EFFECTS OF DISCRETE NUCLEAR LESIONS OF THE AMYGDALA ON BLOOD GLUCOSE AND FOOD INTAKE IN THE RAT

by 1254

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In the rat, the amygdala can be divided structurally and functionally into three areas: the basolateral and the corticomedial nuclear complexes, and the anterior amygdalar region. The amygdala receives projections from the pyriform cortex via the longitudinal association bundle, from the rostral half of the hypothalamus and some projections from the olfactory bulb. Most amygdalar efferents pass to the hypothalamus where they terminate in a rather diffuse array. The stria terminalis carries both the amygdalar efferents and the afferents arising in the hypothalamus; thus, it is a bidirectional pathway between these two structures (Cowan, Raisman and Powell, 1965; Gloor, 1960). The amygdala receives fibers from the medial septal nucleus by way of the diagonal band of Broca (Gloor, 1960; Fox, 1940), and sends efferents to the septum through the supracommissural component of the stria terminalis (Fox, 1940). The amygdala is connected to its contralateral equivalent by fibers passing through the commissural component of the stria terminalis and the anterior commissure, via the external capsule (Fox, 1940).

Recent experimental evidence has established the involvement of the amygdala in the functioning of the endocrine system. Pituitary hormones are under the control of neural factors in the hypothalamus which, as noted above, receives neural input from the amygdala. The hormones of the anterior pituitary, except for prolactin, are secreted in response to stimulation by hormonal releasing factors (RF's) manufactured by cells in relatively discrete hypothalamic areas. These RF's are then conveyed to the anterior pituitary through the hypothalamo-hypophyseal portal system. Hormones of the posterior pituitary, on the other hand, are actually manufactured in the hypothalamus and seep through neural tissue to the posterior pituitary where they are stored for subsequent release elicited by neural stimulation. No RF mechanism seems to exist for hormones of the posterior pituitary (Guillemin, 1967; Martini and Ganong, 1966; Turner, 1966).

The stimulation necessary to activate the secretion of the RF-production sites in the hypothalamus may be altered by amygdalar activity. Eleftheriou has demonstrated an increase in pituitary gonadotropin secretion and hypothalamic gonadotropin-RF secretion following damage to the basolateral nuclear complex of the deermouse (Eleftheriou, 1967; Eleftheriou and Pattison, 1967; Eleftheriou and Zolovick, 1967; Eleftheriou, Zolovick and Norman, 1967). Eleftheriou also demonstrated an increase in the activity of the pituitary-adrenal axis in the deermouse following damage to the medial amygdalar nucleus (Eleftheriou, Zolovick and Pearse, 1966). Eleftheriou and his co-workers have concluded that under normal conditions the amygdalar nuclei exert an inhibiting influence on the RF production sites in the hypothalamus, and that after damage to the amygdalar nuclei, there is an immediate depletion of the RF in the appropriate hypothalamic area and a rise in the plasma concentration of the target hormone. The RF produced subsequently is secreted immediately as a result of constant stimulation of the production site, thus the hypothalamic levels of the particular RF remain low until the cells eventually lose their secretory capability, or "burn out."

Glycosuria, the presence of glucose in the urine, following bilateral amygdalectomy was reported, in the rat, by Metcalf in 1966. Glycosuria results from an elevated blood sugar level (hyperglycemia) which is high enough to surpass the renal threshold for the retention of glucose. At extremely high blood sugar levels, the kidney tubules can no longer re-absorb all the circulating glucose filtered through the glomeruli, resulting in a spilling over of glucose into the urine. Hyperglycemia following bilateral amygdalectomy has been reported in the cat and in the monkey, but just as often there have been reports of no changes in blood glucose levels (Gloor, 1960; Goddard, 1964). The conflict in the reports may well have been due to differences in the size and extent of the damage. The more recent work by Eleftheriou and

his co-workers emphasizes the importance of introducing discrete nuclear lesions, but in the earlier studies cited by Goddard and by Gloor, and in the study by Metcalf, the amygdala was treated as an internally homogeneous structure and was ablated *in toto*.

Hyperglycemia can be brought about by hormonal mechanisms, some of which depend on the hypothalamic-pituitary feedback loop. One mechanism which does not directly depend on the involvement of the feedback loop is that underlying diabetes mellitis. This form of diabetes is produced by a lack of, or an inability to utilize endogenously produced insulin. The full syndrome of diabetes mellitis consists of polyphagia, polydipsia, polyurea, glycosuria, ketosis (formation of ketone bodies due to the breakdown of protein), and severe weight loss due to an inability to metabolize ingested food. Like diabetic rats, rats with total amygdalectomy exhibit extremely high blood sugar levels (sufficient to produce glycosuria), but in contrast to diabetics, Metcalf's amygdalectomized rats exhibited either a transitory hypophagia lasting for a few days, or died of starvation. Thus, amygdalectomy does not cause hyperglycemia by the same mechanisms found in diabetes mellitis.

Growth hormone (GH) also increases the level of blood glucose. In addition to raising blood glucose directly, a high level of circulating GH inhibits the action of, or prevents the production of insulin. Thus GH prevents insulin from lowering blood glucose. Since bone growth does not continue into adulthood, one of the primary actions of GH in the adult may be to regulate blood glucose levels by antagonism to insulin (Turner, 1966).

Adrenal corticotropic hormone (ACTH) serves to produce an increase in blood glucose. ACTH stimulates the secretion of glucocorticoids from the adrenal cortex which in turn raise blood glucose by stimulating gluconeogenesis: the formation of glucose from the amino acids of protein and from glycerol in fat (Guyton, 1961; Martini and Ganong, 1966; Turner, 1966). The work

of Eleftheriou suggests that the effect of amygdalar damage on ACTH secretion and/or GH secretion is a likely cause of blood glucose disruption.

