

Extended Essay

Research Question: *Can One-hour-long Light Pulses Applied During the Night Shift the Phase of Circadian Rhythms in the Locomotor Activity of Siberian Hamsters?*

Subject: Biology

Supervisor: Mrs. Hilde DeSmet

Candidate Name: Sangkyun Cho

Exam Session: May 2007

Word Count: 3981 words

Abstract:

Circadian rhythms are biological rhythms that repeat daily, for example rhythms in sleep and wakefulness, rest and activity, cardiac output, body temperature, and hormone secretion. These biological rhythms are controlled by an internal circadian clock or timing mechanism, which synchronizes the biological rhythms of the body with the rhythms of the environment (e.g. the rotation of the Earth). In mammals, this internal clock is located in the brain, and light is the most powerful and effective cue that resets it daily.

The process of resetting an organism's circadian clock using an external light stimulus or cue is known as photic entrainment. In this essay, the effect of external light on the circadian rhythms of hamsters was investigated through a series of experiments. First, experimental hamsters were subjected to constant darkness, and their rhythms in locomotor activity were monitored (measured by the number of wheel revolutions) for several days to see if their circadian clocks continued to operate without the normal alternations of day and night. Then, the hamsters were exposed to one-hour-long pulses of high-intensity light (at midnight) for one week, and the rhythms in their level of locomotor activity were again recorded to see if the phases of their rhythms have been shifted (advanced or delayed) due to the light pulses.

The results of the experiment showed that (despite some inaccuracies or fluctuations) the hamster's circadian clocks were delayed by two to three hours following the light pulses. This is because information on the changes in the luminance of the environment were perceived by the retina of the hamsters, and were sent to the master mammalian circadian clock, called the suprachiasmatic nucleus (SCN), where this was interpreted and sent to numerous tissues of the body to regulate various circadian rhythms, including the level of their locomotor activity.

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Introduction:

Our lives are based around various recurrent cycles or rhythms. Some of these cycles reflect regular changes in our physical environment, such as the Earth's rotation or the passage of the seasons, whereas others are social, for example alternations between the working week and the weekend or the schedules of night shift work. We are adapted to these natural and social cycles and are able to function properly because our bodies undergo regular rhythmic changes that are synchronized to the rhythms of our environment. Such biological rhythms are generated by an internal "clock" or timing mechanism in our bodies. These biological clocks govern virtually all aspects of metabolism, physiology and behavior, including sleep and wakefulness, rest and activity, cardiac output, body temperature, oxygen consumption and hormone secretion.¹

This does not only apply to humans. In fact, biological clocks seem to be a universal and omnipresent mechanism, existing in practically every organism on the Earth. As humans undergo certain biological rhythms such as sleep and wakefulness, animals, plants, and even some prokaryotic cyanobacteria undergo biological rhythms in order to live in harmony with the rhythms of their environment.² For example, some flowers are able to raise and lower their leaves during specific hours of the day, certain types of fish can lighten the color of their skin when it gets dark, many animals seem to know precisely when to hibernate, and pupas of some insects only emerge at a specific time of day.

These biological clocks regulate a wide range of daily, monthly, seasonal, and even annual rhythms. Of the numerous types of rhythms that are controlled by the biological clocks of organisms, the most noticeable and common are those that occur daily, such as rhythms in sleep/wakefulness, rest/activity, and the rise and fall of body temperature. These rhythms, which repeat themselves every 24 hours according to the Earth's rotation, are called *circadian* rhythms, from the Latin words "*circa*" meaning "about" and "*dies*" meaning "day".

Circadian rhythms were first studied experimentally over a century ago (1729) by a french astronomer, Jean-Jacques de Mairan. In his studies, de Mairan discovered that organisms are able to maintain their rhythmicity even when exposed to constant light or darkness, without any external stimulus.³ The circadian rhythms of his experimental organisms did not disappear, clearly indicating that they were not simple responses to external stimuli, such as the presence of sunlight. He came to the conclusion that these daily rhythms were driven instead

¹ The Millar Research Group, *Circadian Rhythms* (n.d.) <<http://template.bio.warwick.ac.uk/staff/amillar/circad.html>> [21 August 2006], par.1/2.

² The Center for Research on Biological Clocks. *Coordination of Circadian Physiology in Diverse Species* (n.d.). <<http://www.bio.tamu.edu/clocks/program.htm>>[5 September 2006], par.1.

³ "Circadian Rhythm," *Wikipedia*, (2006). <http://en.wikipedia.org/wiki/Circadian_rhythms> [20 August 2006], Introduction.

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by an internal timing mechanism—an endogenous clock.⁴

In a constant environment, these circadian rhythms continue in periods remarkably close to 24 hours. For example, the human clock has a period of roughly 25 hours, with a 1 hour discrepancy and only 4% difference from the Earth's 24 hours.⁵ For wild-type Golden hamsters, the period is almost precisely 24 hours. However, the fact that there is a close match between an organism's endogenous period and that of the Earth is not enough for the organism to maintain synchrony with the Earth's rotation and the changes in its physical environment. Even if the discrepancy between the two periods is minimal, the biological clock requires a cue that enables it to reset daily to agree with the local time of the environment.⁶ This is because after very long durations of time, even a small difference will gradually become large and cause the two periods to completely desynchronize, just as the distance between two racing cars become greater in a car race. Therefore, the ability of circadian clocks to reset their phases according to the physical environment is a crucial and necessary element in their functioning.

