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ACTA BIOLOGIAE EXPERIMENTALIS

PROPERTIES OF SPINAL FROG PREPARATION IN RELATION TO THE LEVEL OF THE SPINAL TRANSECTION

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In the 19th century, studies of the spinal frog were chiefly concerned with the receptive fields of spinal reflexes. Although these investigations already have more than a hundred years' old tradition, the problems involved are not as yet fully solved and an analysis of the spinal reflexes still meets with a number of difficulties.

It has been found that from any point of the frog's skin it is possible to obtain more than one type of reflexes (S a n d e r s - E z n 1867). Vigorous stimulation causes, what some of the investigators have been calling, a generalized reflex, even a general movement of the whole body (V o l k m a n n 1838).

Several receptive fields were differentiated producing antagonistic reactions. For instance, stimulating the digits of the hind limb produces either flexion (Turck's method) of this limb or extensi... (B a g l i o n i ... alm produces or the flexion ..., stimulation produces two flexes, whose of the hind ... clusion that flex arcs as ...cause refle- ...or instance, (1900), and

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... ...t a k e (1912) it was considered that the wisch reflex is the first part of the locomotor reflex and that those two reflexes cannot be separated. B a g l i o n i (1913) maintained that the

order of various reflexes in the frog's spinal cord is functional not segmental". H e r r i c k (1948), on the other hand, on the base of anatomical data, indicated that in the spinal cord of amphibians there is only one motor pattern, namely the locomotor one, and that other reflexes come within this frame.

The starting point of the present work was the observation that two separate groups of chronic spinal frog could be distinguished. One characterised by a definite predominance of the wisch reflexes to stimu-
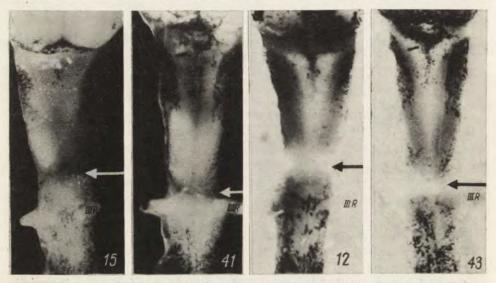

Fig. 1. Chronic spinal frog preparation

Arrows indicate the placement of the transection. III R — dorsal root III. In the upper part of the picture, lobi optici and the cerebellum. The posterior border of the fourth ventricle is well seen in Nos. 12 and 43

lation of the back (A f e l t 1963), the other showing on stimulation of the same field, a predominance of the locomotor reflexes of the jump type (A f e l t, unpublished experiments). A tentative histological analysis showed that the level of the transection within the bulbo-spinal junction differed in these two groups of frogs (Fig. 1). The present series of experiments was carried out to verify these data on acute spinal preparations.

MATERIAL AND METHODS

The experiments were carried out on *Rana esculenta*. No narcosis was used for the operation. The course of the operation was as follows: The smallest possible cut was made on the skin of the back, the dorsal part of the 1st and 2nd vertebrae was removed and the spinal cord transected with knife-scissors. In one group of frogs, the spinal cord was transected just beneath the end of the fourth ventricle, i.e., through the *calamus scriptorius;* in the other group, just above the

third spinal root, i.e., in the lower part of the bulbo-spinal junction. Since in Anura the 1st root appears only in the larva stadium and the 2nd reaches the spinal cord at the level of the *calamus scriptorius,* in our operation technique both preparations retained all roots beginning from the 3rd.

After transecting the spinal cord, the injured spot was covered with skin and almost immediately the reflexes from the receptive fields on the back were verified and then again those from the limbs.

In some experiments, the transection was made first at the higher level and then at the lower, the reflexes being elicited each time. In other animals, the spinal cord was transected only once, either at the higher or at the lower level.

The reflexes from the limb receptive fields were verified in two positions: when the animal was suspended, unable to touch anything with its limbs, and when it was in a sitting position. The reflexes from the limb receptive fields were evoked by light touches with a kind of brush, stimulating with Frey's hair and by pinching with a pincette or squezing manually. About 200 frogs were examined in this way. In a smaller group of frogs, reflexes to stimulation of the receptive field of the wisch reflex of the hind limb were analysed. This group consisted of 9 frogs in which the transection was made at the level of the *calamus scriptorius* and 10 with a lower transection. The reflexes were evoked by a brush or by means of a brass wire 0,09 cm in diameter and 1 cm long (F r a n z i s k e t 1951). The skin areas on the back about 1 cm long were stimulated by scratching with this wire or brush more or less in the same place. The animals were stimulated 100 times every 10 seconds. No qualitative differences in the reflexes to the above mentioned two stimuli were observed. Stimulating with the brush produced a smaller number of reactions to the same number of stimuli without changing the numerical relations between them. After the end of the experiment the frog was sacrificed and the spinal cord removed and photographed (Fig. 2).
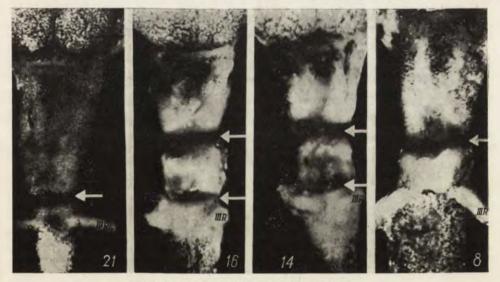

Fig. 2. Acute spinal frog preparation

Arrows indicate the placement of the transections. III R — dorsal root III. In Nos. 21 and 8, single transections at lower or higher level respectively. In Nos. 16 and 14, two transections, that at the higher level followed by the transection at the lower level

EXPERIMENTAL PART

A. Stimulation of various receptive fields
on the frog's skin after spinal cord transection
at the level of the *calamus scriptorius*

*1. Reflexes from the receptive field on the digit tips of the fore
limbs.* Stimulation of the digits of the fore limbs, while the animal was
suspended, evoked the flexion reflex, "Adduktionsreflex". This
form of the reflex (called strictly in refering to the fore limb
"Umarmungsreflex", B a g l i o n i 1913) was always observed in females
and in males out of the mating period. In late Winter and in Spring, the
males, to the same stimulation, performed the embracing reflex, differ-
ing from the previous one in that it is performed with both legs
(Umklammerungsreflex, B a g l i o n i 1913). In cases of very strong and
particularly longlasting stimulation, sometimes, after flexion of the fore
limb, flexion of the ipsilateral hind limb also occurred. In the sitting
position a not very defined reaction of withdrawing from the stimulus
(Retraktionsreflex) was observed.

*2. Reflexes from the receptive field on the palm and back of the
fore limbs.* Sqeezing the palms and stimulating the outside of the limb,
when the animal was suspended, evoked a forward or sideways thrust
of that limb in conjunction with an extensor thrust of the digits
(Streckreflex, B a g l i o n i 1913) with a simultaneous wisch reflex of the
hind limb directed towards the stimulated spot. The same stimulation
when the animal was in the sitting position caused withdrawal, back
thrust of the given limb and a wisch reflex of the hind limb similar to
the one previously described. The movements of the fore limb caused
a change in the animal's position. If they were not too strong, the
front part of the body shifted sideways, in the opposite direction to the
limb movement (Umdrehereflex, B a g l i o n i 1913); very strong move-
ments caused forward shifting.

3. Wisch reflex of the fore limb. The receptive field of the fore limb
wisch reflex stretches beltwise from the nostrils, round the edge of the
mouth, beyond the external ear and reaches to the under side of the
arm and forearm. In our frogs only the latter part was uninjured. Sti-
mulating the nostrils and mouth caused closing of the eyes. From the
rest of the field immediately after the operation it was very seldom
possible to evoke any definite movement. Sometimes, however, a wisch
reflex of the fore limb appeared, directed forward and towards the head.

On the other hand, several hours after operation this reflex could be evoked in all frogs without exception, and irrespective of the position of the animal. When the frog was sitting, the body was often slightly raised at first on the side of the stimulated limb and only later did the animal perform the wisch reflex.

4. *Wisch reflex of the hind limb*. Stimulating the receptive field of the wisch reflex on the animal's back caused two different reactions: the wisch reflex of the hind limb (V o l k m a n n 1838, B a g l i o n i 1913, B i e d e r m a n n 1900, F i c k 1870), or a stronger or weaker backward stretching of that leg (B a g l i o n i 1913, F i c k 1870), a stretch reflex, such as appears while jumping, when the animal pushes itself off the ground. This reaction, in agreement with S a n d e r s - E z n (1867), was recognised as a reaction of the locomotor type. The quantitative relations between these two reflexes (for 100 stimulations) are compared in Table I.

Table I

No. of the frog	1	3	5	8	10	11	14	15	16	
Wisch reflex	14	29	16	26	37	15	36	48	29	mean 28
Locomotor reflex	9	9	12	21	14	6	9	25	15	mean 13

The difference between the two arithmetic means is statistically significant, $p = 0.043$. The method of S t u d e n t - G o s s e t was used (G u i l f o r d 1960).

It thus follows from Table I, that in the spinal frogs in question, stimulation of the animal's back results in a predominance of the wisch reflex.

5. *Reflexes from the receptive field of the digit tips of the hind limb*. Stimulation of the digits of the hind limb caused flexion of that limb (Beugereflex, V o l k m a n n 1838, B a g l i o n i 1913, B i e d e r m a n n 1900, B r u c k e and Y a s u t a r o S a t a k e 1912, S c h l o s s e r 1880) when the animal was suspended, or the above described reaction of stretching the limb backwards when the frog was sitting (Tonischer Streckreflex in dem Fuss, S a n d e r s - E z n 1867, V o l k m a n n 1838, B a g l i o n i 1913, B i e d e r m a n n 1900, B r u c k e and Y a s u t a r o S a t a k e 1912, S c h l o s s e r 1860, G e r g e n s 1877).

B. Stimulation of various receptive fields on the frog's skin after spinal cord transection in the lower part of the bulbo-spinal junction

1.2.3. Reflexes from the receptive field on digit tips, palm, back skin of the fore limb and from the receptive field of the wisch reflex of the fore limb. Stimulation of all the receptive fields of the fore limb evoked only one reflex: a very strong stretching of this limb towards the back of the body, and, sometimes, the wisch reflex of the ipsilateral hind limb. Thus a reflex was evoked, which in the previously described spinal frog was characteristic only for stimulation of the palm and skin of the outside of the fore limb, and this only when the animal was in a sitting position. On the other hand, these reflexes, an element of which was flexion of the fore limb, disappeared. More intensive stimulation produced, in addition, tenseness of the front part of the body (Tonischer Streckreflex des Kopfes und des Rumpfes, G o l t z 1869, V e r w o r n 1896)[1].