Amygdalar lesions have been found to produce changes in food intake as well as changes in blood glucose and in endocrine functioning. In the albino rat, total amygdalectomy was reported to produce depressed food intake ranging from mild hypophagia to complete aphagia and adipsia (Metcalf, 1966; Yamada and Greer, 1960). The results found by Yamada and Greer, however, were attributed by the authors to lateral hypothalamic damage resulting from the extensive size of the lesions. Collier and Gault (1969) reported aphagia following damage to the corticomedial nuclear complex of the amygdala, although their data clearly indicates that some animals received damage to the basolateral nuclear complex.

In the hooded rat, White and Fisher (1969) found that electrical stimulation of the transitional area of the corticomedial amygdala and the pyriform cortex produced a decrease in food intake which could be prevented by damaging the ventromedial hypothalamus or the stria terminalis. Rosen (1968) found no significant changes in intake of either normal or quinine adulterated food following lesions of the corticomedial nuclear complex.

A study on the effects of chemical stimulation of the amygdala did not succeed in resolving these conflicting results. Grossman (1964) found that both hypophagia and hyperphagia could be differentially elicited from the ventral amygdala by application of cholinergic or adrenergic substances, respectively, with an inverse effect on water consumption. Grossman, however, did not anatomically define "ventral amygdala," nor did he report his cannula implantation co-ordinates or the results of histological procedures. It is not possible to ascertain from this study, whether stimulation occurred in the ventral portion of the basolateral complex, the corticomedial complex, or both.

It becomes increasingly clear that discrete nuclear lesions become a vi-

tal aspect of the experimental procedure in the investigation of the functions of the amygdala. The work of Eleftheriou clearly indicates the differential effects on the endocrine system of the various nuclear complexes of the amygdala. The need for discrete lesions is also supported by the conflicting results on changes in food intake following amygdalar damage. It is the purpose of the experiment reported herein to determine the effects of discrete amygdalar lesions on blood glucose and food intake. It has been shown that damage of the medial amygdalar nucleus produces an increase in ACTH secretion and increased levels of circulating adrenal cortical steroids. These increases are produced by a release from inhibition of the RF production sites in the hypothalamus. An increase in adrenal cortical activity produces an increase in blood glucose. Among the RF production sites involved in the function of the pituitary-adrenal axis is the ventromedial hypothalamus. Changes in activity of the ventromedial hypothalamus would also cause observed changes in the regulation of food intake. An increase in the activity of the ventromedial hypothalamus such as that produced by the release of inhibition from the medial amygdala, should then produce a decrease in food intake (Teitelbaum, 1961). This depression of food intake was found following medial lesions by Collier and Gault (1969). In direct conflict to the hypothesis that the medial amygdala inhibits the hypothalamus is the data of White and Fisher (1969) which indicated that the medial amygdala, when stimulated, produced aphagia indicating an excitatory effect of the medial amygdala on the hypothalamus.

The role of the basolateral nuclear complex of the amygdala has not been investigated to the same extent as has the corticomedial nuclear group. When the findings of Metcalf's study are combined with the findings of the study by Collier and Gault on the medial nucleus, the indications are that either: (a) the basolateral amygdala has no role in food regulation, (b) the basolateral may decrease food intake by acting in unison with the medial nucleus, (c) it

may decrease food intake by operating through some mechanism parallel to that through which the medial operates. The only positive statement that can be asserted is that the basolateral does not give any indications of operating in antagonism to the medial nucleus.

The effect of damage of the basolateral nuclear group on blood glucose regulation has also not been studied. Since the effects of total amygdalectomy are similar to those of lesions of the corticomedial group, the same set of assumptions must be drawn regarding basolateral amygdalar function in blood glucose regulation as in food intake.

METHOD

Subjects

Twenty-six male, Long-Evans hooded rats 120-150 days of age and weighing approximately 350-400 gms. were used. The animals were purchased from Rockland Farms, Gilbertsville, Pa., housed in individual wire-mesh cages and maintained on a 9:00 A.M. to 9:00 P.M. reverse daylight cycle. Water was available at all times.

Procedure

Feeding. Each animal was taken off ad lib. Purina Rat Chow and deprived of food for twelve hours. Following the deprivation period, each animal was put on an enriched liquid diet developed by Teitelbaum and Epstein (1962). The quantity of food allowed each animal (40 ml/day) was sufficient to allow a slight increase in weight (approximately 1 gm/day, after an initial loss). The quantity was small enough to be readily eaten by a normal rat within a period of three minutes, when fed in three portions per day. The liquid diet consisted of 240 ml. evaporated milk, 75 ml. whole powdered egg solution, 125 ml. of 50% sucrose (w/v) solution, 0.3 ml. liquid vitamin preparation (Poly-vi-sol, Mead-Johnson Co., Evansville, Ind.), 35 ml. Kaopectate (Upjohn Co., Kalamazoo, Mich.), 100 ml. distilled water, and 1 ml/100 ml of solution of 10% formalin as a preservative. The diet was presented in small stable glass dishes (furniture casters) three times daily: 12 ml. at 9:30 A.M., 13 ml. at 2:30 P.M., and 15 ml. at 8:00 P.M.

Each rat's weight was recorded daily, prior to the morning feeding. In addition, the amount of food left from the previous feeding and the animal's condition (e.g. state of alertness, disposition, general appearance and behavior toward food) were noted at each feeding.

Blood sampling and analysis. Blood samples were taken from the tip of

the tail daily, just prior to the afternoon feeding. Starting on the first day the animal received the liquid diet, the rat was placed in a hemi-cylindrical, plexiglas restraining tube covered with cloth to shut out light. The restraining tube allowed the tail to protrude to its full length. The tail was massaged and pinched gently each day for several days to allow the animal to become accustomed to the situation. After the tail manipulation, the animal was returned to his home cage and fed.