Of the many different types of stimulus that occur daily, sunlight (or light in general) seems to be the most effective and potent cue for circadian clocks to reset itself in most organisms.⁷ This is because light is “inevitably connected to the Earth's rotation, and is completely reliable in its daily experience.”⁸ The process of organisms adjusting or synchronizing to another cycle or period due to a light stimulus is known as photic entrainment. In this essay, the photic entrainment of rodents will be investigated, by exposing Siberian Hamsters to regular one-hour-long pulses of high-intensity light and seeing if the rhythms of their locomotor activity (measured by the number of wheel revolutions) will shift accordingly or not. First, the locomotor activity of the hamsters will be observed and analyzed over several days under constant darkness. Then, the rodents will be exposed to regular light pulses in an attempt to entrain them to a new cycle (see *Method* for details).

If exposed to constant darkness, it is expected that the rodents' biological clocks will continue to generate their circadian rhythms, and therefore, as nocturnal animals, their levels of locomotor activity will continue to be high during subjective night (time that would have been night had the rodents continued in the normal day/night cycle) and low during subjective day (time that would have been day had the rodents continued in the normal day/night cycle). In other words, the regular rhythm of locomotor activity will be maintained.

If pulses of high-intensity light are delivered, however, it is expected that the rodents' phase (the position of a repetitive process in its cycle) of locomotor activity will be shifted (either advanced or delayed) and the

⁴ Ibid.

⁵ Arthur T. Winfree, *The Timing of Biological Clocks* (New York: Scientific American Books Inc., 1987), p.8.

⁶ Ibid.

⁷ The Hormone Shop, *Biological Clocks* (13 December 2006).

<<http://www.thehormoneshop.com/biologicalclocks.htm>>[21 August 2006], par.3.

⁸ Winfree, 8.

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rodents' circadian rhythms will no longer repeat itself from the same position. As circadian clocks of rodents are reset by light every solar day, the regular light pulses will force/stimulate the rodents to adjust to a new cycle. In other words, the rodent will be entrained to a new phase due to the light pulses. However, entrainment would occur only if the pulses of light are delivered during subjective night, since delivering it during subjective day will have no effect—light pulses delivered during subjective day will be perceived simply as normal sunlight.

Equipment/Materials:

- Three Siberian hamsters.
- One data logger that measures wheel revolutions.
- Two cages with hamster wheels (with radii of 5~7cm).
- Ample supply of food and water for the hamsters.
- Thick black cloth/blanket that is large enough to cover the cages.
- Three high wattage (50W) lamps.

Method/Procedure:

- 1) Of the two cages, select one in which the experiment will be conducted.
- 2) Place one of the hamsters in the experiment cage, and the others in the second cage, and place the two cages in two separate rooms
- 3) Let the hamsters adjust to the new environment, for about one week.
- 4) For the following three days, observe the hamster's (the one in the experiment cage) behavior, and record the number of revolutions every three hours for 24 hours by reading off the data logger.
- 5) Average out the recorded results and plot a graph with time as the x-axis, and the number of revolutions as the y-axis, and see if there are any patterns in the graph of locomotor activity.
- 6) Starting from the fourth day, cover the black blanket over the experiment cage, turn off all lights in the experiment room, and cover the windows so that the room is completely dark.
- 7) As done in step 4), record the number of revolutions every three hours, and graph the results. Continue this for three more days.
- 8) Observe if the rhythm of locomotor activity continues in complete darkness, without the alternation of day and night.
- 9) On the eighth day, expose the experiment hamster to a bright pulse of light for one hour during subjective night (from midnight to 01:00), using the three lamps (keeping darkness for the rest of the day). Make sure that the light reaches all areas inside the cage.
- 10) Repeat this everyday for one week.
- 11) During the second week, continue to give the light pulses, but this time, also record the number of revolutions every three hours, as done in steps 4) and 7) (for seven days).
- 12) Average out the results and graph them. Observe if the rhythm in locomotor activity have shifted due to the light pulses given during the two weeks.
- 13) Repeat steps 1) to 12) for the second and third hamsters.
- 14) Pool and average out the results of all three hamsters, and graph the data as done in steps 5) and 12).

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Results:

Hamster I

Table Showing the Number of Revolutions Counted During the Normal Day/Night Cycle

Time/Hours	Number of Revolutions			
	Day 1	Day 2	Day 3	Average
00:00~03:00	7861	7615	7292	*7589.33
03:00~06:00	5632	2961	5259	4617.33
06:00~09:00	861	1512	1476	1283
09:00~12:00	241	1048	21	436.67
12:00~15:00	1339	940	1160	1146.33
15:00~18:00	368	0	717	361.67
18:00~21:00	1974	5959	1569	3167.33
21:00~00:00	7959	12331	4768	8352.67

Example of Calculations:

* $(7861 + 7615 + 7292)/3 = 7589.33$ revolutions

Table Showing the Number of Revolutions Counted When Subjected to Constant Darkness

Time/Hours	Number of Revolutions				
	Day 4	Day 5	Day 6	Day 7	Average
00:00~03:00	8547	10034	8889	7717	*8796.75
03:00~06:00	1331	3220	6223	5542	4097
06:00~09:00	1343	1671	3082	4100	2549
09:00~12:00	902	1991	1071	1367	1332.75
12:00~15:00	1319	1001	740	497	889.25
15:00~18:00	1842	211	768	270	772.75
18:00~21:00	3687	1807	1265	0	1689.75
21:00~00:00	4523	4777	3994	4202	4374

Example of Calculations:

