THE EFFECT OF MELATONIN ON GROWTH AND DEVELOPMENT OF MONGOLIAN GERBILS (*MERIONES UGUICULATUS*)

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Summary

This project researched the effect of melatonin on weight gain, food intake, stages of development, and activity levels of Mongolian gerbils. Melatonin is a hormone secreted from the pineal gland that plays a major part in regulating biological and circadian rhythms. Melatonin was administered through drinking water to five litters, each with six pups, with five litters as controls. There were no conclusive results, because the effect of melatonin was masked by the mother gerbils.

Introduction

The purpose of this project was to determine whether melatonin affected weight gain, food intake, activity levels, and development of captive Mongolian gerbils (*Meriones unguiculatus*). Melatonin is a hormone secreted from the pineal gland that plays a major role in regulating biological and circadian rhythms (1). Secretion of melatonin is stimulated by the suprachiasmatic nucleus (SCN) in the brain, the main biological clock of the body. The SCN, with the help of hormones such as melatonin, regulates circadian cycles. Seasonal reproductive behavior is also controlled by the SCN (2). The pineal gland in gerbils contains nerves connected to the eyes that sense day length (3). In daylight, secretion of melatonin is suppressed, so when days are shorter, more melatonin is secreted (2). Melatonin levels thus correlate with seasonal changes in day length. Therefore, melatonin can be used to mimic ways in which mammals react to the short photoperiod of winter (4).

Because Mongolian gerbils (all gerbils referred to in cited papers are Mongolian) come from the desert regions of Northern Asia where climate changes dramatically with different seasons, it is logical that they are affected by melatonin (5). If gerbils did not respond to melatonin by adjusting their bodies and behaviors, they would not survive harsh seasonal changes. Observations done in the wild show that gerbils do not hibernate, but they do become less active in the winter (6). This is because they need to conserve energy and avoid burning fat stores since less food is available.

Previous studies showed that meadow voles *(Microtus pennsylvanicus)* and Mongolian gerbils decreased body weight when they were on a short photoperiod (4, 6, 7). Other studies showed that by reducing their body weight, gerbils decreased energy requirements for thermoregulation by spending less time foraging for food (8). However, these studies showed that gerbils maintained a delicate balance when seasonally adjusting their body weight. Weight losses were not drastic, because they had to maintain an extra layer of fat for insulation and energy storage (8, 9).

Previous research has shown that young gerbils born late in the season prepared for winter with delayed somatic growth, which is growth that relates to the outer walls of the body and weight gain but does not affect internal organs (4). Gerbils that were raised in short-day environments were observed to eat less than those in long-day environments (4).

In previous experiments, eye opening had been observed to occur later when the gerbils were raised in short-day environments (7). This is because the age at eye opening is correlated to weight. If the gerbils gained weight more slowly, they were observed to open their eyes at a later date (7).

All of these previous experiments studied the effects of melatonin on animals by either observing them in the wild during winter or by simulating a winter environment with altered photoperiods. The project reported here studied how melatonin affected gerbils directly in a controlled environment.

The first hypothesis of this project was that the gerbils would gain weight more slowly when they were given melatonin compared to the controls. It was expected that significant changes in the weight of gerbils given melatonin (from hereon known as "melatonin gerbils/pups") would occur only after weaning, since before weaning the concentration of melatonin would diminish as it passed through the dam's body (4).

The second hypothesis was that melatonin gerbils would initially eat more in anticipation of winter conditions mimicked by the melatonin, but food intake would eventually decrease below that of control gerbils.

The third hypothesis was that the melatonin gerbils would develop more slowly in the stages being considered—nest leaving, teething, and eye opening.

The final hypothesis was that melatonin gerbils would decrease physical activity as they attempted to conserve energy, because melatonin would make them lethargic. It was expected that gerbils would also expend less energy, because when eating less they would not have to forage for food as often.

Methods

Mongolian gerbils were obtained from the University of St. Thomas. Observations and data collection started when the offspring were newborns and continued until they were 50 days old.