4. Wisch reflex of the hind limb. Stimulating the receptive field of the wisch reflex of the hind limb evoked the same two reactions as described for stimulation of the first preparation. But the quantitative relations between the wisch reflex and the locomotor reflexs were different (Table II).

Table II

No. of the frog	1	14	15	16	17	18	19	20	21	22	
Wisch reflex	8	21	59	30	8	24	36	13	24	32	mean 25
Locomotor reflex	29	18	71	62	14	39	103	16	55	47	mean 45

The same statistical calculations as before showed that the differences between the number of these two reactions are also statisticaly significant ($p = 0.011$).

It thus follows from Table II that in the spinal frog in question a predominance of the locomotor type of reactions from the receptive field of the wisch reflex was obtained.

5. Reflexes from the receptive field on the digit tips of the hind limb.

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[1] G o l t z and V e r w o r n considered this reflex to be connected with the trigeminal nerve. In our experiments, it was not observed after cutting the *calamus scriptorius*. On the other hand, it appeared very regularly after the cut at the lower level, even when this cut was a second one. This problem needs further investigation.

Stimulation of the digit tips of the hind limb evoked the same reactions as described for the previous preparation, i.e. flexion of the limb when the frog was suspended and backward stretching when it was sitting.

DISCUSSION

The above described results show that there are differences in the reflexes of spinal frogs with transection at different levels of the bulbo-spinal junction. In spinal frogs with the transection at the *calamus scriptorius*, stimulation of different receptive fields of the fore limb gives a greater number of various reflexes. The same stimuli to a spinal frog transected at a lower level evoke a smaller variety of reactions and, as a rule, only stretch reflexes can be obtained. Stimulating the receptive fields on the back of the animal and on the surface of the hind limbs evokes in both preparations the same reflexes. It was, however, observed that stimulating the back of the spinal frog transected in the lower part of the bulbo-spinal junction produces a greater number of reflexes of the locomotor type, i.e. extensor thrusts, than the same stimulation of the higher cut preparation. In view of the fact that in the lower cut preparation there appears, moreover, the Verworn stretch reflex, it may be stated that this preparation shows a generally increased tendency to react to stimulation by the extensor thrust. It seems that the differences in interpretation of the phenomenon of overlapping of the wisch and locomotor reflexes, which appear in older papers, may be at least partially explained on the base of our results. It seems from the earlier descriptions with a tendency to treat the wisch reflex as an independent unit that the authors used preparations with a predominance of the wisch reflex (higher transection?), while in others, where the wisch reflex was treated as an element of the locomotor reflex, preparations with a predominance of the extensor thrust were used (lower transection?).

The fullest anatomic description of the vicinity of the bulbo-spinal junction in amphibians was given by H e r r i c k (1948). In Urodela, the *calamus scriptorius* extends between the first and second pairs of spinal roots, and the dorsal part of its gray matter contains two important nuclei: the commissural nucleus of Cajal and the nucleus of the dorsal funiculi, extending for a considerable distance anteriorly and posteriorly of the *calamus scriptorius* and considered as comparable with the external cuneate nucleus of mammals. Both these nuclei form the first center of correlation and integration for all exteroceptive, proprioceptive and visceral functions of the body with exception of sight

and smell. It is reached by the cutaneous reception and deep sensibility signals from the head, the trunk and the limbs. Posture, locomotor reaction and other basic components of the mass-movement type are controlled here. Efferent fibres starting from the nucleus funiculi dorsalis proceed both in the cronial and caudaldirection. They are, in the majority, myelinated and partly decussating. The uncrossed pathway leads mainly to the upper parts of the efferent system, the decussating fibres go mainly spinewards and are responsible for the bulbar reflexes. It is not known to what extent this pattern holds good for the Anura, but the probability is that they have a similar configuration.

It can therefore be assumed that the difference between our two preparations was due to the fact that in the spinal frog with higher transection, the nucleus described by H e r r i c k was to a great extent preserved, whereas in the lower cut preparation it was entirely or almost entirely lacking.

Frogs the spinal cord of which transected just below the fourth ventricle can perform the flexion movement of the front limb. Intensification of the stimulation at any point of the body resulted in an increase in the number of rather chaotic movements by all limbs, or in generalized movements of all muscles. In the chronic preparation, as a result of repeated stimulation of the receptive field of the wisch reflex of the hind limb, there appeared an ever increasing number of movements of all limbs up to and including the coordinated reaction of crawling. The exclusion of the coordinating center resulted in the animal being unable to perform certain definite reactions, in this particular case flexion of the fore limb, and consequently of more complex functions. Frogs with spinal transection over the third spinal root could only leap, they did not walk.

As was already stated in the introduction, increase in stimulation produced a generalized reflex. This is probably due to the fact that stimulation involved an increasing number of reflex arcs. Both the anatomic data and our observations point to the fact that different reflex arcs are interconnected in the vicinity of the bulbo-spinal junction. When the nucleus funiculi dorsalis is cut off, the actions of the fore limbs undergo greater changes than those of the hind limbs, and the eliminated functions are those involving the flexion reflex. This indicates the kind of posture control and locomotor reactions that occur in this area.

Producing antagonistic reflexes in frog from the same receptive field is, at least in some cases, connected with the position of the body

when the reflexes are elicited. For instance, from the same receptive field of the digits of the hind limb we obtained a flexion reflex when the limbs were freely suspended, and an extensor thrust when the animal was sitting. It is possible that the stimulation applied by us provoked not so much a flexion reflex or extensor thrust, as rather a reflex of the locomotor type, of which both these reactions are components. The reflex begins from either, depending on the position of the limb and muscle tension. These reflexes do not change in our spinal frogs, so it may be assumed that their reflex arcs are situated below the third roots.

SUMMARY

1. The pattern of the spinal reflexes of frog changes according to the level of the transection at the bulbo-spinal junction.

2. A spinal transection at the *calamus scriptorius* results in a greater variety of reflexes from the receptive fields of the fore limb than if the transection is made at a lower level.

3. Transecting the spinal cord in the lower part of the bulbo-spinal junction causes elimination of the flexion reflex of the fore limb and all the more complex motor functions connected with this movement. A preparation obtained in this way shows an increased tendency to perform the extensor thrusts.

4. A probable cause of the differences in behaviour is the partial or almost total removal of the nucleus funiculi dorsalis which, according to the anatomic data, controls posture, the locomotor reaction and other basic components of the mass-movement.

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CARDIAC AND RESPIRATORY CONCOMITANTS IN CLASSICAL DEFENSIVE CONDITIONING IN CATS *

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It is well known that changes in cardiac activity produced by an unconditioned stimulus (US) are easily reproduced by classical conditioning procedures (cf. G a n t t 1960). Many investigations performed on dogs (cf. G a n t t and D y k m a n 1957, S o ł t y s i k et al. 1961) have shown that after a few pairings of a neutral stimulus with punishment by means of an electric shock, producing tachycardia, the stimulus *per se* also elicits an increased heart rate. On the other hand, there is experimental evidence indicating that in cats a conditioned stimulus followed by electric shock elicits a decrease in the heart rate (J a w o r s k a 1959, F l y n n 1960).

The purpose of the present work was to investigate further the cardiac, respiratory and motor reactions in cats trained in a classical defensive conditioned reflex situation.

MATERIAL AND METHODS

Eight adult cats were trained in a sound-proof conditioned reflex (CR) chamber. During the pre-conditioning period the animals were adapted to the laboratory environment. They were harnessed in order to eliminate artifacts due to movements.

Excitatory CRs were established throughout a training period of about two months by associating the auditory or visual stimuli (conditioned stimuli, CS)

with an electric shock (unconditioned stimulus, US). The auditory CS was a tone or 1000 cps. and the visual CS, a single flash or a series of 5 flashes. Whereas in cat No. 1 all three CSi were applied, in other cats only one of them was used. Intensity of US varying from 1.2 to 4.8 milliamperes with duration 100 msec. was delivered through a bipolar silver electrode applied to the skin of the right foreleg. In the first training session, the CS-US interval was 500 msec. which, thereafter, was gradually increased until it reached 5 sec. in the fifth session, this duration being kept constant until the end of the training. Sessions consisted of twelve trials daily.

Heart rate was measured by recording EKG for ten second periods: before the onset of the CS, during CS-US interval and after the end of the US. In addition, respiration was recorded (Table I).

Table I

Individual results of heart rate concomitant to conditioning (heart rate measured in beats per sec)

Cat No.	Number of sessions	Conditioned stimulus	Heart rate during conditioning		
			Control	Interval CS-US	After US
1a	7	light (1f)	3.2	3.1	3.3
b	15	sound	3.6	2.7°	3.4
c	20	light (5f)	4.0	3.1°	3.9
2	20	light (5f)	3.4	2.9°	3.4
3	36	sound	3.8	3.1°	3.8
4	33	sound	3.4	2.4°	3.4
5	37	sound	3.8	3.0°	3.7
6	14	light (1f)	2.8	3.7	3.8
7	31	sound	3.6	2.9°	3.7
8	12	sound	3.1	2.6°	2.8
		Mean = 3.57		2.97	3.42
		t =	6,2*		

* Significant at the 0.001 level of confidence.

RESULTS

1. Pretaining period. The effects of light, sound and electric shock applied separately were analyzed prior to conditioning.

Cardiac reactions were usually absent during the orienting reflex (OR) elicited by light or sound (Fig. 1 A and B). Only occasionally, when the animal was overtly startled by the sound, could a very transient slowing of the heart rate be observed. Sometimes, a slight alteration of the rate and amplitude of respiratory movements was noticed, as shown

in Fig. 1. The shock was given alone with varying intensities. The cat reacted as if its leg was affected, performing combined head and leg movements to remove the electrodes. The first series of shocks induced only slight movements, but repetition of the shocks caused abrupt movements often accompanied by an increase in the heart and respiratory rates (Fig. 1 C), a decrease in the heart rate was never observed (Fig. 1).
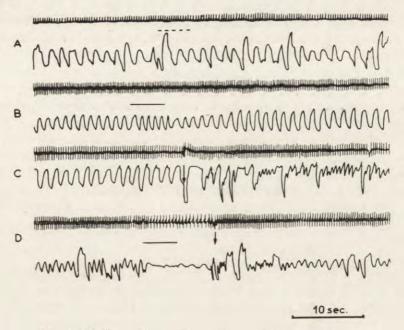

Fig. 1. Electrocardiographic and respiratory recordings
A — control of heart and respiratory movements during pretraining period to a visual stimulus (5 flashes), B — the auditory stimulus (indicated by solid line) and C — the electric shock (indicated by arrow); D — training period: the solid line inidicates auditory CS, and the arrow, onset of shock

2. *Training period.* Upon application of the CS, a localized flexion of the limb corresponding to the motor response commonly described in dogs was never observed. Whenever a motor response appeared, it involved practically the whole body, including slight movements of the limbs, head and trunk, accompanied by vocalization. During the development of this reaction, which appeared only at the beginning of training, the heart rate was either unchanged or slightly increased.