* $(8547 + 10034 + 8889 + 7717)/4 = 8796$ revolutions

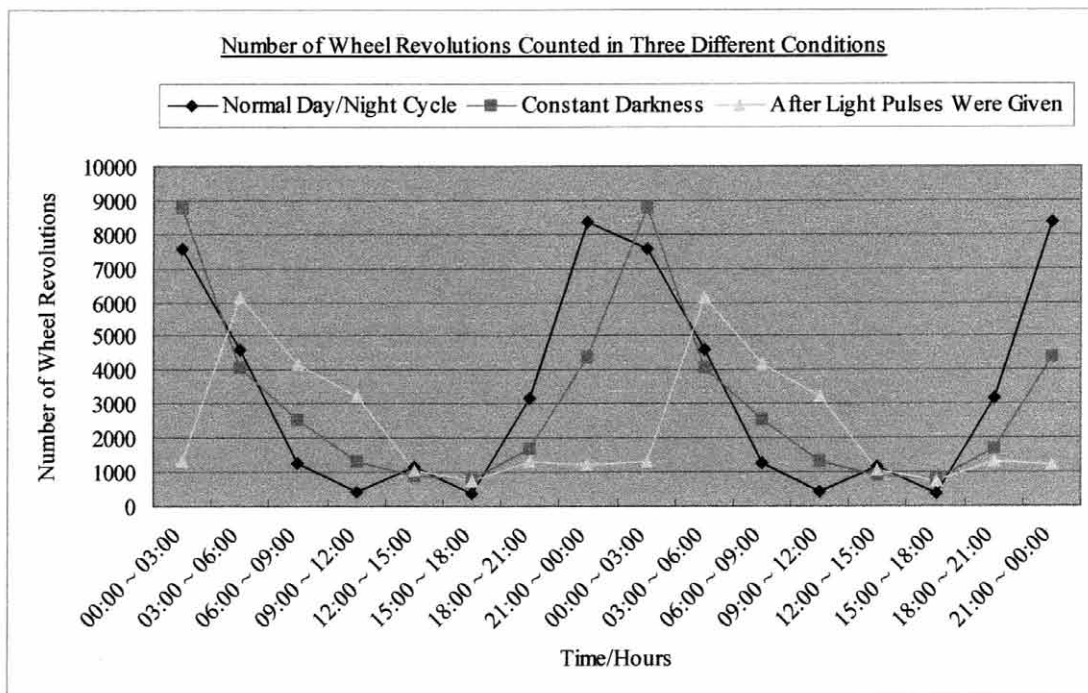
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Table Showing the Number of Revolutions Counted When Subjected to One-Hour-Long Light Pulses During Subjective Night

Time/Hours	Number of Revolutions							Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
00:00~03:00	194	740	1896	1911	1034	2889	717	*1340.14
03:00~06:00	5861	3885	7078	6343	4671	8089	7100	6146.71
06:00~09:00	4046	2548	3015	4331	3220	6223	5782	4166.43
09:00~12:00	2136	602	201	3902	6991	4371	4367	3224.29
12:00~15:00	339	0	2362	319	1009	2740	497	1038
15:00~18:00	109	0	3890	0	321	760	52	733.14
18:00~21:00	1974	33	1	3687	807	1835	1103	1348.57
21:00~00:00	959	53	943	531	1777	3118	1202	1226.14

Example of Calculations:

$$* (194 + 740 + 1896 + 1911 + 1034 + 2889 + 717)/7 = 1340.14 \text{ revolutions}$$



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Hamster II

Table Showing the Number of Revolutions Counted During the Normal Day/Night Cycle

Time/Hours	Number of Revolutions			
	Day 1	Day 2	Day 3	Average
00:00~03:00	7080	6585	4986	*6217
03:00~06:00	6677	4630	6835	6047.33
06:00~09:00	29	4512	5832	1283
09:00~12:00	2384	3023	4899	3457.67
12:00~15:00	5969	2870	1447	3428.67
15:00~18:00	3368	1132	2986	2495.33
18:00~21:00	1663	607	1947	1405.67
21:00~00:00	4394	3693	3053	3713.33

Example of Calculations:

* $(7080 + 6585 + 4986)/3 = 6217$ revolutions

Table Showing the Number of Revolutions Counted When Subjected to Constant Darkness

Time/Hours	Number of Revolutions				Average
	Day 4	Day 5	Day 6	Day 7	
00:00~03:00	8636	6088	8968	8688	*8026.75
03:00~06:00	6763	6542	3960	2850	5028.75
06:00~09:00	4984	5924	6525	5179	5653
09:00~12:00	4532	1982	11	5267	2948
12:00~15:00	319	2650	2879	3690	2384.5
15:00~18:00	2870	3553	1542	1392	2339.25
18:00~21:00	5987	809	3979	2479	3313.5
21:00~00:00	4643	3536	5098	4695	4493

Example of Calculations:

* $(8636 + 6088 + 8968 + 8688)/4 = 8026.75$ revolutions

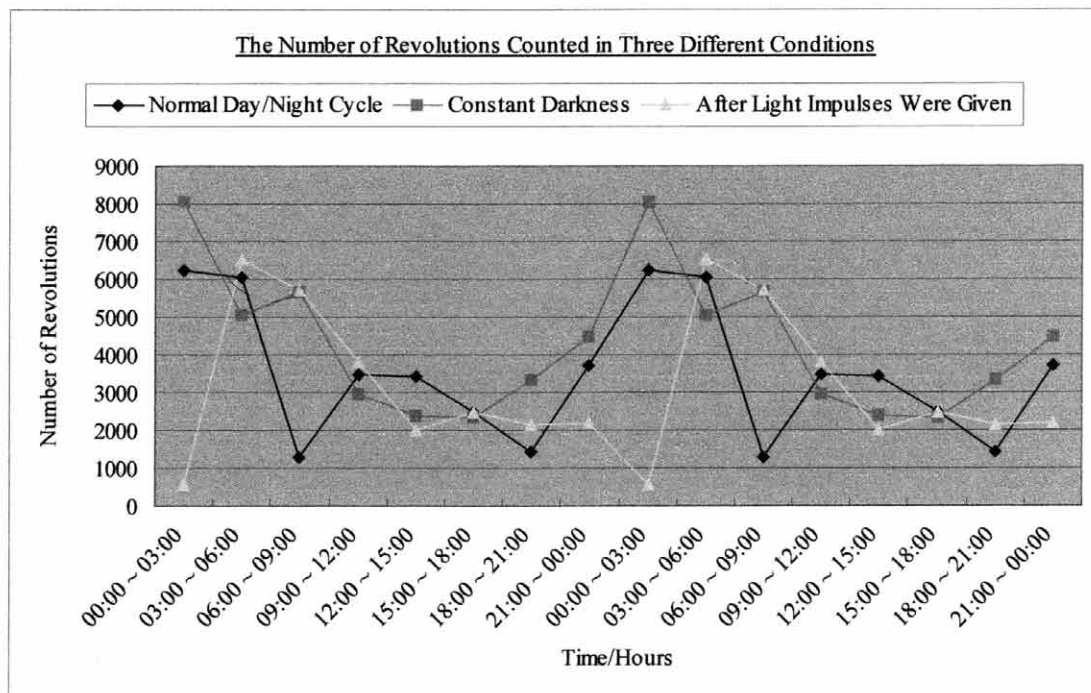
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Table Showing the Number of Revolutions Counted When Subjected to One-Hour-Long Light Pulses During Subjective Night

Time/Hours	Number of Revolutions							Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
00:00~03:00	26	87	0	19	1034	0	2808	*567.71
03:00~06:00	5729	6379	5096	7873	6524	8962	5100	6523.29
06:00~09:00	8863	6039	3983	4657	6552	6229	3782	5729.29
09:00~12:00	5837	98	2765	5752	6991	4639	582	3814.29
12:00~15:00	768	1653	1874	2164	3764	3268	497	1998.29
15:00~18:00	3965	2806	3835	978	339	2652	2793	2481.14
18:00~21:00	1975	2865	4657	687	774	2784	1104	2120.86
21:00~00:00	2985	4427	2879	1265	1654	993	1004	2172.43

Example of Calculations:

$$* (26 + 87 + 0 + 19 + 1034 + 0 + 2808) / 7 = 567.71 \text{ revolutions}$$



Hamster III**Table Showing the Number of Revolutions Counted During the Normal Day/Night Cycle**

Time/Hours	Number of Revolutions			
	Day 1	Day 2	Day 3	Average
00:00~03:00	8035	7747	10019	*8600.33
03:00~06:00	5831	2987	4030	4282.67
06:00~09:00	542	2690	3476	2236
09:00~12:00	1241	1734	21	988.66
12:00~15:00	3450	2129	99	1892.67
15:00~18:00	765	53	1178	665.33
18:00~21:00	3029	5069	4003	4033.67
21:00~00:00	6837	5587	4573	5665

Example of Calculations:

$$* (8035 + 7747 + 10019)/3 = 8600.33 \text{ revolutions}$$

Table Showing the Number of Revolutions Counted When Subjected to Constant Darkness

Time/Hours	Number of Revolutions				
	Day 4	Day 5	Day 6	Day 7	Average
00:00~03:00	7094	5034	9872	5051	*6762.75
03:00~06:00	1239	5008	3223	4418	3472
06:00~09:00	1518	670	1201	4140	1882.25
09:00~12:00	0	1036	72	1367	618.75
12:00~15:00	2364	1876	0	5685	2481.25
15:00~18:00	1916	369	0	703	747
18:00~21:00	5387	5807	124	1107	3106.25
21:00~00:00	6677	4555	4994	5242	5367

Example of Calculations:

$$* (7094 + 5034 + 9872 + 5051)/4 = 6762.75 \text{ revolutions}$$

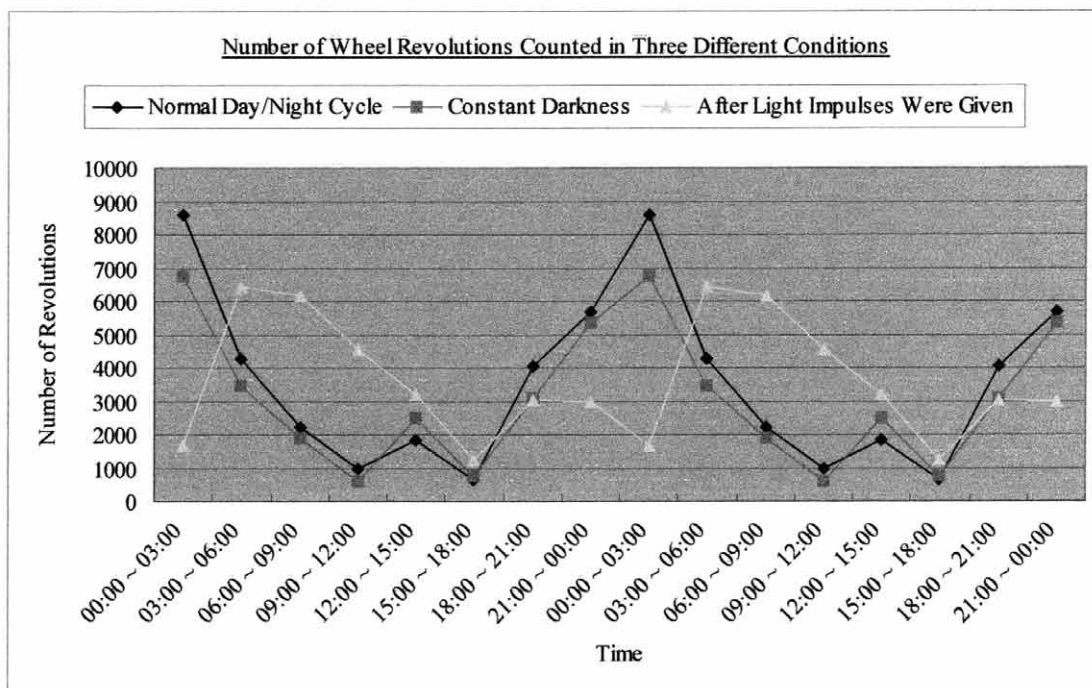
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Table Showing the Number of Revolutions Counted When Subjected to One-Hour-Long Light Pulses During Subjective Night