The gerbils were kept in a ventilated, temperature- and humidity-controlled environment of 70ºF on a 12/12-hour light/dark cycle. The gerbils were given rat chow and water ad libitum and were housed in 7.5 x 16.5 x 7.5 in^3 clear plastic containers with stainless-steel lids. Ten female and ten male gerbils were mated. Males were removed from the cages once females gave birth. Five gerbil mothers received melatonin (0.05 g/mL of water consumed daily) in their drinking water as soon as they gave birth, while the other five served as controls. Each litter was culled to six pups to control for milk availability. Once the pups were weaned at 25 days, they received melatonin directly in their drinking water.

The gerbils were never put in an uncomfortable or unsafe situation. Melatonin is not harmful to gerbils, because it is a hormone already found in their bodies and the amounts given to them were not high enough to be unsafe (9). When the litters were culled to six pups, the pups were cross-fostered to other litters. Cross-fostering is a routine and safe practice. After the project, the gerbils were either donated to a breeder or kept at the University of St. Thomas for further observations. The veterinarian on call was Dr. David W. Wetherill D.V.M. from the Como Park Animal Hospital.

Body Weight: The gerbil pups were weighed on the day of their birth and re-weighed at 15 days. At this point the pups were identified separately, either by their coloration and markings or by artificial marking with a permanent marker or shaving. At 25 days, the pups were weighed then weaned from their dams by placing them in a separate cage. The pups were weighed again at 35, 45, and 50 days.

Food Intake: Before weaning, food intake of dams was measured daily. It was assumed that the amount eaten by the dam correlated to the amount of milk the pups received. On Mondays, the gerbils were given 400 g of rat chow. The amount of food in the tray was then weighed daily between 10 and 11 a.m, including any large chunks that had fallen into the litter. The food was replaced each Monday. After the pups were weaned, their food intake was measured instead of their dams'.

Development: Three events were studied as indicators of whether the development of the gerbil pups was affected by melatonin. The first stage was nest- leaving, which occurred at 7-10 days after birth (10). Nest-leaving was defined as the pups moving themselves half a cage length from the nest. Being pushed or carried out of the nest by their mothers was not considered nest-leaving; the pups needed to exhibit defined, controlled movements. Since all the pups in a litter completed this stage of development over the duration of one day, the same date was recorded for the litter as a whole. Also, at 7-10 days the pups were still hairless, so it was impossible to individually identify them.

The second stage in development was teething, which occurred at 12-14 days after birth (10). This was defined as when the bottom front incisors had fully erupted from gums and was recorded individually for each pup.

The third stage was eye-opening, which usually occurred at 17-22 days of age (10). This was defined as the point when at least one of the eyes was completely open and was recorded individually.

Activity: Observations of the litters were made every day for 20 minutes between 3 and 4 p.m. The activities of the pups were recorded at one-minute intervals as active (A), active near nest (ANN), or inactive (I). Activity was defined as being a half-cage length out of the nest and moving around. Digging was also considered active. If pups were less than a half-cage length from nest and active—either grooming, shredding, feeding, etc.—but not moving, this was recorded as active near nest. If the gerbils were sleeping, nursing, or otherwise inactive in or away from the nest, this was considered inactive. Nursing was considered inactive, because when pups were under the dam it was not possible to tell whether they were actually nursing. Observations were extended to 30 minutes after pups were weaned.

Statistics: Body weight, food intake, and development were analyzed using Minitab General Linear Model ANOVA. Activity was analyzed with Chi-square tests. P-values were determined as significant when less than 0.05. Effect of dams was run as a factor for body weight and development.

Results

Body Weight: Figure 1 shows mean control and melatonin body weight compared over time. On days 15, 25, and 35, gerbils given melatonin had a significantly higher body weight than controls. There was no significant difference between melatonin and control gerbils' body weights on days 0, 45, and 50 (Table 1).

