

uniform in size, and showed varying degrees of fluorescence, from very faint to quite high intensities. Among the fluorescent cells, groups of completely non-fluorescent nerve cells occurred. They were visible only because of the faint non-specific background fluorescence of the tissue, but appeared clearly when the sections were studied by phase-contrast microscopy. Around some of the cells, both of the fluorescent and the non-fluorescent types, adrenergic terminals were seen. However, the majority of cells did not receive any nerve terminals. Scattered in these ganglia small, intensely green-yellow fluorescent cells, isolated or more frequently collected in groups, were located. Several long and richly branching processes extended from the cells. Leaving the peripheral ganglia, bundles of smooth, moderately green-fluorescent as well as non-fluorescent axons could be seen. Furthermore, the peripheral bundles outside the vas deferens and the accessory genital glands could be traced back to them. The nerve cells were dispersed over a rather wide area in the vicinity of the accessory male genital glands, and were regularly seen in the walls of the prostate and the coagulating gland, too. They could not be found within the vas deferens or in the seminal vesicle.

No visible reduction of the peripheral innervation was observed in structures from the side where the hypogastric nerve was cut, as compared with the non-denerated side or with unoperated control animals. No reduction in the vascular innervation was seen, nor could any

decrease in the amount of adrenergic nerve terminals around the peripheral nerve cells be estimated with any certainty.

Further studies on the monoamine-containing structures of the internal accessory male genital organs in different species will appear elsewhere⁹.

Conclusion. The present results confirm the previously mentioned evidence for a peripheral synapse in the adrenergic innervation of the vas deferens and seminal vesicle of the guinea-pig¹⁻³.

Zusammenfassung. Samenleiter und Samenblase vom Meerschweinchen besitzen eine besonders kräftige adrenergische Innervierung, die von Nervenzellen, die in unmittelbarer Nähe der Organe liegen, ausgeht.

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⁹ CH. OWMAN and N. O. SJÖSTRAND, *Z. Zellforsch.*, in press (1964).

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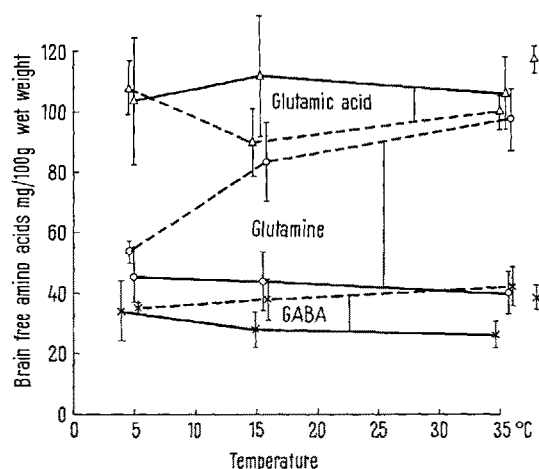
Changes of Cerebral Glutamine, Glutamic Acid and GABA during Arousal from Hibernation

The hibernating state, and especially its most dramatic phase, arousal from hibernation, being highly dynamic and coordinated physiological events, during which the excitability of the brain undergoes profound but reversible modifications, provides exceptional possibilities for evaluating the assumed role of glutamate-GABA system in the regulation of cerebral excitability. Starting from these considerations, the changes in glutamine, glutamic acid, and GABA concentrations of the brain during arousal from hibernation have been investigated and compared to the corresponding alterations occurring during spontaneous reanimation from an artificially induced state of hypothermia.

49 European ground squirrels were used. Arousal from hibernation was initiated by transferring the animals from the cold room (5–10°C) to the laboratory (at a higher temperature). Hypothermia was induced according to the well known method of GJAJA¹. The time needed to cool an animal below 5°C ranged from 2 to 3 h. The container to which an animal was confined in the ice-box was opened and the air was allowed to circulate for 10 sec at each 10th min during the first half hour, at each 20th min during the next hour of refrigeration, and at each 30th min later on, until the desired temperature was attained. Both hibernating and hypothermic animals were decapitated in groups of seven at 5–7°, 15° and 35–37°C respectively, their rate of reanimation being approximately the same. Cerebral amino acids were separated and quantitatively estimated as previously described².

As illustrated in the Figure, the content of all the investigated compounds in the brain of ground squirrels

killed while deeply hibernating, with a rectal temperature of 5–7°C, was significantly lower than the corresponding values found in the brain of control non-hibernating animals sacrificed at the same time of the year. During



Values for hibernation are represented by heavy lines, while those for hypothermia are marked by dotted lines. Symbols at the far right end of the Figure indicate concentrations estimated in the brain of control, non-hibernating animals. o = glutamine, Δ = glutamic acid; x = GABA.