Time/Hours	Number of Revolutions							Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
00:00~03:00	3297	1130	1996	911	0	5670	781	*1684.14
03:00~06:00	6066	5794	8979	7333	4685	4064	8111	6433.14
06:00~09:00	5074	6080	4003	5038	5739	9577	7782	6184.71
09:00~12:00	2131	5949	3618	6881	6497	4371	2369	4545.14
12:00~15:00	2419	1732	4571	4355	2173	2740	4497	3212
15:00~18:00	249	67	2310	615	1650	3560	77	1218.29
18:00~21:00	3989	3198	0	5329	3807	1137	3808	3038.29
21:00~00:00	392	4587	0	3212	4731	4018	3927	2981

Example of Calculations:

* $(3297 + 1130 + 1996 + 911 + 0 + 5670 + 781)/7 = 1684.14$ revolutions



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Combined Results of Hamsters I, II, and III:

Table Showing the Number of Revolutions Counted During the Normal Day/Night Cycle

Time/Hours	Number of Revolutions			
	Hamster I	Hamster II	Hamster III	Average
00:00~03:00	7589.33	6217	8600.33	* 7468.89
03:00~06:00	4617.33	6047.33	4282.67	4982.44
06:00~09:00	1283	1283	2236	1600.66
09:00~12:00	436.67	3457.67	988.66	1627.67
12:00~15:00	1146.33	3428.67	1859.67	2144.89
15:00~18:00	361.67	2495.33	665.33	1174.11
18:00~21:00	3167.33	1405.67	4033.67	2868.89
21:00~00:00	8352.67	3713.33	5665	5730.33

Example of Calculations:

* $(7589.33 + 8600.33 + 6217)/3 = 7468.89$ revolutions

Table Showing the Number of Revolutions Counted When Subjected to Constant Darkness

Time/Hours	Number of Revolutions			
	Hamster I	Hamster II	Hamster III	Average
00:00~03:00	8796.75	8026.75	6762.75	* 7862.08
03:00~06:00	4097	5028.75	3472	4199.25
06:00~09:00	2549	5653	1882.25	3361.42
09:00~12:00	1332.75	2948	618.75	2144.62
12:00~15:00	889.25	2384.5	2481.25	1918.33
15:00~18:00	772.75	2339.25	747	1286.33
18:00~21:00	1689.75	3313.5	3106.25	2703.17
21:00~00:00	4374	4493	5367	4744.67

Example of Calculations:

* $(8796.75 + 6762.75 + 8026.75)/3 = 7862.08$ revolutions

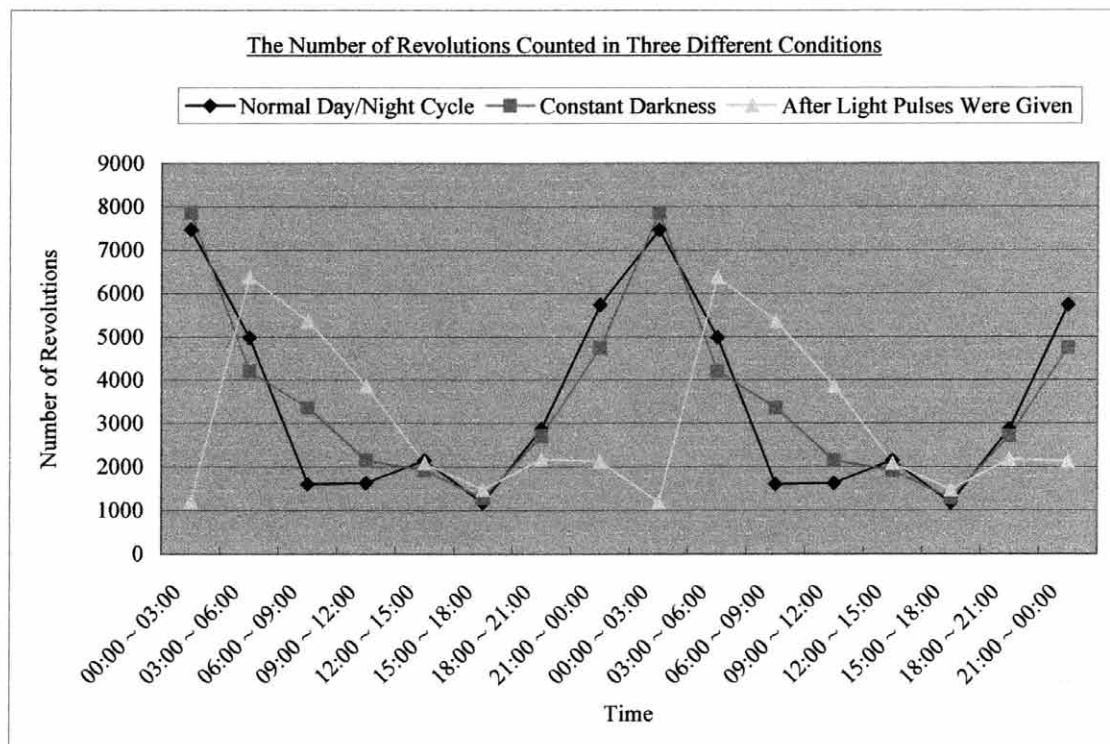
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Table Showing the Number of Revolutions Counted When Subjected to One-Hour-Long Light Pulses During Subjective Night