The most frequent postural reaction of the cats to the CS adopted in later training was to remain motionless with the limbs semi-flexed and the rest of the body arched. The general attitude of the animal was that of expecting the imminent shock.

Concomitant to this behavior, bradycardia and hypopnea appeared. Changes in respiratory amplitude without changes of heart rate were, sometimes, observed, especially during the initial stages of training; but, whenever the heart rate decreased, hypopnea was always present (Fig. 1 D).

Table I shows the average heart rate of each animals in all sessions. The column headed "control", shows the average data obtained during the ten seconds immediately before the onset of the CS. The column headed "Interval CS-US" refers to the average number of heart beats per second obtained in the interval between the onset of the CS and delivery of the shock; finally the column headed "After US" shows the average heart rate after the application of the shock. It may be observed that a slowing down of the heart rate occurs in response to the CS. Only cats Nos. 1 and 6, which were trained with a single flash CS, failed to show this effect, probably because the flash lasted too short. It is interesting that, as compared with the control, the heart rate does not increase after the US even though, as mentioned above, before conditioning tachycardia, usually, appeared after the shock.

To get a fully established bradycardic CR, four to six sessions were required.

DISCUSSION

The results of the present paper demonstrate important differences between the cat and other species. Robinson and Gantt (1947), Gantt (1960) and Sołtysik et ai. (1961) demonstrated that the CR in the dog was accompanied by tachycardia. In the cat, as was described above, there are no cardiac or respiratory concomitants during an OR. Only a startle reaction to a stimulus brings forth a decrease in heart rate and respiratory amplitude.

It is also worthwhile mentioning that the effect of the electric shock is not the same throughout its applications, even though the intensity is maintained. The response to the first electric discharge is a "surprise" reaction. Only after successive stimulation does the well known defensive reaction to a painful stimulus become apparent. The movements brought about by shock include participation of the whole body. It is then possible to record an increase of the cardiac and respiratory frequency. On the other hand, once the association between the CS and US has been accomplished the cardiac frequency turns to normal instead of being increased, as would be expected from recordings of respones to the electric shock alone. A possible explanation could be that the bradycardic reaction has a long-lasting effect which would inhibit the responses to

the US. In other words, a modification brought about by learning. Another possibility could be that the electric shock, through repetition, loses its effect as an US.

The process of conditioning reveals certain interesting features. During the short period in which motor responses are obtained, they are never limited to the stimulated limb, as is the case in defensive conditioning in dogs. When the motor responses disappear, and the "expectant" attitude to the CS sets in, a spectacular decrease in the cardiac rate and hypopnea occurs. In this way we confirmed the results of J a w o r s k a (1959) and F l y n n (1960) who described bradycardia in classical defensive conditioning in cats.

Two aspects deserve special attention: the bradycardia and hypopnea as responses elecited by the CS, and the neurological mechanisms underlying such reactions.

Taking into consideration that an electric shock does not elicit bradycardia or hypopnea, it appears that they do not represent a true type I CR. Bradycardia and hypopnea could be new responses which would tend to protect the animal from the dangerous effects of the shock. It would seem as if the animal „instrumentalized" these autonomic reactions, in other words, that bradycardia and hypopnea would be type II CR's. The problem which emerges concerns the quality of these responses in relation to their excitatory or inhibitory nature. In view of the fact that the „expectant" attitude is characterized by a lack of movement, it is reasonable to suppose that bradycardia and hypopnea are inhibitory responses.

It could also be that bradycardia and hypopnea are concomitant with some other defensive CRs such as the increased flexor tone. This is connected with breath holding which produces a decrease of the heart rate. In this case, the cardiac reaction would be a consequence of a purely muscular phenomenon. In this context, it would be useful to repeat our experiments in cats paralyzed by curare in order to block the motor component of both the orienting and conditioned reflex. Such an experiment was performed by G a n t t (1960) on dogs, in which case the CR tachycardia was not lost.

As to the neurological mechanisms underlying these responses, efferent pathways involved in the heart CR seem to be mediated by the vagus nerve, as indicated by F l y n n (1960). However, the knowledge of the integrative mechanisms remains obscure. It is known that the stimulation of the cortex, particularly of the cingular gyrus and orbitofrontal cortex, produces modifications of the cardiac and respiratory rates (S m i t h 1938,

1945, Delgado, 1960). Yet, dogs in which the cortex has been completely removed on one side and the gyrus cinguli on the other, were able to acquire cardias CR's (Gantt, 1960).

SUMMARY

Eight cats were trained in classical defensive CR's in order to observe cardiac, respiratory and motor responses.

The CS elicited bradycardia in spite of the fact that the US (electric shock) produced either tachycardia or no change.

The meaning and the possible mechanisms of such a reaction is discussed.

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THE INHIBITORY ROLE OF AMYGDALA STIMULATION

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In our previous work with D e l g a d o (1960, 1961 a, b) it was found that stimulation of the baso-lateral portion of the amygdaloid complex in cats inhibits food intake as well as the performance of the instrumental alimentary reactions.

This was recently supported by M o r g a n e (1962) who performed analogous experiments on rats and received similar results. The author also found that damage to the dorsal part of the medial forebrain bundle, just cephalad to the ventromedial nucleus of the hypothalamus obliterated the inhibitory effect of amygdala stimulation. All these findings suggest that the baso-lateral portion of the amygdaloid complex plays the role of inhibitory alimentary center, probably acting through the hypothalamus.

Ablation studies also favor this point of view. G r e e n, C l e m e n t e and d e G r o o t (1957), M o r g a n e and K o s m a n (1957, 1959, 1960), and others (F u l l e r et a l. 1957, W o o d 1958) have shown hyperphagia, and B r u t k o w s k i, F o n b e r g and M e m p e l (1961), disinhibition of the inhibitory alimentary reflexes after lesions of the amygdaloid complex.

In a recent paper by E g g e r and F l y n n (1962) it has been shown, however, that amygdala stimulation interferes also with the a t t a c k reactions elicited by simultaneous stimulation of the hypothalamic attack area. This implies a more general inhibitory role of the baso--lateral amygdaloid nuclei.

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The present paper is a further study of the inhibitory role of the amygdala. It was interesting to see how amygdala stimulation influences fear symptoms produced both by external nociceptive stimuli and direct stimulation of the hypothalamic fear area.

MATERIAL AND METHODS

Experiments were performed on 7 male mongrel dogs. Chronic electrodes were implanted stereotaxically under nembutal anaesthesia into amygdala, hypothalamus and some other structures, according to the methods described by D e l g a d o (1955) and F l y n n et al. (1961), slightly modified by us. Stereotaxic coordinates were designed according to the Stereotaxic Atlas of the dog (L i m, L i u and M o f i t t 1960).

The dogs were divided into two grops. Group I (4 animals) was trained before operation to avoid an electric shock applied to the skin, or an air-puff into the ear; Group II (3 animals) was trained after operation to avoid the stimulation of the hypothalamic fear area. One dog from Group I was subsequently trained also in Group II. Avoidance training was performed according to the K o n o r s k i and M i l l e r method (1933). The animals were trained to perform a particular movement in response to the CS to avoid a noxious reinforcement which otherwise followed the CS. Acoustic and visual stimuli were used as CSi, and the lifting of the hind or fore leg and placing it on the bar was trained as avoidance reaction. During the initial training, CS was in random order either followed by noxious reinforcement, or by passive movement induced by the experimenter. Immediate cessation of the CS followed the passive movement, and the noxious reinforcement was not applied in those trials. Usually, after two or three experimental sessions the animals responded to the CS actively, avoiding noxious reinforcement. After the animal had achieved a criterion of 90 per cent of correct responses in last 100 trials, the avoidance performance was overtrained during at least ten more experimental sessions. In most dogs avoidance performance was 100 per cent.

In the test experiments, the effect of amygdala stimulation on the general fear symptoms and on the performance of avoidance reactions was studied.

In additional experiments, the effect of amygdala stimulation on food intake was controlled.

The experiments were carried out every day. During the training period 20 avoidance trials were given in one experimental session. In the test experiments with Group I, ten control trials were given before amygdala stimulation, ten test trials during stimulation, and ten control trials afterwards. In Group II, only twenty trials were run in one experimental session: ten before amygdala stimulation, and ten during stimulation. CS was applied every 30 sec. in each set of 10 trials, with 2 to 3 min. intervals between the control and test sets. During the whole period of test trials amygdala was stimulated constantly. Parameters for brain stimulation were rectangular square waves 50 to 100 sec., with pulse duration 0.5 to 1.0 msec. The intensity of amygdala stimulation was 0.1 to 0.25 mA. Care was taken to stimulate at the threshold level to avoid amygdala afterdischar-

ges. Intensity of the hypothalamic stimulation to evoke a proper fear reaction varied from 0.2 to 1. O mA. After the experiments had been completed the animals were sacrificed, perfused with formalin, and their brains cut stereotaxically in blocks 10 to 20 mm. Paraffin sections were stained according to Nissl and Klüver techniques for histological analysis and verification of the placement of the electrodes.

RESULTS

During the avoidance training the dogs showed various fear symptoms, such as general restlessness, attempts to escape from the stand, sometimes, whining or screaming. They also performed numerous intertrial responses, the number being clearly related to the other fear symp-
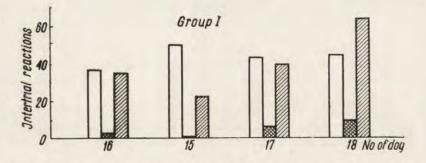

Fig. 1 illustrates the effect of amygdala stimulation on the number of intertrial reactions

Each block of 3 bars is a representative of one typical session for each dog of Group I. Each bar stands for the number of intertrial reactions performed during the five minutes before stimulation (open), during amygdala stimulation (crossed) and after stimulation (striped). It is seen that intertrial reactions are considerably reduced during amygdala stimulation

toms. All these reactions were much more pronounced in Group II which was trained to avoid stimulation of the hypothalamic fear area (cf. F o n b e r g 1963). Most of the time, the dogs were so anxious that they continuously moved chaotically in all directions trying to wriggle out of the harness and escape from the stand (Fig. 2 A). The only exception was dog No. 14 which was much quieter than the others, even when fear area was stimulated.

