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A Study of the Effects of Hypothermia on the Electrical Activity of the Hypothalamus in *Citellus tridecemlineatus*[1]

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Abstract. Deep, chronic electrodes were implanted in the anterior and posterior hypothalamus of seven thirteen-lined ground squirrels. The electrical brain activity and heart rate were recorded as the animal's body temperature was decreased by cooling the animal in a refrigerator. Above 23°C body temperature, amplitude varied, but remained near normal. From 23° to 21°C the amplitude dropped sharply, decreasing by almost 50 per cent. Below 21°C, the amplitude decreased more gradually. Detectable but not easily measurable activity was recorded at 11°C. Frequency showed an initial rise, then decreased between 22° and 26°C, then increased again through 16°C. Heart rate declined slowly and the heart continued to beat even at 15°C where most recordings were terminated.

The purpose of this study was (1) to attempt to determine the effects of hypothermia on the electrical activity of the hypothalamus in the thirteen-lined ground squirrel, *Citellus tridecemlineatus,* and (2) to test the hypothesis that a significant level of hypothalamic activity would be observed at body temperature below 24°C in the ground squirrel, a hibernator.

Lipp (1960) reported that the amplitude of hypothalamic activity in the cat showed little change as body temperature decreased to 30°C. From 30° to 24° a slight increase was reported and below 24°C the amplitude decreased and the activity almost disappeared. He also stated that cerebral activity ceased at a temperature of 22°C and that there was little change in frequency in hypothalamic activity.

Strumwasser (1959) recorded spontaneous brain activity from the motor cortex, hypothalamus, and other areas in *Citellus beecheyi* during hibernation at a body temperature of 6.1°C. He reported finding some repetitive pattern of activity from every brain area investigated.

Equipment and Procedures

Electrodes were made of 32-gauge insulated stainless steel wire. An attempt was made to orient the electrodes with an interelectrode distance of one mm. Placements were made stereo-

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taxically in the anterior and posterior hypothalamus. Since no stereotaxic atlas for the ground squirrel exists, the coordinates were determined experimentally by using two trial animals with two electrode placements in each.

Relative to the data included herein, histological confirmation of anterior and posterior placements was made for animal 2. In animal 1 the anterior electrode was in the optic chiasma, the posterior was in the anterior hypothalamus. Electrode placements for the other animals are not yet available.

Surgery was performed under pentobarbital sodium (Nembutal, Abbott) administered intraperitoneally. An incision was made in the scalp and properly placed holes were drilled in the skull by use of a dental drill. Both wires of an electrode pair were then inserted through one hole and lowered to the correct depth. Electrodes were affixed to the skull with dental acrylic. Leads from the animal to the recording apparatus were attached by using microsocket contacts (donated by Cannon Electric Company, Los Angeles). Animals were kept in an incubator post-operatively until they regained consciousness. No EEG records were taken for at least two weeks post-operatively.

Recordings were made on a Grass Model IIID Electroencephalograph. Muscle filters were on for all EEG records. EEG recordings were taken from three different bipolar electrode combinations. An EKG was taken from the hind toes.

Recordings were made with the animals strapped to a restraining board. The head was held relatively immobile in a balsa block holder. To facilitate restrainment the animals were lightly anesthetized with ether. Subsequently, one hour was allowed for the ether to be blown off.

Rectal temperature, as an approximation of brain temperature, was monitored visually with a Yellow Springs Telethermometer and small-animal rectal thermistor probe. A one-minute control EEG and EKG record was taken prior to placing the animal in a refrigerator. Refrigerator temperatures varied from 2° to 10°C. The body temperature at which these control records were taken was usually several degrees below the normal 36° to 37°C. This was probably due to the ether and the period of waiting while strapped down.

After the animal was placed in the refrigerator, body temperature was monitored and recorded each time the temperature decreased one degree, and a thirty-second EEG and EKG record was taken. Recordings were made until the EEG was either too low to be analyzed effectively for frequency and amplitude, or until it had become masked by amplifier noise. The animal was then removed from the refrigerator and rewarmed.

Eight records from seven animals were analyzed. Six records were taken in August and September; two records, including a repeat on one animal, in December.

The frequency was determined by counting the peaks in thirty one-second samples by record, usually running consecutively. Amplitude was determined by measuring to an accuracy of 0.1 mm with a vernier caliper the distance perpendicular to the line of motion of the paper from the first peak to the first trough in every other second in which frequency was counted. The heart rate was determined by counting the deflections of the EKG for 30 consecutive seconds at each body temperature, and converting these thirty-second counts to beats per minute. Graphs of the mean amplitude, frequency, and heart rate at each degree drop in body temperature were made for each animal. The general trends described below were shown in most of the individual graphs of these means. However, composite graphs were made, one for frequency, one for amplitude, and one for heartbeat.