This procedure was followed until the morning weight measures indicated that the animal's weight had ceased to drop from deprivation and adaptation to the 40 ml/day feeding schedule. Stabilization to the new schedule took from 5 to 8 days depending on the initial size of the animal: the heavier the animal, the longer it took for the animal to stop losing weight on the 40 ml/day schedule. When the animal's weight stopped dropping, the first blood sample was taken. With the rat in the restraining tube, the tip of the tail was wiped with 70% ethanol and dried. With a clean razor blade, the tip was sliced off and a small drop of blood squeezed out and discarded. Then 0.1 ml. of blood was drawn into a scored, un-heparinized, micropipette (Yankee Disposable Micropet, 100 lambda, Clay-Adams). The sample was blown into 10 ml. of dilute tungstic acid solution, and the micropipette discarded. The diluted sample was then frozen for subsequent analysis. The tail was then cleaned, dried, and dusted with a powder comprised of 50% nitrofurazone antibiotic (Furacin, Eaton Laboratories). This sampling procedure could be continued for at least 90 days without deleterious effects to the tail. After sampling, the animal was returned to its home cage and fed.

The method of blood glucose analysis used was the Folin-Malmros colorimetric method utilizing 0.1 ml. of whole blood (Hawk, Oser and Summerson, 1954). Blood glucose data is expressed in mg% or the number of mg. of glucose per 100 ml. of whole blood. For a detailed description of the analysis proce-

dure see the appendix.

Surgery and histology. After at least 7 days of blood sampling the animal was deprived of his evening meal and operated upon at night to facilitate a continuation of the normal blood sampling procedure. The rat was anaesthetized with 1.2 ml. of Equithesin administered intraperitoneally which was followed by 0.1 ml. of atropine sulfate administered intraperitoneally, to suppress secretion of mucous. Lesions were produced electrolytically at the uninsulated tip (0.25 mm.) of a stainless steel insect pin, coated with Epoxylite insulation. Lesions were produced with anodal dc current (1 mA for 15 sec.) at the insect pin. An anal cathode completed the circuit.

The electrode was inserted stereotactically, utilizing the atlas of Pellegrino and Cushman (1967) as a guide. Anterior-posterior measurements were expressed as mm. from bregma, lateral measurements were in mm. from the midline suture, and depth measurements were in mm. from the dural surface. The co-ordinates used were as follows: medial amygdala, 0.8 A-P, ±3.5 L, -8.0 D; basolateral amygdala, -1.0 A-P, ±5.0 L, -7.5 D; combined basolateral and medial, -1.0 A-P, ±3.5 L, ±5.0 L, -7.5 D, -8.0 D. The animal's head was positioned with the incisor bar 5.0 mm. above the interaural line. Following surgery, each animal received 45,000 units of Bicillin (Wyeth) intramuscularly.

After two to eight weeks of blood sampling, the animal was given 1.5 ml. of Equithesin (intraperitoneally) and perfused with physiological saline followed with 10% formalin. The brains were removed and fixed in acid formalin for 1 week. They were then imbedded in celloidin, cut coronally at 25μ, and stained with cresyl violet.

Animals in the Sham-lesion group were treated identically, except for the fact that no current was passed through the electrode after it was placed stereo-

tactically. After removal, the brain was stored in 10% formalin and left unstained.

Urine analysis. Several times during the course of the experiment, each animal's urine was assayed for the presence of glucose. This was accomplished by dipping a strip of clinical Tes-Tape (Lilly) into the urine. Changes in the color of the Tes-Tape indicate roughly the amount of sugar in the urine.

RESULTS

Histology

Diagrams of representative lesions of each of the experimental groups are shown in Fig. 1. Histological grouping and analyses were performed without knowledge of the blood sugar or weight measurements.

Two animals were removed from the data analysis following histological examination. One was removed because there was extensive thalamic damage along the route of the electrode track, the other was removed because the corticomedial group was lesioned on one side of the brain, the basolateral on the other.

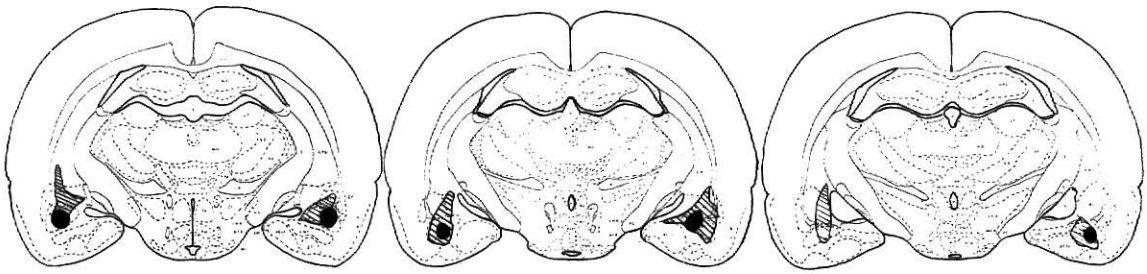
There were seven animals with damage to the medial nucleus (AME Group). In all seven animals, some minor damage was inflicted on the cortical nucleus which lies just lateral to the medial nucleus and bends mediad at the caudal end of the medial nucleus. Three of the AME animals sustained very minor thalamic damage, probably due to current leakage around the shaft of the electrode. Five AME animals sustained very slight damage to the optic tract. The AME animals did not show damage to the basolateral nucleus.

Six animals received damage to the basolateral nuclear group (ABL Group). There was some minimal pyriform cortex damage in approximately half the animals in this group. There were also instances of claustrum and corpus callosum damage in some of the animals. One of the rats sustained caudate damage as a result of current leakage around the shaft of the electrode. No medial damage was seen.