During amygdala stimulation the behavior of the dogs of both groups changed radically. The animals stood quietly, sometimes, sniffing, no attempt to escape was ever noticed. The intertrial reactions were considerably reduced or abolished (Fig. 1).

With the decrease in the number of intertrial reactions, other fear symptoms were also diminished or abolished. Fig. 2 shows the picture of the dog before (A) and during amygdala stimulation (B). We see that while before stimulation the animal was restless and attempted to escape, he stood quietly, and looked comfortable during stimulation.

In spite of the inhibitory effect of amygdala stimulation on the general fear reactions the avoidance performance to the CSi remained unchanged. All dogs reacted to these stimuli properly by the trained avoidance movement. Thus, a clear discrepancy was obtained under
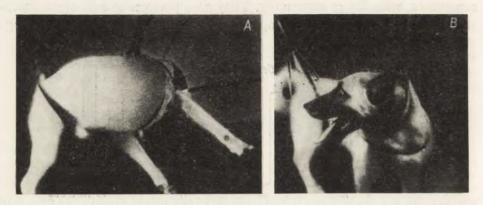

Fig. 2 shows the picture of the dog No. 17, before amygdala stimulation (A): anxious, trying to escape from the harness, and during stimulation (B): quiet, looking content

amygdala stimulation between fear reactions displayed in intervals which were greatly reduced or even completely abolished and the avoidance responses to the CSi which remained unaffected (Figs. 3 and 4). This discrepancy between the amygdalar effect on the general fear symptoms and avoidance performance was more pronounced in Group II trained to avoid the stimulation of the hypothalamic fear area. As mentioned above, in those dogs the fear symptoms evoked by direct cerebral stimulation were more dramatic, therefore the change from the pre-stimulation fear level into the quiet state produced by stimulation was more drastic.

Several control tests performed during amygdala stimulation showed that the dogs preserved the orienting reaction to new stimuli, followed with eyes the moving objects, reacted to touching and petting, and were able to lift their limbs and walk.

When the amygdala was stimulated by the same electrode and with the same intensity during eating, the dogs behaved in exactly the same way as did the cats in our earlier experiments (F o n b e r g and D e l g a- d o 1961). The animals immediately ceased to take food, sometimes spitted it out and turned the head away from the bowl.

Histological verification of dogs Nos. 11, 12, 13, 14, 16, 17 and 18 showed that the tips of the electrodes were placed in the baso-lateral
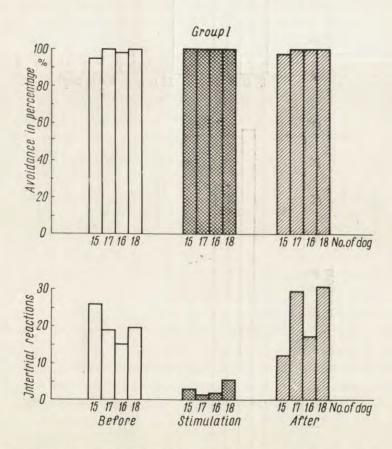

Fig. 3 illustrates the comparison of the effect of amygdala stimulation on both the intertrial reactions and avoidance performance in Group I. The mean values for each dog were obtained from five experimental sessions (50 trials before, 50 trials during and 50 trials after stimulation)

Upper part of the graph represent the percentage of the avoidance performance and the lower part, the number of intetrial reactions. It is seen that in spite of the fact that amygdala stimulation reduces greatly the number of the intertrial reactions it does not affect the avoidance performance
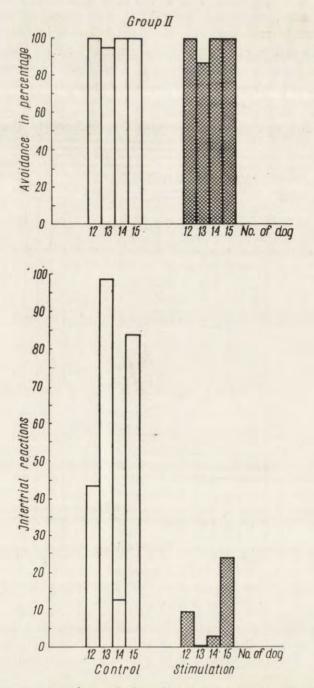
Fig. 4 illustrates a comparison of the effect of amygdala stimulation on the
number of intertrial reactions and the avoidance performance in Group II
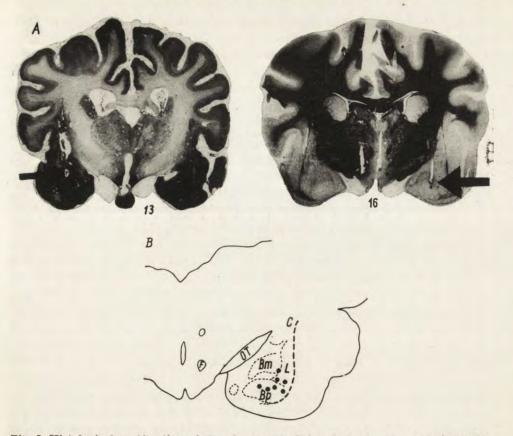For indications see Fig. 3

Fig. 5. Histological verification of the placement of the electrodes. A — Photographs of frontal sections of the brains. Dogs Nos. 13, and 16; B — Composite diagram showing location of points from which during stimulation fear behaviour patterns were inhibited with no interference with the avoidance performance. All points are collected together both left and right and also 1—2 mm posterior or anterior

Indications: OT, optic tract, C — claustrum, F — fornix, L — nucleus amygdaloid lateralis, Bm — nucleus basalis magnocellularis, Bp — nucleus basalis parvocellularis

part of the amygdaloid complex (Fig. 5). The electrodes placed within the amygdaloid complex in the more posterior and dorsomedial regions were uneffective in the inhibition of fear reactions and produced generalized amygdala seizures or other symptoms which will be described elsewhere.

DISCUSSION

As pointed out in the introduction, both alimentary and aggressive reactions are inhibited by baso-lateral amygdala stimulation. Our present experiments show that this holds also for fear reactions which are

greatly reduced or even abolished by amygdala stimulation. These facts indicate that the effect of amygdala stimulation is not limited to the inhibition of alimentary reactions but extends upon other behavior patterns. It must be emphasized, however, that this general inhibition cannot be considered an „arrest" reaction observed, sometimes, during the stimulation of various parts of the limbic system. In our present experiments as well as under conditions described by F o n b e r g and D e l g a d o (1961) and E g g e r and F l y n n (1962) the animals were able to walk and play during stimulation and the orienting reaction to new stimuli was well preserved in all cases.

We suppose that amygdala inhibition is particularly directed on the hypothalamic centers of emotional reactions (drives) connected with fear, attack and hunger. From this point of view it is difficult, however, to explain why amygdala stimulation inhibits the instrumental alimentary responses while it does not affect the avoidance reactions. Possibly, due to the lack of reinforcement the well trained avoidance reactions are based on a somehow different mechanism from that of the instrumental alimentary reactions. We may assume that in the well trained avoidance reaction the connections between CS and avoidance movement, trained to this very stimulus, are relatively weakly related to the emotional fear state (cf. F o n b e r g, B r u t k o w s k i and M e m p e l 1962). In consequence, since - as we suppose - amygdala stimulation particularly inhibits emotional states, it affects rather the alimentary reactions and not avoidance. In accordance with this assumption is the fact that intertrial reactions, which are perfomed independently from the CS, and seem to be (C h u r c h and S o l o m o n 1956, K a m i n 1954 and F o n b e r g 1958, 1960 b, 1961) closely related to the emotional fear state, were also strongly inhibited by amygdala stimulation. The discrepancy of the amygdala effect on the general fear state and avoidance reactions to the CS seems to be in favor of our previous supposition (F o n b e r g 1958 a and b) that there may exist both specific and unspecific kinds of fear related to different brain structures.

It is, however, not excluded that the direct effect of the baso-lateral amygdala stimulation primarily concerns alimentary inhibiton, while inhibition of attack and fear reactions may consist of indirect, secondary effect. In fact, it is well known that carnivorous display aggressive reactions only when hungry, while being satiated they never attack spontaneously their pray. Therefore inhibition of hunger produced by amygdala stimulation may cause lack of aggression. Similarly, it is possible that fear responses are less prominent when an animal is fully satiated since in that state the parasympathetic tonus is predominant.

All these assumptions should be tested by further experiments.

SUMMARY

Chronic electrodes were implanted stereotaxically into the baso-
-lateral amygdala and hypothalamic fear area in the dog. Amygdala sti-
mulation produced inhibition of fear symptoms evoked both by external
noxious stimuli and cerebral stimulation of the fear area. The instrumen-
tal avoidance reactions to the CSi established to avoid both kinds of
aversive stimuli were, however, not affected. Possible explanations of
the nature of the amygdalar inhibitory effects were discussed.

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THE EFFECT OF PARTIAL LESIONS OF THE PREFRONTAL AREA ON ALIMENTARY CONDITIONED REFLEXES IN DOGS

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In a series of papers of this laboratory the effects of prefrontal lobectomies on alimentary conditioned reflexes, both classical and instrumental, were described in dogs. It has been shown that excitatory conditioned reflexes (CRs) are after these operations unchanged or increased and the inhibitory reflexes are disinhibited (B r u t k o w s k i et a l. 1956, B r u t k o w s k i 1957, 1959, 1963, Ł a w i c k a 1957). The degree of the impairment of inhibitory CRs depends on the difficulty of the inhibitory task, and after the postoperative retraining the inhibitory processes are partially restored.

In all these studies, the whole prefrontal region rostrally to the presylvian sulcus was ablated, together with the white matter. However, the problem arose as to whether all parts of this region are in the same degree responsible for inhibitory processes, and, if not, which parts have a crucial role in this respect. The present paper is concerned with this problem.

