

ABSENCE OF CHROMATOLYTIC CHANGE IN THE CENTRAL NERVOUS SYSTEM OF THE WOODCHUCK (*MARMOTA MONAX*) DURING HIBERNATION

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SIX FIGURES (TWO PLATES)

INTRODUCTION

Since the discovery by Nissl in 1885 that the chromophilous granules in the cytoplasm of nerve cells—granules which had already been described by Flemming ('82) and which von Lenhossék later termed *tigroïdes*, though now more generally spoken of as Nissl granules—are intensely stained by basic aniline dyes, especially by methylene blue, the disposition of these granules, under numerous physiological and pathological conditions, has been the subject of much study. From the extensive functional alterations that numerous authors (Valentin, Dubois, Merzbacher, etc.) have reported to occur in the nervous system during hibernation, and from the morphological variations which are said to take place in the nerve cells in certain other conditions, such as sleep and starvation, one would naturally expect to find marked changes, especially in the Nissl granules, during hibernation. This state, as is well known, is attended in some animals by almost continuous sleep and profound torpor for four months and even longer, during which time no food whatever may have been eaten and the body temperature has been reduced to but a few degrees above the freezing point.

HISTORICAL

An examination of the literature revealed the fact that some observers have reported marked structural changes in the nerve cells during hibernation. Levi ('98) in the toad (*Bufo vulgaris*)

found that the Nissl bodies had greatly diminished in size and the acidophil granules had become basophilic during winter-sleep; but in hibernating mammals no changes were observed. A more detailed study by Legge ('99), however, led this author to conclude that the cells of the cerebro-spinal axis of hibernating bats do undergo visible changes. In the cells of the cerebral cortex he found the Nissl granules to have elongated, being more fusiform in shape, and to be displaced towards the periphery, forming a sort of envelope to the cytoplasm. Baroncini and Beretta ('00) also reported morphological changes in the spinal cord and especially in the cerebral cortex of mammals (muscardin and bat) during winter-sleep. They found that the Nissl granules had greatly decreased and stained more diffusely. The chromatic substance of the nucleus was also more diffuse than in active specimens and the nucleolus seemed to have disappeared in many cases. Essentially the same changes in the Nissl granules in the cells of the cerebro-spinal axis and in the Purkinje cells of the cerebellum of hibernating hedgehogs, were reported by Marinesco ('05) who found a distinct decrease in these granules during the torpid state. Those that remained were reduced to fine granules or were diffused in the cytoplasm. A more recent study by Zalla ('10) agrees with the older results obtained by Levi in regard to mammals. Zalla found no appreciable morphological difference in the Nissl substance in the dormouse (*Myoxus glis*) during hibernation as compared with the active state. His results in amphibia were not constant, but in reptiles he found a distinct decrease in this substance in the motor cells of the cord and pons during winter-sleep.

The question of changes in the chromophilous substance of the nerve cells during hibernation in mammals thus seems to be unsettled. Legge, Baroncini and Beretta, and Marinesco apparently have observed marked changes, while Levi and Zalla could not establish any such chromatolysis. However, these authors did not all work on the same species of mammal. In amphibia there is also some disagreement. Levi found a distinct decrease in the Nissl granules during hibernation, while Zalla, whose results were not very constant, believes that there

are no changes. In reptiles, however, Zalla did find a marked decrease during torpor.

Some other morphological changes in the nerve cells during hibernation may be briefly mentioned. Querton ('98) by means of the Golgi method on cerebral neurones found that the protoplasmic prolongations retract and assume a moniliform appearance during the dormant period. This, however, could not be substantiated by Baroncini and Beretta ('00). Some work has been done on the neurofibrillae. Tello ('03) reported that in the motor cells of the spinal cord of lizards the neurofibrillae are much thicker than usually found. Tello and Cajal ('04) found that this was true only during the cold season when the animal is dormant, and that when the animal is warmed up and made active the fibrillae become more numerous and much finer. Cajal thus believes that the hypertrophy of the neurofibrillae is due to the cold and resulting diminished spinal reflexes, because exposure of the animal to a low temperature brought about this giantism of the neurofibrillae while warming it up caused a return to the normal, and because this change is not seen in the telencephalon and mesencephalon whose cells retain their activity to a much greater extent during the lethargy. Marinesco ('05) has repeated these experiments on young cats and dogs and Dustin ('06) has done the same on young rabbits with essentially similar results. A temperature below 10°C., however, was found to be less effective than 10°C. in bringing about these changes in the neurofibrillae, according to Marinesco. This may be related to the fact that a temperature too low excites hibernating mammals and finally wakes them up since the body temperature rises as a result of increased activity. The latter author found no such modification of the fibrillae in hibernating hedgehogs, which fact he interprets as indicating that the activity of the nervous system of hibernating mammals is not reduced to the extent that it is in the lizard and other cold blooded animals. Zalla ('10), on the other hand, found that the neurofibrillae were fewer and farther apart in the dormouse during hibernation.