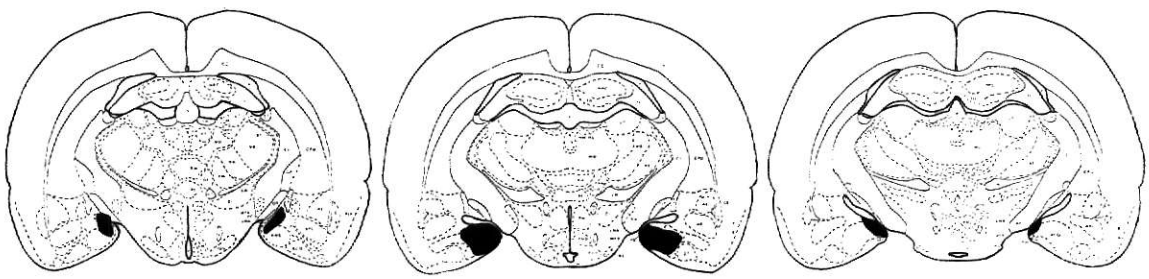
Six animals received lesions of both the medial nucleus and the basolateral nuclear complex (ABL-AME Group). One animal showed some slight unilateral pyriform cortex and external capsule damage. Two animals showed some slight stria terminalis and optic tract damage. One of the ABL-AME subjects showed evidence of unilateral gliosis in the lateral hypothalamus.

Figure Caption

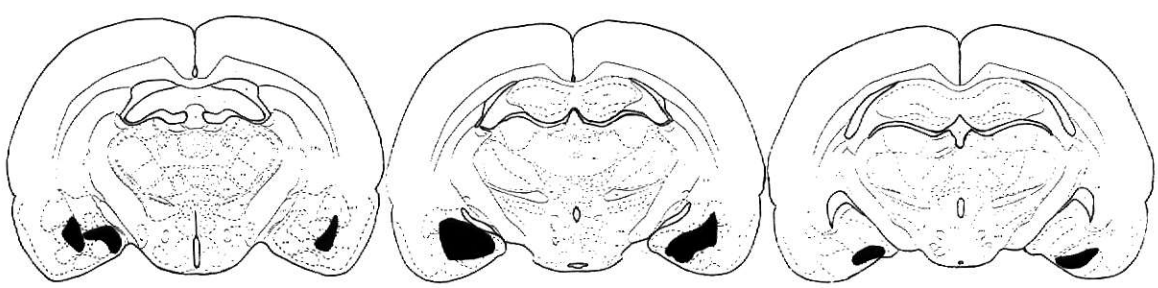
Fig. 1. Diagrammatic representation of one lesion from each of the three experimental groups. The three sections illustrating each lesion indicate the Anterior-Posterior extent of the damage. Solid black represents total destruction; cross-hatching represents dense gliosis.



BASOLATERAL



MEDIAL



BASOLATERAL-MEDIAL

The brains of the five sham-lesioned animals (Sham Group) were fixed in acid formalin and examined for infection both on the cortex and in the region of the electrode placement. Two brains showed mild cortical infection, but no greater than that observed in some of the lesioned brains. One of the Sham brains exhibited slight unilateral infection in the medial amygdalar region.

All histological examinations and analyses were checked and verified by a second observer, experienced in lesion techniques and histological analyses.

Blood glucose

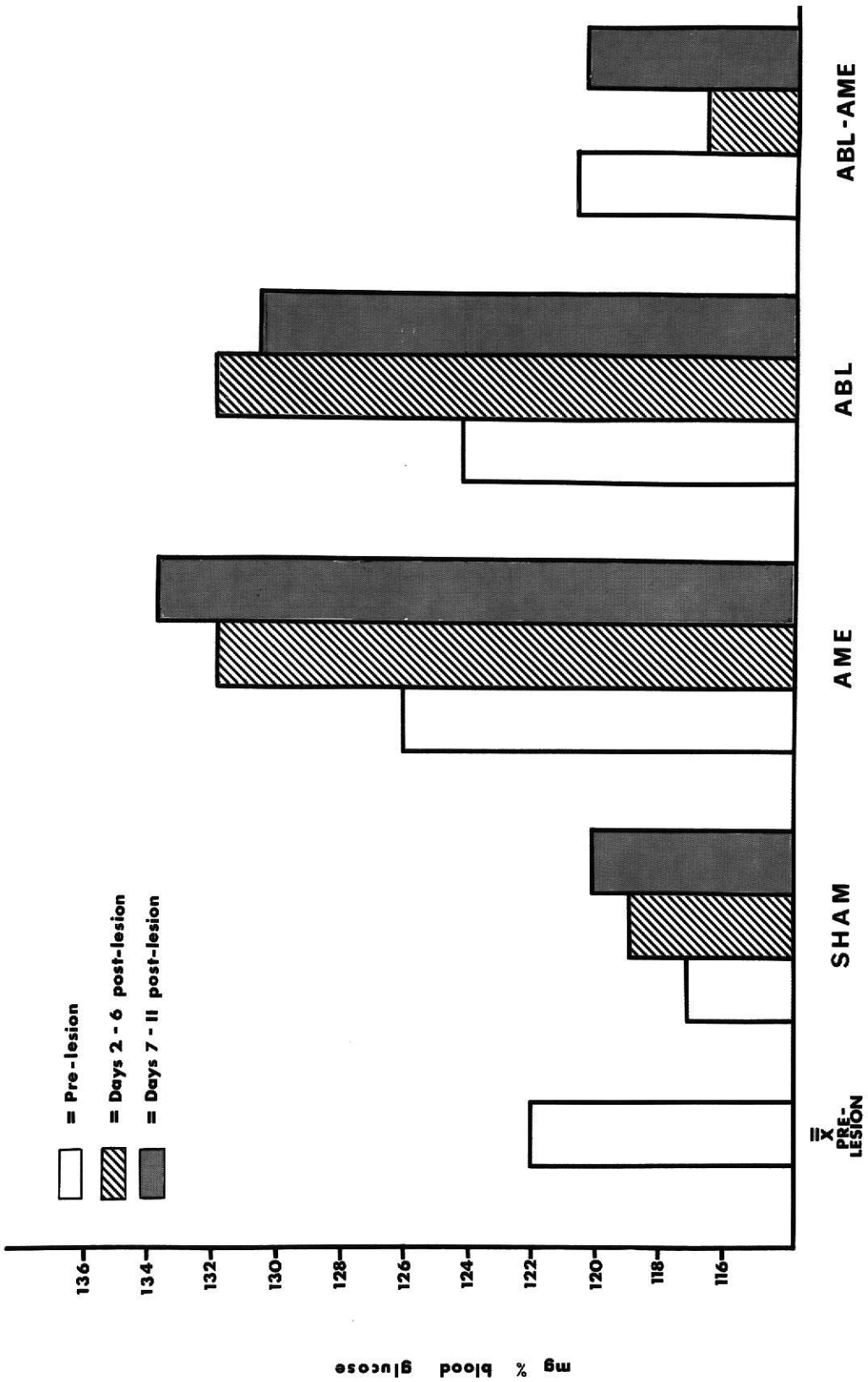
Blood glucose levels were determined for each animal from the time of weight stabilization to at least eleven days postoperatively. The glucose levels for six days prior to surgery were taken to be the base level against which the postoperative glucose levels were compared. Figure 2 shows the mean blood glucose levels for each lesion group. The first blood sample after surgery was discarded due to fluctuations in blood glucose produced by the surgery itself. The next 10 days were divided into five-day blocks to determine any major changes in blood glucose over time.