EXPERIMENTAL PROCEDURE AND METHODS

Experiments were performed in a standard CR chamber on 13 mongrel dogs. Instrumental CRs were used throughout this study, the conditioned response consisting in the animal raising its right foreleg and placing it on the foodtray situated in front of the dog. The conditioned stimulus (CS) was a buzzer. In the preliminary training the animals were habituated to the experimental situation and trained by passive movements to perform the instrumental response to the buzzer. The food reinforcement followed immediately the performance of the trained movement. The reward consisted of a few pieces of bread moistened with broth

which were presented in small bowls put into position by an electrical device. The intertrial intervals were 1 to 2 min., and usually 8 trials were given in each experimental session.

When the experimental responses to the buzzer were firmly established (which took in average 14 sessions) and the intertrial responses disappeared, training of the inhibitory CR was started. In each session, in two trials, dispersed among the positive trials, the compound composed of the conditioned inhibitor (CI), namely the sound of a metronome, and the buzzer was applied without reinforcement (conditioned inhibition).
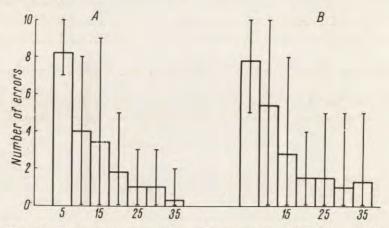

Fig. 1. The average number of errors in inhibitory trials during the preoperative training. Each block represents 5 sessions (i.e. 10 inhibitory trials). The maximal and minimal number of errors in each block is indicated. Only first 35 sessions are represented

A — dorsolateral group; B — medial group

In the beginning of inhibitory training, the metronome was given for 5 sec. and immediately after its cessation the buzzer was applied for another 5 seconds, i.e. the CI—CS interval was zero sec. In response to the metronome the animals hardly performed the learnt movement, but they did so to the buzzer following the metronome. However, after a number of experimental sessions this response gradually disappeared, while the response to the buzzer applied alone was unchanged.

When the criterion of not more than one error in 5 successive sessions (i.e. in 10 inhibitory trials) was reached, an interval of 1 sec. was made between metronome and buzzer. After reaching the same criterion, the interval was prolonged in steps to 2, 4 and 6 sec.

The general course of the inhibitory training is represented in Figs. 1 and 2. It may be seen that there were great individual differences between the dogs, which were clearly manifested in the general animals' behaviour. While in some animals the training ran quite smoothly and uneventfully, in others prolongation of the CI—CS interval produced restlessness, refusal to take food and other neurotic symptoms. In most dogs, these symptoms were only transient and disappeared with further training, some of them (4), however, developed heavy neurotic disorders and had to be eliminated from the present study.

After reaching criterion with 6 sec. CI—CS interval, a break in experiments lasting 6 days was applied. In all dogs but one the inhibitory CRs were unimpaired.

Then the animals were operated on in aseptic conditions under nembutal anaesthesia (35 mg/kg). The cortical tissue was removed bilaterally by subpial suction, the white matter being spared. The postoperational period was uneventful.
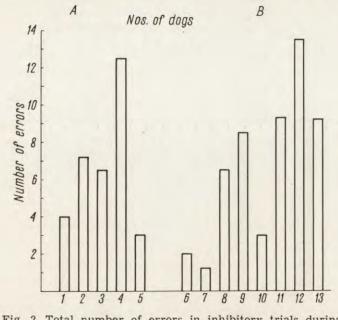

Fig. 2. Total number of errors in inhibitory trials during
the preoperative training
A — dorsolateral group; B — medial group

After 6 days, the CR experiments were started again, the whole schedule of experimental sessions being the same as in the last period before operation, i.e. the CI—CS interval in the inhibitory compounds amounted to 6 sec. When the animals reached the preoperative criterion of performance, they were sacrificed, the brains removed and fixed in formalin. The rostral parts were embedded in paraffin, sectioned and stained by the Nissl, Klüver or oil-red methods, and cortical lesions were reconstructed. Localisations of lesions (Figs. 3 and 5) were based on myeloarchitectonic map of the prefrontal area worked out by K r e i n e r (1961, and in preparation).

RESULTS

I. The effects of dorsolateral prefrontal lesions

Dorsolateral lesions were made in 5 dogs. Their extent is shown in Fig. 3. As seen from this figure in different dogs different parts of this region were removed. The most extensive lesion was made in dogs Nos. 3 and 4 in which both proreal and orbital areas were nearly totally de-
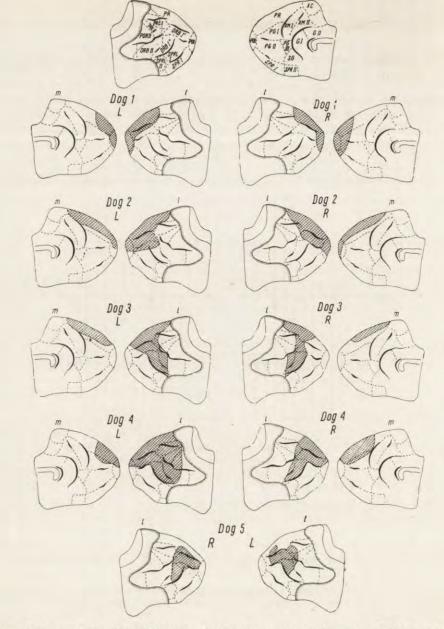
Fig. 3. Extent of dorsolateral lesions in dogs Nos. 1 to 5. Top, maps of dorsolateral and medial surfaces of the prefrontal region. Below, schemes of myeloarchitectonical areas of the prefrontal cortex with the indications, of the placement of the lesions

Heavy lines, sulci; broken lines, borders of areas; dotted line, the rostral border of the anterior composite gyrus which corresponds with the presylvian sulcus; the olfactory bulb and tract and the composite gyrus were removed at the indicated place for convenience of reproduction; 1, lateral aspect; m, medial aspect. POL, area polaris; PR, area prorealis; PRL I, area prorealis lateralis I; PRL II, area prorealis lateralis II; SPR I, area subprorealis I; SPR II, area subprorealis II; SPRL I, area subprorealis lateralis I; SPRL II, area subprorealis lateralis II; ORB I', area orbitalis I', ORB I", area orbitalis I"; ORB II, area orbitalis II; PORD, area paraorbitalis dorsalis; PG I, area praegenualis I; PG II, area praegenualis II; PG III, area praegenualis III; G I, area genualis I; G II, area genualis II; XM I, area praecruciata medialis I; XM II, area praecruciata medialis II; XC, area praecruciata centralis. L, left hemisphere; R, right hemisphere

stroyed. In dog No. 2, the effect was limited to the proreal area which was removed *in toto*, while in dog No. 5 the lesion was limited to the orbital areas. In dog No. 1 chiefly polar area was removed (see Table I).

Independent of the extent and localisation of the lesion within the dorsolateral surface of the prefrontal region the impairment of the CR activity, although present in all the dogs, was quite negligible. The positive CRs were quite normal, while the inhibitory responses were slightly impaired (Fig. 4). This was manifested by the fact that in the
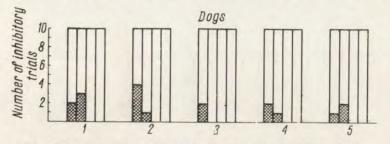

Fig. 4. Effects of dorsolateral lesions on inhibitory CRs.
Each column denotes 5 session blocks (i. e. 10 inhibitory trials).
Crossed parts of the columns denote errors in inhibitory trials.
Note that errors are performed occasionally, only in the first two
5 session blocks

first 10 postoperative sessions the animals occasionally performed the trained movement in response to the CS presented 6 sec. after the CI. The most marked impairment of the inhibitory responses was in dog No. 2 in which the lesion encroached on the medial surface of the cortex. No intertrial movements were observed after operation. The general animals' behaviour was exactly as before operation.

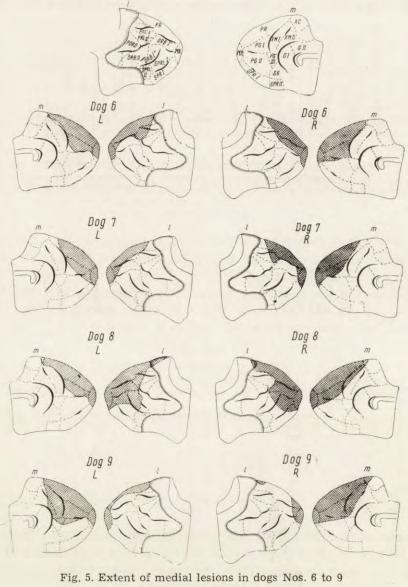**Table I**
Extent of dorsolateral lesions

Dog	POL	PR	PRL I	PRL II	SPRL I	ORB I'	ORB I''	ORB II
1	t	p	—	—	—	p	—	—
2	p	t	—	—	p	t	p	—
3	—	nt	—	—	—	p	t	p
4	p	nt	p	p	—	t	t	p
5	—	p	—	—	—	t	nt	—

t, total lesion; nt, nearly total lesion; p, partial lesion.

It should be noted that similar results were obtained in three other dogs after dorsolateral lesions in unpublished pilot experiments performed in this laboratory by B r u t k o w s k i, Ł a w i c k a and M i s h-k i n (1959).

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II. The effects of medial prefrontal lesions

The lesions were performed in 8 animals. Their extent varied some-what in different dogs (Fig. 5 and 5a). In all of them, the medial part of the proreal area was removed as well as the part of the pregenual area (PG I). While in dogs Nos. 6, 7 and 8 the lesions involved PR and PG I, in dogs Nos. 9 to 13 the impairment encroached on parts of the precrucia-

Fig. 5. Extent of medial lesions in dogs Nos. 6 to 9
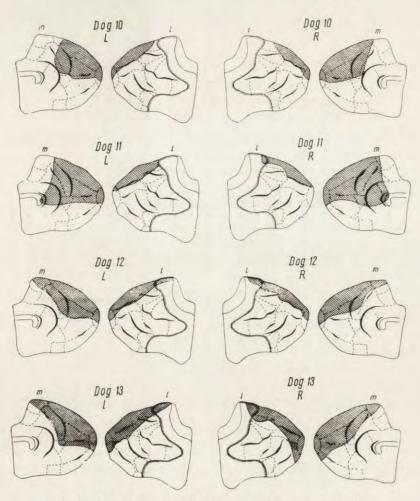Denotations as in Fig. 3

Fig. 5a. Extent of medial lesions in dogs Nos. 10 to 13
Denotations as in Fig. 3

te regions (medial and central). In dog No. 11, the lesion encroached
also on the genual area (see Table II).

