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KAZIMIERZ BIAŁASZEWICZ

THE GENERAL ARCHITECTONICS OF THE SPINAL CORD AND SPINOBULBAR JUNCTION IN THE FROG

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It has recently been reported by A f e l t (1963 a, b) that spinal reflexes in a spinal frog preparation vary depending on the level of a section in the region of the spinobulbar junction. These observations indicate that a mechanism coordinating the locomotor functions of the frog is located in this region. The neuroanatomical investigations of the spinal cord and spinobulbar junction in the frog are scarce and incomplete. On the basis of the morphological studies carried out so far, one cannot form a sufficiently firm opinion as to possible physiological-anatomical correlations pertaining to the structures under study. We have, therefore, undertaken an investigation on the cyto- and myelo-architectonics of the spinal cord and the spinobulbar junction of the frog. It was our intention to correlate the morphological observations with the physiological material available.

MATERIAL AND METHODS

Spinal cords and brains of 9 normal, adult specimens of *Rana esc*. (L.) were fixed in 10 per cent neutral formalin and embedded in paraffin. Sections 10, 15, or 20 microns thick were made in all the three planes. Seven complete frontal, one sagittal and one horizontal series were obtained. All the sections were stained according to the Klüver or the Woelcke method. In addition, the Golgi-Bubenaite method was used in some specimens.

RESULTS

Gross anatomy. Lumbar and cervical enlargements can be distinguished within the spinal cord. The cervical enlargement passes directly, without any distinct boundary, into the medulla oblongata. No structures

fied by the topography and morphology of the cells in question and the direction of their axons, as well as their connection with the dorsal funiculi terminating there.

In the lowest frontal sections of the medulla oblongata, the area containing motoneurons is situated in a position typical of the spinal cord. On higher levels of the medulla oblongata, but still below the calamus scriptorius, a gradual displacement of motoneurons toward the opening of the fourth ventricle occurs, thus forming the hypoglossal nucleus.

On the other hand, the area corresponding to the dorsal horn of the spinal cord takes an intermediary position between the nucleus of dorsal funiculi and motor area in the frontal sections of the medulla oblongata. This area corresponds to the intermediary area, distinguished by H e r r i c k (1948) in Salamander.

Microscopic anatomy of the white substance. Ventrolateral area. This area topographically corresponds to the ventral and lateral funiculi in mammals. The uniform structure of this area, as well as the lack of structural differentiation within this system, substantiates a joint description of this area. The ventrolateral area at different levels of the spinal cord and the lower part of the medulla oblongata appear identical. For example, a similar number of fibers constituting the ventrolateral area is seen in both the frontal sections of various levels of the spinal cord and the lower part of the medulla oblongata. In other words, no accumulation of fibers can be differentiated in a frontal plane. A large number of fibers is noticed in the ventrolateral area of the spinal cord and the bulb in the Golgi preparations. These fibers are situated radially in relation to the long axis of the spinal cord. We could not find them in sections stained for myelin fibers. The connection between neurons and these fibers could not be established either. Myelinated fibers, observed in the ventrolateral area are located parallely, circularly or radially in relation to the long axis of the spinal cord, that is, in all directions. In contradistinction to mammals, type A and B neurons could be distinguished in all sections of this area (Fig. 2B).

Dorsal funiculi. The dorsal root fibers entering directly the dorsal funiculi can be easily traced in the horizontal sections of the dorsal roots. Thus, the sensory character of the dorsal funiculi becomes obvious. The axons of the sensory neurons situated in the spinal ganglia enter the dorsal funiculi and then ramify into the ascending and descending branches (Fig. 3B). Numerous ramifications of such ascending and descending fibers, penetrating the dorsal horn are observed in sagittal sections of the dorsal funiculi (Fig. 3C).

In contrast to the axons from higher levels of the spinal cord, the sensory axons, entering the spinal cord through the dorsal roots at lower sections, keep in the dorsal funiculi a more medial position. This is reminiscent of the arrangement in mammals.

A small but evident frontal accumulation of fibers is visible while comparing the number of axons of dorsal funiculi in frontal sections at various levels of the spinal cord.

The dorsal funiculi may be easily traced in frontal, horizontal and sagittal planes up to the level slightly above the calamus scriptorius (Fig. 3A, D) where they „break up" abruptly in the region which corresponds with the area of nuclei of dorsal funiculi (see above).

DISCUSSION

Dorsal funiculi and nuclei of dorsal funiculi. According to K a p p e r s, H u b e r and C r o s b y (1960) no true dorsal funiculi can be distinguished in the frog. They suggest that all ascending sensory fibers run in the ventrolateral area. W a l l e n b e r g (1907) found a larger number of dorsal funiculi in frontal section (frontal accumulation) fibers at the cervical levels than at the lower levels. He indicates, however, that this is due to the presence of descending trigeminal, vagal and vestibular fibers rather than to an increased number of dorsal root fibers. On the other hand, K ö p p e n (1888), S a n d m e y e r (1892) and G a u p p (1896) found dorsal root fibers in the dorsal funiculi at the cervical level and, on the basis of this coincidence, considered the dorsal funiculi to be homologous with corresponding structures in mammals. This agrees with our findings. First, it is possible to trace dorsal root fibers which penetrate the dorsal funiculi. Secondly, the ascending branches of dorsal root fibers, running in small bundles, can be observed at various levels of the spinal cord and the medulla oblongata — up to the calamus scriptorius. Thirdly, the dorsal funiculi fibers terminate and internal arcuate fibers originate in the area which can be considered the nucleus of the dorsal funiculi. Finally, the topographic relations within the dorsal funiculi and related areas between dorsal root fibers from the lower and upper extremities and the descending trigeminal fibers are closely correlated to those in mammals. The dorsal root fibers from the lower extremities keep a more medial position than those from the upper extremities. On the other hand, the descending sensory branches of the trigeminal nerve are located more laterally in relation to the ascending dorsal root fibers. Considering the above data, an assumption could be made that the frontal accumulation in the frog depends on both the increase in the number of descending fibers sensory in character and the increased number of dor-

sal root fibers. The basic difference in the character of these structures in the frog and in mammals is that of a quantitative nature: the number of dorsal root fibers ascending to the level of the medulla oblongata increases greatly in the course of evolution.

In conclusion, our investigations substantiate the hypothesis that dorsal funiculi and their nuclei are homologous with the corresponding structures of mammals.

The occurrence of the nuclei of the dorsal funiculi in the frontal sections of the spinal cord and the medulla oblongata as well as the internal arcuate fibers, originating at that level, constitute the only remarkable morphological difference between the highest sections of the spinal cord and the lowest sections of the medulla oblongata. On the other hand, the lateral sensory area, and the internal arcuate fibers, originating in this place, equally dispersed throughout all the levels of the bulb, are the most charakteristic feature of the entire medulla oblongata. Accordingly, the level of the appearance of the internal arcuate fibers seems to be a natural boundary between the spinal and the bulbar structures. The distinction of the spinobulbar junction in the frog, although based on tradition, cannot be morphologically justified.

Ventrolateral area. This area corresponds to the ventral and lateral funiculi of the mammals. The following characteristic features of this area in the frog can be established on the basis of the present observations: (1) an absence of a remarkable frontal accumulation of fibers, a condition which cannot be compared to that in the mammals; (2) a complete lack of nerve bundles which could be traced over a number of successive sections; (3) a number of neurons which can be observed in this area, is a condition that would be considered abnormal in mammals; (4) a large number of radially situated fibers, observed in the Golgi preparations. The nature of these fibers was not established. The dendritic character of these fibers could be suspected on the basis of other authors' investigations.

Considering the above specified characters of the ventrolateral area in the frog, there is no reason to believe that these structures are homologues of the ventral and lateral funiculi in mammals. It seems much more probable, that the ventrolateral area constitutes a large, poorly differentiated synaptic area (Herrick 1948) and may be considered a coordinating system of the spinal cord, working on the basis of a high autonomy.

On the basis of the material and methods available, we could not trace long descending motor tracts. However, it is possible that in the ventrolateral area they run scattered.
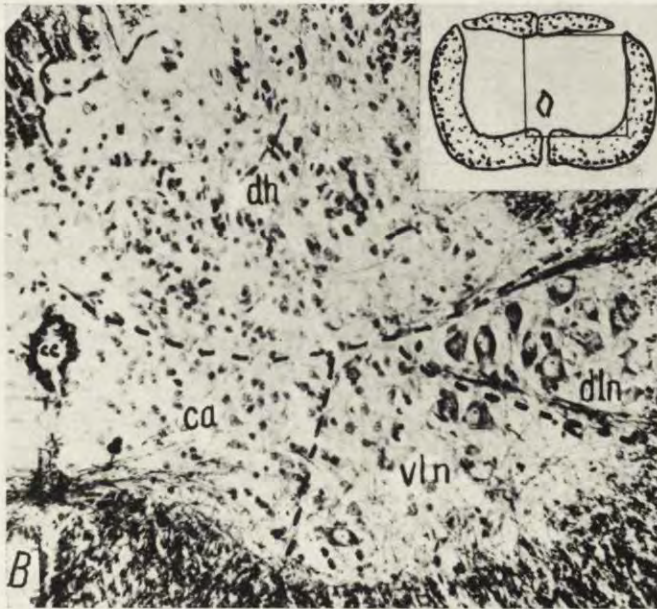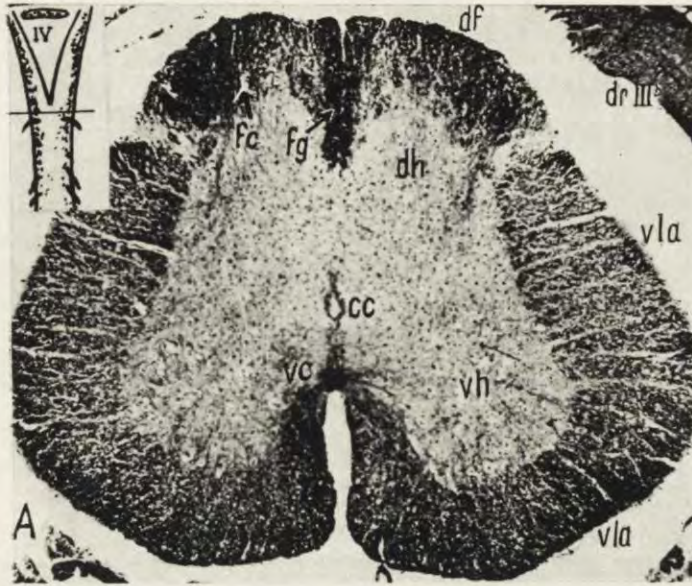
Fig. 1. A: Klüver's method. Low power view. Frontal section of the intumescentia cervicalis. B: Klüver's method ×100. Frontal section of the intumescentia lumbalis. The arrangement of the ventral and dorsal horns

Explanations to Figs. 1—4: The diagrammatic outlines shown in the corner of the microphotographs illustrate the position of the sections taken for microphotography in relation to the spinal cord or the medulla oblongata as a whole. Lines indicate the given level or plane of the section in the figure. Rectangles mark the boundary of the microphotograph against the background of the given section. A, neurons type A; B, neurons type B; C, neurons type C; ca, commissural area; CC, central canal; df, dorsal funiculi; dh, dorsal horn; dln, dorsolateral nucleus; dr, dorsal root; dr III, dorsal root III; iaf, internal arcuate fibers; ndf, nucleus of dorsal funiculi; vc, ventral commissure; vh, ventral horn; vla, ventrolateral area; vln, ventrolateral nucleus; vIV, ventricle IV; XII, hypoglossal nucleus
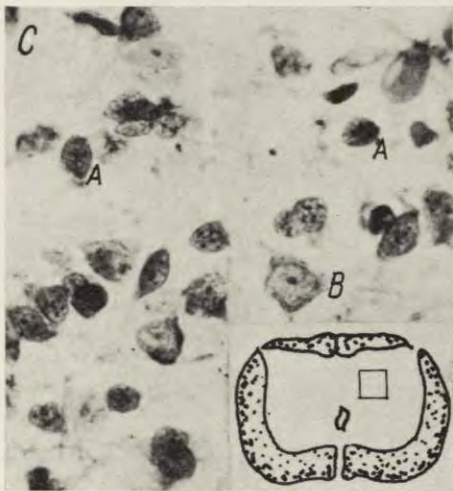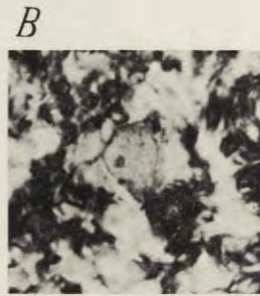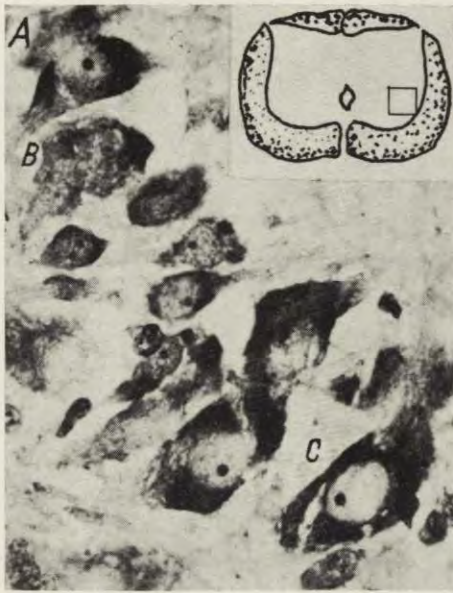
Fig. 2. A: Klüver's method × 400. Motoneurons in the dorsolateral nucleus of the spinal cord. B: Klüver's method × 400. Small size neuron (type B) in the ventro-lateral area. C: Klüver's method, × 400. Type A and B neurons in the dorsal horn. D: Golgi-Bubenaite method × 200. Frontal section of the ventrolateral area of the spinal cord. A large number of radially situated fibers can be observed in this area. The connection between neurons and fibers could not be stated with certainty. Other explanations as in Fig. 1

Fig. 3. A: Klüver's method $\times$ 100. Horizontal section of the dorsal funiculi. The area of termination of the dorsal funiculi fibers in the vicinity of the fourth ventricle B: Woelcke's method $\times$ 200. Horizontal section of the dorsal root. The ramification of the dorsal root fibers into the ascending and descending braches can be observed. C: Klüver's method $\times$ 200. Sagittal section of the do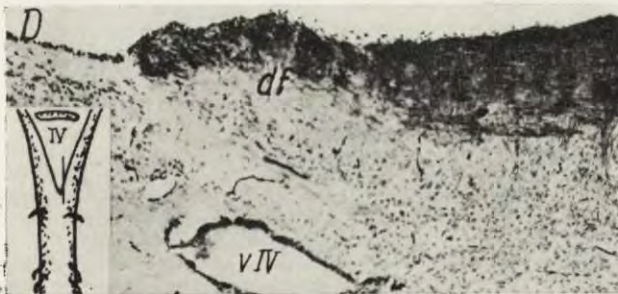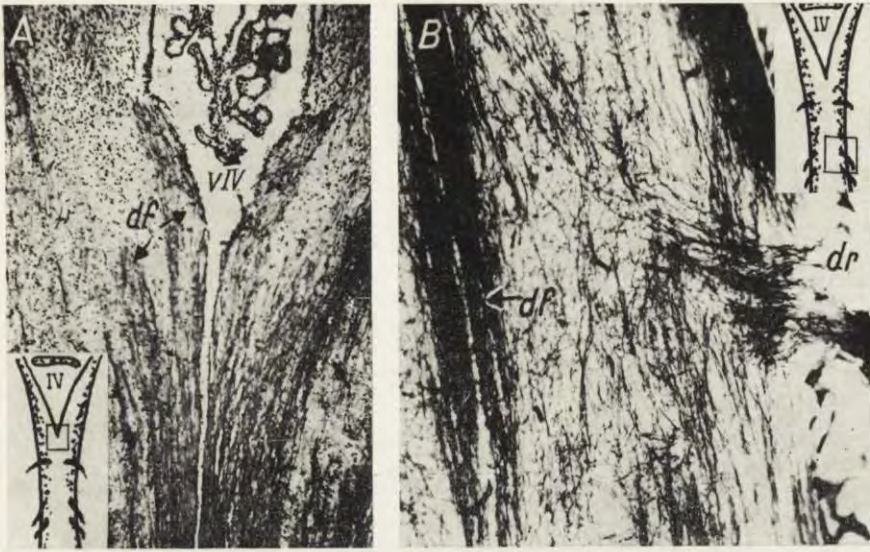rsal funiculi. A large number of ramifications to the dorsal horn. D: Klüver's method $\times$ 100. Sagittal section of the dorsal funiculi. The area of the termination of the dorsal funiculi fibers in the vicinity of the fourth ventricle. Compare Fig. 3A. Other explanations as in Fig. 1
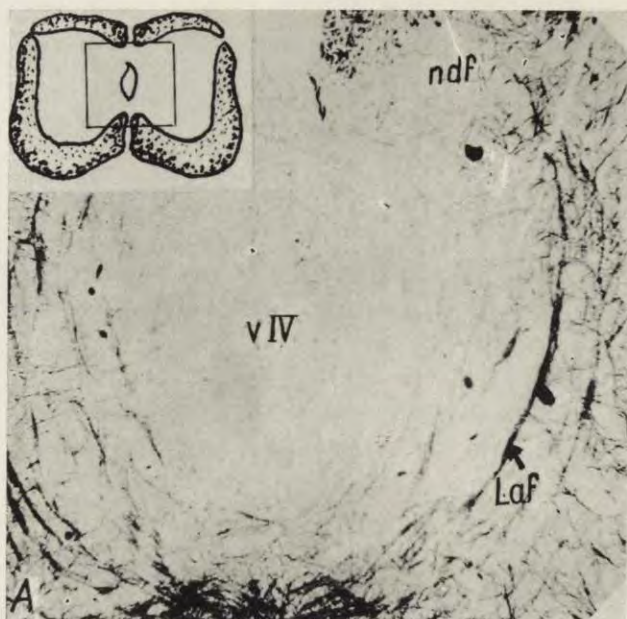
Fig. 4, A: Woelcke's method × 200. Frontal section of the lowest level of the medulla oblongata. The position of internal arcuate fibers. B: Klüver's method × 200. Frontal section of the lateral sensory area of the medulla oblongata. The position and arrangement of the nucleus of the dorsal funiculi and the origin of the internal arcuate fibers can be observed. Other explanations as in Fig. 1

SUMMARY AND CONCLUSIONS

The dorsal funiculi and their nuclei in the frog seem to be the primordium of the corresponding structures in the mammals. This is based on the evidence that (1) small bundles of the dorsal root fibers can be traced to the medulla oblongata; (2) the area of the termination of the dorsal root fibers is, at the same time, the region of the origin of the internal arcuate fibers; (3) the presence of the frontal accumulation of the dorsal funiculi fibers in the frontal sections at higher levels of the spinal cord in comparison with the lower ones.

There is no reason to believe that the ventrolateral area in the frog is a homologue of the ventral and lateral funiculi in mammals. It was found that the ventrolateral area fails to contain in the frog (a) a remarkable frontal accumulation of fibers throughout the various levels of the spinal cord and (b) bundles of nerve fibers which could be traced in the number of successive sections. On the other hand, the ventrolateral area in the frog contains a number of neurons which in mammals, could be considered abnormal. Accordingly, the ventrolateral area in the frog appears a coordinating system of the spinal cord, possibly working on the basis of high autonomy. The level at which the internal arcuate fibers occur seems to be a natural boundary between the spinal and the bulbar structures.

REFERENCES

AFELT Z. 1963a — Properties of spinal frog preparation in relation to the level of the spinal transection. Acta Biol. Exper. (Warsaw), *13*, 155.

AFELT Z. 1963b — Variability of reflexes in chronic spinal frogs. In: Central and Peripheral Mechanisms of Motor Functions. E. GUTMANN, P. HNIK (eds). Prague: Publishing House Czechoslovak Acad. Sci., p. 37.

ARIENS KAPPERS C. U. and HAMMER E. 1918 — Das Zentralnervensystem des Ochsenfrosches *(Rana catesbyana)*. Psychiat. en neurol., Amsterdam, *22*, 368

ARIENS KAPPERS C. U., HUBER G. C. and CROSBY E. C. 1960 — The comparative anatomy of the nervous system of vertebrates, including man. New York: Hafner Publ. Co. Vol. 1.

GAUPP E. 1896 — Anatomie des Frosches by Ecker A., Wiedersheim R. and Gaupp E. 3d ed. Braunschweig.

HERRICK C. J. 1948 — The brain of the tiger salamander. Chicago.

KÖPPEN M. 1888 — Zur Anatomie des Froschgehirns Arch. f. Anat. u. Physiol., Anat. Abt., 1.

von LENHOSSEK M. 1895 — Der feinere Bau des Nervensystems im Lichte neuester Forschungen. Berlin: Fischer, 2nd ed.

SANDMEYER W. 1892 — Secundäre Degeneration nach Extirpation motorischer Centra. Zeitschr. f. Biol. *28*, 177.

WALLENBERG A. 1907 — Die kaudale Endingung der bulbo-spinalen Wurzeln des Trigeminus, Vestibularis und Vagus beim Frosche. Anat. Anz., *30*, 564.

LOCOMOTOR REACTIONS IN A CHRONIC SPINAL PREPARATION OF THE FROG

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There are three different manners of moving in the Anura: swimming, walking and jumping. The latter two reactions occur only when at least one of an animal's extremities is touching a hard ground. When its body is stimulated, an intact animal whose extremities are not in contact with a solid object, takes swimming movements. If an animal's foot is touching, even very slightly, a hard surface that does not move together with it, a response of walking is evoked (G r a y and L i s s m a n n 1946) by stimulation.

When all four limbs in the spinal thoad are simultaneously subjected to passive retraction, they show the same type of diagonal coordination which characterizes ambulation in the intact animal (G r a y and L i s s- m a n n 1940). The third locomotor reaction of the Anura, that is, jumping is, so far, little investigated. Such mechanical properties of this reaction as, the take-off angle, the trajectory, height and length of the jump as compared with the size of an animal, as well as changes in the position of the center of gravity were mostly dealt with. The amphibians' postural reactions are known only superficially (A b b i e and A d e y 1950).

In the present paper, we shall deal with locomotor reactions, occurring in the spinal preparation of the *Rana esc.* Two types of the reaction, jumping and walking will be analyzed and compared with those observed in an intact, adult animal.

METHODS AND RESULTS

Specimens of *Rana esc.* were experimented on. A full transection of the spinal cord at the level of the bulbospinal junction was carried out according to F r a n z i- s k e t's (1951) method, aseptically and without the anesthesia. Sections were mostly
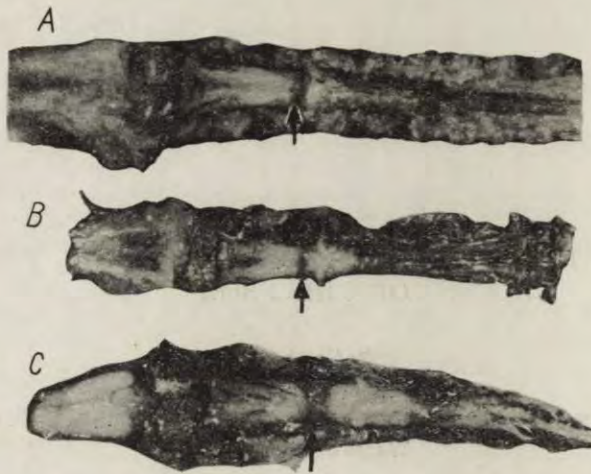
Fig. 1. Chronic spinal-frog preparations. Arrows indicate the placement of the transection. A, Transection above root III. B, Transection in the intermediary level of the spinobulbar junction. C, Transection in the region of the calamus scriptorius

made at two levels, that is, just above root III (Fig 1A) and in the region of the calamus scriptorius (Fig. 1C). Besides, a part of preparations was transected at an intermediary level between those, mentioned above (Fig. 1B). After the operation, the animals lived up to two years. They were kept on a moist ground at a temperature of 15 to 23°C. Their feeding was started 3 months after the operation; they were fed every 2—3 days and even daily, depending on the temperature. The alterations in the motor behavior which occur immediately after the surgery remain throughout the entire period of postoperative observation. The animals lie flat on the ground and a change in this posture may be evoked only by the stimulation. The reactions, described below, were produced by the stimulation with varying strengths and different stimuli (0.1 to 2 V current, touch, pressure, pinching with tweezers, pressing together with fingers), applied to different receptive fields of the trunk and extremities of a frog. In chronic experiments, the same area of the receptive field of the reflex investigated were every day stimulated many times and all motor reactions were recorded.

(1) *Spinal preparation transected in the lower part of the bulbospinal junction:*

(a) Stimulation of different receptive fields of the foreleg. As reported previously (A f e l t 1963 a, b), the stimulation of the foreleg receptive fields evokes in acute spinal preparation a backward extension of this leg, stretched along the body. Frequently, a flexion of the hind ipsilateral leg occurs at the same time. Occasionally, the frog touches with the hind leg the stimulated region on the foreleg. A simultaneous extension of all the

four legs, resembling a sort of a flat leap (Fig. 2) is caused by an increase in the stimulation intensity. An extension of one foreleg is invariably caused by the chronic stimulation of the Abwischreflex receptive field of this leg but it is not always stretched backwards and along the body. No jumping reaction occurred even after long periods of the stimulation of this field in chronic preparations.

Fig. 2. The jumping reaction in a spinal frog. Transection was made just above III root

(b) Hind leg stimulation. Depending on the intensity of stimulation, the following reactions are evoked by stimulating the hind leg, both during the immediate and later period after the operation: (1) the extension of the leg stimulated, (2) the simultaneous extension of both hindlegs, (3) the extension of all the four extremities, described above, that is, a flat leap.

When both hind legs are stimulated simultaneously but with different intensities, the preparation, performing a leap, turns sidewards, that is, towards an extremity which is more intensively stimulated.

