers to start of injections for all other groups. The horizontal line indicates the body weight reference level calculated separately for each treatment group (see text). The shaded area represents the time hamsters maintained a body weight below the reference level. Hamsters which did not respond to the melatonin treatment (see text) are not included in this graph.

for each treatment group) never molted and either maintained initial body weight or lost body weight continuously. The majority of the saline-injected hamsters never molted and continuously lost body weight (Figs. 1f,g). This body weight loss was not followed by a subsequent gain, as would be expected for a physiological, photoperiodic response. However, some hamsters showed a delayed onset of photoresponsiveness, indicated by molt and body weight loss, followed by body weight gain (n=4 for hamsters injected with

saline following 9 weeks and n=3 for hamsters injected following 15 weeks of SD)

Collectively, melatonin injections induced body weight loss and molt in a higher percentage of hamsters (76%) than saline injections (41%, $p<0.02$).

Discussion

Hamsters which were insensitive to SD conditions showed typical body weight and fur color responses, when provided with daily melatonin injections. These results strongly indicate that the lack of photoperiodic responses is not based on melatonin insensitivity, as shown for short day insensitive Peromyscus leucopus (18). Rather, as hypothesized in previous studies (15,16), transduction of daylength into an effective pineal melatonin signal seems to be deficient in nonresponsive Djungarian hamsters.

However, some saline-injected hamsters exhibited typical SD responses as well, after they previously had been insensitive to SD conditions. There are at least 3 explanations for this phenomenon. Given the assumption that short day insensitivity rests with a defect in circadian organization in this species, the daily handling and injections might have served as an additional Zeitgeber which entrained the pineal melatonin rhythm. Secondly, daily injections might have directly affected pineal melatonin production. The short duration of elevated pineal melatonin late in the night, typical for nonresponsive hamsters, might have fused with a stress-induced pineal melatonin rise in the early night (21,22), resulting in a duration of elevated pineal melatonin long enough to induce SD adjustments (23). Thirdly, in a previous study (24), we described considerable variation in the onset of SD responses in otherwise untreated Djungarian hamsters. In that study the majority of hamsters responded instantaneously to SD conditions with body weight loss, followed by molt, the occurrence of torpor and gonadal regression. However, a considerable percentage of the hamsters (7 out of 22) required prolonged SD exposure until the typical body weight loss and molt were elicited. Up to 30 weeks of SD exposure was required for some individuals, which therefore were referred to as "late responders". Such "late responders" certainly were present in this study as well and probably contributed to the high percentage of hamsters which exhibited body weight loss and the winter molt following saline injections. In any event, the fact that some hamsters show SD adjustments following saline injections does not invalidate our conclusion that short day insensitive hamsters are melatonin sensitive and can respond to injections. The percentage of hamsters which responded to melatonin was considerably higher than that of hamsters which responded to saline.

Timing of melatonin-induced body weight adjustments and molt is affected by SD pretreatment. A substantial correlation exists between the duration of SD pretreatment and the duration that hamsters maintain their low body weight (Fig. 2A). Since hamsters lost body weight immediately following melatonin injections or SD exposure (Fig. 1), onset of refractoriness was correlated with SD pretreatment (Fig. 2B). A similar correlation was found for onset

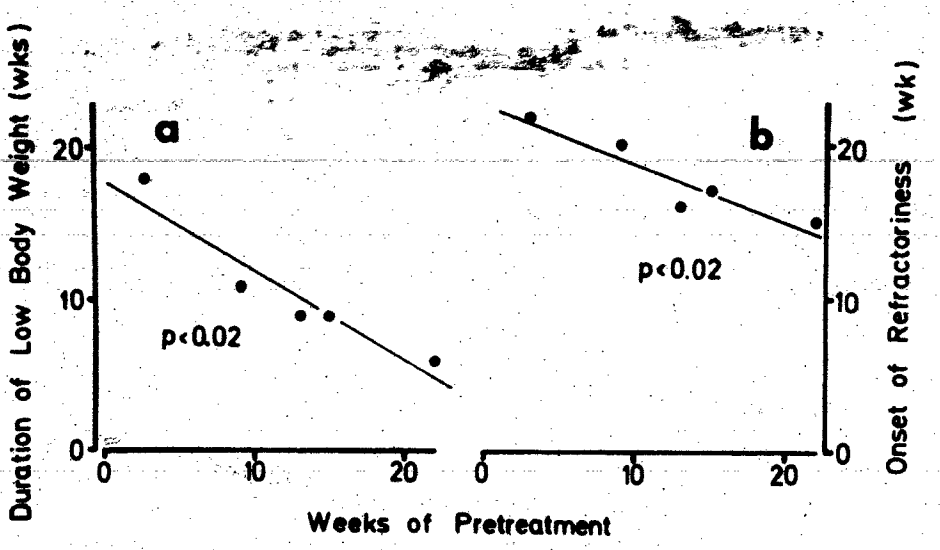

Fig. 2.