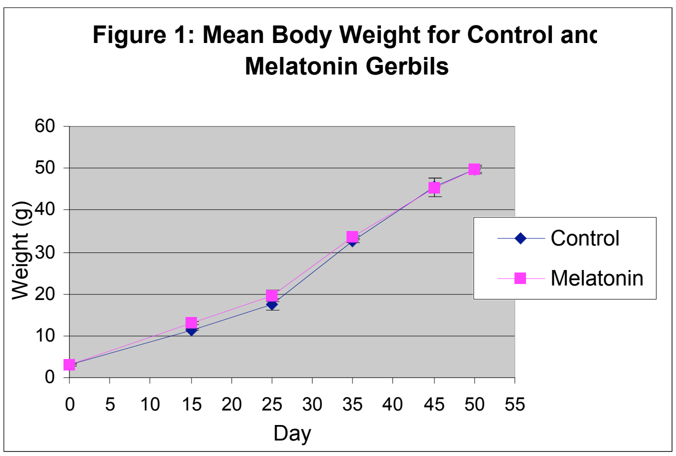


Table 1: Effect of melatonin on body weight.

	Mean Weight (g)			
Day	Controls	Melatonin	f	p
0	3.167	3.099	0.780	0.382
15	11.471	13.007	266.370	0.000
25	17.459	19.445	130.010	0.000
35	32.570	33.562	5.900	0.019
45	45.491	45.343	0.130	0.722
50	49.842	49.682	0.100	0.757

Food Intake: Figure 2 compares mean food intake for control and melatonin gerbils over time. Melatonin had no statistically significant effect on the food intake except at 35 days (Table 2).

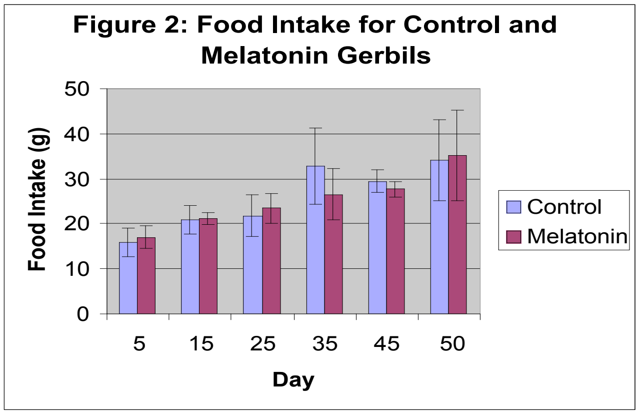
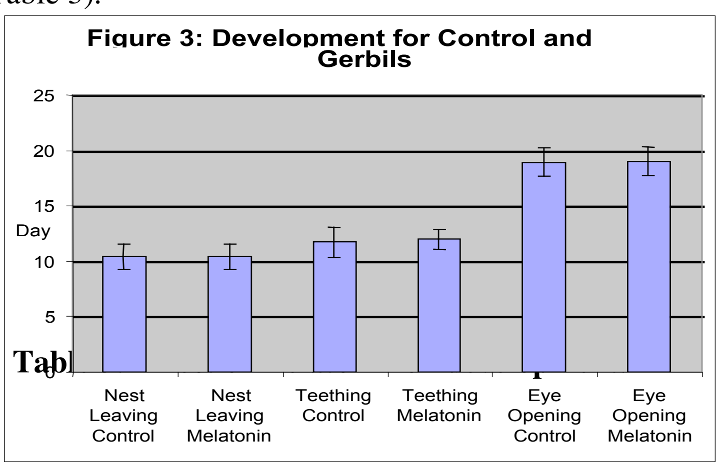


Table 2: Effect of melatonin on food intake

	Mean Food Intake (g)			
Day	Control	Melatonin	f	p
5	15.868	16.956	0.680	0.438
15	20.926	21.182	0.270	0.616
25	21.718	23.460	0.000	0.963
35	32.848	26.558	6.470	0.038
45	29.488	27.738	1.390	0.277
50	34.164	35.210	0.050	0.835