Results

Both the amplitude and frequency changes of these eight records exhibited general trends. From the control record to records taken at 24°C or 22°C, the amplitude varied (Figure 1). Within this variation a general upward trend was seen in channels one and three, while a general downward trend was seen in channel two. From about 22° to about 20°C a sharp drop in amplitude, representing almost 50 per cent decrease occurred in all channels. Below 20°C, the amplitude continued to decrease, although more gradually, until the recording was terminated around 16°C.

Two animals exhibited activity of considerably higher amplitude, and amplitude ranges were large (Table 1). Ranges were especialy wide at 30°, 24°, and 19°C in channel one, and at 32°, 27°, and 25°C in channel two. In both channels the higher amplitude value is from the same animal.

No experiment in this series was ever continued until brain activity ceased. One animal was cooled to 6°C, although no activity below 11°C was of sufficient amplitude to be analyzed. As temperature decreased, time required for cooling increased. Almost four hours were required for this animal to cool from 10° to 6°C, while five hours were required to cool from 31° to 11°C. Because this time-temperature relationship appeared asymptotic, no attempt was made to continue the experiment until activity ceased.

Frequency showed an initial increase which lasted until 34°C in channel one, 31°C in channel two, and 32°C in channel three.

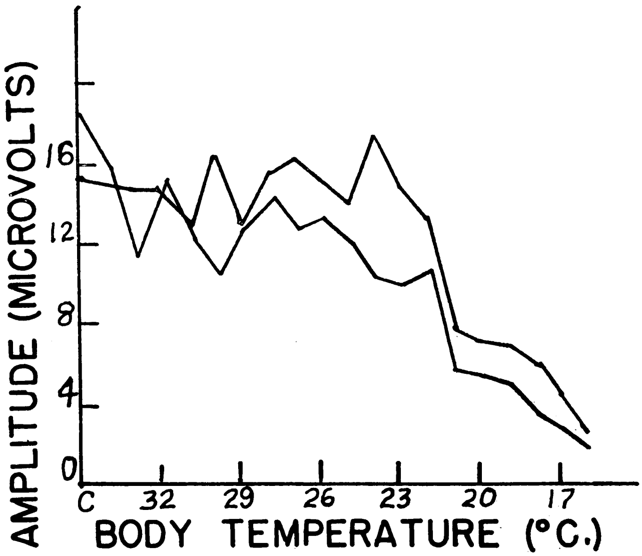

Figure 1. Means of aplitude at each degree body temperature from eight records. Mean amplitude of activity in the control record is represented on the line labeled C. The upper graph shows the amplitude of activity in channel one, recorded from the anterior hypothalamus. The lower graph shows the amplitude of activity in channel two, recorded from the posterior hypothalamus. Activity in channel three (not graphed) was recorded between anterior and posterior hypothalamus.

Table 1. Extremes of amplitude at each degree decrease in body temperature.

Body Temperature in °C	Channel 1 High	Channel 1 Low	Channel 2 High	Channel 2 Low	Channel 3 High	Channel 3 Low
	Amplitude in Microvolts					
Control	24.3	5.5	23.9	4.3	27.1	11.5
35°	30.8	19.8	25.1	14.0	...	...
34°	36.3	13.3	23.9	14.9	...	...
33°	29.7	7.6	25.7	6.7	27.8	7.3
32°	36.3	4.8	31.4	6.9	23.1	9.8
31°	36.3	5.9	24.5	5.2	19.1	11.2
30°	42.9	3.9	20.5	4.0	19.1	11.5
29°	24.2	3.9	25.7	4.5	22.4	11.5
28°	27.5	5.9	27.9	4.8	17.8	14.6
27°	30.8	3.9	28.5	4.9	19.2	12.9
26°	29.7	7.1	26.2	5.8	21.1	14.8
25°	29.7	5.3	27.4	4.5	21.8	6.7
24°	38.6	6.4	22.6	4.1	21.3	9.9
23°	31.1	3.8	25.5	4.3	23.9	8.2
22°	31.1	4.8	25.0	3.0	11.8	7.6
21°	15.1	2.5	13.7	2.3	21.6	7.1
20°	14.6	3.2	13.7	2.9	8.2	3.4
19°	8.5	3.5	8.9	3.1	7.0	6.1
18°	9.9	2.4	6.3	2.5	8.2	3.6
17°	5.8	3.8	5.2	2.1	5.8	5.3
16°	5.9	1.5	3.0	1.9	4.4	3.7

As body temperature lowered to 27°C, the frequency in channels one and two decreased gradually at first, then sharply between 28°C and 27°C. The frequency in activity from channel three decreased until body temperature was 23°C. A final gradual upward trend was then seen in channels one and three. Frequency of activity in channel two increased and then varied, showing a general trend downward from temperatures 19° through 15°C, at which point most recordings were terminated (Figure 2).