Duration of low body weight (a) and onset of refractoriness (b) as a function of pretreatment with SD. Responsive hamsters were taken as pretreated by 3 weeks (see text for details).

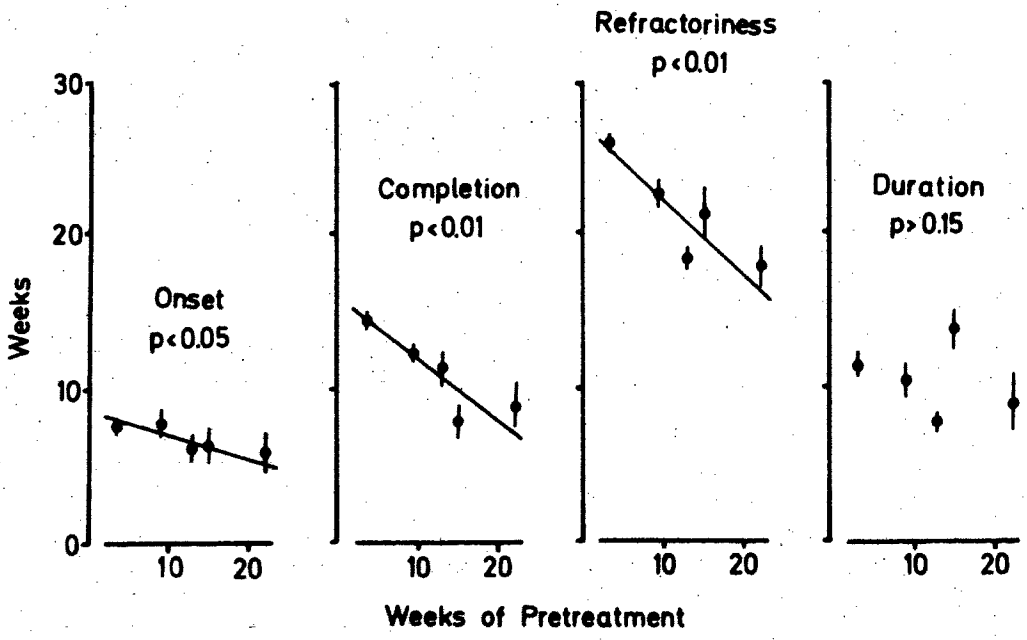

Fig. 3.

Timing for the onset, plateau, refractoriness and duration of molt as a function of SD pretreatment (see text for details). A significant correlation was found for all characteristics, except duration of molt.

of refractoriness for molt (Fig. 3). If only the number of short days had triggered refractoriness, all melatonin-injected hamsters should have become refractory at the same time. In contrast, if only the number of SD melatonin signals had been crucial, onset of refractoriness should have been delayed with respect to responsive hamsters by exactly the time interval that nonresponsive hamsters lacked a proper SD melatonin signal, i.e. by the duration of pretreatment. However, the timing of refractoriness was intermediate between these two extremes, which suggests that refractoriness for body weight and molt is the result of an integration of total time spent under SD conditions and the availability of the SD melatonin signal.

The effect of SD pretreatment on the timing of melatonin-induced molt can not completely be explained by an advanced onset of refractoriness. In contrast to changes in body weight, duration of high fur color index was not correlated with duration of pretreatment (Fig. 3). However, hamsters reached their stable high fur color index earlier if pretreated with SD (Fig. 3). This was due, in part, to an advanced initiation of molt in pretreated hamsters. Collectively, hamsters spent less time in the typical SD state with regard to body weight, but not with regard to molt, if pretreated with SD. This difference might be due to different time requirements for the body weight and molt cycle. On the other hand this might suggest differences in the effect of SD pretreatment on the control of body weight and molt.

Dose of the melatonin application used in this study clearly exceeded physiological levels. However, we consider it unlikely that the observed differences in the timing of physiological SD responses between melatonin injected and SD responsive hamsters are due to the dose or mode of melatonin delivery. If melatonin injections in general result in a specific timing of physiological adjustments different from SD induced responses, we would also expect to see this specific pattern in hamsters injected with melatonin under long-day conditions. However, melatonin injections under long-day conditions resulted in a response very similar to the SD response (9,10) and distinctly different from the response described here for pretreated, melatonin injected hamsters. Furthermore if the mode of melatonin application was solely responsible for differences in the timing of the response to melatonin, we would expect no effect of SD pretreatment. However, we found a correlation between the duration of SD pretreatment and the timing of the melatonin response.

The mechanism by which SD pretreatment affects the melatonin response remains unclear. Presumably, a deficiency in circadian organization prevents proper transduction of SD information in nonresponsive hamsters (15,16). Nevertheless, SD pretreatment was effective, i.e. nonresponsive hamsters are sensitive to SD conditions. There are two possible explanations for this apparent contradiction. First, this deficiency in circadian organization may not be as general as previously thought (16). That is, nonresponsiveness may be confined to an inability to transduce photoperiodic information for only certain behavioral and physiological attributes. Secondly, an additional integrative pathway, independent of the circadian system, might measure daylength.

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