Time/Hours	Number of Revolutions			
	Hamster I	Hamster II	Hamster III	Average
00:00~03:00	1340.14	567.71	1684.14	* 1185.33
03:00~06:00	6146.71	6523.29	6433.14	6367.71
06:00~09:00	4166.43	5729.29	6184.71	5360.14
09:00~12:00	3224.29	3814.29	4545.14	3861.24
12:00~15:00	1038	1998.29	3212	2082.76
15:00~18:00	733.14	2481.14	1218.29	1477.52
18:00~21:00	1348.57	2120.86	3038.29	2169.24
21:00~00:00	1226.14	2172.43	2981	2126.52

Example of Calculations:

* $(1340.14 + 1684.14 + 567.71)/3 = 1185.33$ revolutions



Analysis and Evaluation:

As Siberian hamsters are nocturnal animals, in the normal day/night cycle their locomotor activity peaked during the night and generally fell during the day. In all three hamsters the highest level of activity occurred approximately from 00:00 to 03:00. When the hamsters were placed in constant darkness in the first part of the experiment, this pattern of activity and inactivity continued to repeat itself, which agrees with what was mentioned in the hypothesis: their endogenous (internal) clocks continued to operate in the constant environment, even without an external stimulus or cue. However, when light pulses were given during subjective night and the hamsters were given time to adjust in the second part of the experiment, this pattern of locomotor activity shifted (was delayed) by approximately three hours, as shown on the graph on the previous page.

This is because, as mentioned in the hypothesis, the light pulses reset the biological clocks of the hamsters during every subjective night, entraining them to a whole new cycle. In other words, the light pulses acted as the cue for entrainment, somewhat similar to the experience of jet lag in humans. Like the changed environment that a human adjusts to during jet lag, the light pulses have stimulated the circadian clocks in the hamsters to reset itself to a new cycle. On the surface, this process of photic entrainment may seem to be quite simple and straightforward. However, at the physiological and molecular-genetic level, the photic entrainment of circadian clocks is an extremely complex process that is still not fully known today.

Throughout the years, the circadian clocks of organisms, especially those of “higher” plants and animals, have evolved into a very complex mechanism, composed of many different features and specializations which can precisely align biological (internal) and environmental rhythms. Recent studies, with the help of advanced technology, have shown that in mammals the “master” biological clock is located in the suprachiasmatic nucleus, in short the SCN, a bilaterally symmetric cluster of nerve cells located in the hypothalamus.⁹ The SCN has a discrete structure, and seems to have a single clearly defined function: It appears that it takes light information from the retina, interprets it, and passes it on to various parts of the nervous system—including the pineal gland, which secretes hormones such as melatonin—causing daily fluctuations and rhythms in numerous body traits.¹⁰

The SCN's role as a pacemaker was first discovered through a series of experiments. When the SCN was destroyed in some experimental rodents, the rodents experienced arrhythmicity in various circadian functions, including activity/rest cycles and hormone secretion.¹¹ The destruction of the SCN had similar effects on humans as well, causing disturbance of sleep/wake and neuroendocrine cycles. Furthermore, it was found in a study that

⁹ Peter H. Redfern and Bjorn Lemmer, ed., *Physiology and Pharmacology of Biological Rhythms* (Germany: Springer-Verlag Berlin Heidelberg, 1997), p.9.

¹⁰ Redfern and Lemmer, 9.

¹¹ Redfern and Lemmer, 9.

the SCN exhibits certain circadian rhythms in vitro such as rhythms in glucose uptake and electrical activity, which can also be shifted using external chemical or electrical stimuli.¹² Further evidence of the SCN's function was provided through neural transplantation studies in rodents. When fetal hypothalamic tissue containing the SCN was transplanted into a SCN-lesioned adult rodent, its arrhythmic patterns in its sleep/wake cycle were replaced by a "normal" free-running circadian rhythm.¹³ This restored rhythm followed that of the donor animal, clearly illustrating that the restored circadian rhythms are generated by the transplanted tissue, rather than another part of the recipient's nervous system.¹⁴

The SCN is therefore the major site of circadian clocks in rodents. However, the complete mammalian circadian timing system (CTS) consists of two other significant components: entrainment pathways and the SCN output (efferents) to effector systems that express the circadian rhythms.¹⁵ Entrainment pathways are the neural pathways through which information about the changes in luminance are transduced and passed on to the pacemaker, the SCN, during photic entrainment. Entrainment pathways can also be broken into three main components: photoreceptors, intrinsic retinal pathways and projections of retinal ganglion cells to the brain.¹⁶ During photic entrainment, a specific set of photoreceptors transduce the changes in light, and neurotransmitters pass this information on to the SCN through the intrinsic retinal pathways and projections of retinal ganglion cells.