(c) Stimulation of the hind leg Abwischreflex receptive field. Both in the acute and chronic preparations, in addition to the Abwischreflex, the extension (locomotor type B i c k e l 1900, B i e d e r m a n n 1900, B a - g l i o n i 1913) reactions, described above, are evoked by the stimulation of this receptive field. In the chronic experiments, the number of the reactions of the simultaneous extension of both hind legs increases considerably in the course of training. Moreover, in addition to the reflexes, described above, a gradual increase was observed in the number of movements which were unclassifiable in practice. These were very delicate, indefinite movements of different parts of all the four extremities.

(d) Posture. The frogs, transected at this level, do not change their po-

sture, that is, always lie flat on the ground, even in the late postoperative period (A b b i e and A d e y 1950). In the intervals between experiments, their forelegs were either limp and laid at random, or extended along the body.

(2) *Spinal preparation transected in the central part of the spinobulbar junction*:

(a) Stimulation of different foreleg receptive fields. The preparations, transected at the intermediary level between root III and the calamus scriptorius differ from each other. All types of reaction which can be performed by the acute preparation transected at the level of the calamus scriptorius may be in principle recorded in them. However, they occur irregularly and rather seldom. In stimulating different receptive fields, located on forelegs, the backward extension of the leg along the body was the most frequently observed reaction. The flexion (or, Abwischreflex, cf. paragraph 1a) of the hind, ipsilateral leg was recorded at the same time. In some preparations, the flexion of the hind ipsilateral leg was a sole reaction to the stimulation of all receptive fields of the foreleg. An increase in the stimulation causes jumping or several chaotic movements, performed with all the four extremities. Identical reflexes occurred in the chronic and in the acute experiments.

(b) Hind leg stimulation. In stimulating the hind extremities, identical reactions were recorded with those, evoked in preparations, transected in the lower part of the junction.

(c) Stimulation of the hind leg Abwischreflex receptive field. The reaction of the preparations, transected at the intermediary level, to the stimulation of the hind leg Abwischreflex receptive field is similar to that of the preparations, transected in the lower part of the junction. It differs from the latter in a fact that, in chronic experiments, the tendency to the occurrence of movements of different legs is much stronger. After a long training, in addition to specific reflexes, the following reactions could be recorded: slight movements of particular parts of different legs, more intensive movements of the entire extremity as if the animal was going to lift it off the ground, a flexion (or the Abwischreflex, directed towards the ipsilateral foreleg) of the hind leg with a simultaneous extension of the foreleg. The latter reaction is identical with that, occurring in the stimulated foreleg of the preparation, described above.

(d) Posture. The frogs, transected at the intermediary level, change their posture in the course of the chronic experiments, although it occurs seldom and for brief periods only. They sit up, supporting their bodies on stretched forelegs.

(3) *Spinal preparation transected in the region of the calamus scriptorius:*

(a) and (b) Stimulation of different receptive fields of the fore and hind leg.

The reactions of the acute preparation to the stimulation of the fore and hind extremities have been described previously (A f e l t 1963 a, b). The increase in the intensity of stimulation causes either a leap, or chaotic movements of all the four legs. The leap may be identical in character with that, performed by the preparations, described previously. This takes place when the animal lies flat. If the frog is stimulated in a sitting position, its leap is performed at an appropriate angle to the ground. The intensive stimulation of a foreleg may cause the occurrence of an „umdrehreflex" and only afterwards, a leap. In this case, the animal „turns" sidewards. A similar effect is produced by different intensities of stimulating both hind legs.

c) Stimulation of the hind leg Abwischreflex receptive field. Reactions, occurring as a response to this stimulation in the acute preparation have already been described. We would like to recall that, in addition to a specific reflex, a backward extension of the hind leg occurs. An increase in the stimulation causes that, instead of the extension of one extremity, both hind legs are stretched backwards and the reaction of a leap occurs.

In chronic experiments in which the stimulation of the hind leg Abwischreflex receptive field was systematically applied, this reaction was gradually abolished. At the same time, the movements of the remaining extremities occurred ever more frequently. The animal either „supported" its body on one of the legs, simultaneously slightly changing the position of the trunk, or it lifted this leg somewhat off the ground, also changing the posture of its body. No increase was, however, recorded in the number of the jumping reaction. The fore leg Abwischreflex occurred at the same time. In most instances, simultaneously with the latter reaction (or somewhat later), the animal extended the other foreleg and „leaned" its body on it. In the further stage of the training, the latter extension reflex was ever more frequently associated with the flexion of the hind leg, ipsilateral to it. This was the same reaction, resembling the hind leg Abwischreflex, directed towards the foreleg which we observed in all our preparations as a response to the stimulation of the skin of the posterior surface of the foreleg.

Both the ipsilateral foreleg and the contralateral hind leg Abwischreflexes were gradually replaced by the forward movements of these legs and placing them on the ground. The following set of reflexes
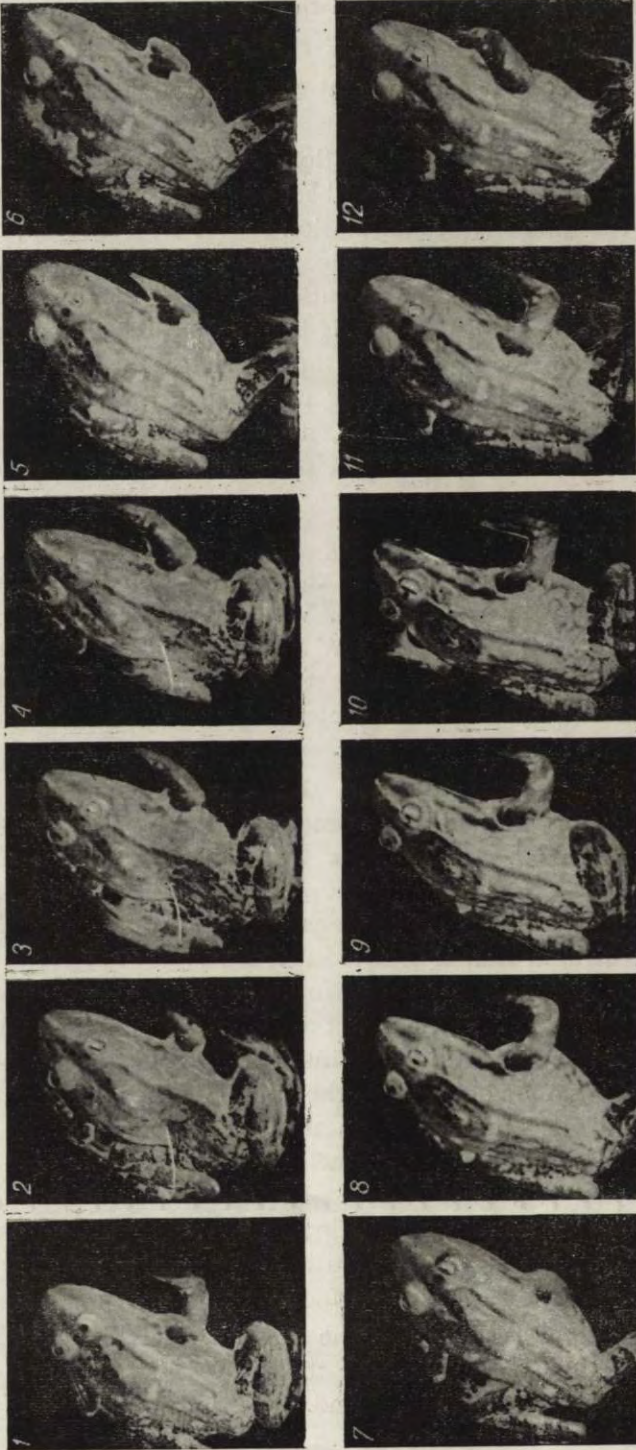
Fig. 3. The walking reaction of the chronic spinal frog preparation. Transection of the cord at the level of calamus scriptorius. Excerpt from a moving picture

occurred, therefore, ever more frequently as a response to the stimulation of the hind leg Abwischreflex receptive field: (1) forward reaching with the foreleg, ipsilateral to the stimulation point and placing it on the ground, (2) simultaneous leaning on the other foreleg, (3) subsequent forward movement of the contralateral hind leg, (4) simultaneous backward movement of the foreleg on which the animal was leaning its body. This reaction became ever quicker and more efficient, producing an irresistible impression as if it was an incomplete „step" of walking. Finally, the last leg (always that one which might perform a specific reflex) was included in this reaction and performed a movement (5) of a backward kick. Afterwards, either the extremities returned to the starting position, or the animal performed an entire series of successive alternate movements and thus, walked (Fig. 3). It is especially in the initial period of the training that the movements of particular extremities may take much time, even to a few seconds. Frequently, they can be distinctly separated in components. Thus, for instance, the Abwischreflex takes place in the following manner: the frog slightly raises the leg, keeps it in this position for a fairly long time (to 10 sec), then, raises it to a still higher position, simultaneously, turning its „hand" inwards, once more keeps motionless and, finally, rapidly puts it down. All these movements particularly those of forelegs, may be associated with a very strong tremor. Multiple movements occur precisely in this training stage. After a single performance of some movement, regardless if it was quick, or slow and clumsy, the animal repeated it several (2—3, sometimes to 7) times in a very efficient manner.

After the final formation of the reaction of walking, the intervals between individual movements of the extremities were different. In some preparations they showed a tendency to become shorter. The latency of the reaction of walking might reach 45 sec. The longer was the average latent period of the reaction, the more frequently the tremor of the extremity, initiating the walking reflex, was observed. The longer were the intervals between particular movements of the extremities, the more frequently a specific reaction was obseved against the background of the locomotor reaction, that is, the Abwischreflex occurred between successive movements of particular extremities. The reaction of walking was not equally effective in different animals (A f e l t and J a n k o w-s k i 1965). The duration of its training was also different. An optimum level of the reaction of walking was reached by individual spinal preparations. After the achievement of this level, the reaction did not alter and no improvement was obtained by the prolongation of the training. On the other hand, nothing was changed by training intervals. A walking animal preserved this capacity also after an interval in experiments.

A slight stimulation, applied to an animal's back, was responded by some preparations with a 20 to 30 cm walk and, by some others, with only 1—2 „steps" at most. The stimulation of any part of the body is responded by a „walking" frog with walking. An intensive stimulation of hind, „walking" legs of preparations evokes either jumping, or walking. The latten reaction occurs more frequently.

(d) Posture. Its body stimulated by tactile stimuli, the chronic spinal preparation of the frog, in which the transection took place in the region of the calamus scriptorius, takes a sitting, „froglike" posture (Franzisket 1963). When undisturbed, the animal lies flat on the ground. Immediately after the operation, the „sitting" reaction may occur only when the forelegs are stiffened by the stimulation of an appropriate receptive field. Thus, for instance, an embracing reflex is performed by a male frog when the skin of the internal part of its shoulder girdle is stimulated. Putting an animal on the ground is also a sort of the stimulation of this receptive field. It evokes the embracing reflex and, the animal, leaning on its clasped, stiff forelegs, takes an apparently sitting position. „Apparently" — because it may easily be overthrown. In identical situation, a chronic preparation does not overturn, but, with its trunk and extremities, it takes several movements which allow it to keep balance. The longer the animal's postoperative survival time and the more intensive its training, the more frequently and easily it takes the sitting posture.

(4) Locomotor swimming reaction. All spinal preparations of the frog, put in the water, were promptly drowning. None of our animals was able to perform in the water the set of movements, characteristic of the swimming reaction. A simultaneous extension of all the four legs, characteristic of the jumping reaction, was evoked at most by the stimulation of both the body and the extremities of an animal. On the other hand, preparations in which the transection of the spinal cord was incomplete, when immersed in the water, started, more or less efficiently, to swim. Such an immersion is the best manner of checking whether or not the operation was a success, that is, transection was complete.

In our material, no occurrence has been recorded of the locomotor reactions. The reaction of walking, as a response to the stimulation of untrained frogs, transected in the region of the calamus scriptorius, was not observed either. On the other hand, the reaction of walking to the stimulation of the extremities was regularly observed in the acute and chronic bulbospinal preparations even when their roots V, VI, VII, VIII and IX were transected, that is, when — in practice — they received an identical information from the periphery with that of our preparations.

DISCUSSION

In the early papers, the locomotor reactions are defined as movements, allowing the animal for changing its position in the space (Ortsbewegungen auf dem Lande oder Wasser). In the more recent ones, the appropriate coordination of the movements of extremities is considered the most essential element of the locomotor reaction. It has been found that, in mammals and birds, the locomotor reaction is apparently absent because an animal is pressed to the ground by its own weight (T e n C a t e 1960, 1962); This hypothesis seems to be confirmed by the results of investigations during which, in chronic spinal preparations of the dog (N e s m e i a n o v a et al. 1964) and cat (K o z a k and W e s t e r m a n n unpubl.), the nerves of extremities were systematically stimulated through the skin. This caused stiffening of the extremities and allowed for walking. It seems that the occurrence of the „true" locomotor reaction in mammals spinal preparation (Mc C o u c h 1947, S h u r a g e r and D y k m a n 1951) may be explained by setting in motion of the stepping reflex mechanism (S h e r r i n g t o n 1910) in an animal leaning its body on stretched extremities.

Intact frogs, kept under conditions identical with those of our operated animals, mostly lie flat on the ground. They take the sitting position when they are influenced by external stimuli. These may be of course both the somatic and telereceptive, mainly visual, stimuli. The chronic spinal preparations, transected in the upper part of the bulbo--spinal junction, take the sitting position also as a response to external stimuli, particularly so after a long period during which reflexes are applied. A fact that these cannot be the telereceptive stimuli makes up a difference.

The relation between the postural and locomotor reactions in the frog remain unknown. Both locomotor reactions that occurred in our spinal preperations, that is, walking and jumping occur, in their full form, only in preparations, transected in the upper part of the bulbospinal junction and it is only in these preparations that the „normal" sitting position may be observed. On the basis of these findings, it may be stated that „propping up" of an animal's body on stiff forelegs constitutes an element of postural reactions which is decisive of locomotor reactions.

It seems that, on the basis of our experiments the following elements may be distinguished in the reaction of jumping:

(1) An appropriate coördination of the movements, taken with all the four legs simultaneously, that is, their simultaneous backward extension. This reaction occurred in all our preparations. Sometimes, being ineffective, it did not cause any movement of the entire animal's body.

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(2) A leap at a certain angle to the ground. It may be evoked only in preparations, transected in the upper part of the junction. Taking the sitting position must precede it.

(3) A leap with a simultaneous sideward turn. It may be evoked either (a) by different intensities of stimulation applied to the hind legs, or (b) by producing the Umdrehreflex which is possible only in preparations, transected in the upper part of the junction.

The spinal preparation, transected, in the region of the calamus scriptorius and stimulated with an appropriate set of the peripheral stimuli, can perform a reaction analogous to the leap of an intact animal while the preparation, transected in the lower part of the junction is not capable of it.

The reaction of walking is the other type of the locomotor reaction, occurring in the spinal preparations of *Rana esc*. It occurs when (1) the preparation is transected in the upper part of the bulbospinal junction and (2) is subjected to a systematic training. In our case, this was a daily, repeated evocation of the Abwischreflex. A very intensive stimulation of different receptive fields does not evoke the reaction of walking both in acute and chronic, naïve preparations. Several reflexes, chaotically following each other or a generalized reaction of the entire body are obtained instead. This phenomenon has been described since the beginning of the investigations on spinal preparations of the frog (V o l k-m a n n 1838).

In early papers, a statement may be encountered that the locomotor reaction of the type of walking occurs only when the spinal cord is cut off together with a piece of the medulla oblongata. B i c k e l (1900) maintains that it is precisely in the medulla oblongata where nerve centers are located destined for the coördination of the movements of the four extremities, necessary in this reaction. A similar statement, referring to the Urodela can be found in the papers by H e r r i c k (1948) and A r i e n s K a p p e r s et al (1960). On the basis of our results, it can be concluded that the reaction of walking occurs in a chronic preparation when the region of the bulbospinal junction is preserved. The accuracy of the reaction depends on a level of the transection (A f e l t and J a n-k o w s k i 1965). The decisive function of the training in evoking it is indicated by the following facts: (1) the reaction does not occur in the acute preparations, even to respond to a very intensive stimulation; (2) the reaction does not occur in the „naïve" chronic spinal preparations; a very intensive stimulation of different receptive fields is responded by the animals with leaps; (3) the reaction cannot be evoked without a systematic training, that is, if, for instance, the Abwischreflex is evoked once in a month (A f e l t 1963).

Two hypotheses may be suggested. The first of them is an assumption that, in an intact animal, an apparatus, coördinating the locomotor reaction of walking exists, located in the region of the bulbospinal junction. This apparatus is triggered by bulbar structures. A normal pattern of this reaction, any influences of the bulb excluded, becomes disturbed and must be reorganized. The other hypotheses might be presented in the form of an assumption that, in an intact animal, there is no special region where the coördinative apparatus of the reaction of walking would be located. The isolated spinal cord may, as an entirety, reproduce this function. In preparations, transected at a low level, this function does not occur because of the arcs of reflexes, containing elements of the flexion of forelegs, become damaged (Silver 1942). Such an animal is not capable of performing all movements necessary in a complex pattern of walking. This problem requires continued studies.

SUMMARY

(1) It was found that the reaction of swimming does not occur in the spinal preparations of the frog.

(2) Jumping and walking occur in their full form only in the preparations, transected in the upper part of the spinobulbar junction.

(3) The reaction of walking occurred in our experiments only in such preparations in which the Abwischreflex was systematically evoked.

REFERENCES

ABBIE A. A. and ADEY W. K. 1950 — Motor mechanisms of the anuran brain. J. Comp. Neurol., 92, 241.

AFELT Z. 1963a — Properties of spinal frog preparation in relation to the level of the spinal transection. Acta Biol. Exper. (Warsaw), 23, 155.

AFELT Z. 1963 — Variability of reflexes in chronic spinal frogs. E. GUTMANN, P. HNIK (eds). (In: Central and Peripheral Mechanisms of oMtor Functions. Prague: Publishing House Czechoslovak Acad. Sci., p. 37.

AFELT and JANKOWSKI K. 1965 — The relation between ambulation-pattern and architectonics of the spinobulbar junction in the frog. (Rana esc.), Acta Biol. Exper. (Warsaw), 25.

ARIENS KAPPERS C. U., HUBER G. C. and CROSBY E. C. 1960 — The comparative anatomy of the nervous system of vertebrates, including man. New York: Hafner Publ. Co. Vol. 1.

BAGLIONI S. 1913 — Die Hautreflexe der Amphibien (Frosch und Kröte). Ergebn. Physiol., 13, 454.

BICKEL A. 1900 — Beiträge zur Rückenmarksphysiologie des Frosches. Arch. Anat. Physiol. Physiol. Abt., p. 485.

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BIEDERMANN W. 1900 — Beiträge zur Kenntnis der Reflexfunction des Rückenmarks. Pflüg. Arch. ges. Physiol., 80, 408.
FRANZISKET L. 1951 — Gewohneitsbildung und bedingte Reflexe bei Rüuckenmarkfröschen, Zeitschr. vergl. Physiol., 33, 142.
FRANZISKET L. 1963 — Characteristics of instinctive behaviour and learning in reflex activity of the frog. Animal Behav., 11, 318.
GRAY J. and LISSMANN H. W. 1940 — Ambulatory reflexes in spinal amphibians. J. Exp. Biol. 17, 237.
GRAY J. and LISSMANN H. W. 1946 — The co-ordination of limb movements in the amphibia. J. Exper. Biol. 23, 133.
HERRICK J. C. 1948 — The brain of the tiger salamander. Chicago.
McCOUCH M. 1947 — Reflex development in the chronically spinal cat and dog. J. Neurophysiol. 10, 425.
NESMEIANOVA T. N. BRAZOVSKAIA F. A. and ARNAUTOVA E. N. 1964 — Facilitation of the regeneration of neural pathways following transection of the spinal cord in the dog. In: Mechanisms of Compensation. Moskva: Nauka, AN SSSR, p. 124 (in Russian).
SHERRINGTON C. S. 1910 — Flexion-reflex of the limb, crossed-extension reflex, and reflex stepping and standing, J. Physiol., 40, 28.
SHURRAGER P. S. and DYKMAN R. A. 1951 — Walking spinal carnivores. J. comp. physiol. Psychol., 44, 3.
SILVER M. L. 1942 — Motoneurons of the spinal cord of the frog., J. comp. Neurol., 77, 1.
TEN CATE L. 1960 — Locomotor movements in the spinal pigeon. J. Exper. Biol., 37, 3.
TEN CATE L. 1962 — L'innervation des mouvements locomoteurs par la moelle lombo-sacrée isolée chez les oiseaux et les mammiferes. J. de Physiol., 54, 310.
VOLKMANN A. W. 1838 — Über Reflexbewegungen. Arch. Anat. Physiol., Lpz., 15.

RELATION BETWEEN THE AMBULATION PATTERN AND ARCHITECTONICS OF THE SPINOBULBAR JUNCTION IN THE FROG *RANA ESC.*

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The section of the central nervous system, contained between the outlet of root III and a place where ventricle IV is closed, is called, the area of the spinobulbar junction. In the frog of a medium size, the maximum length of this section amounts to 1.5 mm. Fairly wide individual fluctuations are observed, that is, this section is either relatively short and wide, or — slender and elongated. Complete spinal transections, made in order to obtain a chronic spinal preparation of the frog were, therefore, marked by a certain dispersion. In the present study, they were mostly located in the lower or upper part of the junction.

As results from the previous papers (A f e l t 1963 a, b, 1965), a reaction of walking was observed in a chronic spinal preparation, transected in the region of the calamus scriptorius. It has been indicated by morphological studies (J a n k o w s k i 1965) that the general bulbar lemniscus which starts below the calamus scriptorius, constitutes a boundary between the spinal cord and the bulb. In this connection, it may be hypothetized that the reaction of walking, observed in our preparations, is related with the preservation of a part of the bulbar lemniscus and the nucleus funiculi dorsalis, corresponding with the former (A f e l t 1963a).

MATERIAL AND METHOD

Six spinal preparations were used for experiments. They were selected on the basis of animals' behavior during chronic experiments. Beginning with the third month after the surgery, every day or every other day, an „Abwischreflex", performed with a hind leg, was repeatedly evoked in these animals. Reactions

of the locomotor type, occurring as a response to the stimulation of the receptive field of the „Abwischreflex" were observed and recorded. Every 10th experiment, locomotor responses were evoked from their specific receptive field, that is, from the region of hind legs. After animals' behavior has settled, that is, after the locomotor reaction has reached the determined, invariable level, animals were killed, their nervous system was fixed in a 10 per cent formalin and embedded in paraffin. A full series of preparations 10 and 15μ thick were made in the coronal plane, through the spinal cord, up to the transection point. Sections were stained by the Klüver method.

RESULTS

Locomotor reactions of spinal preparations and the levels of the spinal cord transection are presented in Table 1.

Reactions, given in Table 1, constituted an upper limit of our animals' motor performance. All preparations, mentioned above, jumped. The more efficient reaction of walking, the less frequent was the reaction of jumping.

In the entire material, degenerations were found in the dorsal and ventrolateral funiculi over the distance from the section to the input of root pair III. In preparations, transected in the lower part of the junction, a medial part of the dorsal funiculi (related with lower extremities) has degenerated to the level below the input of root pair III (Fig. 1). On the other hand, the descending fibers of root pair III remained preserved. No bulbar lemniscus fibers could be found in any preparation below the section, It is very likely that they have entirely disintegrated. In sections of the spinal cord, cellular structures, related to the anterior and posterior horns, remained preserved above root pair III. Morphologically, they differed from structures, occurring in lower sections of the spinal cord (Fig. 2).

DISCUSSION

A hypothesis was presented in the introduction that the reaction of walking, observed in chronic spinal preparations, transected in the region of the calamus scriptorius, is related with bulbar structures. This hypothesis seems to be supported by B i c k e l's (1900) observations which indicate that the locomotor reaction of walking occurs only in the case when, cutting off an animal's head, we leave, together with the spinal cord, a piece of the medulla oblongata. B i c k e l suggested that it was precisely in the lower part of the medulla oblongata that the centers are located, coordinating the movements of all four legs. As we already know on the basis of the previous paper (A f e l t 1965) this is incorrect in respect to the locomotor reaction of jumping. The results of the present work

Table I

Frog. No. 25. Did not walk.

Frog No. 31. An inefficient reaction of walking. Latent period up to 1.5 min. Takes at most 1—2 steps. Long intervals between movements of particular extremities. Tremor.

Frog No. 35. Reactions almost identical with those of frog No. 31.

Frog No. 54. Efficient reaction of walking. Latency averaging 30 sec. Takes average 1—2 steps. No intervals between movements of particular extremities.

Frog No. 15. Reactions almost identical with those of frog No. 54.