The pre-lesion means for each group were combined into a grand mean ($\bar{X}$). This grand mean was found to be 122.34 mg% (se=3.531). By applying a Student's *t* test to the grand mean and the pre-lesion means from each individual group (McNemar, 1962), it was found that the grand mean did not differ significantly from any of the individual pre-lesion means, and thus could be used as a base line for pre-lesion blood glucose. Table 1 illustrates the results of *t* tests applied to the different group means. The degrees of freedom of the *t* tests represented in Table 1 varied from 32 to 75 and were computed from the number of samples per group per treatment, since no subject by days interaction was found.

Homogeneity of variance was tested by an *F* test applied to the estimated standard deviation of the population computed from the data utilized in the *t*

Figure Caption

Fig. 2. Mean blood glucose levels expressed in milligrams percent (mg%).


mg % blood glucose
136
134
132
130
128
126
124
122
120
118
116
= Pre-lesion
= Days 2 - 6 post-lesion
= Days 7 - 11 post-lesion
X PRE-LESION
SHAM
AME
ABL
ABL-AME

test (McNemar, 1962). The results of the F tests are presented in Table 2.

The Sham group showed no differences between the Post-lesion means and either the Sham Pre-lesion mean or the grand mean. The Sham Pre-lesion mean did differ significantly from the AME Pre-lesion mean ($t = 3.606$, $df = 69$, $p < .005$) and from the ABL Pre-lesion mean ($t = 2.624$, $df = 63$, $p < .01$). The variance of the Sham scores was exceedingly small. All the Sham subjects were drawn from the same shipment of rats from the breeding farm whereas the animals in the other groups were drawn randomly from several different stock shipments. Therefore, all the Sham animals were the same age and fairly closely matched in size and weight. The animals in the other groups varied more in size, weight and age which may account for greater variance in blood glucose scores. Metabolic rate varies with age and is a factor in blood glucose levels.

Within the AME group, there was a significant difference between the Pre-lesion grand mean and days 2-6 post-lesion ($t = 3.519$, $df = 37$, $p < .01$) and between the Pre-lesion grand mean and days 7-11 ($t = 4.176$, $df = 37$, $p < .01$). There was no significant difference between the mean of days 2-6 and the mean of days 7-11. The mean of days 2-6 was significantly different from the mean of Sham days 2-6 but any comparisons involving Sham days 7-11 showed no significance due to the lack of homogeneity of variance (see Table 2).

Tests of significant differences involving the ABL group showed the same pattern as the AME group. The ABL group showed a significant increase in blood glucose in both of the five-day blocks above the Pre-lesion grand mean (ABL 2-6 vs. $\bar{\bar{X}}$: $t = 3.503$, $df = 32$, $p < .01$; ABL 7-11 vs. $\bar{\bar{X}}$: $t = 2.722$, $df = 32$, $p < .02$). There was no significant difference between the mean of ABL days 2-6 and the mean of ABL days 7-11. In comparing the ABL data with the Sham data, the lack of homogeneity of variance negated the differences between Sham days 7-11 and both of the ABL Post-lesion blocks, and between Sham days 2-6 and

TABLE 1

Results of t Tests Applied to Group Means

		ABL			AME			ABL-AME			SHAM			$\bar{X}$
		Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion
ABL	Pre-Lesion	—												
	Days 2 - 6	*	—											
	Days 7 -11	*	NS	—										
AME	Pre-Lesion	NS	*	NS	—									
	Days 2 - 6	*	NS	NS	*	—								
	Days 7 -11	*	NS	NS	*	NS	—							
ABL-AME	Pre-Lesion	NS	*	*	*	*	*	—						
	Days 2 - 6	*	*	*	*	*	*	NS	—					
	Days 7 -11	NS	*	*	*	*	*	NS	NS	—				
SHAM	Pre-Lesion	*	*	*	*	*	*	NS	NS	NS	—			
	Days 2 - 6	*	*	*	*	*	*	NS	NS	NS	NS	—		
	Days 7 -11	NS	*	*	*	*	*	NS	NS	NS	NS	NS	—	
$\bar{X}$	Pre-Lesion	NS	*	*	NS	*	*	NS	NS	NS	NS	NS	NS	—

NS Represents No significant difference

* Represents $P < .05$

TABLE 2

Tests of Homogeneity of Variance Between Groups

		ABL			AME			ABL-AME			SHAM			$\bar{\bar{X}}$
		Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion	Days 2 - 6	Days 7 -11	Pre-Lesion
ABL	Pre-Lesion	—												
	Days 2 - 6	NS	—											
	Days 7 -11	NS	—	—										
AME	Pre-Lesion	—	NS	—	—									
	Days 2 - 6	NS	—	—	NS	—								
	Days 7 -11	NS	—	—	NS	—	—							
ABL-AME	Pre-Lesion	—	NS	NS	NS	NS	NS	—						
	Days 2 - 6	NS	NS	NS	NS	NS	NS	—	—					
	Days 7 -11	—	NS	NS	NS	NS	NS	—	—	—				
SHAM	Pre-Lesion	NS	NS	NS	NS	NS	NS	—	—	—	—			
	Days 2 - 6	NS	NS	*	NS	NS	NS	—	—	—	—	—		
	Days 7 -11	—	*	*	NS	*	*	—	—	—	—	—	—	
$\bar{\bar{X}}$	Pre-Lesion	—	NS	NS	—	NS	NS	—	—	—	—	—	—	—

NS Represents No significant difference

* Represents $P < .01$

ABL days 7-11 (see Table 2). Comparisons of the respective blocks of days in the ABL and AME groups yielded no significant differences.