As light information from the retina follows the entrainment pathways and reaches the pacemaker, SCN, it is interpreted and carried on to various areas of the nervous system, including the pineal gland, through SCN efferent nerves. Such output from the SCN project most densely to the hypothalamus, particularly to the area that extends dorsal to the SCN and ventral to the paraventricular nucleus.¹⁷ The SCN expresses circadian rhythms by generating rhythms in its neuronal firing rate to these parts of the body. The rhythmic neuronal output of the SCN may in turn modulate the rhythms of peripheral oscillators or directly influence effector systems.¹⁸ Although evidence is yet insufficient, studies have shown that there is also a possible relationship between the SCN's high neuronal firing rate during subjective day and the release of peptides.¹⁹

In mammals, one of the major recipients of SCN neural inputs is the pineal gland, which is a small endocrine gland in the brain responsible for the secretion of the hormone melatonin. Melatonin is a hormone that can only be produced in the dark because one of its precursors, N Acetyl Serotonin, can only be formed in the

¹² Redfern and Lemmer, 9.

¹³ Redfern and Lemmer, 9.

¹⁴ Redfern and Lemmer, 9.

¹⁵ Redfern and Lemmer, 73.

¹⁶ Redfern and Lemmer, 80.

¹⁷ Redfern and Lemmer, 87.

¹⁸ Jurgen Aschoff et al., *Vertebrate Circadian Systems* (Germany: Springer-Verlag Berlin Heidelberg, 1982), p.70.

¹⁹ Redfern and Lemmer, 87.

dark.²⁰ In all species it is therefore “an important endocrine representation of darkness.”²¹ However, the precise role of the pineal gland and melatonin in the photic entrainment of mammals is not yet known. Unlike in lower vertebrates, it seems that the mammalian pineal gland does not generate biochemical circadian rhythms on its own. Instead, the activities of the mammalian pineal gland are regulated by the circadian system, ultimately the SCN, so that the secretion of melatonin is exclusively nocturnal.²² The SCN connects to a nerve ganglion located in the neck, which is in turn connected to the pineal gland, and via this connection the SCN coordinates the biochemical rhythms generated by the pineal gland.

However, it seems that melatonin itself can also directly regulate clock functioning. Several areas of the brain and the pituitary have binding sites that have high affinities for melatonin, and through these sites the hormone can exert a direct effect on nocturnal biological processes that are clock-dependent.²³ For example, exogenous melatonin can reduce both sleep latency and core body temperature. Even the major clock regulator that controls the circadian rhythms of the pineal gland, the SCN, has melatonin receptors,²⁴ which suggests that the relationship between the functions of SCN and melatonin secretion in the circadian system is a two-way-relationship. The SCN controls the rhythm of melatonin secretion by the pineal gland so that it is exclusively nocturnal, and melatonin in turn affects the SCN to regulate other circadian functions and rhythms in the body. This has been further confirmed by the observation that melatonin applied during late subjective day can advance the circadian rhythm.²⁵

At the molecular-genetic level, the process of photic entrainment and the functioning of the mammalian circadian system are slightly more complex. As mentioned before, in mammals, the pacemaker SCN stimulates and coordinates the rhythmic activity of peripheral tissues and organs through neural inputs. However, it seems that like the pacemaker (the “master” clock), every other cell of the body also contains a circadian timing system, which behave as “slave” clocks. These “slave” clocks seem to exist as a specific mechanism that generates circadian rhythms through interconnected feedback loops, which control the transcription and translation of clock genes.²⁶

In *Drosophila*, the feedback is accomplished by the “clock proteins” PER and TIMELESS (TIM). The genes that code for these clock proteins, *per* and *tim*, turn on in the morning, and the two proteins gradually

²⁰ Aschoff, 6.

²¹ Aschoff, 6.

²² Redfern and Lemmer, 11.

²³ Redfern and Lemmer, 11.

²⁴ Redfern and Lemmer, 18.

²⁵ Redfern and Lemmer, 11.

²⁶ The Millar Research Group, *Circadian Rhythms* (n.d.)

<<http://template.bio.warwick.ac.uk/staff/amillar/circad.html>> [21 August 2006], par.1/2.

accumulate in the cytoplasm during the day.²⁷ When they reach a critical concentration in the evening, they pair up and enter the nucleus to deactivate their own genes. This feedback mechanism causes the protein levels of PER and TIM to rise and fall every 24 hours.²⁸ Another protein, called CLK, oscillates oppositely to PER and TIM. When CLK levels rise, the levels of the other two fall and vice versa. Unlike PER and TIM, CLK is a positive regulator that pairs with a protein called CYC to activate the *per* and *tim* genes.²⁹ When the *Drosophila* are exposed to external light, another key protein, called CRYPTOCHROME (CRY), absorbs the light to help reset the circadian clock and thereby facilitate photic entrainment.³⁰

Mammalian circadian clocks use many of these same proteins, but mammals have three different PER proteins and two types of TIM proteins.³¹ Some of these proteins also play different roles, for example, in mammalian clocks, the duty of the CRY protein is to shut down a set of genes. Also, the CYC's counterpart in mammals is a protein called BMAL, and it is BMAL whose levels rise and fall oppositely to PER's.³² Researchers have come up with two interacting feedback loops that regulate circadian rhythms in mammalian clocks. In one loop, PER2 (the second type of PER protein) turns on the *bmal* gene. The BMAL protein, after a slight delay, returns to the nucleus to activate the *cry* and *per* genes, triggering the second loop. In the second loop, CRY and PER proteins accumulate and then pair up to enter the nucleus, where CRY turns off the *cry* and *per* genes while PER2 once again turns on the *bmal* gene.³³

This mammalian system of interrelated feedback loops therefore causes the level of clock proteins in each cell to rise and fall every day, and by doing this it controls the cells' biochemical reactions. During photic entrainment, the master clock, SCN, receives light information from the retina and sends neural signals to numerous tissues of the body, where these clock genes work to regulate various circadian rhythms in cellular activity.