Frog No 42. Efficient reaction of walking. Direct response of walking to single stimulation. The animal can walk 20—30 cm.
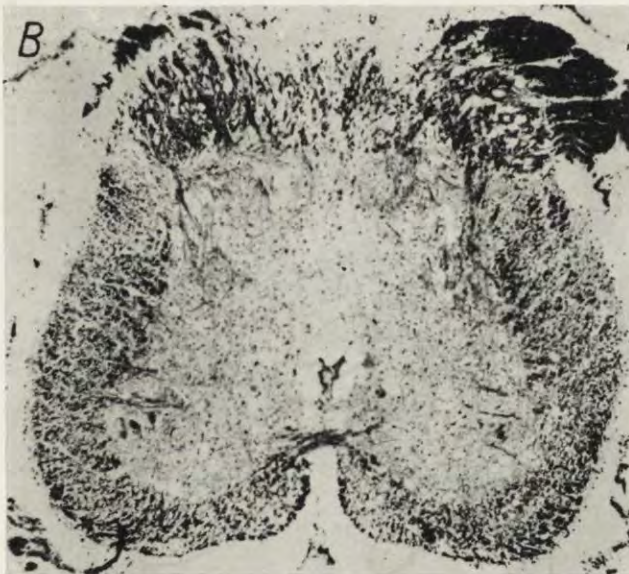
Fig. 1, A: A frontal section just below the lesion. Complete degeneration of the dorsal funiculi and advanced destruction of the ventrolateral areas observed. B: Successive frontal sections taken out of the levels situated every 200 microns below the level of the section shown in Fig. 1A. The desintegration of the frontal funiculi can be observed up to the level shown in Fig. 2A. Some decrease in the number of fibers of the ventrolateral areas can be observed also, particularly in Fig. 1B. Low power view photomicrograph (Klüver's method) of the frontal sections of the spinal cord below the level of the experimental transsection
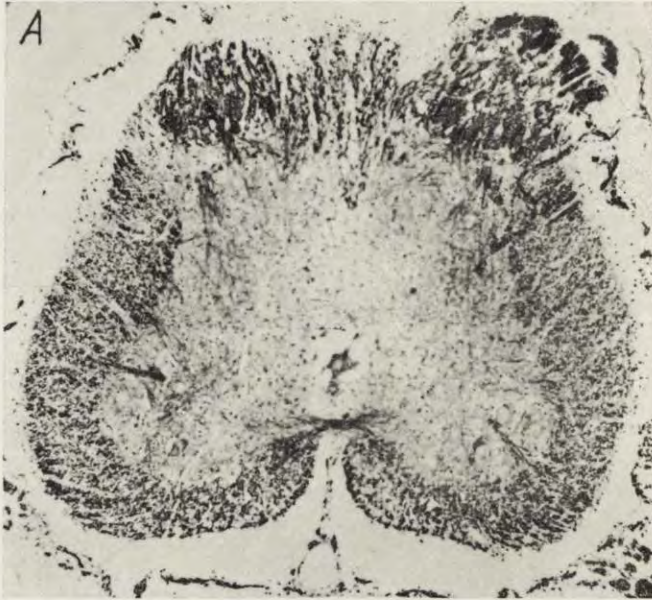
Fig. 2A, B: Explanations as in Fig. 1

exclude the participation of bulbar structures from the reaction of walking that we observed in our preparations. All fibers, crossing each other and constituting the general bulbar lemniscus degenerated in a point of the junction, situated below the section point. This is the only bulbar structure in which the junction differs from the spinal cord. It seems, therefore, that the reaction of walking may be reckoned in frogs among the spinal reactions. On the other hand, on the basis of our material, we cannot answer the question whether or not the cells of the nucleus funiculi dorsalis of this region degenerated.

A supposition that, following the degeneration, a dissociation occurred between an information, incoming from the upper extremities and that — from lower extremities, might serve as a hypothesis, explaining the absence of the reaction of walking in preparations, transected in the lower part of the junction. Degenerations in fasciculus gracilis were observed on larger area than in fasciculus cuneatus. The fibers of the former bundle did not reach the level of root pair III, related with upper extremities. One could suppose, therefore, that an integration of functions of lower and upper extremities, necessary for locomotor movements of the walking type, takes place in a section of the spinal cord, located in the area of root III. The preserved descending fibers of the sensory root pair III do not play any major role in this function.

An assumption that a difference in the number and, maybe, also in the quality of motoneurons (S i l v e r 1942) preserved in the anterior horn is decisive for the performance of the walking reaction, might constitute one more hypothesis, explaining differences in the behavior of preparations, transected at a higher and lower level. An obserwation that acute spinal preparations, transected in the lower part of the junction, do not carry out any reflex, containing elements of the flexion of the foreleg (A f e l t 1963a), speaks in favor of this assumption. It is possible, therefore, that, in preparations, transected in the lower part of the junction, we damage the motoneurons of the flexors of the foreleg which, of course, makes their participation in the complex ambulation pattern impossible. This problem requires a further investigation, however.

SUMMARY

(1) The locomotor reaction of walking in a chronic spinal preparation of the frog, transected in the region of the calamus scriptorius is a spinal reaction.

(2) The precision of the ambulation pattern changes according to the level of the transection.

(3) Hypotheses, referring morphological changes, observed in the transected region of the junction, to the behavior of the preparation, were subjected to an analysis.

REFERENCES

AFELT Z. 1963a — Properties of spinal frog preparation in relation to the level of the spinal transection. Acta Biol. Exper., (Warsaw), 24, 155.

AFELT Z. 1963b — Variability of reflexes in chronic spinal frogs. In: Central and Peripheral Mechanisms of Motor Functions. GUTMANN E., HNIK P. (eds.) Prague: Czechoslovak Acad. Sci. Publishing House, p. 37.

AFELT Z. 1965 — Locomotor reactions in the chronic spinal cord preparation of the frog. Acta Biol., Exper (Warsaw), 25, 161.

BICKEL A. 1900 — Beiträge zur Rückenmarksphysiologie des Frosches. Arch. f. Anat. Physiol., Physiol. Abt. 485.

JANKOWSKI K. 1965 — The general architectonics of the spinal cord and spinobulbar junction in the frog. Acta Biol. Exper., (Warsaw), 25, 159.

SILVER M. 1942 — The motoneurons of the spinal cord of the frog. J. Comp. Neurol., 77, 1.

REPRESENTATION OF AGGRESSION AND FLIGHT REACTIONS IN THE HYPOTHALAMUS OF THE CAT[1]

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It has previously been demonstrated that the motor and autonomic symptoms related to the aggression and flight are integrated by the hypothalamus (C a n n o n and B r i t t o n 1925, B a r d 1928, P a p e z 1937, H e s s and B r ü g g e r 1943). However, the localization of the aggression and flight in the hypothalamus has not been sufficiently explained and discrepant data are met with in the literature.

After the electrolytic lesions of the hypothalamus in cats, the aggression or irritation was found by W h e a t l e y (1944). These reactions occurred as a result of bilateral lesions of the ventromedial nucleus of the hypothalamus or of the areas situated somewhat dorsally to this nucleus.

Discrepanciens as to localization of aggression and flight reactions in the hypothalamus are related to the experiments in which the electrical stimulation of the unanesthetized and freely moving animals' brains was used. By stimulating anterolateral areas of the diencephalon, including the basal septal nuclei, the preoptic area and the basal part of the central thalamus, H e s s (1949) evoked the rage and attack reaction, while an increased restlessness and flight was produced by stimulating the area of the posterior hypothalamus. H e s s' results were confirmed by H u n s p e r g e r (1956) who, in addition, found that the aggression and flight can be also evoked by the stimulation of the central gray of the mesencephalon. Furthermore, H u n s p e r g e r (1956) noticed that,

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depending on the parameters applied and on the time of stimulation, in some cases, the reaction of aggression and, in other cases, the reaction of flight, or even passing one into the other, can be evoked by stimulating always the same „point". Recently, a different and even opposite localization of the reaction of aggression and flight to that of H e s s (1949) and H u n s p e r g e r (1956) was indicated. Thus N a k a o (1958) obtained the reaction of flight by stimulating the anterior hypothalamus and the preoptic area from points, situated somewhat laterally to ventricle III and, the reaction of aggression by stimulating middle and ventral parts of the central hypothalamus. The reaction of fear and flight caused by the stimulation of the anterior hypothalamus and the reaction of aggresion as a response to the stimulation of the central hypothalamus were also observed by Y a s u k o c h i (1960). In the latter case, particularly violent was the aggression produced by stimulating the area of the ventromedial nucleus of the hypothalamus. W a s m a n and F l y n n (1962) evoked the reaction of both the aggression and flight by the stimulation of lateral regions of an area, stretching from the anterior to the posterior hypothalamus. In experimenting on rabbits (K o z l o v s k a i a 1964), the reaction of aggression was produced by the stimulation of the medial areas of the posterior hypothalamus.

In our previous experiments (R o m a n i u k 1962), on the basis of the results of electrolytic lesions of the hypothalamus in rabbits and preliminary investigations during which an electrical stimulation was applied to this structure in cats (R o m a n i u k 1963), a hypothesis was put forward that the „aggression center" is located in the ventral part of the medial hypothalamus, while the „flight center" is located in the dorsal part of the medial hypothalamus. Both „centers" are situated in the area, including the anterior, central, and posterior hypothalamus.

The purpose of the present experiment was to verify this hypothesis and to accurately determine the areas of the hypothalamus in cats responding with the reactions of aggression and flight to the electrical stimulation with the use of the stereotactically implanted electrodes. In addition, we wanted to explain the causes of the discrepant data met with in the literature.

MATERIAL AND METHOD

Experiments were carried out on 35 adult cats, males and females, 3 to 3.5 kg in weight, in which chronic electrodes were implanted in the hypothalamus. The operation was made under Nembutal narcosis (40 mg/kg, injected intraperitoneously), local anesthesia (2 per cent Polocaine) and semiaseptic conditions. Each cat was placed in a Horsley-Clarke stereotactic apparatus. The skin was cut along the midline and holes were drilled in the skull through which unipolar or bipolar

electrodes were introduced according to the D e l g a d o technique (1955). Electrodes were distributed according to the following stereotactic coordinates of the J a s p e r and A j m o n e — M a r s a n atlas (1954): A = 8.5 to 13.5, L = 1.0 to 2.0, H = −2.0 to −7.0. Each cat was implanted with three electrodes in each hemisphere. Stainless steel electrodes, 150μ in diameter and Teflon coated over their entire lenght except for a 0.5 mm tip were used. After introducing the electrodes to the brain, the holes in the skull were cemented with Duracryl (SPOFA-United Pharmaceutical Works, Prague, Czechoslovakia) which thus fixed the electrodes in place. Opposite ends of electrodes, protruding over the skull surface, were fastened, also with Duracryl, to the skull surface. The indifferent silver wire electrode was attached with one end to the crista of the occipital bone and, with another, to the socket. Then, the skin was sutured in such a manner as to leave the socket slightly protruding over the skin surface.

Following a recovery period of about 10 to 15 days, the experiments were started in a 100 × 100 × 100 cm testing cage. During experiments, the stimulator cables were connected with the socket by means of a plug. These cables, passing through a system of pulleys, were suspended from the cage ceiling thus leaving the animals a full freedom of movements within the cage during experiments.

In the first testing, stimulation started as a rule from an intensity of 0.2 mA and then gradually was increased to reach level for threshold, optimal and maximal reactions. Stimulation experiments were performed several times at intervals of 2 to 3 days. The details of the animal's behavior were carefully noted and occasionally, photographed. Unipolar or bipolar stimulation with rectangular impulses (Type 5/60 stimulator, Laboratory of Electromedical Equipment, Warsaw Engineering College) of a frequency of 10 to 100 cy/sec, a pulse duration of 0.5 to 10 msec and an amplitude of 0.2 to 2.0 mA was used. Impulsing was monitored on the screen of a cathodic oscillograph.

After completion of the stimulation 100 experiments the points explored were coagulated for 15 sec with a 3 mA D.C. After 20 days, the animals were killed and their brains perfused with a 10 per cent formalin. Sections 20μ in thickness, stained according to the Nissl method, were made subsequently.

RESULTS

Applying the electrical stimulation to the hypothalamus, a total of 210 points, distributed over the anterior, central and posterior portions of the medial hypothalamus, from the preoptic area up to corpora mamillaria, were examined. The following emotional reactions were observed as a result of the stimulation:

Reaction of aggression. This type of behavior was obtained from 87 points, located in the ventral part of the anterior, central and posterior subdivisions of the medial hypothalamus, contained in the frontal planes Fr. 10.0 to 13.0, which are situated 1 to 2 mm laterally to ventricle III (Figs. 1 and 2). When these points were stimulated, the following autonomic and motor reactions were recorded: pupil dilation, pilo-erection, back arching, growling and snorting, salivation, baring teeth, pushing out claws and scratching the floor, locomotor reaction and violent rushing
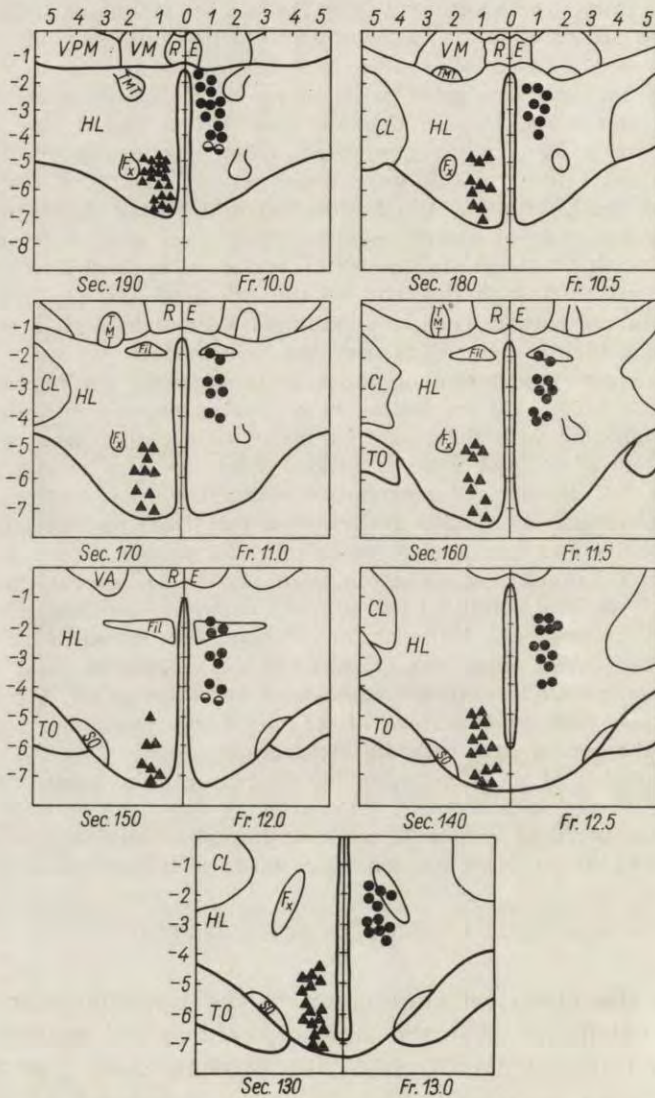
Fig. 1. Summary diagrams of cross sections through the cat's hypothalamus at seven levels (after J a s p e r and A j m o n e-M a r s a n, *A stereotaxic atlas of the diencephalon of the cat, 1954*) to show the histologically verified topographic arrangement of points from which during stimulation emotional behavior patterns were obtained. All points are collected from 35 cats. Large black dots are points from which flight reaction was recorded; triangles show points concerned with aggression; flight reaction associated with some components of the aggression reaction appears as half black dots. CL, nucleus centralis lateralis; Fil, nucleus filiformis; Fx, fornix; HL, hypothalamus lateralis; RE, nucleus reuniens; SO, nucleus supraopticus; TMT, tractus mamillo-thalamicus; TO, tractus opticus; VA, nucleus ventralis medialis; VPM, nucleus ventralis postero-medialis; Fr, frontal plane; Sec, frontal section number
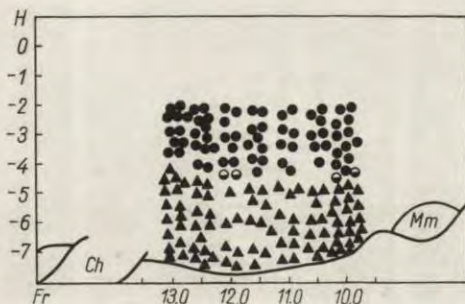
Fig. 2. Electrical stimulation mapping of representation of aggression and flight reactions of the longitudinal view of the hypothalamus of cat. The longitudinal view in this figure and the cross-sectional views of the hypothalamus in Fig. 1 are correlated. Large black dots, flight points; triangles, aggression points; half black dots, flight contaminated with aggression; Ch, chiasma opticum; Mm, corpora mamillaria; Fr — frontal plane; H — horizontal plane

on all objects within the animal's reach. The entire pattern of symptoms occurred when optimal parameters of stimulation were applied which, for the reaction of aggression, were 1 mA, 50 cy/sec, 10 msec. Basically, these symptoms occurred gradually, in the order in which they were specified above and separated from each other by brief (1 to 2 sec) latent periods. When a weak stimulation was applied or when its duration was shortened, only certain elements of the aggressive behavior were observed and latency was prolonged. For instance, when a 0.2 mA, 50 cy/sec, 1 msec stimulation was applied, only the pupil dilation and pilo-erection were observed. With the use of a 0.2 mA, 50 cy/sec, 10 msec current, the back arching, snarling and snorting occurred in addition to the pupil dilation and pilo-erection. Thus, selecting adequate parameters, the entire aggressive behavior pattern could be divided into elements. With maximum stimulation, no seperation of symptoms could be obtained since, immediately after switching-in of the stimulation, the animal violently rushed forward with extended claws, snorting and attacking all objects it met on its way. In such a moment, its pupils were maximally dilated, the pilo-erection and abundant salivation occurring simultaneously (Fig. 3). This attack could be termed as a ,,blind" rush since the cat's movements were violent, uncoordinated and oriented in all directions without avoiding any obstacles. After the stimulation had been switched-off, an after-effect was always observed. The animal stopped and took a taut position with an arched back, dilated pupils, bristled fur and bared teeth. The duration of this after-effect amounted to 0.5 to 3 min depending on the intensity of stimulation. It should be emphasized that the course and character of the reaction of aggression was identical in all points in-
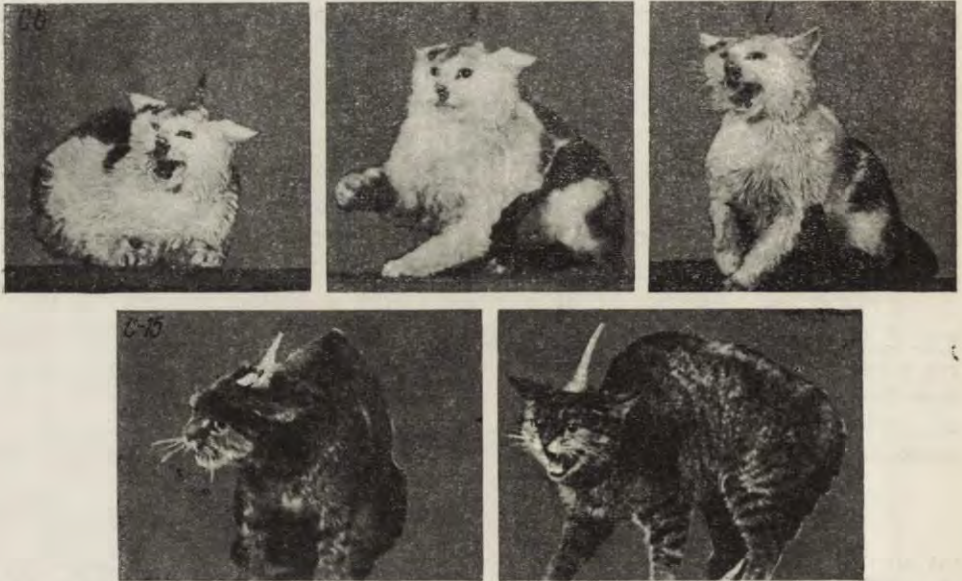
Fig. 3. Progressively increasing aggressive behavior patterns in cats C-6 and C-15 obtained by stimulating the ventral portions of the medial hypothalamus

vestigated which slightly differed from each other only in the excitability threshold. It was found that the excitability threshold of a given point was lower with the bipolar than with the monopolar stimulation.

Reaction of flight. This reaction was evoked by the stimulation of 73 points, located in the dorsal part of the anterior, central and posterior subdivisions of the medial hypothalamus, contained in the frontal planes Fr. 10.0 to 13.0 and situated 1 to 2 mm laterally to ventricle III (Fig. 1, 2). The following symptoms characterized the reaction of flight when optimal conditions of stimulation (0.5 mA, 50 cy/sec, 10 msec) were applied: pupil dilation, increase in the respiratory rate, acoustic effects in the form of shrill mewing, urination, sometimes also defecation, violent locomotor reaction, jumping on the walls of the cage and attempts to get out of it. The latter consisted in violent wedging the body in the cage door aperture or in the upper cover aperture. These attempts were continued all the time of the stimulation. If only the cage door was deliberately left open, the animal instantly took its chance and jumped out of the cage, escaping in panic (Fig. 4). When a less intensive stimulation (0.5 mA, 50 cy/sec, 1 msec) was applied, the behavior of the animal was slightly different, namely, there occurred the dilation of pupils, increase in the rate of respiration, mewing, but — instead of a typical reaction of flight — the cat retreated to the back wall, crouched on the floor and, in such a position, remained motionless till the end of the

Fig. 4. Flight reaction in cat C-7 produced by the stimulation of the dorsal portion of the medial hypothalamus

stimulation. When maximal stimuli were applied, the reaction of flight was chaotic, the animal rushed to and fro all over the cage, running its body againts the walls. After the stimulation was stopped, a rapid respiration, dilated pupils and mewing were still recorded for a certain time. After a few experimental sessions during which flight points were stimulated, the conditioning of the experimental situation was found. The animals started to defend themselves against placing them into the cage and, before experiments were started, they meved and restlessly paced the cage. It was found that the excitability threshold of a given point is lower with the bipolar than with the monopolar stimulation.

The remaining reactions. The stimulation of the hypothalamic points, situated in the frontal planes Fr. 8.5 to 9.5 and Fr. 13.5 did not cause any changes, or produce effects different from the reactions of aggression and flight. The orientation reaction, that is, turns of the head, smelling about, licking lips and closing eyes was observed after the stimulation of 38 points. The remaining 12 points gave no response at all.

DISCUSSION

It has been shown by the results of this experiment that the stimulation of the medial hypothalamus evokes the reactions of aggression and flight with their autonomic and motor component symptoms and

that they are similar to the reactions, obtained by other authors who stimulated various points of the hypothalamus (H e s s 1949, H u n s- p e r g e r 1956, N a k a o 1958, and others). The reactions of both the aggression and flight, evoked by the electric stimulation of the hypothal- amus were identical with those of an intact animal. The present results fully confirm our previous suggestion (R o m a n i u k 1962) that the hypothalamic ,,aggression center" is located in the ventral part of the medial hypothalamus, while the ,,flight center" is located in the dorsal part of the medial hypothalamus. Both ,,centers" are situated in the area of the anterior, central and posterior subdivisions of the medial hypothalamus, contained in the frontal planes between Fr. 10.0 and 13.0.

The results of the present experiment suggest that the discrepancies met with in the literature may be due to the fact that in the earlier in- vestigations on the relationship between the hypothalamus and emo- tional behavior, the location of the points, associated with emotional reactions, was considered basically in anteroposterior and not in dorso- ventral aspect. Thus, from the illustrations presented in the papers by H e s s (1949) and H u n s p e r g e r (1956), it becomes evident that the reaction of flight was produced by stimulating the dorsal portion of the posterior hypothalamus, while the ventral portion of the posterior hypo- thalamus was not explored at all. From the illustrations represented in the papers by N a k a o (1958) and Y a s u k o c h i (1960) it is clear that the reaction of aggression was produced basically by stimulating the ventral and central portions of the posterior hypothalamus. On the other hand, the dorsal portion of the posterior hypothalamus appears less explored. It is also possible that some of these discrepancies result from the application of different techniques and parameters of electrical stimulation. Thus, D e l g a d o and A n a n d (1953) applied different parameters and techniques from those of H e s s (1949), stimulating regions from which the reaction of rage was obtained by H e s s, they could not repeat his result but, on the contrary, they produced a general restlessness and vocalization.

Our experiments have shown that the stimulation of the ,,aggression points" always evokes the reaction of aggression and the reaction of flight occurs always as a response to the stimulation of the ,,flight points'.' The stimulation of a given point was never followed by both the aggres- sion and flight. When, with the threshold parameters, the reaction of aggression occurred, the increase in the amplitude, frequency or dura- tion of stimulation did not cause a change in the character of the reaction. It continued to be an aggression and it was only the latency and intensity of reaction that changed. Similar results were obtained with regard to

the reaction of flight. A combination of aggression and flight in terms of a generalized defensive reaction, was observed occasionally when a very strong stimulation was used and this occurred only when the electrodes were located on the boundary of the ventral and dorsal subdivision of the medial hypothalamus. These results are contradictory to the results of Hess (1949) and Hunsperger (1956) who maintained that an increase in the intensity of stimulating the „aggression point" is associated with the reaction of flight and an increase in the intensity of stimulating the „flight point" is associated with the reaction of aggression. Hess (1949) and Hunsperger (1956) also found the passage of the aggression in flight or vice-versa, caused by the prolonged stimulation of a given point. These contradictions seem to originate from a fact that, in the experiments of Hess (1949) and Hunsperger (1956), a relatively strong stimulation was applied. The electrodes used were thick which interfered with an exact location of the stimulation. With such a technique, a wide area is likely to be covered by the range of stimulation, causing the stimulation of the points, situated outside the zone of the electrode tip. This is supported by the most recent result of Kozlovskaia (1964) who obtained a development of the reaction of aggression into the reaction of flight only under conditions of a very strong stimulation. It is worth mentioning that Wasman and Flynn (1962) found the passage of the reaction of aggression into the reaction of flight in one cat out of 14.

The emotional reactions, described in this paper, were typical of the defensive behavior, noted in intact animals. It appears reasonable to believe that both the reaction of aggression and the reaction of flight are components of defense-type behavior: the reaction of flight might be considered a passive defensive reaction in a sense that an animal defends itself avoiding a harmful situation, while the reaction of aggression might be considered an active defensive reaction in a sense, that an animal defends itself by attacking.

SUMMARY

(1) Defensive reactions related to both the aggression and flight are evoked by the electrical stimulation of the anterior, central and posterior subdivisions of the medial hypothalamus.

(2) The reaction of aggression is obtained by the stimulation of the ventral part of the medial hypothalamus and the reaction of flight by the stimulation of the dorsal part of the medial hypothalamus.

(3) The discrepancies in the data of the present investigation and those reported by other authors, as to the location of these reactions

in the hypothalamus are probably due to the application of different experimental techniques and parameters of stimulation and to the fact that earlier workers considered the location of the points, associated with emotional reactions in anteroposterior rather than in dorsoventral aspect.

REFERENCES

BARD P. 1928 — A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system. Am. J. Physiol., *84*, 490.

CANNON W. B. and BRITTON S. W. 1925 — Studies on the conditions of activity in endocrine glands: pseudoaffective medulliadrenal secretion. Am. J. Physiol., *72*, 283.

DELGADO J. M. R. 1955 — Cerebral structures involved in transmission and elaboration of noxious stimulation. J. Neurophysiol., *18*, 261.