The ABL-AME group did not show significant differences between Pre- and Post-lesion data, nor were there any significant differences between the ABL-AME scores and the Sham scores. The ABL-AME Post-lesion scores each differed significantly from the Post-lesion scores of both the AME group and the ABL group (Tables 1 and 2).

Weight

There were no significant differences between the Pre-lesion and Post-lesion mean weights within groups although the different experimental groups had different baseline weights. Tables 3 and 4 present the mean group weights and percent weight change during days 2-6 post-lesion and days 7-11 post-lesion. The mean weight for each group indicates that all groups have a mean for days 7-11 slightly above that of the pre-lesion level, except for the ABL-AME group. The ABL-AME group is considerably lower in weight in the days 7-11 block than in the Pre-lesion block.

A Spearman rank-order correlation (McNemar, 1962) was run between the Pre-lesion mean weight and the Pre-lesion mean blood glucose level of each of the 24 animals used in order to determine if there was a significant correlation between weight and blood glucose level. There was no significant correlation between the Pre-lesion weight and the corresponding level of blood glucose ($\rho = 0.298$, $t = 1.460$, $df = 22$, $p > .1$).

Food intake

All groups showed a slight decrease in food intake during the eleven days following surgery. This decrease was not large nor consistant. There were no significant differences between the groups in mean food intake. In addition, there were no significant differences between the groups in regard

TABLE 3

Mean Group Weights

Group	Pre-lesion	Post-lesion days 2-6	Post-lesion days 7-11
AME	411.3	411.1	411.6
ABL	410.6	413.4	412.4
ABL-AME	405.5	401.8	396.0
Sham	372.3	372.2	373.4

TABLE 4

Mean Percent Weight Changes

Group	Days 2-6 from Pre	Days 7-11 from Pre	Days 7-11 from 2-6
AME	-0.1 %	0.0 %	+0.2 %
ABL	+0.8	+0.5	-0.3
ABL-AME	-0.9	-2.3	-1.5
Sham	0.0	+0.4	+0.3

to the proportion of the group exhibiting a decrement in food intake (Fisher Exact Probability Test, Siegel, 1956). Table 5 shows the mean food intake per animal for each group over the six days prior to surgery and days 2-11 following surgery, as well as showing the percentage of the group showing a food intake decrement.

The decrease in food intake of the ABL group is larger than that of the other groups but reflects a mean decrement of only 2.4 ml. per day per animal (i.e. each animal ate 37.6 ml/day rather than 40 ml.). One third of the animals in this group showed no decrement at all, and among those that did, only one showed an extreme decrease. This animal accounted for most of the group deficit by showing an average decrease of 8.7 ml/day. Most of the decreases in food intake resulted from an animal's skipping a meal occasionally, rather than a constant decrement.

In summary, while none of the groups differed statistically from the Sham group in regard to the magnitude of the food intake decrement, or the number of animals in a group showing a decrement, a greater proportion of ABL animals showed depressed food intake, and the decrease in food intake was larger in the ABL group than in the other groups.

Urine analysis

The urine of each animal was tested several times at random throughout the experiment. The animal was held around the middle and squeezed gently until a small amount of urine flowed. The urine was tested with a strip of Tes-Tape. No instance of sugar in the urine (glycosuria) was found during the course of the experiment.

Other observations

In general, the AME animals showed little change in overall activity. Some of the animals in this group tended to be slightly jumpy and overactive

TABLE 5

Mean Food Intake per Animal (in ml.)

Group	given Pre	eaten Pre	given Post	eaten Post
AME	240	240	400	395.4 (28.6%)[1]
ABL	240	240	400	376.0 (66.7%)
ABL-AME	240	240	400	396.7 (33.3%)
Sham	240	240	400	396.0 (20.0%)

[1]Number in parentheses represents the percentage of animals in the group showing an intake deficit.

during the early part of the post-lesion period. On the other hand, the ABL animals were lethargic and did not groom. Their coats became dirty and rough looking. These animals also showed extreme reactions on being touched. They would squeak and jump several inches when touched. When not disturbed, they appeared to be almost asleep. The blood samples of the ABL animals were different in their appearance than the blood samples of other groups. Instead of being bright red, rich, and barely translucent, the blood samples of the ABL animals looked weak, watery and rust colored when placed in tungstic acid. After precipitation of the protein, the sediment of red blood cells at the bottom of the vial was only a fraction of the volume of the sediment in the samples from other groups of animals.

After 10 days or so, many of the ABL animals which had previously been lethargic became more active, until no differences in the behavior of ABL and AME animals could be observed.

ABL-AME animals showed either the lethargy of the ABL animals or the hyperactivity of the AME animals but to a lesser extent. Generally, the behavior of the ABL-AME animals was little changed after surgery.

DISCUSSION

A significant elevation in blood glucose can be produced by destruction of either the medial or basolateral amygdala. The elevation of blood glucose is not produced when both areas are damaged. The extreme hyperglycemia leading to glycosuria in animals with massive total amygdalectomy (Metcalf, 1966) cannot be attributed solely to the combined destruction of the basolateral and corticomedial nuclear complexes, although these comprise the main mass of the amygdala.