The first three dogs of this group (Nos. 6, 7 and 8), in which only
pregenual area (PG I) was added to the proreal lesion, the effects of the
surgery were more manifest than in the dorsolateral group (Fig. 6).
In the first 5 postoperative sessions, the inhibitory CR was disinhibited
in about 70 per cent of trials, and in dog No. 6 intertrial movements
occurred. However, the general behaviour of animals was not changed,
and the positive CRs were performed in exactly the same way as before
operation. As in the first group, the impairment of inhibitory reflexes
was transient, so that in the second 5 session block they were only
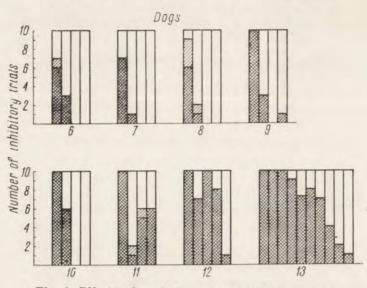
3*

Fig. 6. Effects of medial lesions on inhibitory CRs

Denotations as in Fig. 4. Stripped parts of the columns denote incomplete responses. Note that the disinhibition of inhibitory CRs in these dogs is stronger and more longlasting than in dogs represented by diagrams in Fig. 4

slightly disordered, and in the following sessions the whole CR activity returned to normal.

Much different was the picture in those animals in which the lesion included the part of the precruciate areas. Here, the disorder involved not only inhibitory CRs but also excitatory ones, and, in addition, the whole stereotype of animals' behaviour during experimental sessions was disturbed. The latent periods of the positive conditioned responses

Table II

Extent of medial lesions

Dog	POL	PR	PG I	PG II	PG III	XM I	XM II	XC	G I	G II
6	t	t	t	—	—	—	—	—	—	—
7	t	t	nt	—	—	—	—	—	—	—
8*	t	t	nt	—	—	—	—	—	—	—
9	t	t	t	p	p	t	p	—	—	—
10	p	t	t	p	—	t	p	—	—	—
11	t	t	t	p	nt	t	nt	—	t	p
12	p	t	nt	—	—	t	—	p	—	—
13	t	t	t	p	p	t	—	p	---	—

Denotations as in Table I.
* in addition dorsolateral areas: POL, PR, ORB I', ORB I", SPRL I were removed

were prolonged: while normal dogs performed the trained movement immediately after the onset of the CS, the operated animals did so after a 2 or 3 sec. delay.

In the intertrial intervals, they displayed increased restlessness, often performed the trained movement, or occasionally jumped on the foodtray with both forelegs. However, the most prominent disorder appeared in their reactions to the inhibitory compound. In the first 5 postoperative sessions the disinhibition was complete, and the dogs displayed the positive response not only to the CS following the CI, but, sometimes, also to the CI itself. Licking the empty bowl was also observed. In the following sessions, in dogs Nos. 9 and 10 compensation occurred rather quickly, while in dogs Nos. 11, 12 and 13 the disorder of inhibitory CRs lasted much longer (see Fig. 6).

DISCUSSION

The problem of the present paper was to find out which part of the prefrontal region is responsible for inhibitory processes in alimentary CRs. In consequence, the cortex of various parts of this region was removed while the white substance was spared.

According to the Kreiner's division of the prefrontal region based on its myeloarchitectural structure, the dorsolateral surface may be subdivided into the proreal areas, orbital areas, subproreal areas and polar area (Fig. 3). On the medial surface, there are parts of the proreal, subproreal and polar areas, and also pregenual, genual and precruciate areas (cf. Figs. 3, 5, and 5a).

In the present paper the lesions involved orbital areas on the lateral surface, proreal areas on the dorsal surface, and pregenual and precruciate areas on the medial surface (see Tables I and II). The results obtained appear to be quite clear. While removal of the proreal area, orbital areas and polar area, separately or jointly, produced only a negligeable impairment of inhibitory CRs, the removal of medial parts of the region was followed by more serious defects. The removal of the pregenual area (PG I), jointly with proreal area, produced a quite marked deficit of inhibitory processes which seemed to increase when the lesion encroached on the precruciate area.

One may ask whether, and how much, the postoperative impairment was related to the preoperative training, and also to the extent of lesion.

The first question may be answered by comparing Fig. 2 with Figs. 4 and 6. Among the dorsolateral animals, the dogs Nos. 1 and 5 were bright learners while dog No. 4 was rather dull. Nevertheless, the postoperative scores are exactly the same. Similarly, in the medial group quite analogous effects were obtained in dogs Nos. 6, 7 and 8 in spite of

the fact that dog No. 7 was the brightest of all our dogs, while dog No. 8 was rather a poor learner. Thus, we cannot find any relation between the preoperative scores in learning and the postoperative deficit in our animals.

We have also no evidence to show that the degree of defect depends on the extent of lesion. In the dorsolateral group far the greatest lesion was performed in dog No. 4 who was also a poor learner. A very small lesion was made in dog No. 5 who was a good learner. Nevertheless, the effects of the surgery were much the same. Similarly, in the medial group the extent of lesion in dog No. 12 was certainly smaller than that in dog No. 8, in which the great part of the dorsolateral surface was removed. Nevertheless, the impairment of the inhibitory processes was much greater. And so, it seems that the deficit obtained after our lesions depends mainly on their localisation. This does not exclude that within a given crucial area the degree of disorder may be related to the extent of lesion.

The strongest impairment of inhibitory processes was observed in dogs Nos. 12 and 13. If we compare the lesions made in these dogs with those in dogs Nos. 9 and 10, in which the impairment of inhibitory processes was much more shortlasting, we see that the only difference between these two lesions is that in dogs Nos. 12 and 13 the removal of the cortical tissue extended more caudally and involved a part of premotor region just in front of the cruciate gyrus. This area was denoted by K r e i n e r as central precruciate area. Of course, it is difficult to judge whether this very area is crucial for our test, or whether its addition to our lesion simply makes the ablation of the medial precruciate region more complete.

Our results are in agreement with other studies of this laboratory. S t ę p i e ń, S t ę p i e ń and K r e i n e r (1962) have recently found that removal of the premotor area produces a very strong and longlasting impairment of the inhibitory processes, and also disorders of behaviour mentioned briefly above. These authors have also found that only the lesions made in medial (Kreiner's medial and central precruciate areas) but not in lateral part of the premotor region are followed by disinhibition. This is exactly the same area whose removal in our animals Nos. 9 to 13 produced a strong impairment of inhibitory processes.

Analogous results were also recently obtained by B r u t k o w s k i and D ą b r o w s k a (1963). Their experimental procedure was somewhat different from ours in that the tone differentiation was used instead of conditioned inhibition, and the number of positive and inhibitory trials was balanced. They found that under conditions of 1 min. intertrial interval schedule the removal of medial surface of the prefrontal area pro-

duced an impairment of inhibitory CRs while that of the lateral surface did not.

In another paper by B r u t k o w s k i and M e m p e l (1961) it was reported that ablations of the genual area situated just in front of the genu of the corpus callosum are also followed by disinhibition. To sum up, it seems that, in dogs, the medial surface of prefrontal-premotor region plays a significant role in inhibitory CRs.

It would be also useful to compare our data with those of B r u t-k o w s k i et a l. (1960, 1963) and B u t t e r et a l. (1961) obtained in monkeys. It has been shown that, first, dorsolateral prefrontal lesions produce only a quite negligeable disinhibitory syndrome comparable with that received in our animals after dorsolateral lesions, and, second-ly, that orbital lesions produce a marked disinhibition. Whether or not the orbital surface of the monkey' brain can be considered a homologue of the medial part of dog's brain is a matter to be elucidated.

In recent years there was much discussion about the intimate nature of disinhibitory syndrome (cf. R o s v o l d and M i s h k i n 1961, K o n o r s k i 1961). It was argued that at least two different mechanisms may be involved in it, namely the impairment of inhibition of drives and/or impairment of inhibition of motor acts. It is nearly sure that when after premotor lesions the animals incessantly perform the instrumental response, this conditioned hyperactivity is due to disinhibition of motor acts (cf. S t ę p i e ń, S t ę p i e ń and K o n o r s k i 1960). On the other hand, if a positive response to the inhibitory CS is strictly accompanied by salivation, as was the case in B r u t k o w s k i' s experiments (1959), we have every reason to believe that we deal with drive disinhibition.

The present paper does not supply any evidence as to what sort of disinhibition we are dealing with after medial prefrontal lesions. This should be the subject of future investigation.

SUMMARY

1. The present paper deals with the effect of partial prefrontal lesions on instrumental inhibitory CRs in dogs.

2. Ablation of the dorsolateral surface of the prefrontal region (proreal and orbital areas) produces only a slight and shortlasting impairment of inhibitory reflexes.

3. Lesions situated on the medial surface of the prefrontal region (pregenual area) are followed by a much stronger disinhibition. The disinhibition is even more pronounced when the lesion is extended upon the medial precruciate area. The impairment of inhibitory processes is reversible so that it disappears after postoperative retraining.

4. Positive CRs are not impaired after prefrontal ablation, but they

are slightly impaired when the lesion encroaches on the medial precruciate area.

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RHYTHMIC CHANGES IN FOOD INTAKE AND BLOOD SUGAR LEVEL IN THE RABBIT AS A FUNCTION OF FOOD DEPRIVATION INTERVALS

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Many processes of a living organism such as changes in body fluids, temperature, some of the electrical phenomena of heart and brain functioning (EKG and ECG), sleep and waking states, locomotion, ingestion of food etc. (S i e g e l and S t u c k e y 1947, M a i z e l i s and R u t t e n b u r g 1949, S l o n i m and Š č e r b a k o v a 1949, Š č e r b a k o v a 1949 a, b, K u k s o v a 1961, S a v v a t e e v 1957, W e h m e y e r and C a s p e r s 1958, K a r n o p 1959, D o e, V e n n e s and F l i n k 1960,, S i e g e l 1961, A l t m a n 1962) undergo diurnal rhythms.

In general, the rhythms determined by heredity are of remarkable regularity, and only, under certain conditions, do they vary or show a tendency to extinction. The common observation, on the other hand, that rhythms developed during the animal's lifetime may change indicates that the acquired periodic activity belongs to the category of characters which are relatively fitted to their environment (M a r g o l i n a and B r a n d t 1949, S l o n i m, O l n i a n s k a j a and R u t t e n b u r g 1949, Š č e r b a k o v a 1949 c, M a i z e l i s 1958, 1959, H u r w i t z and A p p e l 1959).