DELGADO J. M. R. and ANAND B. K. 1953 — Increase of food intake induced by electrical stimulation of lateral hypothalamus. Am. J. Physiol., *172*, 162.

HESS W. R. 1949 — Das Zwischenhirn: Syndrome, Lokalisationen, Funktionen. Basel: Benno Schwabe.

HESS W. R. and BRÜGGER M. 1943 — Das subcorticale Zentrum der affectiven Abwehrreaktion. Helv. physiol, acta, *1*, 33.

HUNSPERGER R. W. 1956 — Affektreaktionen auf elektrische Reizung im Hirnstamm der Katze. Helv. physiol. acta, *14*, 70.

JASPER H. H. and AJMONE — MARSAN C. 1954 — A stereotaxic atlas of the diencephalon of the cat. Ottawa: The National Research of Canada.

KOZLOVSKAIA M. M. 1964 — Structural and functional organization of responses of a defensive type evoked by hypothalamic stimulation (in Russian). Fiziol. Zhurn., *50*, 1218.

NAKAO H. 1958 — Emotional behavior produced by hypothalamic stimulation. Am. J. Physiol., *194*, 411.

PAPEZ J. W. 1937 — A proposed mechanism of emotion. Arch. Neurol. Psychiat., *38*, 725.

ROMANIUK A. 1962 — The effects of lesions of the medial hypothalamus on the conditioned reflexes type II and emotional behaviour. Acta Biol. Exper. (Warsaw), *22*, 59.

ROMANIUK A. 1963 — Emotional responses evoked by hypothalamic stimulation in cats. Bull. Acad. Pol. Sci., sér. sci. biol., *11*, 437.

WASMAN M. and FLYNN J. P. 1962 — Directed attack elicited from hypothalamus. Arch. Neurol., *6*, 220.

WHEATLEY M. D. 1944 — The hypothalamus and affective behaviour in cats. Arch. Neurol. Psychiat., *52*, 296.

YASUKOCHI G. 1960 — Emotional responses elicited by electrical stimulation of the hypothalamus in cat. Folia psychiatr. neurol. jap., *14*, 260.

STUDIES ON THE VISUAL FIXATION REFLEX
I. GENERAL PROPERTIES OF THE ORIENTATION FIXATION REFLEX IN PRETRIGEMINAL AND INTACT CATS[1]

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The fixation reflex consists in bringing the images of an object from periphery to the areae centrales of both eyes and maintaining the eyes in this position for some time. This reflex may be evoked either by the so called „indifferent" visual stimulus and then it is a component of the Pavlovian orientation reflex („orientation fixation reflex"), or by the conditioned visual stimulus („conditioned fixation reflex"). The latter is also called the „voluntary eye movement".

The conditioned fixation reflex was extensively investigated on human beings (cf. W o o d w o r t h and S c h l o s b e r g 1958, A l p e r n 1962). On the other hand, our information on the orientation fixation reflex is very scanty: according to the authors' knowledge this reflex was only preliminarily investigated by B e r k s o n and F i t z-G e r a l d (1963) in infant chimpanzees, and by V o r o n i n and S o k o l o v (1960) in human beings. Therefore in the present paper the orientation fixation reflex was submitted to the careful experimental analysis.

The problem of the experimental animal arose. As it is well known, the fixation reflex is absent in the anaesthetized preparation. On the other hand, in the awake animal observation of the eye movements is difficult. The study of the orientation fixation reflex in human beings

is obviously also inconvenient. However, it was recently shown by several authors (B a t i n i et al. 1959, A f f a n n i, M a r c h i a f a v a and Ż e r n i c k i 1962a, K i n g and M a r c h i a f a v a 1963) that the vertical fixation reflex is preserved in the rostral part of cats with midpontine pretrigeminal transection; the presence of this reflex may be considered as a sign that the isolated cerebrum of the pretrigeminal animal is in a good condition (Ż e r n i c k i 1964). In that preparation investigation of the fixation reflex is easier that in an intact animal. First, due to the immobilization of the head the movements of the eyeballs may be precisely recorded, and the fixation reflex is enhanced (the synergic movement of the head is eliminated). Furthermore, the eye movements are produced by visual stimuli only (the olfactory stimuli are excluded by a trachaeal cannula). Finally, the optokinetic reflex is eliminated (K i n g and M a r c h i a f a v a 1963). In the present paper therefore, the pretrigeminal cat was used in the study of the orientation fixation reflex. However, as a necessary control, some experiments were performed also on intact cats.

The objects used for elicitation of the fixation reflex had basically the steady position in the visual field of the animal; the only possible movement of the objects was the rotatory one. The investigation of the orientation fixation reflex to the objects changing their position will be reported elsewhere (Żernicki and D r e h e r, in preparation). The findings on the neural mechanism of the fixation reflex are described in a separate paper (D r e h e r, M a r c h i a f a v a and Ż e r n i c k i 1965)

MATERIAL AND METHODS

Experiments on pretrigeminal cats. Acute and semichronic (survival of two days) preparations were used. A brain stem transection was performed under ether anaesthesia. A spatula oriented stereotaxically 60° from the horizontal plane was used. In order to avoid brain oedema, the middle part of the cerebellum was removed by suction immediately after the transection. The nictitating membranes and the upper eyelids were partially removed in order to allow better observation of the eyeballs.

The experiments were performed in a small, optically isolated chamber. The animal remained in a Horsley-Clarke holder designed so that the visual field remained unobstructed. The chamber was illuminated by a strong diffused light in order to allow filming (the illumination of the eyes was 650 luxes). A white screen was placed 120 mm in front of the animal's eyes and in some experiments 320 mm. On the screen, in the saggital plane of the animal, two black X shaped figures were located (Fig. 1). One figure was 25° above the horizontal plane through the nodal point of the eye (upper figure X) and the other 25° belw this level (lower figure X)[2]. The angular size of the figures was either 12° (large

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[2] The nodal point of the cat's eye is 9,9 mm above the basihorizontal plane (B i s h o p, K o z a k and V a k k u r 1962).
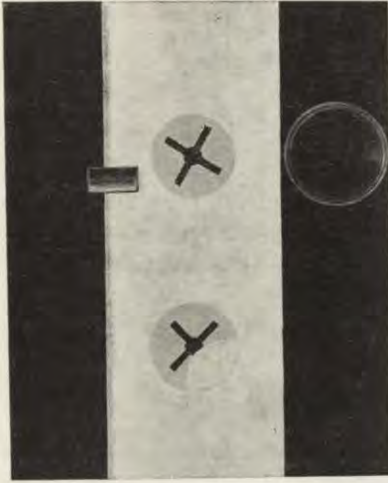
Fig. 1. The screen with the figures X. The lower figure X is partially covered by the board. On the left is an aperture for direct observation; on the right is the tube of the film camera. The lateral parts of the screen are black to eliminate the asymetry in brightness of the screen produced by the black camera

figures X) or 3° (small figures X). The figures could be covered or uncovered by means of small paper boards (see Fig. 1. below). In some experiments the figures X were replaced by the neon lamps of diameter 6°. For evoking the fixation reflex the following stimuli were used: i) the rotation of a permanently exposed figure X at a rate of 1 cycle/sec, ii) the temporary uncovering of the stationary or rotating figure X, and iii) the continuous or intermittent, at rate 1/sec, lighting of the neon lamp. The duration of the stimulus was usually 2—4 sec, and in some cases 10—20 sec.

The eye movements and the EEG activity were recorded by an inkwriter (the EOG record was taken by an A.C. channel and the course of the eye movement was distorted). Beside this the ocular behaviour was filmed and the movement of either the centre of the pupil or the ink spot on the cornea was analysed. A point visible on the stereotaxic head holder served as a reference. Generally the film camera was located in front of the eyes but for control in some experiments the eyes were filmed from the side and the calculation of the eye movements was preceded by establishing the location of the centre of rotation of the eye[3]. The rate of filming was usually six and sometimes 24 frames per second.

The nursing care of our preparations was limited to a maintenance of the body temperature within 37°—39°C, sporadic injections of 5 per cent glucose and protecting the eyes from drying. In semichronic experiments antibiotics were administered and the urinary bladder was manually evacuated.

Experiments on intact cats. Under ether anaesthesia the head of the animal was attached in the holder. After recovery from the anaesthesia the cats were located in the experimental chamber in the same position as the pretrigeminal preparations. The rotation of the permanently exposed large figure X was used for evoking the fixation reflex. The murmur accompanying the visual stimulus was masked by the continuous sound of the buzzer. The eyes of the cats were observed directly.

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[3] In the pretrigeminal cat the centre of rotation is located on the average 10 mm behind the cornea (Dreher, Kozak and Żernicki, in preparation).

RESULTS

I. Experiments on pretrigeminal cats

The time-course of the fixation reflex. The amplitude and the duration of the fixation reflex varied considerably and depended on several factors (cf. next Section). The fixation reflex consisted either of one fixation or of more than one fixation in a series. The former was called the ,,single fixation reflex" and the latter, observed in about 20 per cent of preparations, the ,,serial fixation reflex". In the single fixation reflex, the fixation could be either complete or abortive: we distinguished, therefore, the ,,full single fixation reflex" and the ,,abortive single fixation reflex" respectively (Fig. 2). In the serial fixation reflex at least the first fixation was a full one.
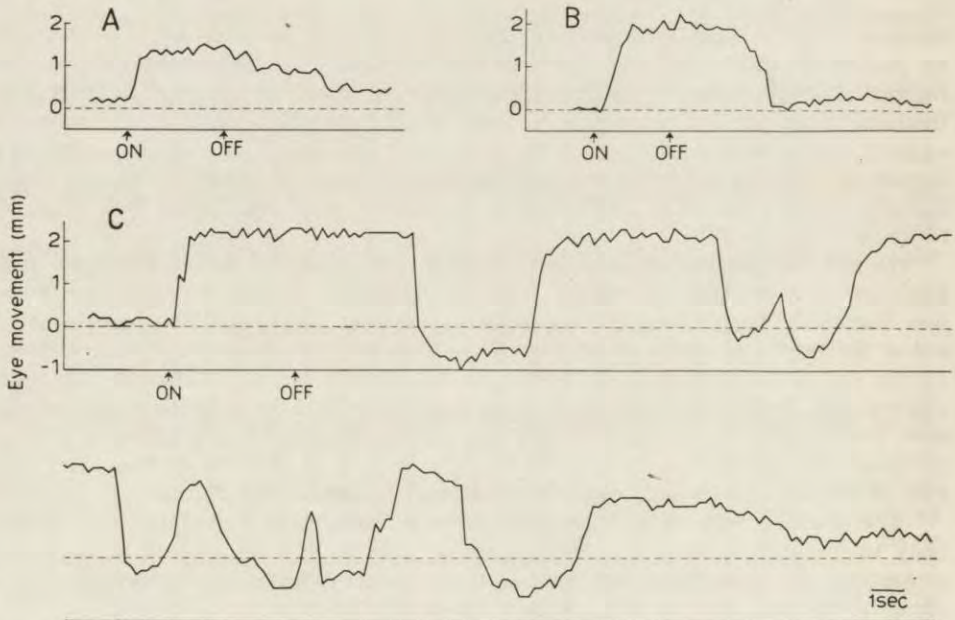
Fig. 2. Fixation reflexes in response to rotation of the large upper figure X. A, an abortive single fixation reflex. B, a full single fixation reflex. C, a serial reflex. The eyes were filmed from the front and the movement of the centre of the pupil analysed. 1 mm corresponded approximately with 10° of rotation of the eyeball. The position of the eyeballs before application of any stimuli (the position of rest) was accepted as the zero level

I. The full single fixation reflex. The latency of the reflex varied considerably and was usually between 130 and 180 msec. The time-

-course of the reflex may be divided into four phases: 1) the eye movement towards the object, 2) the maintenance of the fixation, 3) the return movement, and 4) the „tonic fixation".

Phase 1. The eye movement towards the stimulus consisted with either one or several saccadic movements, and lasted from about 120 msec. to 1 sec.

Phase 2. The fixation was usually maintained for several seconds independently of the duration of the stimulus (see next Section).

Phase 3. The time-course of the return movement was similar to that of the movement towards the object, but the return movement was usually incomplete and sometimes excessive (Fig. 3B).

Phase 4. When the return movement was incomplete, which was usually the case, the residual inclination of the eyeballs was gradually decreased and could be usually detected for as long as a few minutes; this inclination was called the tonic fixation (Fig. 2B and 4B)[4]. When the return movement overshoot the original position, the eyeballs were usually inclined temporally in the direction away from the stimulus source.

II. The abortive single fixation reflex. In this reflex all the above described phases could also be distinguished. However, some differences in the time-course of the abortive single fixation reflex in comparison with that of the full one may be noted: i) the average velocity of the movement towards the stimulus was usually slower, ii) the phase of maintenance of the fixation was usually shorter, iii) the return movement was usually also slower, iv) the tonic fixation was stronger, and v) the „off effect" of the stimulus was sometimes observed: when the termination of the stimulus took place during the phase of maintenance, a return movement could be observed, and when the stimulus was terminated during the return movement a transient increase of the amplitude of the fixation could appear. On the other hand, it is worth noting that the latency of the abortive fixation reflex was similar to that in the full one.

III. The serial fixation reflex. The number of the successive fixations varied in different reflexex from 2 to 30; the rate of their appearance usually gradually diminished. The first fixations were always full, but

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[4] The term „tonic fixation" corresponds to the term „tonic form of the orientation reflex" introduced by S o k o l o v (1958) for denoting the long shift in the galvanic skin response and EEG activity. On the other hand, the „tonic EEG arousal" of S h a r p l e s s and J a s p e r (1956) seems to have a different meaning (see also B e r l y n e 1960), and may be compared rather to the long reaction observed by us in the serial fixation reflex.

in the end of the reflex the abortive ones could also be observed. The time-course of the full fixation in the serial reflex was basically similar to that in the full single reflex. However, the return movement was always rapid (consisting of one or a few saccadic movements) and excessive; before the next fixation the eyes were inclined temporarily in the direction away from the stimulus source. The time-course of the abortive fixation in the serial reflex was also similar to that in the abortive single reflex. After the last fixation the tonic fixation usually developed.

It is worth noting that in the course of the fixation reflex the eyes performed small (from 1° to 3°) irregular movements. Our graphs show that in the „position of rest" the eyeballs were also not completely still, but then the movements were clearly smaller and they appeared with less frequency.

The factors influencing the intensity of the fixation reflex. Several factors may be enumerated: i) the individual properties of a preparation, ii) the physical properties of the stimulus, iii) the duration of the stimulus, and iv) the number of the previous presentations of the stimulus (see p. 200).

i) It is well known (Ż e r n i c k i 1964) that in pretrigeminal preparations there are strong individual differences in behavioural responsiveness, and the latter may also vary in a given preparation. Actually in response to the same stimulus we could observe different types of fixation reflex.

ii) The uncovering of a large rotating figure X was the strongest stimulus and it usually evoked either the full single fixation reflex (Fig. 3B) or the serial one. A similar but clearly weaker effect was usually produced by the rotation of a permanently exposed large figure X. The uncovering of the large stationary figure X (Fig. 3A), the uncovering of either rotatory or stationary small figure X, and the intermittent or continuous light of the neon lamp produced the abortive fixation reflexes; the last stimulus often did not evoke fixation reflex at all.

iii) The fixation reflex was only slightly enhanced by the prolongation of the stimulus (Fig. 4). The phase of the maintenance of the fixation could be then a little prolonged and the amplitude of the tonic fixation increased. When stimulus was short (1—4 sec), the fixation lasted usually longer than the stimulus itself (Fig. 2B, 3B, 4A), and when the stimulus was longer (10—20 sec), the fixation usually terminated before the end of the stimulus (Fig. 4B). It is worth noting that the serial fixation reflex could be evoked by both long and short stimulus.
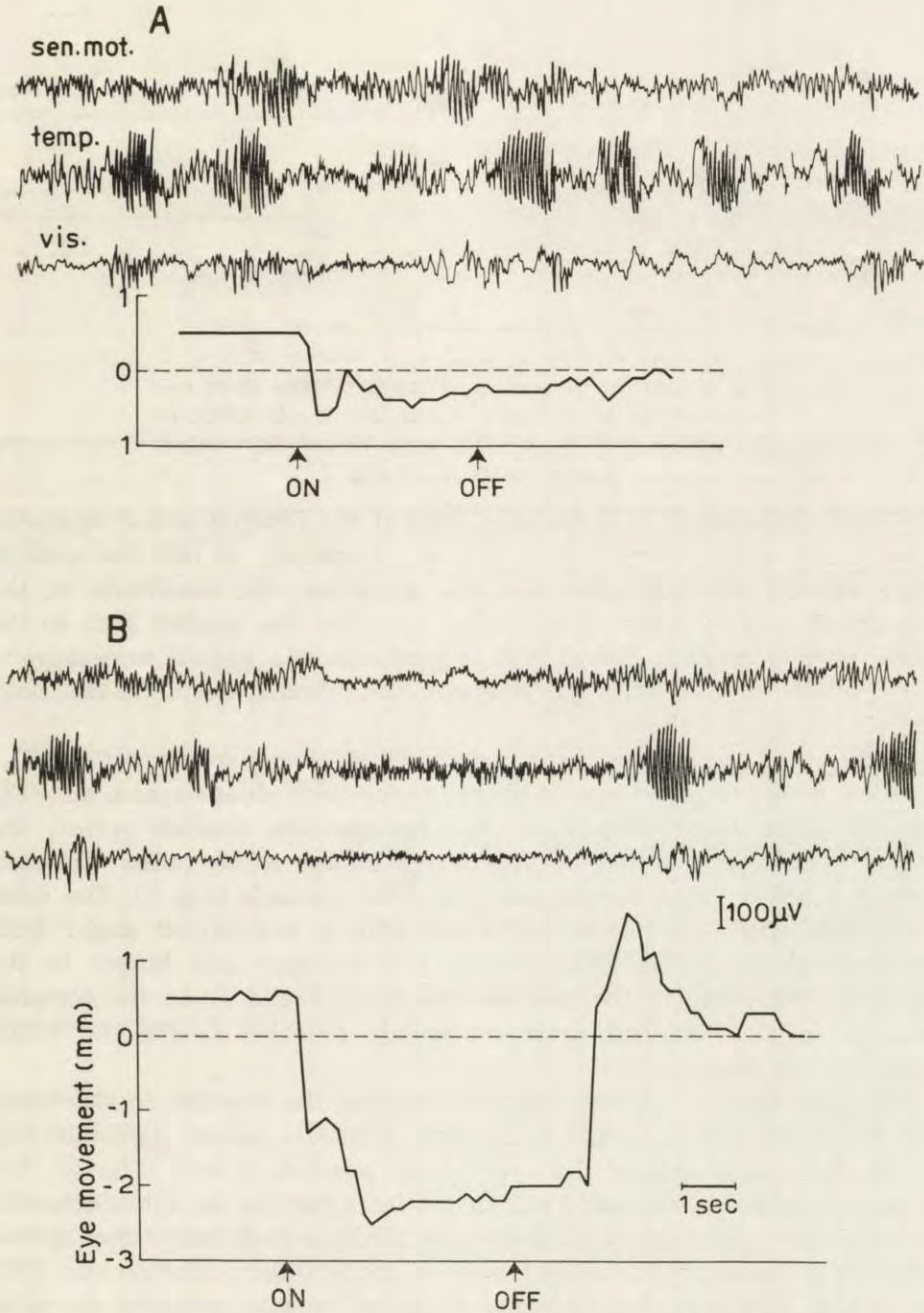
Fig. 3. Fixation reflexes in response to the uncovering of the large figure X, either while stationary (A) or, 2 min after, while rotating (B). Other explanations as in Fig. 2
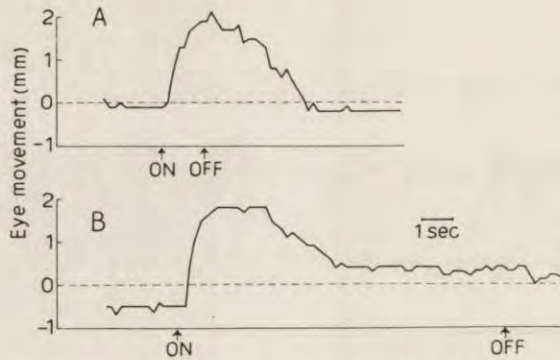
Fig. 4. Fixation reflexes in response to the short rotation of the large figure X (A) and, 2 min after, to the longer rotation of the same figure (B). Other explanations as in Fig. 2

It should be noted that the amplitude of the fixation reflex depended also on the position of rest of the eyeballs. Generally, at rest the eyeballs were slightly inclined upwardly and, therefore, the amplitude of the full fixation in response to the upper stimulus was smaller than to the lower one. It was also found that in some cats the stimuli were beyond the fixation field of the preparation, and consequently evoked only abortive fixation reflexes.

The unspecific responses associated with the fixation reflex. The fixation reflex was accompanied by a pupillary dilation and an EEG arousal (Figs. 3, 5A, 6 and 7). The stronger the fixation reflex, the stronger were the unspecific reactions[5]. In the serial reflex the successive fixations were accompanied by EEG arousals (Fig. 7). The tonic fixation seemed also to be associated with a maintained slight EEG desynchronization. The EEG arousal was stronger and longer in the occipital leads than in the frontal ones (Figs. 3 and 7); in the occipital records the EEG arousal could be usually detected during the whole period of fixation.

In some control experiments we compared the reaction to the upper and lower stimulus with that to the same stimulus located approximately within the visual axis of the eyes in the position of rest (Fig. 5). The fixation reflex was obviously eliminated in respect to the latter stimulus although the up and down movements of the eyes could sometimes appear after its termination (Fig. 5B). However, the pupillary dilation and EEG arousal to this stimulus appeared to be of similar intensity as those produced by the upper or lower stimulus. These unspecific reactions,

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[5] Under our experimental conditions small changes in the pupillary diameter could not be accurately recorded during the eyeball movements.

temp. **A**

EOG

stimulus

B

$\llcorner$____$\lrcorner$ 100 µV
1 sec
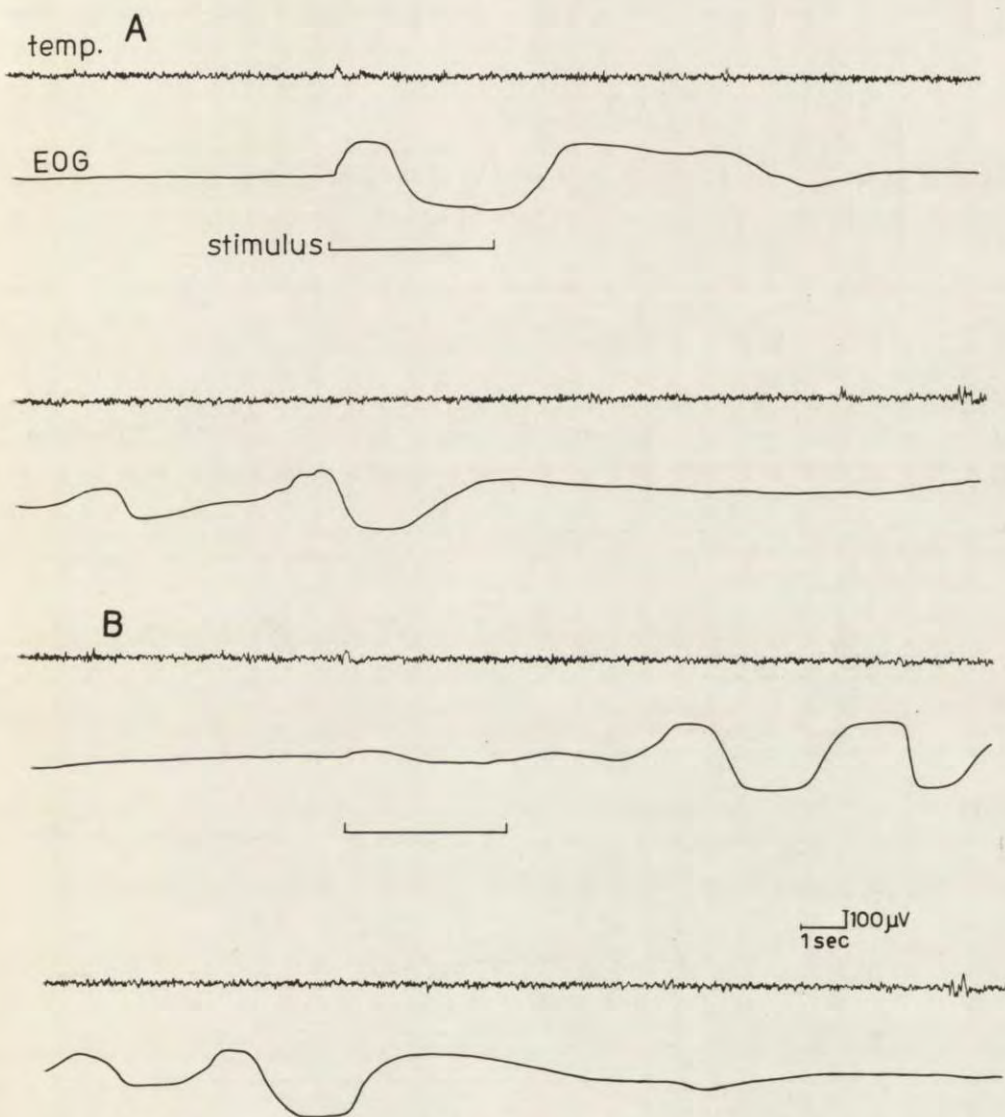
Fig. 5. The fixation reflexes to the rotation of the upper large figure X (A) and, 2 min after, the response to the same stimulus located approximately within the visual axis of the preparation (B)