PRESENT INVESTIGATION

In view of the conflicting reports as just reviewed and the more recent observations by Crile on changes in the brain cells under various emotional and other conditions, much less striking than the phenomenon of hibernation, the work we have done on the woodchuck in this regard, seems worthy of a brief note. Woodchucks, or ground hogs, which represent the American marmots, are some of the best examples of hibernating mammals in this country. All species remain dormant for four to six months each year, and hence constitute good material for a study of hibernation. This work was commenced early in January 1913 by J. A. Myers, who fixed and imbedded the central nervous system of six woodchucks, four of which were killed at various intervals while hibernating (January 18, February 6, March 15 and April). One was killed shortly after waking up (March 15) and another, during the following summer.

In addition to studying the above series by means of the Nissl stain, the other co-author prepared another series consisting of the brain and spinal cord of fifteen woodchucks killed during the autumn, winter and spring of 1913-1914. This series includes one animal killed about a month before hibernation (October 25), one just before hibernation (November 22), five during hibernation (February and March)—one of these was partly awake when killed on February 16, but was sluggish and had a rectal temperature of 19°C.—one within two days after waking up (March 16) and seven others which had been awake from three days to more than a month. Three of this last group had been fed for one, two and three weeks respectively. These animals were kept in the artificial burrows which were designed by Professor Simpson of this laboratory and which have already been described elsewhere.¹ The rectal temperature of the dormant animals varied from 8°C. to 12°C., whereas, the temperature of the active animals ranged from 32°C. to 38°C.

¹ Rasmussen, A. T., *Amer. Jour. Physiol.*, 1915, vol. 39, p. 20.

All the animals of both series were killed quickly by transfixing the heart through the chest wall. No anaesthetic was used, except in five cases where only sufficient ether was given to keep the animal quiet. These five animals were all killed after hibernation while awake and active. The amount of ether given did not seem to have any noticeable effect on the Nissl granules. If, however, these five cases are excluded from consideration because of the introduction of this additional factor, the two series involve as strictly comparable cases two before hibernation, nine during hibernation and five after hibernation. The blood was washed out immediately after death with normal saline solution by injection through the aorta. The saline was followed by a saturated aqueous solution of bichloride of mercury to which had been added 10 per cent of formalin. Thus the central nervous system was fixed very quickly *in situ*. The whole brain and cord were then removed and cut into transverse sections a few millimeters thick. The desired levels were further fixed in a saturated aqueous solution of bichloride of mercury for 48 hours, washed in running water 36 hours, and dehydrated in graded alcohols containing iodine in the usual manner. The tissue was cleared in xylol and imbedded in paraffin melting at 54°C. The levels thus imbedded were: olfactory bulb, motor cortex, mid-thalamus, midbrain at the level of the superior colliculus, mid-cerebellum and pons, medulla oblongata at extreme inferior border of fourth ventricle, first cervical, sixth cervical, sixth thoracic, second lumbar and lower lumbar segments of the spinal cord.

Sections were cut five microns in thickness, except in the case of the spinal cord where the sections were six microns thick, and stained on the slide for ten minutes in a large quantity of hot (70°C.) solution of 1 per cent methylene blue in water saturated with aniline oil. The excess stain was washed off rapidly in water and decolorization carried on by transferring the slides directly to 95 per cent alcohol for two to ten minutes. Dehydration was completed in absolute alcohol and clearing in cajuput oil followed by xylol. The sections were mounted permanently in Canada balsam dissolved in xylol. Where the size

permitted, corresponding sections from all animals of a series were fixed on the same slide to insure equal staining. In other cases several sections from the same block were placed on one slide and all the corresponding slides stained together by carrying them through the reagents by means of a basket, or rack, which would contain the entire lot.

RESULTS

The nerve cells of the woodchuck are essentially typical, containing the usual large round nucleus, with one nucleolus. The chromophilous substance in the cytoplasm has the usual appearance and arrangement so well known that no description will be necessary here. In spite of all precautions there are noticeable variations in the size, distinctness and arrangement of the Nissl bodies in homologous cells of animals in the same state and even in the cells of the same group in a particular section. These variations are found in both dormant and active animals. We can detect no modification in the Nissl granules characteristic of the hibernating as compared with the non-hibernating state. Certainly in these woodchucks there is not the difference indicated by the figures given by Marinesco in the case of the hedgehog. The chromophilous substance is present during hibernation in at least as great a quantity as at other times and presents the usual appearance when stained with methylene blue. The arrangement of the granules varies somewhat even in cells of the same group, being more abundant in the periphery of the cells in some cases and in others being grouped more densely around the nucleus. The size and shape of the bodies vary from fine irregular granules to larger elongated ones; but when a large number of cells are examined the extreme variations may be found in animals in the same state and often in the same section. A predominance of a particular variation in either state can not be established. The accompanying figures and explanations will suffice to indicate the general cell picture before, during and after hibernation. The larger types of nerve cells were selected as illustrations because in them the Nissl bodies are more distinct and make better photographs.