¹ J. GJAJA, *Bull. Acad. Royale Serbe* 6, 65 (1940).

² LJ. KRŽALIĆ, V. MANDIĆ, and LJ. MIHAILOVIĆ, *Exper.* 18, 368 (1962).

arousal from hibernation, glutamine and glutamic acid content remained practically unaltered. Concentrations of GABA, however, exhibited a small but steady and significant tendency to decrease as the animal approached full arousal from the hibernating state ($p > 0.01$). Concentrations of the investigated amino acids, estimated in the brain of animals artificially refrigerated to 5–7°C, were also significantly lower than the corresponding values found in the brain of euthermic animals. As is seen in the Figure, their decrease was of approximately the same order as in the hibernating animals sacrificed at the same rectal temperature. In contradistinction to the arousal from hibernation, the process of warming from hypothermia was characterized by a highly significant increment ($p > 0.001$) of glutamine in the brain. The sharpest increase of this amino acid, observed between 5 and 15°C, was accompanied by an important decrease of cerebral glutamic acid. As the warming process proceeded, concentrations of both compounds increased and, with restitution of euthermia, attained the control values. The increase in the concentrations of GABA, although less noticeable, proved to be statistically highly significant ($p > 0.001$) once the animal reached the temperature of 35–37°C.

The results described call for a few brief comments. Unless it is believed that the differences observed in our experiments are due to the use of the specific method of cooling, one is tempted to infer that the glutamate-GABA

metabolism in the brain of ground squirrels waking from hibernation, and those spontaneously warming up from an artificially induced state of hypothermia, follows two different pathways. Experiments using other methods of inducing hypothermia are at present being carried out in order to check this assumption. The significant tendency of GABA to decrease as the animal wakes up from the hibernating state also merits special attention. It provides another (among many) reasons for not rejecting the possibility that GABA might indeed play an important role in the regulation of cerebral excitability⁸.

Résumé. Chez le spermophile, la teneur en glutamine et en acide glutamique cérébral au cours du réveil du sommeil hivernal était inchangée. Pendant le réchauffement des animaux refroidis, la glutamine et GABA étaient remarquablement élevés.

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Body Temperature Cycling of Winter Little Brown Bats in the Cold Following Heat Exposure¹

Heat and cold acclimation studies have been conducted for a number of homeotherms, including white rats^{2,3}, brown rats⁴, and rabbits⁵. In general, homeotherms which have been heat-acclimated for 3–5 weeks reveal lower metabolic rates and poorer body temperature regulation at low ambient temperatures when compared to cold-acclimated homeotherms⁶. HART further states that acclimation in homeotherms is developed in about 2–4 weeks under laboratory conditions, and that the primary function of cold acclimation is to extend the survival range for a limited period of time.

Little is known about acclimation in heterotherms with the exception of spermophiles⁷ and dormice⁸. These two mammals and acclimatized (natural climatic exposure) bats⁹, have shown a body temperature metabolism pattern similar to true homeotherms. The aim of the present investigation was to determine the effect of progressive heat exposure on body temperature cycling and arousal patterns for winter-captured little brown bats, *Myotis lucifugus*, in the cold. An attempt was made to determine if changes in body temperature could be used as an index for heat acclimation in hibernating species of bats.

Methods. Equal numbers of male and female little brown bats were captured from a Southern Indiana cave in December 1962. The bats were transferred to the laboratory, individually housed at a neutral temperature of 33°C (heat exposure) and fed daily on adult mealworms. A day-night cycle of 14 h of light (05.00 to 19.00) and

10 h of dark (19.00 to 05.00) accompanied the heat exposure. Relative humidity was 18–20%.

At four-day intervals (4–48 days) a group of two males and two females was taken from the heat (33°C) and exposed for three days (72 h) to the cold (10°C) in total darkness. 10°C was selected as the cold-test temperature as it approximates the upper limit of most winter cave hibernaculae¹⁰, but still permits winter hypothermic bats to arouse and raise their body temperature to the homeothermic level¹¹.

Body temperature of the bats was measured using 30-gauge copper-constantan thermocouples implanted subcutaneously in the upper mid-abdominal region in conjunction with an automatic, strip chart recording potentiometer.

¹ This investigation was supported by the National Institutes of Health Grant GM-10811 and the Purdue Research Foundation X-R Grant 3269.

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