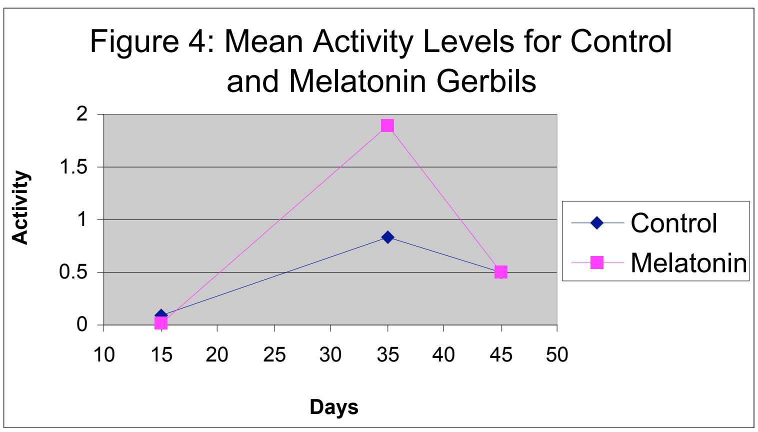
Development: Figure 3 compares the development of control and melatonin gerbils. Melatonin did not affect the development of nest leaving, teething, and eye opening of melatonin gerbils (Table 3).



	Mean Age (days)			
	Controls	Melatonin	f	p

Nest-Leaving	10.400	10.400	0.000	1.000
Teething	11.700	11.967	2.000	0.163
Eye-Opening	18.933	19.000	0.130	0.722

Activity: Figure 4 compares activity levels for control and melatonin gerbils over time. Day 15 both control and melatonin gerbils differed significantly from random ($\aleph^2 = 0, \mathrm{p} > 0.25, df = 2$). Both groups differed in the same way—mostly inactive—showing that melatonin did not have a significant effect. At 35 days, the controls were significantly different from random, with the trend toward inactivity, however, the melatonin gerbils were not significantly different from random, with a trend towards higher activity levels than the controls ($\aleph^2 = 6.64, \mathrm{p} < 0.05, df = 2$). This shows that the melatonin had an effect. At 45 days, both control and melatonin gerbils were significantly different from random with a trend towards inactivity ($\aleph^2 = 0.61, \mathrm{p} > 0.25, df = 2$). Melatonin did not have a significant effect at 45 days.



Effect of Dams: Dams had a significant effect on body weight except at day 50 (Table 4). Dams also had a significant effect on development (Table 5).

Table 4: Effect of dam on body weight

Day	F	P
0	4.680	0.000
15	41.780	0.000
25	33.330	0.000
35	11.900	0.000
45	3.600	0.002
50	1.720	0.118

Table 5: Effect of dam on development.

	F	P
Nest-leaving	n/a	n/a
Teething	15.140	0.000

Eye-opening	19.200	0.000

Discussion

The results were opposite to the hypotheses. It was expected that control gerbils would weigh more than melatonin gerbils, but melatonin gerbils actually weighed more at 15, 25, and 35 days. This makes sense that the melatonin gerbils gained weight since they are preparing for winter and are putting on a layer of fat. Food intake was also expected to decrease for melatonin gerbils, but there were no differences between melatonin gerbils and controls, except at 35 days when melatonin gerbils ate less. Melatonin was expected to delay development, but it had no effect. Melatonin gerbils were hypothesized to be less active, but there were no differences between control and melatonin gerbils except at 35 days, where the melatonin gerbils were more active. It makes sense that the gerbils were mostly inactive, since this is a common behavior among mammals.

Overall, melatonin did not have a strong effect on gerbils, except at 35 days. This may be because it is shortly after weaning, and in the wild the gerbils would be on their own or more independent at this point.

The effect of dams may have masked the effect of the melatonin since dams did have a significant effect on the gerbils' weight gain and development. In future studies dams could be made more genetically constant by using sisters or closely related females. Another option would be to use a larger sample size, i.e. 50 instead of 10, to randomize genetic differences. More data might minimize dam effects so effects of melatonin, if any, could be seen. It would also be important to weigh dams prior to the experiment to determine if their size has an effect.

Another study could be done by repeating this project using a species of gerbils that is native to a region near the equator, such as Egyptian or Indian gerbils. In these areas day length remains more constant with the change of seasons. It would be interesting to see if these species respond differently to melatonin than Mongolian gerbils.

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