therefore, seem to be essentially produced not by the stimulation of the proprioceptors in the extraocular muscles, but by the visual stimulus itself. The stronger and the longer the fixation reflex, the ,,better" and longer was the object seen and the greater the arousal of the animal.
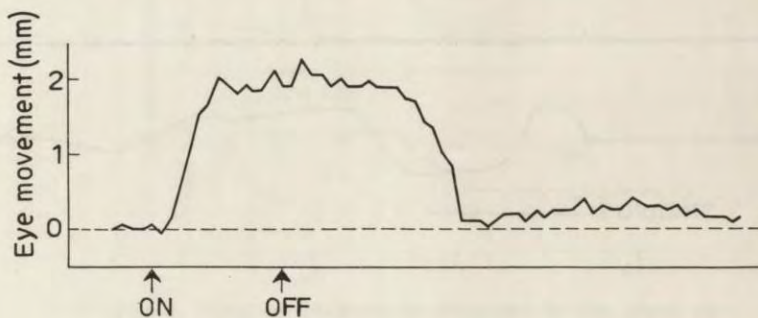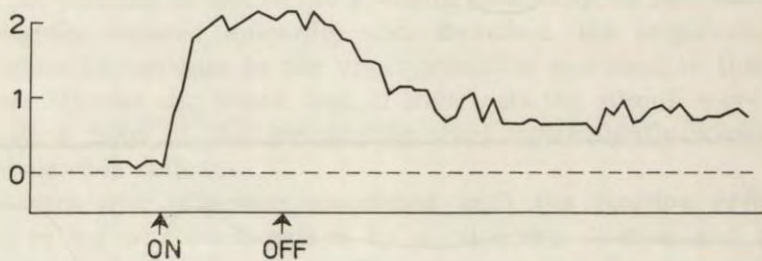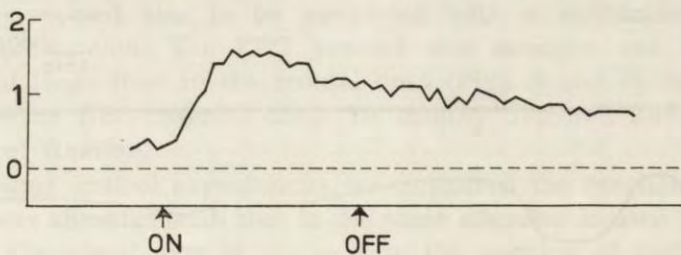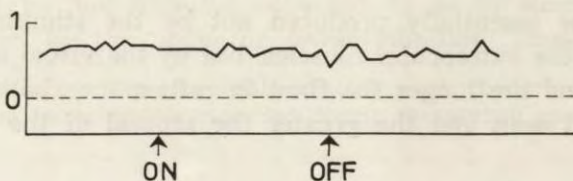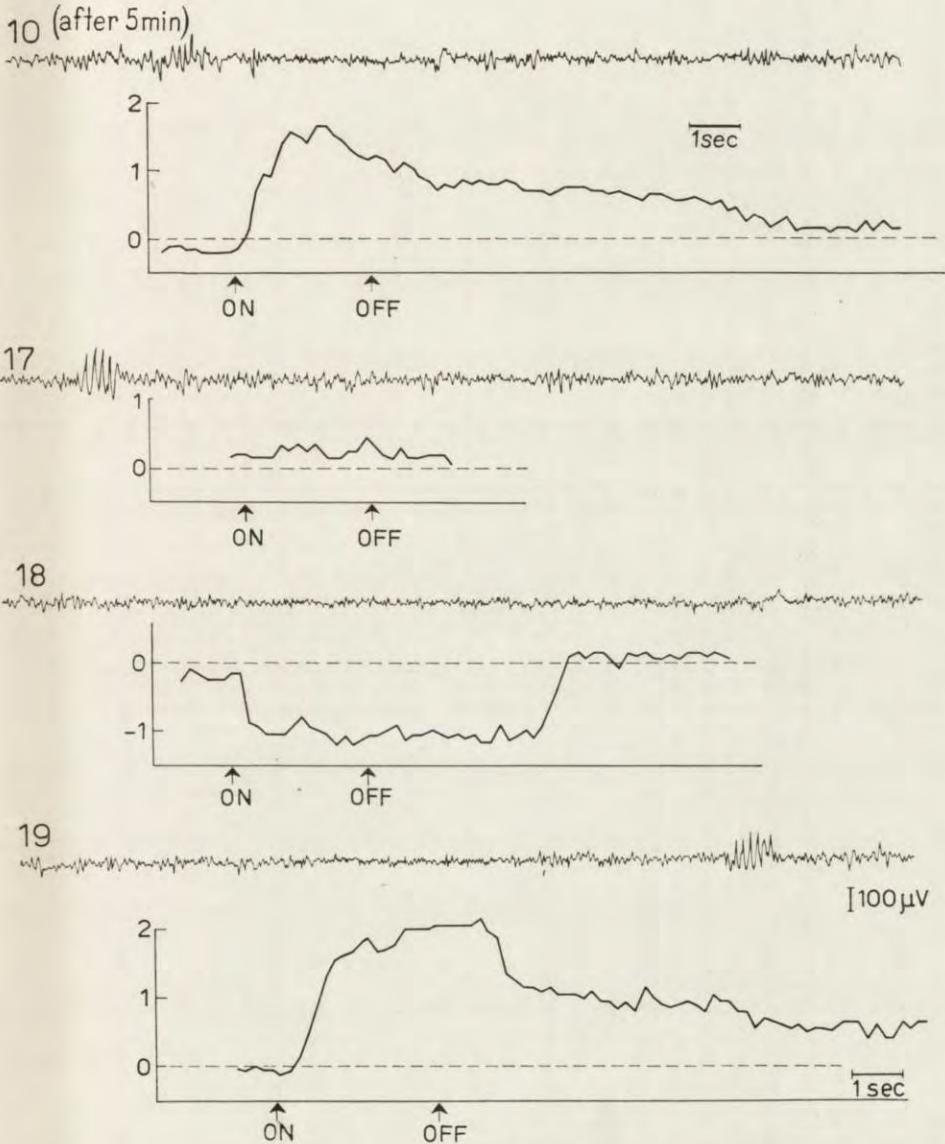
Fig. 6. Habituation of the full single fixation reflex to the rotation of the upper large figure X. On the left, the successive number of the trial. Intertrial intervals 30 sec. Note, that the habituation of the EEG arousal had similar course to that of the habituation of the fixation reflex. Other explanations as in Fig. 2

front. par.

par. occ.

EOG

stimulus

1
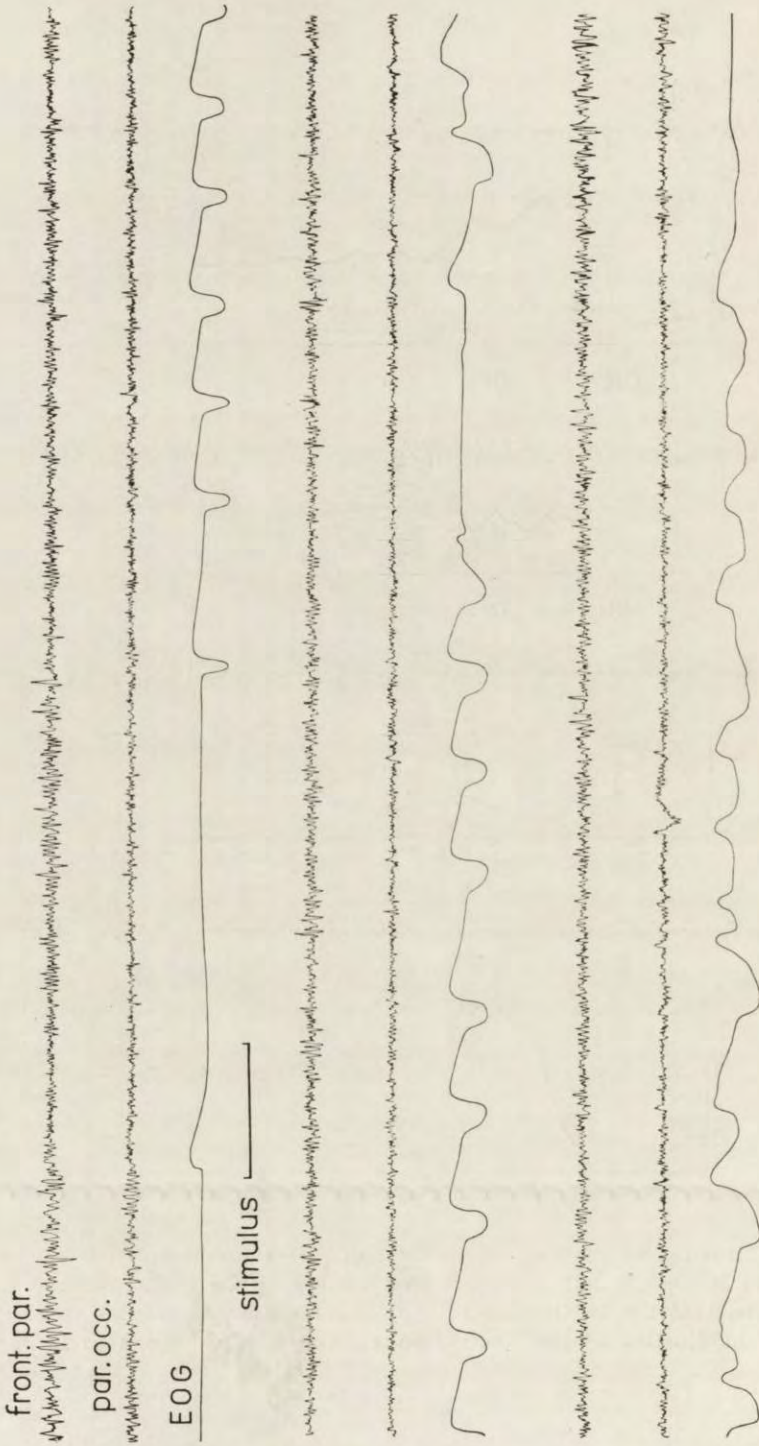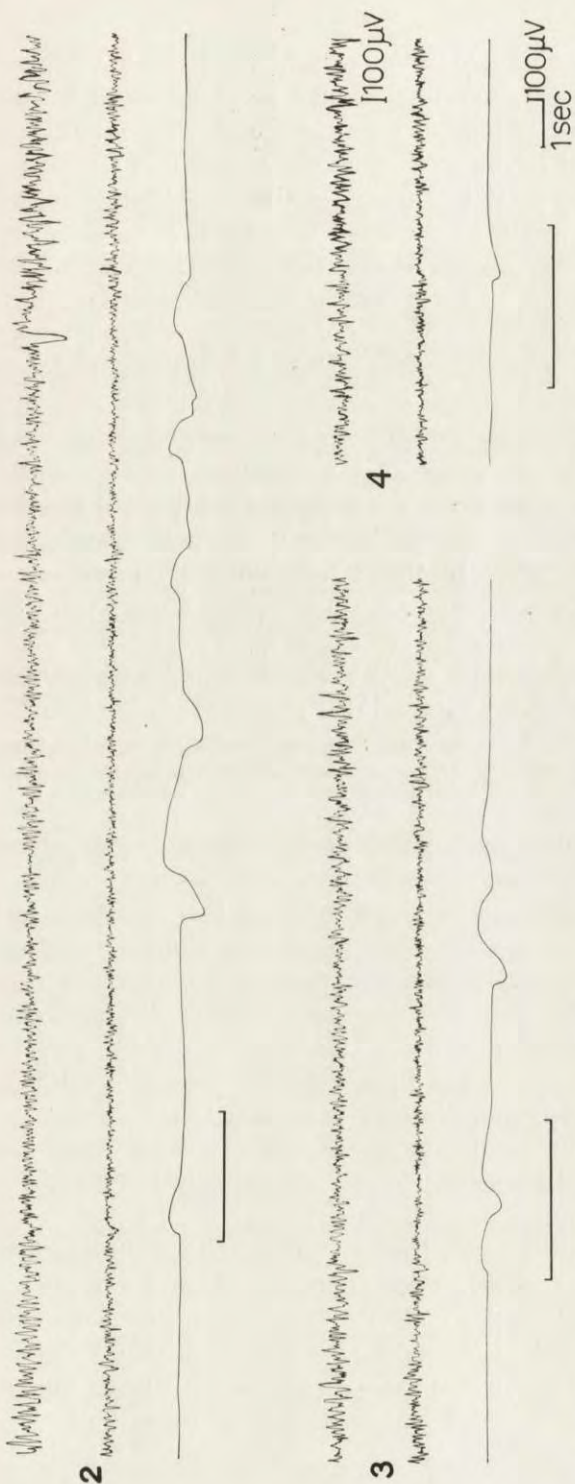
Fig. 7. Habituation of the serial fixation reflex to the rotation of the upper large figure X. On the left, the successive number of the trial. The first interval was 3 min and the next intervals were 1 min. Note the rapid decrease in the number of the following fixations in the course of habituation

Effect of repetition of the stimulus.

1. Habituation of the fixation reflex. When the stimulus was applied repeatedly at short intervals (from 15 sec to 3 min), the duration and the amplitude of the fixation reflex diminished gradually (Fig. 6, trials 1—9); in the serial reflex first of all the number of the fixations quickly diminished (Fig. 7). It is worth noting that the diminishing of the reflex was not accompanied by a clear prolongation of its latency. As will be shown below the diminution of the fixation reflex had an enduring character and, therefore, it could be due neither to fatigue nor to sensory adaptation. Thus it should be considered as a result of habituation.

The resistance of the fixation reflex to habituation depended on several factors. First of all, the stronger fixation reflex was more resistant to habituation. Furthermore, the shorter were the intertrial intervals, the quicker was the decrease of the response. Finally, there were considerable individual differences: a complete habituation of similar fixation reflexes evoked with the same intervals could be obtained after a few trials in some cats, while in others not earlier than after about 50 trials.

In the fully habituated state, a pause of 5 to 10 min. resulted in a partial recovery of the fixation reflex (Fig. 6, trial 10). The recovered reaction could be, however, quickly rehabituated with further repetition of the stimulus at short intervals (trial 17). A dishabituation of the fixation reflex could be often evoked by a single application of an extraneous stimulus, as e.g. the rotation of the opposite figure X (trial 18 and 19).

If after a complete habituation of the reaction a rest of one hour was allowed, the spontaneous recovery of the fixation reflex was always strong. However, the responses then were less resistant to habituation than during the first session. When several sessions of habituation following each other at 1-hour intervals were conducted, the fixation reflex recovered less and less and in some cats it disappeared completely (chronic habituation), (Fig. 8). At the same time, the fixation reflex to the rotation of the opposite figure X was preserved although it could be also diminished (generalization of habituation).

The habituation of the fixation reflex was accompanied by habituation of the pupillary dilation and EEG arousal. There was a striking similarity in the course of the habituation of the fixation reflex and EEG arousal. On the other hand, in the first trials the diminution of the pupillary dilation was clearly stronger than that of the fixation reflex.

2. „Summation" of the tonic fixation. Simultaneously with the acute habituation of the fixation reflex the tonic fixation became larger and larger (Fig. 1, trial 1—6). The increase was relatively strong and could not be the result only of the above mentioned stronger tonic fixation in the

I

EOG

stimulus

$]100\mu V$
1sec

IV   **VII**
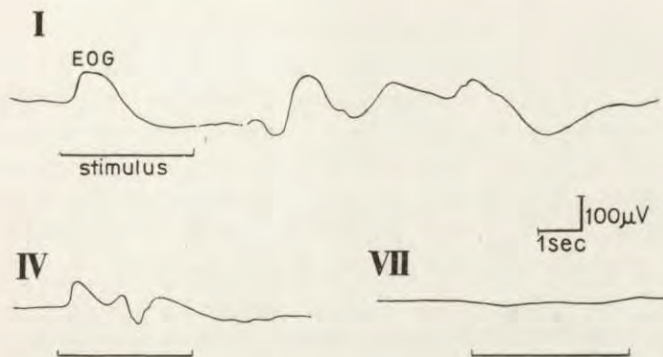
Fig. 8. Chronic habituation of the serial fixation reflex
to the rotation of the upper large figure X. The reactions
in the first trials of the Ist, IVth and VIIth sessions are
shown. In every session the stimulus was repeated 20 times.
The intersessional intervals were 1 hour

abortive fixation reflexes. This gradual increase was called the ,,sum-
mation" of the tonic fixation. Because the tonic fixation usually conti-
nued to occur when the next stimulus was applied, due to the summation
of the tonic fixation the diminution of the amplitude of the fixation re-
flex produced by habituation could be partially compensated.

II. Experiments on intact cats

Although in intact cats the observation of the eyeballs could not be
precise, there was little doubt that the general time-course of the fixation
reflex was similar to that in the pretrigeminal preparation. However, in
intact cats the individual differences in the intensity of the fixation
reflex were less pronounced and the rotation of figure X usually pro-
duced the full single fixation reflex: the serial reflex could be evoked
only by a long-lasting stimulus and the fixations appeared only during
its duration. As in the pretrigeminal cat, the fixation reflex was accom-
panied by the pupillary dilation.

The course of habituation of the fixation reflex seemed to be also
similar to that in the pretrigeminal cat. Simultaneously with the diminu-
tion of the amplitude and shortening of the fixation reflex the summation
of the tonic fixation was observed. The spontaneous recovery of the
reflex, a rapid rehabituation and chronic habituation could be also ob-
served.

DISCUSSION

*The similarity of the fixation reflex in the pretrigeminal and intact
cat.* Our results indicate that the fixation reflex is similar in the intact

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and pretrygeminal cat but its intensity shows stronger individual variation in the pretrigeminal preparation. This variability is obviously not the result of the disturbance in some pretrigeminal cats of the mechanism of the fixation reflex itself, because similar variability show also pupillary and EEG responses to the stimulus located within the visual axis of the preparation. The individual variation in the behavioural responsiveness of the pretrigeminal cat seems to be mainly the result of the slight differences in the level of the transection.

The similarity between the fixation reflex in the pretrigeminal and intact cat is quite understandable because there is much evidence to indicate that the functions of the isolated cerebrum of the pretrigeminal cat are basically retained (A f f a n n i et al. 1962ab, Ż e r n i c k i and O s e t o w s k a 1963, Ż e r n i c k i 1964). This similarity has practical value suggesting that the data on the fixation reflex obtained in the pretrigeminal cat are fundamentally also valid for an intact animal.

The time-course and the mechanism of the fixation reflex. Our results have clearly shown that in the fixation reflex the role of the retinal feedback is limited. Actually the duration of the fixation reflex depends only slightly on the duration of the stimulus and the ,,off effect" of the reflex is usually absent. Furthermore, the phase of the maintenance is also present in the abortive fixation reflex. Finally, the tonic fixation develops in every kind of the fixation reflex. Therefore we may conclude that the fixation reflex of a given pattern is triggered by the onset of the stimulus and later on it develops to same extent independently of the stimulus.

Some changes in the visual field during the fixation reflex are worth mentioning. First of all, due to the eye movements towards the object and those in the opposite direction, the territory of the external world controlled visually by the animal is considerably enlarged. Furthermore, as a result of the tonic fixation the part of the external world associated with the previously applied stimulus is controled better than the opposite one.

The role of the physical properties of the stimulus. Our data showing that the angular size of the object and its motion (rotation) plays an essential role in the intensity of the fixation reflex are in agreement with numerous observations concerning different reflexes. The finding, however, that the temporary uncovering of the rotating figure X produces a stronger effect than the rotation of the permanently exposed figure X is less obvious. On the one hand, the former stimulus may be considered as a stronger one because it consists of several components (the movement of the board, the sight of the figure X itself and its rotation).

On the other hand, however, although the latter stimulus consists in the rotation of the figure X only, the figure X remains after the termination of the stimulus in the visual field of the animal and may play some role in the further development of the reflex.

The problem of the small (from 1° to 3°) eye movements. It is well known that, when the image of the fixation point is not on the central area of the retina, the eyeballs are not motionless. Therefore, the small movements of the eyes observed by us in the „position of rest" and during the abortive fixation are quite understandable. However, an explanation is needed for the small eye movements, which were observed during the full fixation. First of all, it should be noted that these movements were obviously not the so called „physiological nystagmus", which has a much smaller amplitude and which could not be demonstrated with our technique. It may be recalled that under our experimental conditions the full fixation could be evoked only by the large figure X and the small eye movements were probably a result of a changing of the fixation point within this object. Another explanation of the movements during the full fixation may be that the image of the object is not brought precisely on the area centralis due to the absence of the convergence of the eyes in the pretrigeminal cat. However, the convergence of the eyes does not seem to play a major role in the mechanism of acute vision in the cat, as shown by the presence of a rather poor convergence in the intact cat.

The comparison of the time-course in the orientation and conditioned fixation reflex. This comparison is difficult because the investigators of the conditioned fixation reflex were not interested in its general time-course but in some particular problems such as the time-course of the saccadic movement or physiological nystagmus. However, it may be noted that the latency of the reflex and the velocity of the saccadic movement is comparable in the orientation and conditioned fixation reflex (Westheimer 1954, Yarbus 1956, Hyde 1959, Robinson 1964).

SUMMARY

1. The orientation fixation reflex of the eyeballs was investigated in the midpontine pretrigeminal cat.

2. Single and serial fixation reflexes were distinguished according to the number of fixations occuring. The single reflex consisted of four phases: i) the eye movement toward the object, ii) the maintenance of the fixation, iii) the return movement, and iv) the tonic fixation. The single fixation reflex was further subdivided according to whether it

attained complete amplitude (full single fixation reflex) or it was incomplete (abortive single fixation reflex).

In the serial reflex, in the time between the repeated fixations the eyeballs were inclined away from the stimulus source. After the last fixation the tonic fixation was developed.

3. The intensity of the fixation reflex depended mainly on the behavioural responsiveness of the preparation and the physical properties of the stimulus.

4. The fixation reflex was triggered by the onset of the stimulus, and later on the reflex developed to some extent independently of the stimulus.

5. The fixation reflex was accompanied by pupillary dilation and an EEG arousal.

6. The acute and chronic habituation of the fixation reflex was obtained. In acute habituation the diminution of the amplitude of the fixation was partially compensated by the summation of the tonic fixation.

7. For control the fixation reflex was observed also in restrained intact cats. The time-course of the fixation reflex, the development of habituation and of increase of the tonic fixation appeared to be similar in intact and pretrigeminal cat.

REFERENCES

AFFANNI J., MARCHIAFAVA P. L. and ŻERNICKI B. 1962a — Orientation reactions in the midpontine pretrigeminal cat. Arch. ital. Biol., *100*, 297.

AFFANNI J., MARCHIAFAVA P. L. nad ŻERNICKI B. 1962b — Conditioning in the midpontine pretrigeminal cat. Arch. ital. Biol., *100*, 305.

ALPERN M. 1962 — Movements of the eyes. In: DAVSON H. (ed.). The eye. New York and London: Academic Press, *3*, 3.

BATINI C., MORUZZI G., PALESTINI M., ROSSI G. F. and ZANCHETTI A. 1959 — Effects of complete pontine transections on the sleep-wakefulness rhythm: the midpontine pretrigeminal preparation. Arch. ital. Biol., *97*, 1.

BERKSON G. and FITZ-GERALD F. L. 1963 — Eye fixation aspect of attention to visual stimuli in infant chimpanzees. Science, *139*, 586.

BERLYNE D. E. 1960 — Conflict, arousal and curiosity. New York: McGraw-Hill Book Company, p. 93.

BISHOP P. O., KOZAK W. and VAKKUR G. J. 1962 — Some quantitative aspects of the cat's eye: axis and plane of reference, visual field co-ordinates and optics. J. Physiol., *163*, 466.

DREHER B., MARCHIAFAVA P. L. and ŻERNICKI B. 1965 — Studies on the fixation reflex: II. The neural mechanism of the fixation reflex in the normal and pretrigeminal cat. Acta Biol. Exper. (Warsaw), *25*, 207.

HYDE J. E. 1959 — Some characteristic of voluntary human ocular movements in the horizontal plane. Amer. J. Ophthal., *48*, 85.

KING F. A. and MARCHIAFAVA P. L. 1963 — Ocular movements in the midpontine pretrigeminal preparation. Arch. ital. Biol. *101*, 149.

ROBINSON D. A. 1964 — The mechanics of human saccadic eye movement. J. Physiol., *174*, 245.

SHARPLESS S. and JASPER H. 1956 — Habituation of the arousal reaction. Brain, *79*, 655.

SOKOLOV E. N. 1958 — The orientation reflex and its structure and mechanism. In: The orienting reflex and exploratory behaviour (in Russian). VORONIN L. G., LEONTIEV A. N., LURIA A. R., SOKOLOV E. N. and VINOGRADOWA O. S. (eds). Moscow: Acad. Pedag. Sci. RSFSR, p. 111.

WESTHEIMER G. 1954 — Mechanism of saccadic eye movements. A. M. A. Arch. Ophtal., *52*, 710.

WOODWORTH R. S. and SCHLOSBERG H. 1958 — Experimental Psychology. New YORK: Henry Holt and Company, p. 492.

VORONIN L. G. and SOKOLOV E. N. 1960 — Cortical mechanisms of the orienting reflex and its relation to the conditioned reflex. Electroenceph. clin. Neurophysiol., Suppl *13*, 335.

YARBUS A. L. 1956 — The motion of the eye in the process of changing points of fixation (in Russian). Biofizika, *1*, 76.

ŻERNICKI B. 1964 — Isolated cerebrum of midpontine pretrigeminal preparation: a review. Acta Biol. Exper. (Warsaw), *24*, 247.

ŻERNICKI B. and OSETOWSKA E. 1963 — Conditioning and differentiation in the chronic midpontine pretrigeminal cat. Acta Biol. Exper. (Warsaw), *23*, 25.

STUDIES ON THE VISUAL FIXATION REFLEX
II. THE NEURAL MECHANISM OF THE FIXATION REFLEX IN NORMAL AND PRETRIGEMINAL CATS[1]

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In the first paper of this series (Ż e r n i c k i and D r e h e r 1965) the general properties of the fixation reflex of the eyeballs were described (the investigation was limited to the study of the fixation reflex to so called „indifferent" visual stimuli, i.e., the orientation fixation reflex). The observations were chiefly made on the pretrigeminal preparation, where eye movements may be precisely recorded. Depending on the number of fixations the single and the serial fixation reflexes could be distinguished. The single reflex was subdivided into the full fixation reflex and abortive fixation reflex. The fixation reflex was usually accompanied by several other reactions. Some of these have a directional character, as, for example, conjugate turning of the head (this component of the fixation reflex is absent in the pretrigeminal preparation); while others are non-directional: pupillary dilation and EEG arousal (see also A f f a n n i, M a r c h i a f a v a and Ż e r n i c k i 1962).