Diurnal cycle of light and dark, and feeding schedules are among the main factors causing the rhythm of activity. In carnivores, the periodic activities which are clearly dependent on feeding mode become relatively easy adopted to the changes of feeding schedules (F i l a t o v a 1949). On the other hand, rhythmic behavior in monkeys is primarily associated with light-dark cycle. Č e r k o v i č (1959) found that monkeys repeatedly exposed to dark daytime and lighted nighttime periods separated by in-

tervals of natural diurnal cycles of light and dark showed severe neurotic disorders.

It has also been found that physiological functions undergo diurnal rhythms under conditions of food deprivation. Observations on the effect of food deprivation on the diurnal rhythmicity of the body temperature in man have been made by O l n i a n s k a j a and P o p o v a (1949). K a n f o r (1949) pointed out that diurnal alterations of blood sugar level in food deprived monkeys are correlated with locomotor of activity.

There appears to be relatively little systematic data concerning food intake as a function of deprivation intervals and feeding schedules. Rhythms of feeding activities under circumstances of food deprivation were mainly described in rats who starved up to 48 hours (L a w r e n c e and M a s o n 1955, B a r e 1959, B a r e and C i c a l a 1960). These investigations revealed that the normal rhythm in feeding behavior might mask the increase in food intake produced by deprivation.

The purpose of the present investigation was to measure the level of blood sugar and ingestion of three kinds of food (carrot, potatoes or oats) in relation to the previous feeding experience of rabbits deprived of food up to 84 hours.

MATERIAL AND METHODS

Experiments were carried out on two groups of experimentally naive rabbits in the early spring months (February, March and April). In each group there were six male and female animals approximately 2 years old at the time of the experiment. They all were housed in individual living cages. The body weight was measured once a week. The groups were divieded into three subgroups: A, B and C of two animals. At 9 : 00 a.m. each animal was fed a basal diet and it was allowed to eat ad libitum. The amount of food consumed was measured. After cessation of eating, the Group I animals were again provided with ad libitum food and had free access to it overnight. The Group II animals were also fed supplementarily except that the additional ration was fixed. Water was offered in neither group.

Details of the procedure in Experiment I

G r o u p I. At. 8 : 30 a.m. the unconsumed food was removed and weighed. At 9 : 00 a.m. subgroup A was exposed to an overdose of carrot, while subgroups B and C received an excess of cooked purée-type potatoes and oats respectively. After the animals had ceased to return to the food, the spillage was removed, and each animal was again exposed to ad libitum diet, consisting of carrot, potatoes, oats and hay, and left with the food overnight. In other words, Group I had free access to the food at all times.

G r o u p II. In contrast to Group I, no unconsumed food could be collected in the morning because the food given on the day before had all been consumed within approximately 4 hours. At 9 : 00 a.m. subgroup A was placed on ad libitum diet of carrot, while subgroups B and C had free access to potatoes and oats respectively. After the animals had stopped eating, the unconsumed food was removed and measured, and food of fixed size was offered. Thus the subgroup A rabbits received supplementarily purére-type potatoes, oats and hay of standard size weighed 50 gms each; subgroup B was fed 100 gms carrot, 50 gms oats and 50 gms hay, and subgroup C was permitted to eat 100 gms carrot, 100 gms potatoes and 50 gms hay. In other words, Group II was provided with food every 24 hours.

After about one month of this adaptation, Group I was placed on 6-hr., 12-hr., 24-hr., 36-hr., 48-hr. or 60-hr periods of deprivation of food and water. The intervals between feedings in Group II were extended by 6, 12, 24, 36, 48 and 60 hours, i.e. the deprivation periods were about 30-hr., 36-hr., 48-hr., 60-hr., 72-hr., and 84-hr. Following the appropriate period of deprivation the animals were returned to their regular feeding until they recovered their pre-deprivation body weight.

Experiment II

Following this experiment, feeding in Group II rabbits was shifted to 9 : 00 p.m. From the next day onward, the animals were submitted to deprivation procedures. In animals Nos. 6 and 26, the intervals between feedings were four times extended by 12 hr., in Nos. 12 and 24, three times by 24 hr., and in Nos. 17 and 19, three times by 36 hr. After each deprivation period, the 12-hr. group was permitted to eat for four days, and the 24-hr. and 36-hr. groups, for six days. Consequently, in the 12-hr. group the first feeding after deprivation intervals occurred on days 3, 8, 13 and 18; in the 24-hr. group, on days 3, 10 and 17, and in the 36-hr. group, on days 4, 12 and 20 from shifting the feeding schedule to evening hours.

The blood sugar level was determined with Hagedorn-Jensen technique. Measurements were taken before feeding from the ear marginal vein. In the pre-deprivation period the measurements were taken several times on each animal. During the deprivation period the measurements were taken at the time which was associated with pre-deprivation feeding period, and, subsequently, after deprivation interval prior to feeding. During the 60-hr. (in Group I) and 84-hr. (in Group II) deprivation intervals, the level of blood sugar was measured every 12 hours.

S t a t i s t i c s. Significance of differences between arithmetic means was calculater using the Mosteller's "A_1" and the "u" tests.

RESULTS

The scores presented in Table I are the average amount of food consumed during the first morning feeding (basal diet) for the entire observation period, i.e. 3 months. It is seen that the mean weight of animals of Group I was superior to that of Group II animals, while consumption

4*

Table I

Group I								
Subgroup A			Subgroup B			Subgroup C		
Animal	Mean body weight kg	Carrot gms	Animal	Mean body weight kg	Potatoes gms	Animal	Mean body weight kg	Oats gms
54 ♀	3.60	80±5	52 ♂	3.40	61±9	50 ♂	4.15	14±3
55 ♂	3.40	50±7	53 ♀	3.65	75±10	51 ♀	4.60	14±3
Mean		65			68			14
Group II								
Subgroup A			Subgroup B			Subgroup C		
Animal	Mean body weight kg	Carrot gms	Animal	Mean body weight kg	Potatoes gms	Animal	Mean body weight kg	Oats gms
6 ♀	2.38	144±11	17 ♂	3.10	94±5	26 ♂	2.48	29±4
12 ♂	3.30	99±13	24 ♀	3.00	112±14	19 ♀	2.30	23±4
Mean		122			1C3			26

of the basal diet in Group I was significantly lower than that in Group II. It is interesting, however, that the intake of the basal diet in the Group I animals, who had food available at all times, was also relatively high. On the average, Group I ate 450 gms., and Group II, 250 gms. daily.

Experiment I
Food intake and blood sugar level in rabbits after food deprivation

Food deprivation procedure produced marked differences in feeding behavior for both groups.

G r o u p I. As seen in the upper part of Fig. 1, in all instances the greatest consumption was obtained after 12-hr. deprivation. After 24-hr. deprivation interval an apparent decrease of the average of food consumed was noticed. Over the 36-hr. and 48-hr. deprivations, the consumption of carrot and potatoes showed relatively slight alterations, while that of oats decreased below the pre-deprivation level. After a 60-hr. deprivation experience, the oats consumption returned to the initial value, and the intake of carrot and potatoes increased, although the maximum level was not attained. In contrast to the intake of two re-

maining kinds of food, slight 24-hr. rhythmic changes occurred in the consumption of potatoes.

A comparison of the data shown in the upper and lower portions of Fig. 1 reveals a negative correlation between the mean intake of basal diet and the level of blood sugar throughout various deprivation periods. Originally, the mean sugar level was 114 mg%/o. After 6-hr. deprivation it was significantly reduced by 19%/o. Over the next deprivation periods of 12-hr., 24-hr. and 36-hr. a stabilization level with only minor fluctuations was noted which was followed by an increase after 60-hr. deprivation to 110 mg%/o.

G r o u p II. In contrast to Group I in which slightly developed or no feeding cycles with the increase of deprivation intervals were found, Group II showed definite periodic changes in eating behavior revealing

Fig. 1. Food-intake and blood-sugar level in rabbits who had free access to the food at all times (Group I) in relation to number of hours of food deprivation.
Top: Mean intakes of carrot (from 2 animals), potatoes (from 2 animals) and oats (from 2 animals) after food deprivation periods as per cent. of mean pre-deprivation intake based on 10 experimental days (indicated at 100). C, carrot; P, potatoes; O, oats. Bottom: Mean blood sugar level in mg%/o during food-deprivation periods. Data from 6 animals. The vertical lines represent standard deviation

the highest intake of oats after extending the 24-hr. feeding intervals by 12-hr., and that of carrot and potatoes by 36-hr. Fig 2). Every 24 hours the animals returned to the pre-deprivation level of eating (cf. upper part of Fig. 2).

In the lower part of Fig. 2, the mean blood sugar level as a function of the length of deprivation periods is shown. It is clearly seen that
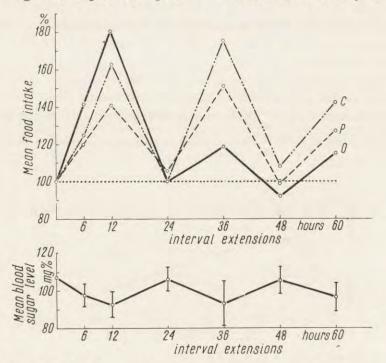

Fig. 2. Food-intake and blood-sugar cycles in rabbits who were provided with food every 24 hours (Group II) in relation to number of hours by which the 24-hr. feeding intervals were extended

Top: Mean intakes of carrot (from 2 animals), potatoes (form 2 animals) and oats (from 2 animals) after the 24-hr. feeding intervals extended by 6-hr., 12-hr., 24-hr., 36-hr., 48-hr., and 60-hr. as per cent. of mean pre-deprivation intake based on 10 experimental days (indicated at 100). C, carrot; P, potatoes; O, oats. Bottom: Mean blood sugar level in mg% during food deprivation periods (after 24-hr. feeding intervals extended by 6-hr., 12-hr., 24-hr., 36-hr., 48-hr., and 60-hr.). Data from 6 animals. The vertical lines represent standard deviation

a perfect negative correlation exists between food consumption and sugar level. Similarly to the feeding rhythms, periodic changes in blood sugar level occurred every 24 hours: a slight but statistically significant decrease after 6-hr., 12-hr., 36-hr. and 60-hr., and a return to the original level after the 24-hr. and 48-hr. deprivation periods were noted.