The frequency range was smaller in channel two than in channel one, and the frequency range was much less than that for amplitude. Frequency extremes are shown in Table 2.

Table 2. Extremes of frequency at each degree decrease in body temperature.

Body Temperature in °C	Frequency in CPS Channel 1 High	Channel 1 Low	Channel 2 High	Channel 2 Low	Channel 3 High	Channel 3 Low
Control	35	26	39	27	42	33
34°	40	30	43	26	41	41
33°	37	26	40	32	40	38
32°	39	30	42	26	42	37
31°	36	30	42	25	43	35
30°	37	28	44	25	45	35
29°	36	28	46	25	45	33
28°	36	27	45	24	42	34
27°	31	27	35	22	42	33
26°	32	25	34	22	42	29
25°	32	23	35	19	44	28
24°	33	21	33	20	41	27
23°	39	20	33	21	44	25
22°	40	25	38	23	43	31
21°	34	21	35	25	45	32
20°	38	19	39	23	42	33
19°	42	18	40	30	47	34
18°	37	19	39	29	39	31
17°	43	18	42	28	49	29
16°	40	27	37	24	42	33
15°	42	31	40	27	38	38

Heartbeat showed a slow but steady decrease in rate from the control record to the final thirty-second record, interrupted only by an increase of 20 beats per minute at 28°C (Figure 3).

Discussion

These results would seem to indicate that the brain of the thirteen-lined ground squirrel does react to hypothermia in a way slightly different from that of a non-hibernator. The observed difference was, however, less than expected. While activity was observed at a temperature of 18°C in four animals and at 11°C in one animal, there is a striking similarity to Lipp's findings in the cat in that the voltage decreased dramatically

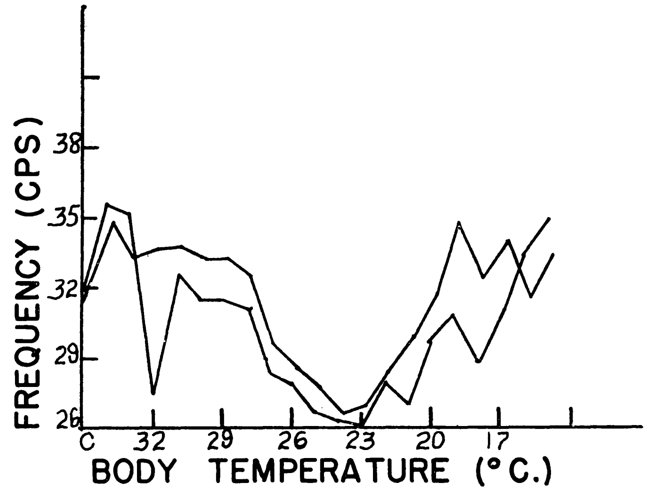

Figure 2. Mean frequency of activity from eight records. Upper graph from channel 2, lower from channel 1.

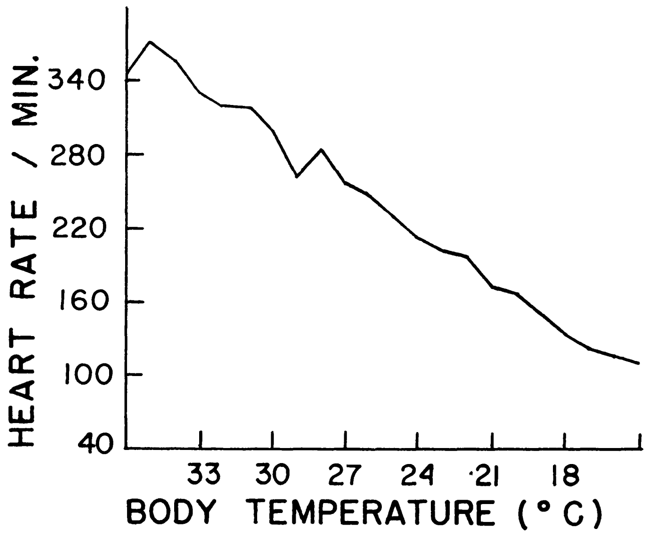

Figure 3. Mean EKG from seven records.

below 24°C. In contrast to Lipp's findings, activity was never seen to cease. Lipp reported finding little or no heart activity below 20°C in the cat. No cessation of heart beat was seen in the ground squirrel.

The findings from the hypothermic *Citellus tridecemlineatus* are similar to Strumwasser's from the hibernating *Citellus beecheyi* in that no cessation of activity was seen.

It is to be emphasized that this is a progress report, and much more work remains to be done. Some proposals are: that finer electrodes be used with more and better placements throughout and on the brain, more precise records be taken, and more precise analyses be made. It might also be suggested that a more natural or realistic state of hibernation be used while records are made.

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