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² In regard to the general problem of the correlation of structural changes to activity in nerve cells, attention should be called to the experiments carried out on six different species of animals by R. A. Kocher, reported in this journal (vol. 26, No. 3, p. 341) since this article was submitted for publication. This author could find no correspondence between the size or structural characteristics of nerve cells and various grades of activity.

PLATE 1

EXPLANATION OF FIGURES

The three photographs in this plate were taken from the nucleus hypoglossus. The sections from the various animals were mounted on the same slide and hence stained together. $\times 320$.

1 Before hibernation. Woodchuck killed October 25, 1913, without any anaesthetic. Animal active. Rectal temperature 37.6°C .

2 During hibernation. Woodchuck killed March 7, 1914, without any anaesthetic. Animal very dormant and had been so nearly all the time for at least three months. Rectal temperature 9°C .

3 After hibernation. Woodchuck killed April 11, 1914, without any anaesthetic. Animal active. Had been fed for two weeks. Rectal temperature 37°C .

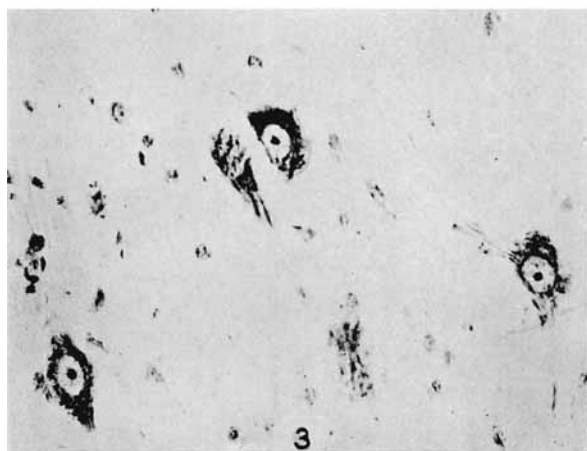
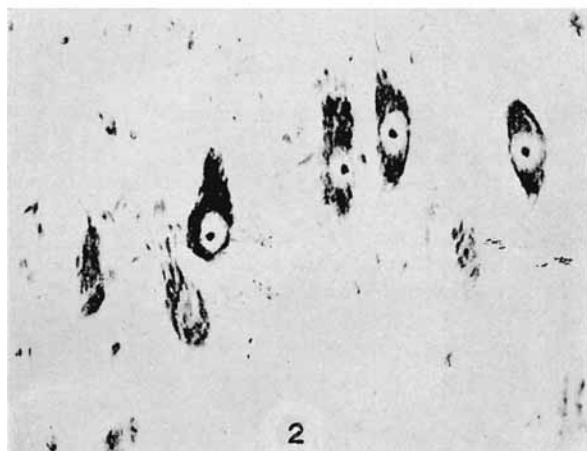
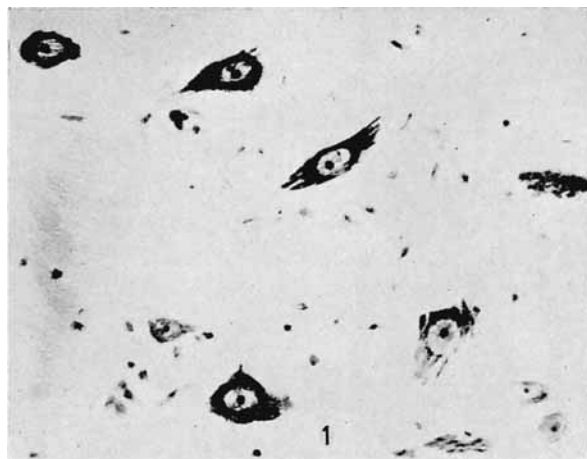


PLATE 2

EXPLANATION OF FIGURES

Photographs of motor cells from the antero-lateral group in the sixth cervical segment of the spinal cord. The sections were mounted on the same slide and hence stained together. $\times 320$.

- 4 Before hibernation. Same animal as in figure 1.
- 5 During hibernation. Same animal as in figure 2.
- 6 After hibernation. Same animal as in figure 3.