The hypothesis that hyperglycemia would result from medial damage due to the effect on the pituitary-adrenal axis demonstrated by Eleftheriou et al. (1966) was supported by the blood glucose data. Further evidence for hyperfunction of the adrenal cortex was contributed by the general observations which indicated that the animals with medial damage showed greater activity or "nervousness," which is symptomatic of excess circulating adrenal steroids (Miller, 1969).

It is possible that both the basolateral and corticomedial lesions act to elevate the level of blood glucose by the same mechanism. Eleftheriou et al. (1966) did not test the effect of basolateral damage on the pituitary-adrenal axis. The assumption of one mechanism for both nuclear groups is not a reasonable one though, since the combined effect of damage to both centers would be at least as large as that produced by either single effect. The absence of glycosuria in the present study indicates that blood sugar could have been considerably higher, i.e. that it had not reached the renal threshold. A higher blood glucose concentration was not seen with combined lesions eliminated hyperglycemia produced by either lesion. Furthermore, the general behavior of the animals with basolateral damage was quite different from those with medial lesions. The ABL animals were slow, lethargic, groomed poorly, ate slowly and showed a rather extreme "startle" response when touch-

ed. The AME animals, on the other hand, showed none of these behaviors. They showed no decrement in speed of food consumption and exhibited slight hyperactivity or a generally higher level of excitement. The ABL animals also showed changes in the color and other qualities of the blood after surgery, which was not present in the AME group. The changes related to a decrease in the concentration of red blood cells, and the change in blood color was readily apparent to all observers. Guyton stated (1961) that an increase in the activity of the pituitary-adrenal axis or the pituitary-thyroid axis produces a large increase in the proliferation of red blood cells, and conversely, a decrease in function of the adrenal cortex or the thyroid produces a decrease in the concentration of red blood cells. Although no conclusions can be drawn from the empirical blood glucose data, the addition of the supplementary observations provides evidence for two separate routes of influence.

The effect of ABL and AME damage on food intake was quite different indicating that each lesion may have a seperate action. First, it is important to note that in contrast to the studies of amygdalar control of feeding such as that by Metcalf (1966) and by Yamada and Greer (1960) on total ablation of the amygdala, and by Collier and Gault (1969) on ablation of the medial nucleus, no aphagia and/or consistant hypophagia was observed in this experiment. As stated earlier, the aphagia found by Yamada and Greer (1960) was attributed to damage of the lateral hypothalamus. The present study was not designed to test changes of food intake in a positive direction, since on the basis of previous lesion studies, none were expected.

Rosen (1968) reported no differences in food intake following damage to the medial amygdalar nucleus. The lack of effect was attributed to possible strain differences. Of the studies cited above, only those by Rosen and the author were performed on the hooded rat. Aphagia following total amygdalectomy in the hooded rat was observed however, by King and Mitchell

(J.C. Mitchell, personal communication).

Statistically, no differences were found in this study regarding food intake between groups. One of the five Sham animals showed a slight decrease in food intake which could probably be attributed to the effects of surgery. Two of the seven AME animals showed a decrease which was of the same magnitude as that seen in the Sham animal. The ABL group, on the other hand, showed some decrease in 66.67% of the animals (4 out of 6). The decrement was larger, on the average, than that of the other groups. As stated, these results are not statistically significant, but do indicate a possible trend.

There are no differences between ABL and AME groups if the blood glucose and weight data are considered exclusively. There is no reason to suspect that the basolateral nuclear complex acts through any mechanisms other than those through which the medial or corticomedial group functions. The increased activity of the hypothalamic-pituitary axis following medial damage, and the increased activity of the ventromedial hypothalamus following medial amygdalar stimulation could be explained by the following relationships between amygdala and hypothalamus: both the corticomedial and basolateral amygdalar groups normally have an excitatory rather than inhibitory influence on the regions of the hypothalamus. The relationship between the nuclear complexes however, is one of mutual inhibition. Thus, if the AME were damaged one would expect increased stimulation of RF production sites in the hypothalamus by the ABL. This is consistant with the data on changes in the activity of the pituitary-adrenal axis following AME damage found by Eleftheriou et al. (1966) and would explain the hyperglycemia resulting from AME damage in the present experiment. If the AME were stimulated, an increased stimulation of the hypothalamic RF-production sites would be expected. This is consistant with the results of stimulation of the corticomedial region of the amygdala by White and Fisher (1969).

Destruction of the ABL would release inhibition of the AME causing the same result as destruction of the AME, i.e. hyperglycemia, due to increase stimulation of the RF-production sites by the AME. This was found in the present study. Finally, destruction of both the basolateral and corticomedial regions simultaneously eliminates stimulation of the hypothalamus by either structure, preventing hyperglycemia. In this situation, the animal could continue to regulate blood glucose at normal levels by insulin and GH. The absence of hyperglycemia following destruction of the combined AME and ABL areas was another major result of the present study.

If one takes into account the behavioral observations and the trends indicated in the food intake data, it appears as though the different nuclei of the amygdala might possibly be operating through two separate hormonal axes. The existance of two separate hormonal axes does not preclude the model presented above involving mutual inhibition between the nuclear groups of the amygdala and stimulation of the hypothalamus by the individual nuclear complexes, but raises the question as to the terminus of the amygdalo-hypothalamic fiber tracts. The hyperglycemia elicited by medial damage may be mediated by the pituitary-adrenal axis. The hyperglycemia following basolateral damage is accompanied by changes not seen following medial damage, e.g. differences in general activity, changes in blood composition other than blood glucose concentration, and indications of a greater decrease in food intake.

The absence of hyperglycemia following damage to the combined ABL and AME areas might be attributed to destruction of both excitatory centers as stated above, or it may be due to an antagonism between the effects of the lesions of each of the separate areas. This antagonism might exist as a result of neural properties of different areas within the hypothalamus itself, or might be a result of antagonism between different hormones or effects of different hormones within the hypothalamus or the system, differentially re-

leased after damage to the two amygdalar areas.