Little is known about the neural mechanism of the fixation reflex. Although considerable knowledge of the nervous pathways subserving eye movements has been accumulated by anatomical and physiological investigations in anaesthetized preparations we do not know, however, which ones of these pathways are involved in the fixation reflex. On

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the other hand, some results concerning the eye movements, which were obtained in unanaesthetized animals, are incomplete (Ke n n a r d and E c t o r s 1938, R a d e m a k e r and t e r B r a a k 1948, B l a k e 1959).

In the experiments described in the present paper the effects of lesions of cerebral structures (involved in mechanism of eye movements) upon the fixation reflex were separately investigated in different groups of cats. The postoperative examination was made both before and after midpontine pretrigeminal transection.

METHODS

Three types of experiments were carried out on cats. In the first group (10 cats) the superior colliculi were bilaterally removed (tectal cats) — Fig. 1A. In the second group (4 cats) the visual cortical areas were ablated (occipital cats), and in the third group (4 cats) the frontal oculomotor areas (Claes 1939) were removed (frontal cats).

The operations were performed in sterile conditions under nembutal narcosis. The surgical removal of the colliculi was preferred to electrocoagulation because injury of the visual cortex by the electrodes was thus avoided and the operation
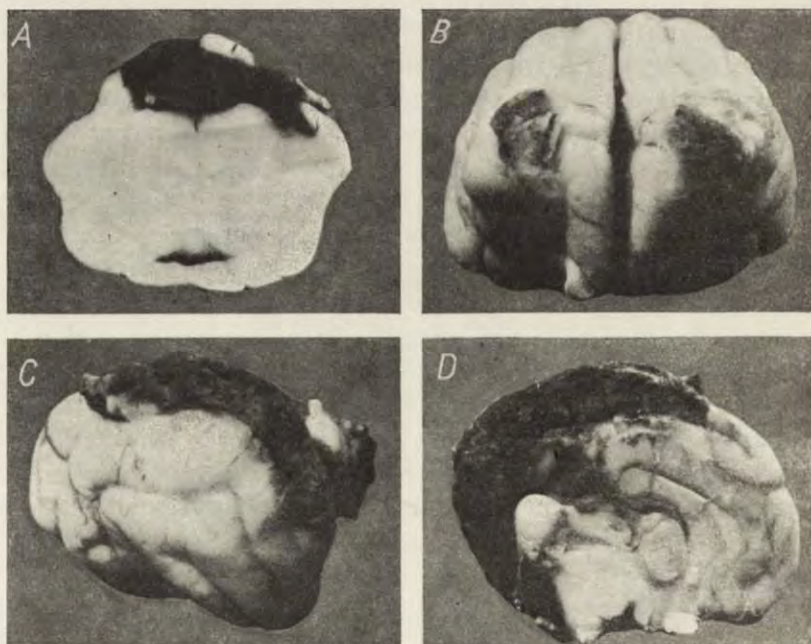
Fig. 1. The brains of the representative cats. A, Tectal cat. B, Frontal cat. C, Occipital cat, lateral aspect, D, Occipital cat, medial aspect

was visually controlled. After a wide craniotomy on the left side, the dura matter was excised and the whole parietal and occipital lobes exposed. The occipital lobe was gently moved aside, the caudal part of the inferior sinus coagulated, the inferior part of the falx cerebri cut, and then the superior colliculi aspirated. In order to limit any accidental damage to the ampulla Galeni the most superficial part of the tectum was left in place, but it was disconnected from the tegmentum by a complete aspiration of the underlying tissues (Fig. 1A).

Examination of the animals started a week after the collicular or cortical removals. The locomotor behaviour based on visual cues was tested in an experimental room, in which several boxes of different size were placed. During the examination of ocular activity the head of the cat was restrained by hand. A piece of white cotton wool, a piece of meat and a live mouse were used as stimuli for evoking the fixation reflex. The objects were either stationary or they were rhythmically moved in front of the cat's eyes at distance of about 40 cm, at a rate of 0,3 c/sec (the fixation reflex to the latter stimuli is called the following fixation reflex, cf. Ż e r n i c k i 1964).

Two weeks after the initial operation the pretrigeminal section was performed in these cats under ether narcosis (B a t i n i et al. 1959, Ż e r n i c k i and D r e h e r 1965). Two hours later, the animals were placed in a small, optically isolated chamber (for a detailed description of the chamber, see Ż e r n i c k i and D r e h e r 1965). For evoking the fixation reflex X shaped rotating figures of angular diameter 12° were used. The figures were positioned on a white screen located in front of the animal's eyes. One of the figures was 25° above the horizontal plane through the nodal point of the animal's eyes and the other 25° below this level. The ocular responses were filmed and the EEG simultaneously recorded.

Finally, the occipital and frontal oculomotor areas were exposed and stimulated using small bipolar silver ball electrodes with 1 msec, 2-6V electric shocks at a rate of 100/sec.

<p style="text-align:center">RESULTS</p>

I. Tectal cats

Before pretrigeminal section. When placed in the experimental room, the animals sat quietly. When pushed and forced to walk, however, they avoided obstacles in a normal manner. The cats also retained the visual and tactile placing reactions.

Labyrinthine rotational and postrotational nystagmus, both vertical and horizontal, appeared to be normal in frequency and amplitude in all our cats. The pupillary light reflex was present.

Vertical ocular spontaneous activity was absent. On the other hand, horizontal spontaneous activity was marked in all preparations.

Similar differences were also observed for the evoked ocular activity. The vertical fixation reflex to all visual stimuli, both stationary and moving in front of the animals eyes, seemed to be completely lost; while the horizontal fixation reflex, although seriously affected (long latency, small amplitude and quick disappearance with repetition of the stimulus)

was occasionally present. Furthermore, in some cats the lateral following reflex did not show the usual pattern but the eyes either moved in a direction opposite to that of the object or moved without any relation to the stimulus.

The usual associated responses of the fixation reflex also appeared to be seriously affected. Turning of the head towards the stimulus was abolished both in the vertical and in the horizontal plane, and pupillary dilation disappeared almost completely.

After pretrigeminal section. The results obtained with the pretrigeminal cats placed in the experimental chamber were in agreement with those obtained before the transection. Neither the upper nor the lower figure X produced any fixation reflex (Fig. 2A), but only a small
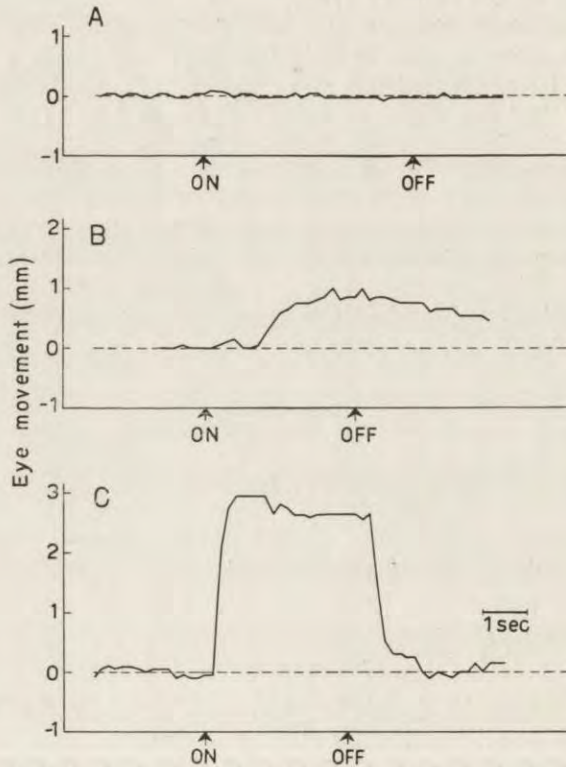
Fig. 2. Graphs of the fixation reflexes to the rotation of the figure X located above the animal's eyes. A, no fixation reflex in the tectal cat. B, abortive single reflex in the occipital cat., C, Full single reflex in the frontal cat. The movement of an ink spot on the cornea in relation to the reference point visible on the stereotaxic apparatus is shown. On the ordinates 1 mm corresponds approximately to 10° of the angular eye movement

pupillary dilation accompanied by an EEG arousal. However, during a state of increased alertness, following stimulation of the hypothalamus for 2 sec or an injection of ephedrine, the figure X could usually elicit an abortive single fixation reflex.

The EEG records appeared to be permanently desynchronized in all animals.

Stimulation of the visual cortex did not produce any eye movements, as it usually does in normal pretrigeminal preparations, and evoked only a weak pupillary dilation. Small vertical eye movements could be evoked, however, in those animals, in which the post-mortem histological examination showed an incomplete ablation of the colliculi. Stimulation of the frontal cortex always elicited clear-cut conjugate vertical movements (for the summary of the results see Table I).

Table I

The comparison between the tectal, occipital and frontal cats

Tests / Lesions	Avoidance of obstacles	Visual placing	Ocular spontaneous activity		Fixation reflex		Pupillary dilation	Effect of occipital stimulation	Effect of frontal stimulation
			vertical	horizontal	vertical	horizontal			
Tectal	good	good	absent	good (vivid)	absent	poor	poor	absent	good
Occipital	poor	absent	good	good	poor	poor[1]	po'r	—	good
Frontal	good	good	good	good	good	good	good	good	—

[1] better than in tectal cats.

II. Occipital cats

Before pretrigeminal section. The spontaneous behaviour of the animals was more lively in comparison with tectal cats. However, the cats often collided with obstacles. The visual placing reaction was absent, while the tactile one was present. Vertical and horizontal labyrinthine rotational nystagmus and the pupillary light reflex were present.

Spontaneous ocular activity was present both in the vertical and horizontal planes. Both the vertical and the horizontal fixation reflexes were seriously impaired. The fixation reflex to the stimuli contrasting better with a dark background (white cotton-wool, white mouse) seemed to be less impaired. Although the amplitude of the response seemed to be comparable to the normal, the fixation reflex nevertheless was

irregular and disappeared after a few presentations of the stimulus. Turning of the head towards a visual stimulus was affected in a similar way. Pupillary dilation was small.

After pretrigeminal section. Figure X evoked, after a long latent period, the abortive single fixation reflex, which was followed by a small pupillary dilation (Fig. 2B). The reflex dissappeared after a few presentations of the stimulus.

Stimulation of the frontal cortex elicited clear-cut conjugate vertical movements of the eyes.

III. Frontal cats

Before pretrigeminal section. The general behaviour of these animals seemed to be normal, apart from the obvious defects related to the ablation of the motor cortex.

Spontaneous ocular activity was marked. Both the vertical and horizontal fixation reflexes were present. Turning of the head towards a visual stimulus was similarly unaffected.

After pretrigeminal section. The figures X elicited a full single fixation reflex followed by a pupillary dilation and an EEG arousal (Fig. 2C). The reflex was no less resistant to habituation than in the „normal" pretrigeminal preparation. The serial fixation reflex was never observed. However, we may not conclude that this reflex may not be obtained in the frontal cat, because it appears only in 20 per cent of normal pretrigeminal preparations. The stimulation of the visual cortex elicited conjugate vertical movements of the eyes.

DISCUSSION

Fixation reflex in frontal cats. The persistence of a strong fixation reflex, with a normal time-course, after bilateral ablation of the frontal oculomotor areas seems to indicate that the neural pathway for the fixation reflex does not pass through this cortical relay. Moreover, in tectal and occipital cats, in which the fixation reflex was impaired, stimulation of the frontal cortex still produced eye movements similar to those characteristic of the „normal" pretrigeminal cat (M a r c h i a f a v a, unpublished observations). In the frontal cats, on the other hand, stimulation of the occipital visual areas clearly produced eye movements. Our findings seem to be in agreement with some observations on humans (H o l m e s 1938) and monkeys (K e n n a r d and E c t o r s 1938) that after frontal lesions the fixation reflex is preserved, and with those obtained by C r o s b y, Y o s s and H e n d e r s o n (1952) who showed

that a pathway from the frontal areas to the oculomotor nuclei does not pass through the superior colliculi.

Fixation reflex in the tectal and occipital cats. Our results are in general agreement with some data of other authors suggesting that the pathways for the fixation reflex pass through the visual cortex and superior colliculi. B l a k e (1959) observed a disappearance of the vertical fixation reflex and a strong impairment of the lateral one in cats with collicular lesions. A p t e r (1946) obtained a fixation reflex to light after local aplication of strychnine sulphate on the surface of the superior colliculi in lightly anaesthetized cats. R a d e m a k e r and t e r B r a a k (1948) reported the disappearance of the fixation reflex after ablation of the visual cortex in dogs. Finally, it is well known that the fixation reflex disappears in human beings with occipital lesions (H o l-m e s 1938).

Anatomical studies in the cat (B a r r i s, I n g r a m and R a n s o n 1935) showed that retinal impulses reach independently both visual cortex and superior colliculi. Furthermore, experiments performed on monkeys and observations on humans (which seems to be also true for the cat) show that the efferent pathway from the visual cortex subserving vertical eye movement run to the superior colliculi, while most of the pathways for the horizontal movements bypass the colliculi going directly to the oculomotor nuclei region (H o l m e s 1921 and C r o s b y 1953). Combining these anatomical results with our results, we may conclude that there are two separate pathways subserving horizontal fixation reflex one via the cortex, the other via the tectum (Fig. 3).

Fig. 3. Schematic diagram illustrating the nerve pathways of the vertical and horizontal fixation reflex. Solid line, the pathway for both vertical and horizontal fixation. Dashed line, „the privet" pathway for the vertical fixation. Dotted line, „the privet" pathway for the horizontal fixation. a, lateral geniculate nucleus; b, visual cortex; c, superior colliculus; d, oculomotor nuclei; e, oculomotor muscles
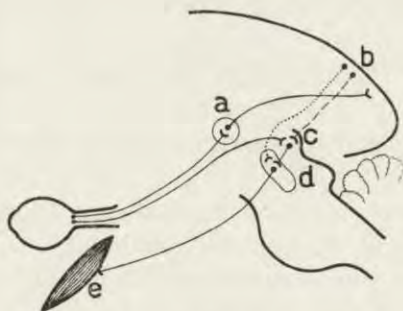
In the occipital cats the destruction of the cortical path produced an impairment of the horizontal reflex, but this reflex could still be evoked by using the remaining tectal path. Furthermore, in the tectal cats, in which the subcortical path was interrupted, only a very small horizontal fixation reflex was retained, which may have been mediated by the

occipital path. The fact that the horizontal fixation reflex was clearly smaller in the tectal cats than in the occipital ones may be explained in two ways: either the tectal path is functionally more significant than the occipital one, or the great majority of the cortifugal fibers cross the superior colliculi.

Concerning the vertical fixation reflex arc, the superior colliculus is a relay for the occipital path as well, thus the efferent collicular pathway directed to the region of the III Nucleus represents the final common path for motor impulses coming both from the cortex and the colliculi themselves (Fig. 3). Such anatomical arrangement was strongly supported by our results which showed that in the occipital cats the vertical fixation reflex was smaller although it had normal time-course, while it was completely lost in the tectal cats.

It seems probable that the roles of the tectal and occipital branches of the reflex arcs are qualitatively different. We may expect that the integration of the sensory information in the cortex is more elaborate than in the tectum. In fact, the defect of vision appeared to be more pronounced in our occipital cats as shown by a much greater impairment in the ability to avoid obstacles. On the other hand, recent experiments by Blake (1959) have shown that pattern vision seems to be no less affected in tectal cats than in occipital ones (Smith 1938). However, it is possible that the impairment of pattern discrimination in tectal cats may be due to a different factor. In these animals oculomotor activity is as a rule seriously affected. We may suppose, therefore, that the so called „physiological nystagmus" of the eyes can be affected too. It is generally accepted that the presence of these small movements of the eyes opposes the adaptation of the receptor mechanism, consequently their absence in the tectal cats could explain the defects in their pattern vision. Of course, such a thesis needs experimental support.

Fixation reflex to various visual stimuli. In both tectal and occipital cats the fixation reflex to the so called „indifferent" stimuli (cotton wool and rotating figure X) was affected in a way similar to this to other stimuli such as a live mouse or a piece of meat. Although the latter stimuli may have an unconditioned component, they are usually considered to be the „natural" conditioned stimuli (Pavlov 1940)[3]. Consequently, our results may indicate that the reflex arcs of the orientation and conditioned fixation reflex are similar. However, it should be noted that under our experimental conditions the meat and the mouse were not strong conditioned stimuli. Actually, although the animals were deprived of food for 24h, they sometimes did not eat the

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[3] The appearance of meat has been shown to be a conditioned stimulus for the dog (Tsitovich 1911).

meat or kill the mouse. On the other hand, we cannot reject the hypothesis that the orientation reflex is included in the conditioned response as a triggering mechanism. Thus every kind of stimulus, either conditioned or indifferent, would first evoke an unconditioned orientation reaction, and later on would serve as a recognized conditioned stimulus while eliciting a true conditioned response. According to the latter hypothesis the impairment of the fixation reflex to the conditioned stimuli would not be a result of the interruption of the conditioned reflex arc, but rather would be due to the abolition of the triggering mechanism of the reaction. It is also worth noting that in humans the reflex arcs of the orientation and conditioned fixation reflexes are different passing through the occipital and frontal lobes respectively (H o l m e s 1938).

The fixation reflex to the stationary objects seemed to be affected similarly to that to the moving ones. We may conclude, therefore, that the reflex arc of the following fixation reflex has similar course to that of the fixation reflex to the stationary object.

Responses associated with the fixation reflex. Our results show that the impairment of the fixation reflex is always accompanied by a similar defect in turning of the head. Since both these responses are abolished by the destruction of the same structures, we may suppose that the pathways of the reflex arc subserving them have a similar course. Furthermore, it is well known that the stimulation of either the superior colliculi (H e s s 1954) or of the frontal areas (for review see C r o s b y e t a l. 1952) may produce eye movements accompanied by turning of the head in the same direction.

Direct relations have been demonstrated recently (Ż e r n i c k i and D r e h e r 1965) between the amplitude of the fixation reflex, and the EEG and pupillary reactions. It does not seem surprising, therefore, that the pupillary and EEG responses were also defective in our preparations, in which a severe impairment of the fixation reflex was found. We cannot imagine a visual stimulus which is not seen sharply but evokes a marked unspecific response. Some anatomical results exist which suggest an explanation of the impairment of the pupillary functions in the tectal cats. In fact, in these animals it may be ascribed to the interruption of the direct colliculo-reticular pathways (A l t m a n and C a r p e n t e r 1961), which in normal conditions may be partially responsible for the unspecific reactions occurring simultaneously with eye movements.[4]

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[4] When this paper was in press a new report describing ocular deficits after lesions of the superior colliculi was published (S p r a g u e J. and M e i k l e Th. 1965 — Exptl. Neurol., *11*, 115).

SUMMARY

1. The effects of tectal and cortical lesions on the fixation reflex of the eyeballs were investigated in cats before and after midpontine pretrigeminal transection.

2. The ablation of frontal oculomotor cortex did not affect the fixation reflex.

3. After the ablation of the visual cortex the fixation reflex was clearly impaired.

4. Removal of the superior colliculi produced a complete disappearance of the vertical fixation reflex and a very strong impairment of the horizontal one.

5. The fixation reflex to stationary and moving objects were affected in a similar way. There was also no difference in the impairment of the fixation reflex to either „indifferent" or natural conditioned stimuli.

6. Any impairment of the fixation reflex was always followed by an impairment of the associated responses: the movement of the head towards the object, pupillary dilation and EEG arousal.

7. Stimulation of the frontal oculomotor areas elicited vertical eye movements in both occipital and tectal cats. Stimulation of the visual cortex produced eye movements in frontal cats. These effects could not be elicited in tectal cats, while only pupillary dilation resulted from such stimulation.

8. Our results seem to indicate that the fixation reflex arc is subdivided into an occipital and a tectal branch. Both branches seem to be partially independent as far as the horizontal fixation reflex is concerned. However, the occipital branch of the vertical fixation reflex crosses also the superior colliculus.

REFERENCES

AFFANNI J., MARCHIAFAVA P. L. and ŻERNICKI B. 1962a — Orientation reactions in the midpontine pretrigeminal cat. Arch. ital. Biol., 100, 297.
ALTMAN J. and CARPENTER M. B. 1961 — Fiber projections of the superior colliculus in the cat. J. comp. Neur., 116, 157.
APTER J. T. 1946 — Eye movements following strychninization of the superior colliculus of cats. J. Neurophysiol., 9, 73.
BARRIS R. W., INGRAM W. R. and RANSON S. W. 1935 — Optic sonnections of the diencephalon and midbrain of the cat. J. comp. Neur., 62, 117.
BATINI C., MORUZZI G., PALESTINI M., ROSSI G. F. and ZANCHETTI A.

1959 — Effects of complete pontine transections on the sleep-wakefulness rhythm: the midpontine petrigeminal preparation. Arch. ital. Biol., 97, 1.

BLAKE L. 1959 — The effect of lesions of the superior colliculus on brightness and pattern discrimination in the cat. J. comp. physiol. Psychol., 52, 272.

CLAES E. 1939 — Contribution à l'étude physiologique de la fonction visuelle. II. Étude des centers oculomoteurs corticaux chez le chat non anesthèsie. Arch. int. Physiol., 48, 238.

CROSBY E. C. 1953 — Relations of brain centers to normal and abnormal eye movements in the horizontal plane. J. comp. Neur., 99, 437.

CROSBY E. C., YOSS R. E. and HENDERSON J. W. 1952 — The mammalian midbrain and isthmus regions. J. comp. Neur., 97, 357.

HESS W. R. 1954 — Diencephalon, New York: Grune Stratton.

HOLMES G. 1921 — Palsies of the conjugate ocular movements. British J. Ophthalmol., 5, 241.

HOLMES G. 1938 — The cerebral integration of the ocular movements. Brit. Med. J. No. 4045, 107.

KENNARD M. and ECTORS L. 1938 — Forced circling in monkeys following lesions of the frontal lobes. J. Neurophysiol., 1, 45.

PAVLOV I. P. 1928 — Lectures on conditioned reflexes. New York: Intern. Pubs. Chapter II.

RADEMAKER G. G. J. and ter BRAAK J. W. G. 1948 — On the central mechanism of some optic reactions. Brain, 71, 48.

SMITH K. U. 1938 — Visual discrimination in the cat: VI. The relation between pattern vision and visual acuity and the optic projection centers of the nervous system. J. Genetic Psychol., 53, 251.

TSITOVICH J. S. 1911 — Origin and elaboration of the natural conditioned reflexes. Diss., SPb. Quoted: PAVLOV I. P. 1928 — Lectures on conditioned reflexes. New York: Intern. Pubs. Chapter II.

ŻERNICKI B. 1964 — Isolated cerebrum of midpontine pretrigeminal preparation: a review. Acta Biol. Exper. (Warsaw), 24, 247.

ŻERNICKI B. and DREHER B. 1965 — Studies on the visual fixation reflex: I. General properties and habituation of the orientation fixation reflex in pretrigeminal and intact cats. Acta Biol. Exper. (Warsaw), 25, 187.

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SOME OBSERVATIONS ON SLEEP-LIKE BEHAVIOUR AND AROUSAL PRODUCED BY ELECTRIC STIMULATION OF THE MEDIAL THALAMUS IN RABBITS

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Neuronal organization of the medial thalamus has been studied by many workers from anatomical, electrophysiological and behavioural points of view. This area appears to play an important rôle in regulating the processes of sleep and wakefulness. The sleep centre of H e s s (1954) has been localized here, more exactly in the region of massa intermedia. However, experiments, in which different points within the medial thalamus were stimulated electrically or electrolytic lesions were produced therein, gave contradictory results as reported by various authors. S c h r e i n e r et al. (1953) described effects both excitatory and inhibitory in character due to electrolytic lesions made in the medial thalamus of cats. H u n t e r and J a s p e r (1949) were able to elicit either motor hyperactivity or somnolence in cats by stimulating electrically various points within this region and using different parameters of the current.

The just presented data indicate that the medial thalamus should be considered as a functionally heterogeneous area. On the basis of anatomical (M c L a r d y 1956, P a p e z 1956) and physiological (M o r u z z i and M a g o u n 1949) findings this region, properly the complex of midline and intralaminar nuclei, appears to constitute a diencephalic prolongation of the midbrain reticular system. On the other hand, it contains neurons belonging to the recruiting system described by D e m p s e y and M o r i s o n (1942, 1943) and M o r i s o n and D e m p s e y (1942). Several observations support the point of view that the latter system is involved in the process of falling asleep. A k e r t et al.

(1952) and H e s s et al. (1953) found in cats that low frequency stimulation of the medial thalamus with rectangular 10 msec or longer impulses produced behavioural and electroencephalographic patterns of sleep. Similar results were obtained in rabbits. The nonspecific thalamic system of these animals seems to exert a constant inhibitory action, for after making lesions within the medial thalamus an increased motor activity ensues which is accompanied by a persisting activation of the EEG tracing (H ö s l i and M o n n i e r 1963). Effects elicited in rabbits by electric stimulation of the medial thalamus vary depending on the parameters of the current. Low frequency stimulation with low amplitude and sufficiently long (10 msec or more) impulses decreased motor activity of the animals and produced behavioural sleep patterns with a pronounced synchronization of the EEG. High frequency stimulation with brief impulses elicited behavioural and EEG arousal (H ö s l i and M o n n i e r 1962). These authors believe that the most important factor in producing hypnogenic effects is a long duration of the impulses used for the stimulation, whereas brief impulses arouse the animals. M o n n i e r et al. (1960) demonstrated in rabbits a functional antagonism between the activating and the recruiting system of the thalamus. According to T i s s o t and M o n n i e r (1959) the dual function of the nonspecific thalamic system is not due to anatomical division of the thalamus into functionally different areas, but because neurons with antagonistic physiological properties are intermixed in this region. On the basis of these findings, M o n n i e r et al. (1963) included the medio--central thalamic region to „moderating" systems of the brain.

In the present study, it has been noted that hypnogenic action of thalamic stimulation does not depend necessarily on long duration of the impulses, but rather relates to the frequency and strength of the current. For this reason various frequencies were tested, particularly those insufficiently investigated by other authors. Furthermore, an attempt was made to find a correlation between the behavioural effect and electrophysiological phenomena elicited by the stimulation.