Experiment II
Food intake and blood sugar level in rabbits (Group II) after shifting the feeding schedule to evening hours

After shifting the Group II rabbits to evening hours feeding schedule, the food intake for the first day exceeded the average prior to the shift by 10 to 40 per cent, and, then it gradually returned to the former level (Fig. 3, days 6, 11 and 16 in 12-hr. group; days 8 and 15 in 24-hr. group; and days 9 and 17 in 36-hr. group).

With the introduction of the deprivation schedules (Fig. 3, days 3 and 8 for 12-hr. group, and days 3 and 4 for 24-hr., and 36-hr. groups respectively), changes in eating behavior were noted, trends of which appeared to be determined by the previous feeding experience: irrespective of the length of the deprivation periods, at evening hours an increase and at morning hours a decrease of food intake occurred. Due to the shift, the 12-hr. and 36-hr. extensions of interval between feedings were associated with morning hours, and, instead of an increase (cf. Experiment I), a decrease of food intake was observed. In contrast, the 24-hr deprivation was followed by an increased food intake.

Subsequent privations, which for 12-hr., 24-hr. and 36-hr. groups ended on days 13, 10 and 12 respectively, showed that regardless of the length of deprivation intervals no or slight differences in the level of food intake before and after food deprivation could be detected.

Finally, privation which for 12-hr. and 36-hr. groups ended on days 18 and 20, showed a reversed picture, viz. a marked increase in food consumption occurred at morning hours. The privation which for the 24-hr. group terminated on day 17 showed that food intake remained at the pre-deprivation level (cf. Exp. I).

After shifting the animals to evening hours feeding schedule, changes in blood sugar level also occurred. On the first day after the shift, the level before feeding was low and in 12-hr. group it amounted to 93 mg%, in 24-hr. group, 99 mg%, and in 36-hr. group, 105 mg%. Measurements taken on days 6, 8, 9 and 11 revealed a slight average increase to 105 mg% in blood sugar level. Similar results were obtained on days 15, 16 and 17.

Like in the feeding behavior, with the introduction of the deprivation schedules changes in blood sugar level occurred which were determined by the previous feeding experience. In other words, irrespective of the length of the deprivation periods the sugar level remained lower at evening hours and increased in the morning.

Measurements made on days 8 and 13 in 12-hr. group, and on days 10 and 12 in 24-hr. and 36-hr. groups respectively showed that the blood sugar level equaled that obtained prior to privation. On the other hand, blood sugar level which was measured after privation on days 17, 18 and 20 indicated a reversed picture: similarly to the findings obtained in Experiment I, after 12-hr. food deprivation the blood sugar level tended to decrease, while following 24 hours it approximated the level which had been noted initially; after 36 hours it was again significantly diminished (Fig. 3).

Fig. 3. Food intake and blood sugar level after shifting the 24-hr. group (Group II) from morning to evening hours feeding schedule

Abscissa: number of days from shifting the feeding schedule (0, mean pre-deprivation food intake from 10 experimental days). Open areas depict food intake prior to privation; shaded areas, food intake following privation. The heavy solid lines indicate blood sugar level before privation. The broken lines, blood sugar level after privation (means from 2 animals). Ordinate in A (top and middle): food intake in gms; in B (bottom): blood sugar level in mg%

DISCUSSION

The above findings indicate that in rabbits fed every 24 hours (Group II) with the increase of deprivation intervals definite periodic changes, consisting of rising and falling phases, boths in eating behavior and blood sugar level occurred. In contrast, in the animals who had unobstructed access to the food at all times (Group I) rhythmicity failed to occur except for slight cyclic changes in the group provided with potato diet. A negative correlation between food consumption and blood sugar level throughout deprivation periods was found in all animals.

After shifting the 24-hr. feeding group from morning to evening hours feeding schedule (Experiment II) rhythmicity in boths food consumption and blood sugar level was found to disappear in nearly 2 weeks, and then a new periodic pattern in either measure was established which became highly fitted to the new feeding conditions.

The results of these experiments suggest that food intake as a function of length of deprivation periods is primarily dependent upon the previous feeding experience. Thus periodic changes in Group II were obviously determined by the 24-hr. feeding schedule. At the same time, failure of rhythmicity in Group I was a reflection of the situation in which the animals were exposed to the food available at all times. Furthermore, Experiment II indicates, that periodicity associated with food consumption in rabbits is very "plastic" and becomes modified as the result of change of the feeding schedule.

Rats, unlike rabbits, reveal a natural monophasic diurnal rhythm of feeding activities associated with the dark-light cycle. S i e g e l and S t u c k e y (1947), and B a r e (1959) found that maximum food consumption in rats maintained under circumstances of free access to the food occurred during the evening and nighttime hours, and was not at all affected either by uniform illumination maintained both day and night or water deprivation (G i l b e r t and J a m e s 1956). Only application of both "disturbances" did become efficient. In contrast, B a k e r (1953) and S i e g e l (1961) reported that rhythmicity was affected solely by uniform ambient illumination.

The effect of fast up to 24 hours on subsequent food intake in rats under conditions of free access to the food was investigated by B a r e (1959) and B a r e and C i c a l a (1960). The authors found that the rate at which the animals ate was determined by the time of day at which the measures were obtained even after deprivation. L a w r e n c e and M a s o n (1955) examined the relationship between an established eating rhythm and the amount of food ingested by rats after varying intervals of deprivation. One animal group had previous experience with

a 24-hr. feeding schedule. Depite differences in experimental procedure the general trend of periodicity in feeding following 36 and 48 hours of food deprivation was in accordance with that obtained in rabbits except that rats suffering deprivation longer than 24 hours did not increase their food consumption. As shown in the present experiments the 24-hr. group increased its mean intake even after extension of the interval between feedings up to 36 hours what equaled about 60-hr. fast.

As seen in Table I, the food intake in the group, which had free access to the food throughout the entire experiment, was significantly smaller than that in the 24-hr. feeding group. This might be related to the retention of a relatively large energy surplus stored in the form of neutral fat. It is also likely that the relatively low food intake in Group I was determined by the previous feeding experience which was associated with the presence of the food at all times and thus failure of adjustment to eat large quantities of food at a time. The latter statement is based on the results reported by G h e n t (1957), B a k e r (1955), and L a w r e n c e and M a s o n (1955).

However, changes in food consumption after varying periods of deprivation cannot be explained only as a result of previous feeding experience. They also appear to be a consequence of the kind of food ingested. In contrast to the standard solid food offered to the animals by other investigators, the animals of the present study were fed three different diets. It was found that, generally, the consumption of carrot and potatoes was superior to that of oats, and this was particularly evident after longer periods of fasting. Increased consumption of carrot and potatoes in the animals deprived of food was presumably associated with a considerable amount of water accumulated in these foodstuffs. Until recently, it was accepted that alterations in food intake were accompanied by parallel changes in drinking behavior (S i e g e l and S t u c k e y 1947, S t r o m i n g e r 1947, V e r p l a n c k and H a y e s 1953). Accordingly, when the food consumption was restricted, the acceptance of water was decreased. This was established upon rats, but this supposition does not appear to be justified with regard to rabbits. Under conditions of free access to water, rabbits deprived of food exhibit polydipsia and polyuria (H u a n g 1955), C i z e k 1954, 1961). The evidence is reasonably convincing that these disturbances in fasting rabbits derive from dehydration and the loss of considerable quantities of sodium chloride. Thus, it seems probable that the increased consumption of food containing relatively great amount of water after deprivation occurs as a result of the disturbance of water balance.

The present studies show that increased food intake following privation in rabbits fed every 24 hours was associated with the time of the

day (evening hours) at which no food was offered previously. On the other hand, at the time which corresponded to the usual feeding period (morning hours), e.g. following extension of feeding intervals by 24-hr. and 48-hr., the subsequent food intake was at the pre-deprivation level. In this connection, it is interesting to consider the data pointing to the inversive relationship between food consumption and blood sugar level in food deprived rabbits, which showed that the peak food intake was associated with a decrease of sugar level, while the falling phase of food intake was linked to the pre-deprivation level of blood sugar. It seems that this negative relation was more than an incidental finding and might be explained as follows. Under conditions of 24-hr. feeding schedule, a sequence of metabolic processes related to ingestion, absorption and utilization of food elements apparently followed the energy intake. Changes in blood sugar level were among the main variable factors involved in these mechanisms. In consequence, ingestion of carbohydrate brought about a period of hyperglycaemia which gradually ceased to be replaced by a decrease of the level of blood sugar. Accordingly, it might be expected that sugar level would be lower at evening hours than in the morning when the animals were fed. Such a result was obtained in the present study and is generally consistent with the findings reported earlier by Mišnajevskij and Voronov (1931) and Derevjagin (1936).

Offering the food to the animals was persistently correlated with a number of components of both the internal and external environments, such as rising (or setting) of the sun, sight of the people entering the room etc., which resulted in an increase in blood sugar level. In consequence, a relatively stable association between certain features of surroundings and the cycle of blood sugar level was established which was hardly to be masked and remained over a period of days under conditions of enforced fasting.

It is to be emphasized that even very slight changes of blood sugar level were associated with marked changes of food consumption. This finding is in accordance with the glucostatic theory of Mayer (1952).

SUMMARY

1. A group of 6 rabbits, which had free access to the food at all times (Group I), was successively deprived of food for 6 hr., 12 hr., 24 hr., 36 hr., 48 hr. and 60 hr. Another group of 6 animals, which was fed every 24 hours (Group II), was deprived of food by extending the intervals between feedings for 6 hr., 12 hr., 24 hr., 36 hr., 48 hr. and

60 hr. Food intake and blood sugar level were measured before and after appropriate privation periods.

2. Following privations in Group II, cyclic changes both in food intake and blood sugar level occurred, which were determined by the 24-hr. feeding schedule: at the time, which had previously been associated with feeding period, the level of blood sugar increased whereas food intake became reduced. In the Group I rabbits, rhythmicity failed to occur.

3. A negative correlation between blood sugar level and food intake after privation intervals was found. Both measures were determined by the previous feeding experience and the length of privation.

4. Increase in food intake after privation in Group II was superior to that in Group I. The consumption of food containing considerable amount of water (carrot and potatoes) increased more than that of the food with less water (oats). This was particularly evident for long food deprivation periods.

5. After shifting the feeding schedule from morning to evening hours in Group II, the pattern of rhythmicity both in food intake and blood sugar level became reversed, and then it got fitted to the new feeding conditions.

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