The two main problems discussed, mainly that of the action of amygdalar nuclei on each other and the action of each on the hypothalamus, and the problem of the hypothalamic loci modified by activity of amygdalar nuclei need further investigation. Eleftheriou's hypothesis concerning the direct inhibition of a hypothalamic RF-production site by an individual amygdalar nucleus or nuclear complex is now in doubt. The hypothesis of White and Fisher regarding the direct stimulation of the hypothalamus by amygdalar nuclei is not consistant with the data of Eleftheriou's studies. To further elucidate the amygdalar-hypothalamic relationships discussed, it seems necessary to monitor the neural activity of the structures in response to the simulation of the other structures involved, as well as an extensive analysis of endocrine changes taking place.

APPENDIX

Folin Malmros determination for blood glucose (Hawk, Oser and Summerson, 1954, pp. 575-577.

1) 0.1 ml. of whole blood is added to 10 ml. of dilute tungstic acid solution to precipitate the protein in the blood. The sample is then frozen for subsequent analysis.

2) The sample is thawed and spun in a centrifuge at about 1500 rpm for about 30 min.

3) 4.0 ml. of the supernatant is transferred to a Folin-Malmros or Folin-Wu sugar tube (Pyrex, Fisher Scientific). 2.0 ml. of potassium ferricyanide solution and 1.0 ml. of sodium cyanide carbonate solution (buffer) are then added to the sugar tube.

4) The sample is then placed in boiling water for 20 min. to promote the redox reaction (ferricyanide to ferrocyanide).

5) The sample is then cooled in ice-water for 1½ min. to stop the reaction.

6) 5.0 ml. of ferric iron solution is added to the sugar tube.

7) The now blue solution is diluted to 25 ml. with distilled water, placed in a cuvette and read in a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 520 nm.

Two methods of calibration of the colorimeter can be used: (a) a standard solution of 0.01 mg/ml of reagent grade glucose can be prepared and run through steps 3 through 7 of the procedure. The resulting %-transmittance is then converted to optical density (absorbance) by the following equation:

O (optical density)= $-\log T_s$; where T= transmittance. The resulting optical density (O) is then plugged into the following formula:

$$\frac{O_{(sample)}}{O_{(standard)}} \times 0.04 \times \frac{10}{4.0} \times \frac{100}{0.1} = \text{mg\% glucose in whole blood}$$

Since the 0.01 mg/ml sample also serves as a standard, the resulting ratio is 1. When run through the rest of the formula which corrects for the dilutions the blood sample has undergone, the final concentration is 100 mg%. The optical density then of the 0.01 sample is taken to be that of a 100 mg% standard.

A known concentration of glucose amounting to 0.012 mg/ml would be run as follows:

$$\frac{O_{(0.012\ mg/ml)}}{O_{(0.01\ mg/ml)}} \times 0.04 \times \frac{10}{4.0} \times \frac{100}{0.1} = 120\ \text{mg\% glucose}$$

(b) The second method of calibration is to prepare a set of serial dilutions of reagent grade glucose consisting of 0.009 mg/ml, 0.01 mg/ml, 0.012 mg/ml, 0.014 mg/ml, etc., corresponding to blood glucose concentrations of 90, 100, 120, and 140 mg% respectively. Then, the optical densities obtained from the colorimeter readings are plotted on a graph using glucose concentration as the abscissa and optical density as the ordinate. This plot should yield a monotonic function. The optical densities then obtained from the experimental samples are plotted on the graph to determine the corresponding blood glucose concentration.

It was found that both methods worked equally well, but the first method was easier in respect to recalibration, thus it was the one employed throughout the experiment.

Recalibration of the machine was conducted every week, and more often whenever stock reagents were replenished.

Precautions were taken throughout the experiment to insure against contamination of samples by glassware in contact with other samples, and against contamination or spoilage of the reagents.

The reliability of the determination procedure was checked by testing a

series of ten samples drawn from the same stock solution of freshly prepared 0.01 mg/ml reagent grade glucose. This concentration, as stated above, corresponds to a blood glucose concentration of 100 mg%. The results of the test indicated a mean concentration of 100 mg%, with sd= 1.897 mg%.

Preparation of Reagents

Standard Glucose Solution- 0.01 mg. reagent grade glucose/ ml. of distilled water. Unless picric acid is added, the shelf life of this solution is only 2 or 3 hours.

Dilute Tungstic Acid Solution- Add 20 ml. of 10% sodium tungstate solution to a volumetric flask(1 l.). Dilute to about 800 ml. Add, with shaking, 20 ml. of 2/3N sulfuric acid. Dilute to volume.

Potassium Ferricyanide Solution- Dissolve 2 g. of c.p. potassium ferricyanide in distilled water and dilute to a volume of 500 ml. (0.4 % solution). Must be kept in a brown bottle and stored in the dark, when not in use.

Sodium Cyanide Carbonate Solution- Place 8 g. of anhydrous sodium carbonate in a 500 ml. volumetric flask. Add 40-50 ml. of distilled water and shake until dissolved. Add 150 ml. of freshly prepared 1% sodium cyanide solution. Dilute to volume.

Ferric Iron Solution- Dissolve 20 g. of sodium lauryl sulfate in 1 l. of distilled water. To this solution, add a solution of 5 g. of anhydrous ferric sulfate in 75 ml. of 85% phosphoric acid, plus 100 ml. distilled water.

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EFFECTS OF DISCRETE NUCLEAR LESIONS OF THE AMYGDALA ON BLOOD GLUCOSE AND FOOD INTAKE IN THE RAT

by

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AN ABSTRACT OF A MASTER'S THESIS

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To test the effect of amygdalar damage on blood glucose level, food intake, and body weight, lesions were placed in either the medial nucleus, the basolateral nuclear complex, or both. Lesions of the medial nucleus and of the basolateral group each caused a significant increase in blood glucose. Lesions damaging the medial and basolateral areas simultaneously did not produce an increase in blood glucose. No significant changes in food intake or weight following surgery were observed in any experimental group.