METHODS

Experiments were performed on 10 male adult rabbits bearing chronically inserted intracranial electrodes. Before the operation nembutal in a dose of 60 — 80 mg/kg was introduced intraperitoneally and the rabbit's head was immobilised in an attachment of S a w y e r et al. (1945) to a stereotaxic instrument of R e i n o s o (1954). Cortical electrodes, made up of a silver wire, were fixed in the skull over the sensorimotor cortex of either side. Subcortical electrodes were concentric ones. They consisted of a stainless steel tube 0,4 mm in diameter insulated except for a tip of about 0.5 mm. Into the tube a tungsten wire 0.1 mm in diameter was

introduced which had been insulated except for a 0.3 mm tip protruding for a distance of about 0.5 mm beyond the end of the tube. Coordinates were taken from stereotaxic atlases of the rabbit's brain of S a w y e r et al. (1954) and F i f k o v á and M a r š a l a (1960). The electrodes were introduced into the right dorsal hippocampus, the left medial thalamus and midbrain reticular formation on the left side. After fixing the electrodes in the bone with an acrylic cement their outer ends were connected to a radio valve plug. A distant electrode consisting of a silver plate was placed on the nasal bone. Antibiotics were administered in the postoperative period.

Experiments began after a complete recovery of the animals. The rabbits were put into a 50×70 cm cage placed in a sound-proof room. Electrodes were connected to an Alvar Reega XV electroencephalograph with long flexible wires not hindering the movements of the animals. Electric activity of the neocortex was recorded between the left and the right sensorimotor area. For subcortical leads mainly unipolar recording was used, between the outer part of the concentric electrode and the distant one. Sometimes the potentials were recorded bipolarly, between the outer and the inner part of the electrode. In order to study some electrophysiological phenomena a Cossor oscilloscope model 1049 MK III A, equipped with a Cossor camera, was connected to the third amplification stage of one channel of the electroencephalograph. In this way, a simultaneous ink--written and cathode-ray registration was possible.

The medial thalamus was stimulated with rectangular impulses from a stimulator through a radio frequency stimulation unit. The duration of every impulse was always 0.8 msec, the amplitude varied from 1 to 20 V. The following frequencies: were used: 1) single impulses applied at a frequency of 1/5 ces or less; 2) low frequency (mainly 5 cy/sec); 3) middle frequency (30 cy/sec); 4) high frequency (100 — 300 cy/sec. Mesencephalic reticular formation was stimulated at a frequency of 300 cy/sec.

In each rabbit 5 — 10 experiments were made every 1 — 3 days. First, the animals were placed in the cage in order to familiarize them with the conditions of the experiments. Spontaneous electrical activity of the brain was recorded. Then a series of experiments with the stimulation of the brain was made. The rabbits were observed through a window, their behaviour was noted in the protocol and photographed.

After the experiments the animals were sacrificed, their brains fixed in 10 per cent formalin and embedded in celloidin. 50 — 100 µ thick sections were made in frontal plane and stained after Weil and Weigert.

RESULTS

I. Spontaneous electrical activity of the brain. Electrical activity of the brain in preliminary experiments was recorded from the sensorimotor cortex, dorsal hippocampus, medial thalamus and reticular formation. It was found that the first two leads, i.e., the neocortical and the hippocampal one, were essential to evaluate the tracing.

Generally, the following types of EEG record were observed:

1. Activated tracing. This tracing was characterized by 25 — 35/sec waves of an amplitude not exceeding 20 µV in the neocortical lead and

by 5 — 7/sec waves in the dorsal hippocampus, their amplitude being very high and ranging sometimes up to 300 — 400 µV. These so called theta waves appeared, too, in the medial thalamus and mesencephalic formation, but were less regular and of a lower amplitude than in the dorsal hippocampus (Fig. 1A). This type of EEG accompanied behavioural arousal characterized by a rabbit walking in the cage and performing searching and sniffing movements. During a motor overactivity or a prompt orientation reaction the frequency of hippocampal theta rhythm increased up to 7 — 9 cy/sec. A state of alertness, but with a diminished motor activity, was accompanied by a less regular theta activity in the hippocampus, of a frequency falling to 4 — 6 cy/sec (Fig. 1B).
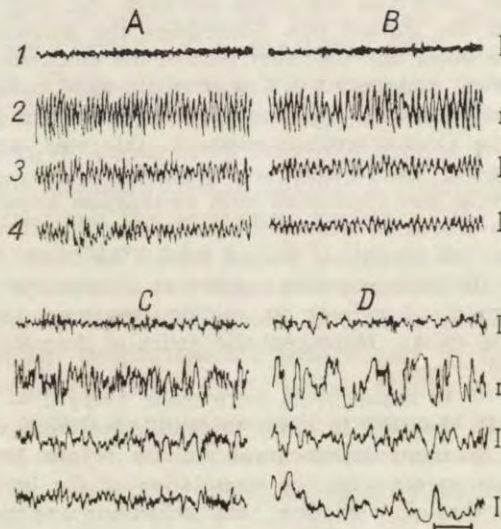
Fig. 1. Spontaneous electrical activity of the rabbit's brain. Leads: 1. Sensorimotor cortex. 2. Dorsal hippocampus. 3. Medial thalamus. 4. Midbrain reticular formation. Tracings: A. Activated tracing. B. Activated tracing, with a lowered frequency of the theta waves. C. Synchronized tracing D. Hypersynchronous tracing. Calibration: 100 µV, 1 sec

2. Synchronized tracing. A synchronized tracing consisted of spindle--like bursts of 8—15/sec waves in the sensorimotor cortex. The hippocampal theta rhythm was disrupted and some high voltage 1—3/sec waves were seen. The animals presenting this type of EEG record usually remained motionless with forelimbs stretched foreward. A sample of this record in shown in Fig. 1C.

3. Hypersynchronous tracing. This type of EEG activity was seen occasionally when recording spontaneous electrical activity of the brain. It consisted of high voltage 1—3/sec waves in both cortical and subcortical leads, the bursts of spindles in the sensorimotor cortex being reduced or absent (Fig. 1D). Behaviourally, the rabbit presented a state of adynamy lying on the floor of the experimental cage.

II. Electrographic and behavioural effects obtained upon electric stimulation of the medial thalamus.

1. Single impulses. In response to a single electric impulse stimulating the medial thalamus an evoked potential appeared in the neocortex and was followed by a burst of 8 — 15/sec waves of an increasing and decreasing amplitude, resembling thus a spontaneously appearing spindle. Duration, amplitude and regularity of this burst depended upon the state of EEG record. In the state of arousal the spindles failed to appear or were only marked (Fig. 2). The best spindles could be evoked during a synchronization of the EEG characterized by spontaneously occurring spindles (Fig. 3). In the periods of hypersynchrony the spindles became less evident (Fig. 4).

From the behavioural point of view, stimulation of the medial thalamus with single impulses every 5 — 10 sec diminished gradually the motor
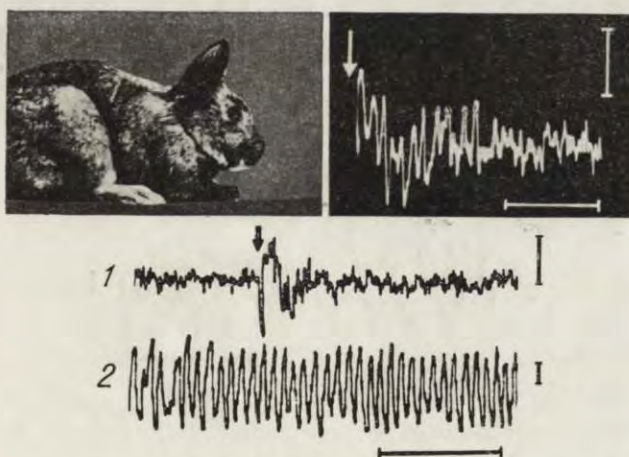
Fig. 2. Single impulse stimulation of the medial thalamus during an EEG and behavioural arousal. Above on the right — a cathode-ray registration. Record taken from the sensorimotor cortex. Calibration: 100 μV, 500 msec. Below — an ink-written registration. Leads: 1. Sensorimotor cortex. 2. Dorsal hippocampus. Calibration: 100 μV, 2 sec. Arrows indicate the stimulus
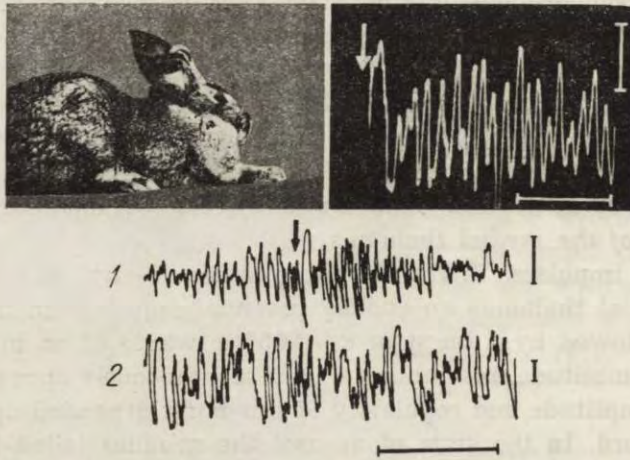
Fig. 3. Single impulse stimulation of the medial tha-
lamus in the stage of a synchronization of the EEG.
Explanations as in fig. 2
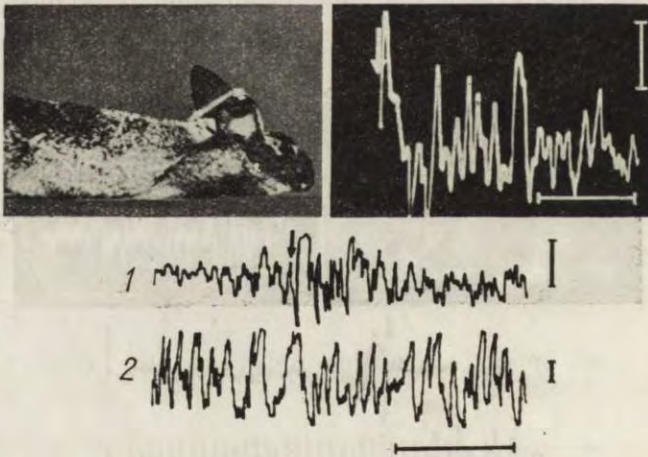
Fig. 4. Single impulse stimulation of the medial tha-
lamus the stage of a hypersynchronous activity. Ex-
planations as in fig. 2

activity of the rabbits. After several minutes of such stimulation the
animals became motionless, often lying on the floor and presenting
behavioural sleep patterns. Even overexcited animals became calm and
quiet in the period of the stimulation. Concomitantly with these findings
a synchronization of the tracing ensued presenting, in some experiments,
a high degree of hypersynchrony.

2. Low frequency stimulation. Stimulation of the medial thalamus at a low frequency elecited a recruiting response in the cortex. Every impulse evoked a biphasic potential. The first deflection appeared after a latent period of less than 10 msec and was followed by a second one, opposite in polarity, with a peak time of 20 — 30 msec. The second component showed mainly a recruiting phenomenon, i.e. its amplitude increased with every successive impulse at the beginning of the stimulation and then waxed and waned periodically (Fig. 5). The best recruiting

Fig. 5. Two potentials taken from a waxing and waning recruiting response. Stimulation at a frequency of 5 cy/sec. On the left — a minimal and on the right — a maximal amplitude. Calibration: 100 μV, 50 msec
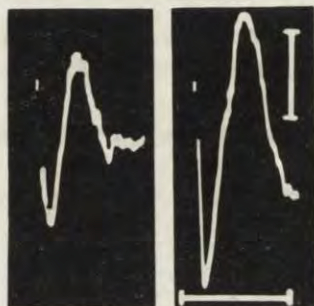
with waxing and waning of the response, was seen when applying slightly above threshold stimuli of an amplitude ranging from 1 do 5 V (Fig. 6). With a frequency of 5 cy/sec, often two potentials appeared to every impulse of the series. A synchronization of the tracing ensued, concomitantly with decreased motor activity of the animals. When, how-
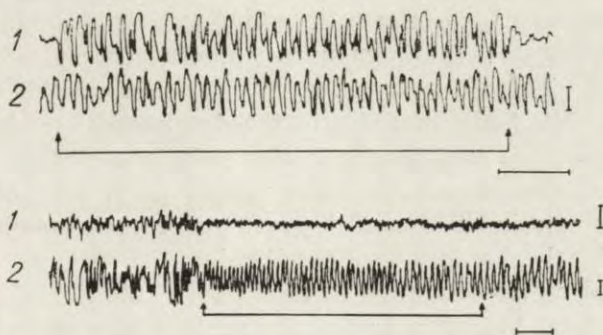
Fig. 6. Electrographic effects of stimulation of the medial thalamus. Upper tracing — a recruiting response. Stimulation at a frequency of 5 cy/sec between the arrows. Lower tracing — activation of the EEG due to stimulation at a frequency of 300 cy/sec (between the arrows). Leads: 1. Sensorimotor cortex. 2. Dorsal hippocampus. Calibration: 100 μV, 1 sec

ever, the amplitude of the impulses was much higher than the threshold value, a series of evoked potentials appeared in the cortex, without waxing and waning often with EEG and behavioural patterns of arousal. Inhibitory phenomena did not appear, when 10 cy/sec stimulation was applied.

3. Middle and high frequency stimulation. EEG and behavioural effects elicited by electric stimulation of the medial thalamus at a middle (30 cy/sec) and a high (100 — 300 cy/sec) frequency were essentially the same. A state of arousal occurred which was very similar, both from the EEG and behavioural point of view, to that obtained with stimulation of the mesencephalic reticular formation. This was particularly evident, when the background electrical activity was synchronized either spontaneously or due to a previous single impulse or low frequency stimulation. Upon the stimulation a prompt activation of the trancing appeared, with a desynchronization of the neocortical activity and a highly regular theta rhythm in the hippocampus (Fig. 6). The rabbit stood up immediately or after several seconds from the onset of the stimulation and showed an increased motor activity. This EEG and behavioural arousal persisted for several minutes after the end of the stimulation. Synchronization of the tracing could be easily restituted by subsequent single impulse or low frequency stimulation of the medial thalamus. It thus was possible to induce alternatively a state of sleep and arousal many times in every experiment by using a varying stimulation frequency.

It should be stressed that the just described effects may be elicited in the given rabbit for a period of several weeks or even months.

III. Localization of the electrodes. In five of the rabbits electrodes reached to the complex of intralaminar nuclei. In one of the animals the end of the electrode was found in the anteromedial nucleus. The remaining 4 rabbits had electrodes in the dorsomedial nucleus.

Localization of the mesencephalic electrodes was within the reticular formation or on the boundary of the central gray. Two of the rabbits had electrodes within the central gray. All mesencephalic electrodes were on the level of the superior colliculus.

Hippocampal electrodes were found to be in the dorsal hippocampus or, in 4 rabbits, on the boundary of the fimbria hippocampi.

DISCUSSION

The present work confirmed results reported by T i s s o t and M o n n i e r (1959), M o n n i e r et al. (1960) and H ö s l i and M o n-n i e r (1962) indicating a functional duality of the medial thalamus in

rabbits. Some data, however, have not been sufficiently demonstrated by other workers and should be emphasized.

EEG and behavioural effects elicited by selective excitation of both functional systems in the medial thalamus appear to depend, for a great part, on the frequency of the stimulating current. With middle and high frequencies arousal is observed. The recruiting system, on the other hand, is sensitive to single impulses and a low frequency stimulation. The most interesting finding in the present study is the possibility to evoke spindles and recruiting response in freely moving rabbits.

Two phenomena observed with single impulses should be stressed: (1) the character of the spindles in different states of the brain electric activity and (2) a sleep-like behaviour developing with single impulse stimulation.

The most typical and well developed spindles may be evoked, when a degree of synchronization consisting of spontaneous spindling in neo-cortical lead is noted. Both in the periods of EEG activation and hyper-synchrony the spindles fail to appear or are scarse and composed only of a few irregular oscillations. This means that for the described phenomenon a definite state of excitability of nonspecific systems is necessary. A similar finding has been reported by T r a c z y k and S a d o w-s k i (1962). Spindles due to caudate nucleus stimulation in cerveau isolé cats were elicited neither after administration of eserine in a dose provoking a desynchronization of the EEG nor after atropine inducing slow high voltage hypersynchronous activity of the brain. In V u-t r i e n--a n's (1964) experiments on conscious rats, spindles evoked by electric shocks stimulating the caudate nucleus were better pronounced in the state of wake-fulness than during sleep.

Stimulation of the medial thalamus with single impulses has been found to be most effective in eliciting sleep-like behaviour which was always accompanied by a synchronized EEG tracing. Single impulses should be thus considered as a powerful factor inducing a general inhibition of motor activity. An inhibitory effect upon a bar pressing response was observed in cats in which single shocks were applied to the caudate nucleus as to evoke the so-called „caudate-spindles", very similar to those obtained with thalamic stimulation (B u c h w a l d et al. 1961).

E v a r t s and M a g o u n (1957) were the first to report the recruiting response in waking cats. Y a m a g u c h i et al. (1963, 1964) found, also in cats, that recruiting phenomena varied with the state of wakefulness. Best recruiting was seen during the stage of EEG synchronization. In waking cats the recruiting response was present, but was depressed during increased motor activity or attention. In rabbits

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the recruiting response was evident both during the activation and synchronization of the EEG tracing, its character was different, however. While in the activation period to every impulse only one potential appeared, during synchronization often two potentials were evident, similarly as in J a s p e r's (1949) acute experiments on cats. After the last impulse a series of oscillations appeared in some experiments similar to that described by L o n g o (1962). From the just presented data it may be concluded that in arousal recruiting is not abolished. Behavioural effect accompanying the recruiting response depended on the strength of the stimulation. Weak, slightly above threshold stimuli provoked a gradually developing sleep-like behaviour, whereas stong ones aroused the animals.

The character of the recruiting response was in agreement with that observed by other authors in rabbits (T i s s o t and M o n n i e r 1959) and other animals (V e r z e a n o et al. 1953). It has been established that the site of origin of these waves comprises the area of intralaminar and midline thalamic nuclei. It should be noted, however, that in rabbits a great portion of the medial thalamus is occupied by the dorsomedial nucleus (A r i ë n s K a p p e r s et al. 1960). There is no agreement upon the rôle of this nucleus in recruiting phenomena. K e r r and O'L e a r y (1957) found it to be not essential in transmitting impulses of the recruiting response to the cortex in rabbits. S t a r z l and M a g o u n (1951) were able to elicit a recruiting response from this nucleus in cats, particularly from its lateral part bordering with the complex of intralaminar nuclei. The rabbits, in which the electrode tip was found within this nucleus, had always good recruiting. Taking into consideration the relatively small dimensions of the medial thalamus in rabbits and the size of electrodes used in these experiments, spread of the current to the neighbouring nuclei cannot be excluded, however.

It seems necessary to analyse more exactly the nature of sleep-like behaviour induced in rabbits by thalamic stimulation.

D e m e n t (1958) described in cats two stages of natural sleep: a synchronized and an activated one. This problem was investigated thoroughly by J o u v e t (1962). According to the latter author the synchronized sleep is related to the thalamo-cortical system of D e m p-s e y and M o r i s o n (1942), whereas the activated one involves the nucleus reticularis pontis caudalis and its connexions with the limbic system. The two stages of sleep have been recently described in rabbits by R o l d á n and W e i s s (1963), though K h a z a n and S a w y e r (1964), on the basis of pharmacological investigations, found the paradoxical sleep in rabbits to resemble rather a state of alertness. It should be emphasized that thalamic stimulation with single or low frequency

repetitive stimuli induces a sleep-like behaviour which is always accompanied by a synchronized EEG tracing. This pattern thus corresponds to the stage of synchronized sleep („sommeil lent") of J o u v e t (1962). Similar behavioural and EEG phenomena have been elicited from the brain stem reticular formation by applying a low frequency stimulation. (F a v a l e et al. 1961) and from other structures (P a r m e g g i a n i 1962). The latter author believes, however, that the thalamus is most important for these effects.

The final problem to be discussed is the mechanism, through which the sleep-like phenomena appear. The EEG tracing accompanying this type of behaviour, particularly a disruption of the regular theta activity in the dorsal hippocampus, is typical for a state of depression of the ascending reticular system (G r e e n and A r d u i n i 1954). One of the possible explanations is that of J o u v e t (1962) that the medial thalamus exerts an inhibitory influence on the brainstem activating system through the cerebral cortex. This point of view is based first on the lack of slow wave activity in EEG records taken from totally decorticated rabbits (T e n K a t e et al. 1940, S e r g i o and L o n g o 1960), cats (J o u v e t 1962) and monkeys (K e n n a r d 1943) and secondly, on the failure to evoke a synchronized sleep through thalamic stimulation in such cats (J o u v e t 1962). M o r i s o n and B a s s e t t (1945) were able, however, to record spindles from the basal ganglia of cats during three days following decortication. Nevertheless, it may be concluded from the present results that the nonspecific system of the medial thalamus is a structure essential for sleep-like behaviour characterized by synchronous electrical activity of the brain.

SUMMARY

1. In freely moving rabbits bearing chronically implanted cortical and subcortical electrodes the medial thalamus was stimulated electrically and EEG records were taken.

2. Stimulation of the medial thalamus with single impulses produced spindles in the sensorimotor cortex, consisting of 8 — 15/sec waves. The spindles were best pronounced in a period of synchronization of the EEG tracing characterized by spontaneous spindling. From a behavioural point of view, sleep-like patterns ensued accompanied by a deep synchronization of the tracing.

3. Low frequency stimulation of the medial thalamus (5 cy/sec) produced a recruiting response in the cortex. With weak stimuli, an inhibition of motor activity and sleep-like behaviour developed.

4. Middle (30 cy/sec) and high (100 — 300 cy/sec) frequency stimulation of the medial thalamus produced an EEG and behavioural arousal.

5. Functional properties of the nonspecific thalamic system and its rôle in sleep mechanisms are discussed.

REFERENCES

AKERT K., KOELLA W. P. and HESS R. jr 1952 — Sleep produced by electrical stimulation of the thalamus. Am. J. Physiol., *168*, 260.

ARIËNS KAPPERS C. U., HUBER G. C. and CROSBY E. C. 1960 — The comparative anatomy of the nervous system of vertebrates, including man. New York: Hafner Publ. Co.

BUCHWALD N. A., WYERS E. J., LAUPRECHT C. W. and HEUSER G. 1961 — The „caudate-spindle". IV. A behavioral index of caudate — induced inhibition. Electroenceph. clin. Neurophysiol., *13*, 531.

DEMENT W. 1958 — The occurence of low voltage, fast electroencephalogram patterns during behavioral sleep in the cat. Electroenceph. clin. Neurophysiol., *10*, 291.

DEMPSEY E. W. and MORISON R. S. 1942 — The production of rhythmically recurrent cortical potentials after localized thalamic stimulation. Am. J. Physiol., *135*, 293.

DEMPSEY E. W. and MORISON R. S. 1943 — The electrical activity of a thalamo--cortical relay system. Am. J. Physiol., *138*, 283.

EVARTS E. V. and MAGOUN H. W. 1957 — Characteristics of cortical recruiting responses in unanesthetized cats. Science, *125*, 1147.

FAVALE E., LOEB C., ROSSI G. F. and SACCO G. 1961 — EEG synchronization and behavioral signs of sleep following low frequency stimulation of the brain stem reticular formation. Arch. ital. Biol., *99*, 1.

FIFKOVA E. and MARSALA J. 1960 — Stereotaxic atlases for the cat, rabbit and rat brains. Prague: State Medical Publishers, (in Czech).

GREEN J. D. and ARDUINI A. A. 1954 — Hippocampal electrical activity in arousal. J. Neurophysiol., *17*, 533.

HESS R. jr, KOELLA W. P. and AKERT K. 1953 — Cortical and subcortical recordings in natural and artificially induced sleep in cats. Electroenceph. clin. Neurophysiol., *5*, 75.

HESS W. R. 1954 — Das Zwischenhirn. Syndrome, Lokalisationen, Funktionen. Basel: B. Schwalbe Verlag.

HÖSLI L. and MONNIER M. 1962 — Schlaff- und Weckwirkungen des intralaminären Thalamus. Pflügers Arch, ges. Physiol., *275*, 439.

HÖSLI L. and MONNIER M. 1963 — Wirkung der bilateralen Koagulation des mediozentralen intralaminären Thalamus auf die elektrische Hirnaktivität und das Verhalten des Kaninchens. Helv. physiol. pharmacol. Acta, *21*, 109.

HUNTER M. and JASPER H. H. 1949 — Effects of thalamic stimulation in unanesthetized animals. Electroenceph. clin. Neurophysiol., 1, 305.

JASPER H. 1949 — Diffuse projection systems: the integrative action of the thalamic reticular system. Electroenceph. clin Neurophysiol., 1, 405.

JOUVET M. 1962 — Recherches sur les structures nerveuses et les méchanismes responsables des différentes phases du sommeil physiologique. Arch. ital. Biol., 100, 125.

KENNARD M. A. 1943 — Electroencephalogram of decorticate monkeys. J. Neurophysiol., 6, 233.

KERR F. W. L. and O'LEARY J. L. 1957 — The thalamic source of cortical recruiting in the rodent. Electroenceph. clin. Neurophysiol., 9, 461.

KHAZAN N. and SAWYER C. H. 1964 — Mechanism of paradoxical sleep as revealed by neurophysiologic and pharmacologic approaches in the rabbit. Psychopharmacologia, 5, 457.

LONGO V. G. 1962 — Rabbit brain research — volume II. Electroencephalographic atlas for pharmacological research. Amsterdam — New York: Elsevier Publishing Co.

McLARDY T. 1956 — Some midbrain-thalamic long-fiber connections in monkey. In: Progress in neurobiology. Proceedings of the First International Meeting of Neurobiologists. ARIËNS KAPPERS J. (ed.). Amsterdam — London — New York — Princeton: Elsevier Publishing Co., p. 384.