However, this is only the theoretical explanation. In the actual experiment, there were many sources of error that affected the results significantly. As one can see from the results, the data collected each day (each trial) did not exactly show the trend that was expected or desired. Although the highest number of wheel revolutions occurred at a similar time period (approximately from midnight to 03:00) in most trials, there were many fluctuations, sudden rises or falls in the level of locomotor activity, and irregular patterns. Also, in some cases the number of revolutions was well above or below the expected number, reaching over 12000 revolutions in three

²⁷ Marcia Barinaga, "Circadian Rhythms: Two Feedback Loops Run Mammalian Clock," *Science AAAS* (12 May 2000). <<http://www.sciencemag.org/cgi/content/full/288/5468/943a>> [3 September 2006].

²⁸ Barinaga.

²⁹ Barinaga.

³⁰ Barinaga.

³¹ Barinaga.

³² Barinaga.

hours, and sometimes as low as 0 revolutions. It was only after averaging the results of all the trials that the trend of the graph resembled the desired results.

It is very likely that such inaccuracies were caused by non-photic factors. The hamsters' locomotor activity or alertness could have been affected by many external stimuli other than light, such as changes in temperature or humidity. As the experiment did not take place at a laboratory prepared specifically for the experiment, large sounds or vibrations caused by humans (including myself) could have also distracted or scared the hamsters, possibly resulting in slight inaccuracies in the results. Also, the hamsters' food and water had to be provided and the cage washed and cleaned regularly, which may have also disrupted the hamsters' activity significantly. Additionally, as the data logger used in the experiment was not automatic, the results had to be hand-recorded by reading off the device each time, and therefore in complete darkness a dim light was necessary. Although the light intensity was probably too low to have had significant effects on the hamsters' circadian rhythms, it is worth considering the potential effects it may have had on the results.

Another significant weakness of the experiment is the fact that locomotor activity cannot be measured solely by wheel revolutions, as hamsters do not run on the wheels during the whole time they are awake. All three hamsters spent most of their time moving around the cage, climbing the cage walls, scratching and cleaning their bodies, eating, or searching for food in the cage. Although theoretically, the number of revolutions should be proportional to the hamsters' level of activity, in some cases this may not have been true. Therefore, the number of wheel revolutions per a given time period cannot fully represent the hamsters' total level of locomotor activity.

Another possible explanation for the fluctuations is the fact that the time given to each hamster to fully reset its circadian clocks following the light pulses may not have been long enough. Researchers have found that the circadian rhythms of the pacemaker, SCN, is shifted almost immediately after exposure to light. However, it appears that more time is required for the rhythms of the peripheral oscillators to shift.³⁴ As behavioral rhythms, including locomotor activity, are controlled by these peripheral tissues and areas of the brain other than the SCN, shifts in the level of the hamsters' activity occur gradually. Although one week was given to the hamsters before recording the number of wheel revolutions, this may have not been sufficient for the effects of photic entrainment to show completely.

By solving such problems and minimizing errors, the experiment can be improved and more precise results may be obtained. First of all, conducting the experiment in a laboratory, where the hamsters' activity will be less distracted, would minimize the effects of various non-photic, human-induced stimuli such as sounds,

³³ Barinaga.

³⁴ Circadiana, *Phase-Shifting Effects Of Light* (10 April 2005).

<circadiana.blogspot.com/2005/04/phase-shifting-effects-of-light.html> [3 September 2006], par.12.

vibrations, or temperature. Keeping the temperature constant by using an air conditioner and measuring temperature every time the results are recorded would also be helpful in keeping the controlled variables constant. Additionally, using an automatic data logger that records the number of wheel revolutions itself every hour instead of every three hours would not only give more precise and detailed data, but would also reduce the effects of the dim light that had to be used in the original experiment. Giving sufficient time for the hamsters' circadian clocks to reset is also critical. Finally, increasing the number of hamsters used in the experiment (at least five) as well as the number of trials for each hamster would give far more accurate results.

Conclusion:

Having looked at both the physiology and molecular basis of circadian clocks, the process of photic entrainment in hamsters (or other mammals) that was investigated in the experiment can be summarized as follows. When the hamsters were exposed to pulses of high intensity light during subjective night, information about the changed luminance of the surrounding was perceived by photoreceptors (mainly in the retina), and was passed down through various entrainment pathways, ultimately to the master circadian clock, SCN. This was then interpreted and passed on to numerous peripheral oscillators (various parts of the nervous system and other organs of the body), to reset the circadian rhythms of the body. These rhythms include not only overt behavioral rhythms, but also biochemical rhythms and cycles in all cells of the body. In each cell, a mechanism of interrelated feedback loops involving clock genes such as *per* and *tim* were regulated by neural inputs from the SCN to adjust to the new cycle induced by the light pulses.

Although the experiment did have some significant weaknesses, with some improvements and modifications in the method (particularly increasing the number of experimental hamsters and the number of trials), more accurate, reliable results would be obtained. Further development of this rather “basic” experiment and a more in-depth, professional-level research on the topic would lead us to a better understanding of the process of photic entrainment and the complex functioning of the mammalian circadian clock. More advanced research on the effects of light on circadian clocks would especially aid scientists in developing methods for improving the treatment of serious sleep disorders such as jet lag or wintertime depression using artificial high-intensity light.

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