MONNIER M., HÖSLI L. and KRUPP P. 1963 — Moderating and activating systems in medio-central thalamus and reticular formation. Electroenceph. clin. Neurophysiol., suppl. 24, 97.

MONNIER M., KALBERER M. and KRUPP P. 1960 — Functional antagonism between diffuse reticular and intralaminary recruiting projections in the medial thalamus. Exper. Neurol., 2, 271.

MORISON R. S. and BASSETT D. L. 1945 — Electrical activity of the thalamus and basal ganglia in decorticate cats. J. Neurophysiol., 8, 309.

MORISON R. S. and DEMPSEY E. W. 1942 — A study of thalamo-cortical relations. Am. J. Physiol., 135, 280.

MORUZZI G. and MAGOUN H. W. 1949 — Brain stem reticular formation and activation of the EEG. Electroenceph. clin. Neurophysiol., 1, 455.

PAPEZ J. 1956 — Central reticular path to intralaminar and reticular nuclei of thalamus for activating EEG related to consciousness. Electroenceph. clin. Neurophysiol., 8, 117.

PARMEGGIANI P. L. 1962 — Sleep behaviour elicited by electrical stimulation of cortical and subcortical structures in the cat. Helv. physiol. pharmacol. Acta, 20, 347.

REINOSO F. 1954 — Anatomisch-cartesianischer Atlas zur Anwendung bei experimentellen Arbeiten am Katzengehirn durch Lokalisierungsmethoden von Horsley, Clarke und Hess. Anat. Anzeiger, 100, Ergänzungsheft, 151.

ROLDÁN E. and WEISS T. 1963 — Excitability changes of the reticular ascendent activating system during sleep cycle in the rabbit. Arch. int. Physiol., 71, 518.

SAWYER C. H., EVERETT J. W. and GREEN J. D. 1954 — The rabbit diencephalon in stereotaxic coordinates. J. comp. Neurol., 101, 801.

SCHREINER L., McK. RIOCH D., PECHTEL C. and MASSERMAN J. H. 1953 — Behavioral changes following thalamic injury in cat. J. Neurophysiol., 16, 234.

SERGIO C. and LONGO V. G. 1960 — Azione di alcuni farmaci sul tracciato elettrico cerebrale e sul comportamento del coniglio decorticato. Arch. int. Pharmacodyn., *125*, 65.

STARZL T. E. and MAGOUN H. W. 1951 — Organization of the diffuse projection thalamic system. J. Neurophysiol., *14*, 133.

TEN CATE J., WALTER W. G. and KOOPMAN L. J. 1940 — Electro-encephalography in rabbits after removal of the neopallium. Arch. Néerl. Physiol., *24*, 578.

TISSOT R. and MONNIER M. 1959 — Dualité du système thalamique de projection diffuse. Electroenceph. clin. Neurophysiol., *11*, 675.

TRACZYK W. and SADOWSKI B. 1962 — Electrical activity of the „cerveau isolé" during caudate nucleus stimulation and its modification by eserine and atropine. Acta physiol. pol., *13*, 521.

VERZEANO M., LINDSLEY D. B. and MAGOUN H. W. 1953 — Nature of recruiting response. J. Neurophysiol., *16*, 183.

VU-TRIEN-AN 1964 — The caudate spindle in rats during sleep and arousal and under the effect of atropine and physostigmine. Physiol. bohemoslov., *13*, 478.

YAMAGUCHI N., LING G. M. and MARCZYŃSKI T. J. 1963 — Differences between cortical recruiting responses observed during wakefulness and natural sleep. Nature (Lond.), *199*, 189.

YAMAGUCHI N., LING G. M. and MARCZYŃSKI T. J. 1964 — Recruiting responses observed during wakefulness and sleep in unanesthetized chronic cats Electroenceph. clin. Neurophysiol., *17*, 246.

Book reviews

Photochemistry of proteins and nucleic acids. By A. D. Mc LAREN and D. SHUGAR. Published by Pergamon Press, Oxford — London — Edinburgh — New York — Paris — Frankfurt, 1964, pp. 449.

In view of the increasing interest in the biological effects of various types of radiation the book on photo-chemistry of proteins and nucleic acids is very welcome. This interest stems from the wide recognition that new techniques, such as column and paper chromatographies, lead to the isolation and identification of minute quantities of radiation products which play an important role in biological systems. By the use of electron spin resonance, flash spectroscopy and nuclear magnetic resonance much valuable material concerning the excited states of molecules and photoproduct structures has been compiled. In addition, the knowledge of the structure of proteins and nucleic acids and that of the role of the nucleid acids in protein biosynthesis and genetics helps to understand, at the molecular level, the influence of radiation exerted on the chemical modifications of the fundamental cellular constituents.

This monograph surveys critically the present-day knowledge of the photobiology. After a brief introduction to the principal laws of photochemistry, spectroscopy and kinetics a major section of the monograph covers the topics of the effect of ultraviolet light on proteins, nucleic acids, enzymes and viruses. The emphasis is on the explanation of photochemical processes in macromolecules and viruses in terms of the photochemistry of monomers and individual bonds.

Chapter VIII is devoted to the inactivation of viruses by ultraviolet light. Some material is also presented on photosensitized inactivations with visible light. Particular attention is paid to inactivation studies with a plant virus.

Chapter IX contains interesting remarks on the origin of life on the earth as a result of photochemical reactions. The interest in this field has been increasing and does not represent a speculation only but, currently, has an experimental foundation. The rapid development of space satelite and interplanetary space vehicle program promises the rate of increase in our knowledge with regard to the possible existence of organic material and perhaps some forms of microorganisms in outer space and on the planet Mars.

A comprehensive appendix provides a selection of modern techniques in photochemistry and photobiology. The authors have accomplished this by careful descriptions of the techniques and apparatus used. There is also a list of over thousand references to the more important publications up to the end of 1963.

This volume is highly recommended to instructors of advanced courses in photobiology, biochemists, biophysicists, virologists and molecular biologists as well as to all thoseengaged in research on photochemistry.

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Advances in Enzyme Regulation. Volume 2. Proceedings of the Second Symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Liver held at Indiana University School of Medicine. Indianapolis, Indiana. September 30 and October 1, 1963. Edited by George Weber. Published by Pergamon Press, Symposium Publications Division, Oxford — London — Edinburgh — New York — Paris — Frankfurt, 1964, pp. 405 + VII.

This interesting and informative volume which contains a series of lectures presented at the 2nd symposium on Regulation of Enzyme Activity in Normal and Neoplastic Liver suffers slightly from the absence of a discussion. One may think that the discussion must have been of considerable interest and, presumably, as intriguing as the lectures recorded. Despite this minor shortcoming, this volume nevertheless is a successful presentation and an excellent source of the topics treated. The material presented has been gathered and arranged in such a way for the first time, and the participants deserve a great deal of credit.

In session 1, papers are presented on the regulation of gluconeogenesis through enzyme activity and synthesis. G. W e b e r, R. L. S i n g h a l, N. B. S t a m m, E. A. F i s c h e r, and M. A. M e n t e n d i e k, present an impressive evidence that the enzymes involved in gluconeogenesis are affected by adrenal cortical hormones (synthesis of new enzyme); the authors conclude that insulin may act as a physiological repressor of a sequence of gluconeogenic enzymes in the liver. H. A. L a r d y, D. O. F o s t e r, E. S h r a g o and P. D. R a y describe the synthesis of phosphopyruvate emphasizing the major role of this substance in carbohydrate synthesis and show that phosphopyruvate synthesis is regulated via phosphoenol-pyruvate-carboxykinase, with malic enzyme playing a minor role. Finally, M. F. U t t e r, D. B. K e e c h and M. C. S c r u t t o n discuss the possible role of acetyl — CoA in the control of gluconeogenesis. The authors demonstrate that the carboxylation of pyruvate to oxaloacetate by pyruvate carboxylase is fully dependent on the presence of acetyl-CoA. The mechanisms of this reaction are treated together with the properties of the enzyme itself.

A special symposium lecture by Sir Hans A. K r e b s, E. A. N e w s h o l m e, R. S p e a k e, T. G a s c o y n e and P. L u n d, which follows section 1, is devoted to factors regulating the rate of gluconeogenesis in animal tissue. It describes a pigeon liver homogenate preparation that readily synthesizes glucose from various substrates. So far, liver preparations have not been proved satisfactory. The authors, on the basis of their experiments (including kidney slices), conclude that feedback control of gluconeogenesis occurs at least at two stages, one being the fructose diphospate, the other the pyruvate carboxylase.

Session 2 is entitled Gluconeogenesis in Various Physiological and Pathological Conditions, and the first paper presented by O. W i e l a n d, L. W e i s s and I. E g e r-N e u f e l d t outlines the regulation of liver acetyl-CoA metabolism in relation to ketosis. This work further completes the picture we now have of the

relationship between carbohydrate and lipid metabolism. The paper by J. A s h m o-
r e, S. R. W a g l e and T. U e t e, which in the opinion of the reviewer is more
closely allied with papers of section 1 than of section 2, deals with changes in
selected enzyme activities (e.g., glucose-6 phosphatase and transaminases) fol-
lowing steroid administration with special reference to glucose formation in
the liver. F. R o s e n and C. A. N i c h o l discuss the specificity of adaptive
enzyme induction in response to cortisol. This section concludes with the paper
by G. C a h i l l on hypoglycaemia in man.

Session 3 is devoted to the regulation of glucokinase. The first paper is by
V. S. I l y i n on the hormonal regulation of liver hexokinase. Since glucokinase
has only recently been discovered, some confusion cannot be avoided, but V. S.
I l y i n does his best to relate his findings to this recent discovery. He describes
the development of hexokinase activity in different parts of the liver cell in the
rabbit and the effect of corticosteroids and insulin on diabetes. Cortisone is shown
to be very effective; in some cases, it is effective only if dissolved in beta lipo-
protein. A. S o l s, M. S a l a s and E. V i n u e l a discuss their findings on induced
biosynthesis of liver glucokinase, and C. S h a r m a. R. M a n j e s h w a r and S.
W e i n h o u s e consider this subject in relation to hormonal and dietary factors.

Session 4 is entitled Feedback Regulation of Enzyme Activity and Synthesis
in the Liver. Here, N. O. K a p l a n and T. L. G o o d f r i e n d draw attention to
the two types (L and M) of lactic dehydrogenase and their possible significance
for function. The regulatory control of pyrimidine biosynthesis is thoroughly
covered by E. B r e s n i c k. H. C. P i t o t, C. P e r a i n o, N. P r i e s and A. L.
K e n n a n describe glucose repression and induction of enzyme synthesis in rat
liver, the enzymes concerned being among others threonine dehydrase and ornithine
transaminase. M. D. S i p e r s t e i n and V. M. F a g a n present a very interesting
paper on the feedback regulation of cholesterol synthesis in which they show that
exogenously supplied (but not endogenously produced) cholesterol suppresses
cholesterol synthesis in the liver, the step that is suppressed being mevalonate
formation from beta hydroxybeta-methylglutarate. This may have an important
bearing on other aspects of lipid metabolism. J. V. P a s s o n n e a u and O. H.
L o w r y follow with their paper on the role of phosphofructokinase in metabolic
regulation where the various metabolic factors (ATP, Mg, citrate, etc.) controlling
its activity are described.

Session 5 deals with Enzyme Regulation by Cofactor Levels. The first paper
is by O. G r e e n g a r d on the regulation of apoenzyme levels of tyrosine trans-
aminase and tryptophan pyrrolase, the heme coenzyme being considered of major
importance for pyrrolase. J. E. W e r g e d a l, Y. K u and A. E. H a r p e r report
on the dietary effects on ammonia and glycine catabolism and glutamic dehy-
drogenase activity in the liver. W. E. K n o x presents further data on the sub-
strate type and hormone induction of tyrosine transaminase and puts forward
a model on how this works.

The final session of this symposium covers enzyme regulation in hepatoma. It
is of much interest that there is no effect of the diet on enzymes in hepatoma.

The rearder will find this volume a valuable source of current information
for enzyme regulation.

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Thirst. Proceedings of the First International Symposium on Thirst in the Regulation of Body Water. Held at the Florida State University in Tallahassee, May 1963. Edited by Matthew *J. Wayner. Published* by Pergamon Press, Oxford —London—Edinburgh—New York—Paris—Frankfurt, 1964, pp. 570.

The First International Symposium on Thirst In the Regulation of Body Water, which was held at the Florida State University in Tallahassee, U. S. A., in May 1963, contributed considerably to the problem of water regulation in living organisms. Undoubtedly, together with air and food the role of water in the life of virtually all living matter is clearly basic to an understanding of both behavior and survival. Traditionally, thirst has been treated as a sensation of oropharyngeal origin due to a general cellular dehydration or osmotic pressure gradient, accompanying fluctuations of body fluids. Thirst, as a motivational force, develops, according to this view, as a direct consequence of this sensation. In this respect, an extensive research is being carried out at the molecullar and cellular levels, at the level of body system, and finally, at the level of an intact freely moving animal. The participants of this symposium presented experimental material concerning water content in living organisms, the balance of body fluids and the role of water in both phylogenetic and ontogenetic aspect, as well as relationships between body fluids and the behavior of an intact organism. The problem of fluid intake was also considered from the clinical point of view. Finally, new findings were reported with regard to neurohormons and chemical mediators within the peripheral and central nervous system.

E. F. A d o l p h found that water intakes vary remarkably among species and among individuals. Neither structural nor functional correlates of this variability among species have been discovered. However, the water ingestion participates in the regulation of body water content as a fixed response to body water deficit. The satiation becomes permanent when an amount of water equal to the deficit is allowed to reach the stomach and to be absorbed from the gut. Distention of the stomach itself supplies sensory information that leads to an early phase of this satiation. The effect of distention is abolished by a bilateral disconnection of the vagus nerve and other sensory pathways originating in the stomach wall. Furthermore, A d o l p h found that water intake varies linearly with either body water volume or dilutation since, there are more ways of inducing drinking than water deprivation and solute addition. This fact leads to the notion that there is no unique stimulus for drinking.

E. J. T o w b i n called attention to the role of the gastrointestinal tract in the regulation of water intake. He found that the stomach and duodenum serve as the sites for the production of isotonicity of the ingestinal fluid. There is a very rapid and active biderectional movement of water and ions across the gastric mucosa. Gastric distention per se lengthens the intervals between drinking periods but permanent satiety ultimately depends upon the absorption of water from the gut. The author concluded that water receptors lie deeper in the mucosal membrane than salt receptors. On the other hand, D. N o v i n in his investigation on the effect on insulin injection on water intake reported that insulin increases water intake. Depleting an animal of salt causes a decrease of the extracellular osmotic pressure together with an entrance of water into the intracellular space from the extracellular. The increase in drinking as a result of salt depletion vould seem to contradict the classical cellular dehydration theory of thirst as salt depletion.

It hydrates rather than dehydrates the cells. It is possible that in addition to an osmotic control of thirst there are volume receptors which affect water intake.

Ch. K u t s c h e r investigated the adaptation of rats to a water deprivation schedule. Reduction of the extracellular volume of the water can increase antidiuretic hormone in plasma. The physiological mechanisms are still obscure, but the increased retention of water cannot be explained wholly on the basis of high plasma antidiuretic hormone. G. C o l l i e r investigating thirst as a determinant of reinforcement found that the reduction of food intake in thirsty animals cannot be accounted for the assumption that rats are still hungry but become satiated more rapidly because of the reduction of water available for dilutation of the ingested load.

J. L. F a l k presented data on water intake and bar-pressing rats for food in an operant conditioning. The lateral hypothalamic lesions can attenuate or completely impair the polidipsia in animals with primary polidipsia. This is a motor effect because during the postoperative phase the animals sometimes make the same number of licks on the freely available water tube. Deficit in licking efficiency occurs with ventromedial lesions and in animals with median eminence lesions too.

H. L. J a k o b s investigated hunger and thirst and problem of their relationship and the locus of the detector system involved in food and water intake. A series of experiments was described which was designed to help separating taste and gastric factors from general systemic dehydration in controlling the intake of glucose, dry food and water output. In general, osmotic analysis was not found adequate to explain those phenomena. The results strongly suggested the importance of neural information initiated in the oral and gastric cavities in the control of glucose intake, the intake of dry foods and in water output.

B. A n d e r s s o n, Ch. G. G a l e and J. W. S u d s t e n in their investigation on goats found that local warming of medial or lateral part of the hypothalamus may act as a nonspecific stimulus; local cooling, on the other hand, may inhibit all neuronal activity in the close vicinity of the thermodes. Nevertheless, taken together the alimentary effects of anterior hypothalamic lesions and those due to hypothalamic warming and cooling indicate that a thermostatic mechanism really exists. Central warm detectors may be regarded as influencing feeding and drinking mechanism via direct neural connections or via changes in the humoral status of the organism.

R. W. S m i t h and S. M. M c C a n n in their experiments on rats with lesions and stimulations in the lateral hypothalamus found that lesions of the lateral hypothalamus area can abolish both food and water intake. Large lesions in this area can result in a permanent adipsia. This means that the lateral hypothalamic area is essential for drinking and feeding behavior. Stimulation studies indicated that the feeding and drinking centers in the lateral hypothalamus are separable, although they probably overlap. A similar problem was discussed by A. N. E p s t e i n and P. T e i t e l b a u m who also studied the behavior of lateral hypothalamic rats. The drinking of the recovered lateral rats is controlled by local oropharyngeal stimuli that signal a dry mouth, but the animal does not drink in response to the internal stimuli that signal inbalances in the distribution of body water.

B. W. R o b i n s o n elaborating the forebrain alimentary response in rhesus monkeys found that although food intake reaches its highest sampling rate in the

lateral hypothalamus, many of the occurrences of this response were found outside this structure. Though food responses inherent in the lateral hypothalamic area are quantitatively superior, the extra hypothalamic structures must be assumed to ply a major functional role in organizing this response and integrating it with other aspects of adaptive behaviour.

P. J. M o r g a n e examined the limbic forebrain system as well as cortical and subcortical system as a common structure for a variety of emotional and viscerosomatic reactions in animals. G. J. G i l b e r t, and L. T. C r o w found that subcommissural organ plays an endocrine role in the aldosteron secretion and may participate in volume regulation with functions paralleling those of the hypothalamic receptors.

S. P. G r o s s m a n found that central regulation of water intake as well hypothalamic as amygdalar may have common neurochemical properties. His data suggest that the central regulation of feeding behavior is mediated by neural mechanisms which may propagate impulses of adrenergic transmitter substances at the level of the hypothalamus and the amygdaloid complex. The central regulation of thirst, on the other hand, seems to be cholinergically mediated. A. E. F i s h e r and J. N. C o u r y investigating the effect of adrenergic and cholinergic transmitter substances on the hippocampal, thalamic and hypothalamic structures came to the same conclusion.

Generally, the proceedings of the „Thirst" symposium appear a most valuable position in the recent neurophysiological and behavioral literature which may be of interest both to biologists and medicals.

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Eksperimentalnoe issledovanie tsentralnykh mekhanizmov zritelnoi funktsii (*Experimental investigation of central mechanisms of the visual function*). By M. M. KHANANASHVILI. Edited by Z. A. VASILEVA. Published by Medgiz, Leningrad, 1962, pp. 180.

This book furnishes data on visual function in the dog, obtained by means of the method of conditioned reflexes (CRs) in association with the ablation technique and anatomical control.

The sections of the book devoted to the review of the literature are dealing with the following problems: (a) the anatomic organization of the visual system in primates and subprimates, (b) the anatomy of the occipital cortex, and (c) the contribution of the occipital cortex to vision.

K h a n a n a s h v i l i employed the salivary and classical defensive conditioning methods as well as the method for studying food-reinforced motor spatial CRs, devised by P. S. K u p a l o v.

The author's goal was to study: (1) the role of the occipital areas 18 and 19, parietal areas and sensorimotor areas in analyzing and synthesizing simple and complex visual patterns; (2) neural mechanisms responsible for the interaction between the visual and other cortical projection areas; and (3) the relation of the lateral geniculate body to complex visual behavior.

In an attempt to realize this goal K h a n a n a s h v i l i carried out selective
lesions of the neocortex as well as a variety of incisions of different depth in order
to isolate cortical fields. Also complete and partial lesions of the lateral geniculate
bodies were carried out.

It was found that following one-stage bilateral lesions of areas 18 and 19
the salivary CRs to the presentation of visual conditioned stimuli (CSi) consi-
derably decreased and their latencies increased. In addition, the performance on
a difficult visual pattern problem, consisting of a circle vs. octagon differentiation,
was disturbed throughout a 4-month postoperative period and the relationship
between inhibitory and facilitatory phases in a salivary delayed CR was affected
for 2.5 months.

On the basis of these findings, K h a n a n a s h v i l i has suggested that the
impairment of a difficult visual differentiation and delayed CR as well as the
disturbance in the interrelation between the visual and sensory cortical receiving
areas result primarily from a damage to areas 18 and 19. However, it has been
indicated that area 17 is also related to complex visual behavior. In conclusion, the
author points out that the three cortical visual areas are specific in functioning.

The second part of this book contains experimental material concerning the
influence exerted on visual behavior of lesions of cortical areas other than those
of the occipital region. It was found that a lesion of area 7, carried out in two
stages, produced a persistent impairment of a difficult visual differentiation and
delayed CR performance together with a disturbance in the interaction between
the visual and sensory projection areas. On the other hand, a lesion of area 5
produced a temporary impairment of a difficult visual differentiation.

By a study on the sensori-motor cortex involvement in the visual function it
has been established that after a bilateral lesion of the sigmoid gyrus (the motor
cortex) an impairment occurred not only with regard to the motor performance
but also with regard to the visual performance. Thus it was found that during
a 3-month postoperative period dogs with lesions of the sigmoid gyrus were
markedly impaired on a circle vs. cross differentiation, which is an easy type
of differentiation. On the other hand, a damage to the sensory cortex was associ-
ated with a slight and transient impairment of the visual differentiation.

Considerable attention is given to the role of the associative fiber connections
within the occipital cortex in the analysis and synthesis of visual patterns. It was
found that after two saggital and three coronal incisions within area 17 the in-
tegration between the motor and visual behavior was disrupted in terms of an
impairment of the motor response to the presentation of moving objects. In a few
dogs, the occipital cortex was incised to isolate 20 or 40 small sectors. As a result
of these lesions the motor response was impaired for a period of more than half
a year. K h a n a n a s h v i l i interprets this in terms of a disturbance in the ana-
lysis and synthesis of complex visual patterns due to the sectioning of intracortical
association fibres in the visual projection area. However, some of the operated
animals showed a very slight and transitory impairment of complex visual per-
formance. This was particularly true for object pattern discriminations.

Much experimental material is presented on the functional interrelation be-
tween the visual and other cortical projection areas. In one experimental series,
the major intracortical association fibres were severed by an incision isolating the
entire visual projection area. In another experimental series, the visual cortex
was isolated from the motor cortex by disrupting the interconnecting fibres.

These lesions increased the latency of the CRs in response to the presentation of visual CSi and impaired the object differentiation performance throughout a 3-week period. However, the interaction between the visual and motor cortex was not lost since the positive salivary and motor CRs were either preserved or recovered promptly.

An interesting material has been collected to show alterations in the complex CR activity following a complete disruption of the association fibres of the cortical projection areas. K h a n a n a s h v i l i lays recurrent emphasis on the evidence that both a system of intracortical fiber connections and an arrangement of association fibres running through the white matter afford a basis for interaction between the cortical projection areas. He indicates that the neural circuit „cortex-subcortex-cortex" is of outstanding value and, therefore, an acceptance of an integrating subcortical superstructure cannot be justified.

Part of the book is devoted to the relation of the lateral geniculate body to vision. Under conditions of chronic experiments, K h a n a n a s h v i l i studied the effects of partial or complete ablating the lateral geniculate bodies upon visual function. He found that following a bilateral subtotal lesion (made by means of electrocoagulation) of the geniculate bodies in the dog object vision was entirely destroyed: the animal did not avoid obstacles and recognized no objects by sight. A classical defensive CR to the presentation of a visual CS, reinforced by an electric shock administered to the animal's limb, the training of which had been started one month after the bilateral removal of the geniculate body, was established within 130 trials. Moreover, intervals between the testing sessions decreased the size of the CR. On the other hand, the CR to the presentation of an acoustic CS was established within 6 trials. A complex visual behavior pattern was disturbed throughout a 15-month postoperative period of observation.

A partial damage to the lateral geniculate body, largely confined to its dorsal portion, produced a considerably smaller defect in vision. Thus two weeks after surgery the dog was able to avoid obstacles. Furthermore, a classical defensive CR to the presentation of a visual CS was established within 50 trials despite the fact that the latent periods varied from 3 to 10 sec. Interestingly enough, the CR to an acoustic CS was formed within 6 trials and the latent periods varied from 1 to 3 sec.

Further research is necessary to elucidate the role of the ventral portion of the lateral geniculate body in vision.

A control operation, consisting of an introducing the electrode in the lateral geniculate body and leaving it in the place for 15 sec, did not affect vision.

It must be emphasized that K h a n a n a s h v i l i is the first to study the function of the lateral geniculate body under chronic experimental circumstances, using conditioning and ablation techniques.

Careful examination of the material reviewed reveals a few minor shortcomings. It is regrettable that the author does not consider the role of the frontal eye-field for vision, although he apparently included this area in the lesion of the sigmoid gyrus.

A more serious fault is the use of a delayed CR to the presentation of a visual CS as an index of visual defects after both occipital and other cortical lesions. In the opinion of the reviewer, under these conditions an impairment of delayed CR does not necessarily reflect a deficit in vision.

Throughout the book, there are few errors in spelling and an occasional place where the meaning is not entirely clear.

Ot note is the fact that numerous findings recently obtained with the aid of electrophysiological techniques are in a good agreement with those obtained by Khararashvili by means of the conditioning techniques. This underlines the usefulness of the conditioning techniques. On the other hand, it is to be hoped that the author will consider necessary to use modern electrophysiological techniques in his future research to have more accurate insight in the brain localization.

All in all, this book is an extremely valuable addition to the literature dealing with vision and an outstanding contribution to the knowledge on the higher nervous activity. It may be recommended to both physiologists and clinicians.

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