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1. The director of the Missouri Basin Project, Dr. R. Stephenson, lent generous assistance, as did the Earth Science Division of the National Science Foundation and the Biology Department of the University of Louisville.
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Disinhibition of Inhibitory Conditioned Responses following Selective Brain Lesions in Dogs

Abstract. Discrete lesion of the genual portion of the anterior cingulate gyrus in three dogs produced temporary disinhibition of preoperatively trained inhibitory food conditioned responses. This disinhibition was accompanied by increase in general behavior motivated by food reinforcement. Lesion of the posterior cingulate gyrus in three other dogs did not produce such impairment.

It has been reported that a prefrontal lobectomy anterior to the presylvian sulcus in dogs produced an increase in some positive conditioned responses (CR's) and temporary disinhibition of inhibitory food and drink as well as classical defensive CR's (that is, fear-like responses). Furthermore, marked increase in emotionality and in the unconditioned responses was noticed (*1*). It was also found that if the lesion extended into the depth of the prefrontal lobe and involved the cortex in front of the genu of the corpus callosum the impairment was even more conspicuous. Hence the probability arises that the genual portion of the anterior cingulate area is one of the critical forebrain regions for the inhibition of some affective responses. A second report dealt with dogs in which, following genual cingulectomy, violent rage and angry behavior occurred (*2*). This evidence also indicates that the genual area serves to inhibit some of the major emotions. To verify this hypothesis with measurable methods the CR procedure was used. The present report describes a series of experiments which were carried out under food conditions.

Six naive dogs were used. All animals were trained in a Pavlovian frame. The animal's task was to place his right foreleg on the food tray to get a food reinforcement whenever the excitatory (positive) conditioning stimulus (CS), a 1000-cy/sec tone, was used, and to refrain from this response when hearing the inhibitory (negative) CS, a 700-cy/sec tone. During the preliminary training, which consisted of 30 daily trials, either the positive CS was not followed by food or, on CS, the experimenter put the animal's leg on the food tray, whereupon the food was presented. These two types of training trials occurred at random. With time the animal responded to the positive CS actively and received food. As soon as the active CR occurred in 30 consecutive trials, the inhibitory CS was introduced which was never followed by food. From then on 15 positive and 15 negative trials, separated by 15-second intervals, were presented in balanced order daily. An error was defined as failing to place the leg on the food tray when the positive CS sounded, or placing the leg on the food tray during the 5 seconds that the negative CS sounded.

Preoperatively, the animals were trained to a criterion of 45 correct responses in 50 successive inhibitory trials. After attaining this criterion all animals were subjected to bilateral, one-stage resections. Three dogs received rostral cingulate lesions which involved the cortex immediately above and in front of the genu of the corpus callosum, that is, the regio genualis or the precallosal sector of the cingulate gyrus according to Yakovlev *et al.* (*3*). It was similar to the lesion that produces angry behavior (*2*), but it was not extended as far ventrally (see Fig. 1). Three other dogs received the posterior cingulate lesion which included all cortex between the medial subdivision of the cruciate fissure (if it had been extended downwards to the corpus callosum) and the splenium. The histological analysis of the brains will be reported elsewhere. Gross verification revealed that the lesions were as accurate as attempted.

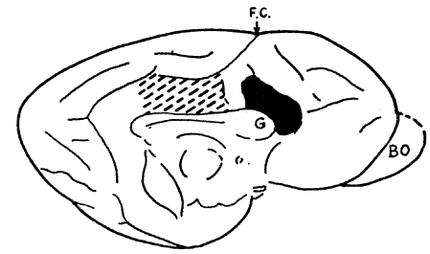

Fig. 1. Medial surface of the dog's brain. The lesion of the genual area is shown in black. The lesion of the posterior cingulate area is shown by hatching. *G*, the genu of the corpus callosum; *BO*, the olfactory bulb; *FC*, the cruciate fissure.

The postoperative results of both animal groups are presented in Table 1. After ablation, all animals with lesions in the genual area showed an impairment in the inhibitory trials, that is, they were disinhibited. To attain the same criterion as before operation they had to be retrained for 4 to 15 days. The behavior of animals with posterior cingulate lesions was not impaired postoperatively.

Two dogs with genual lesions exhibited rage occasionally. However, in contrast with dogs with genual lesions previously described (*2*) angry behavior in these dogs disappeared very soon. This was because of the intentional difference in the extent of the genual lesions in the two sets of dogs, which was calculated to avoid rage in the animals used in this experiment. It seems that the cortical area which suppresses the angry behavior in dogs is located rostral to the genu of the corpus callosum and extends ventrally towards the posteromedial portion of the subproreal region. The latter is in turn the area which, when undercut, "releases" rage in cats (*4*).

Neither septal nor subcallosal areas

Table 1. Scores of pre- and postoperative trials and errors including the criterion (45 correct CR's in 50 inhibitory trials).

Lesion and animal	Preoperative				Postoperative			
	Trials		Errors in		Trials		Errors in	
	Excitatory	Inhibitory	Excitatory trials	Inhibitory trials	Excitatory	Inhibitory	Excitatory trials	Inhibitory trials
Genual								
Dog-23	330	330	0	208	115	115	1	33
Dog-24	290	290	6	188	225	225	2	77
Dog-25	420	420	14	305	265	265	5	73
Posterior cingulate								
Dog-26	420	420	5	291	50	50	3	5
Dog-27	295	295	0	171	55	55	0	7
Dog-28	435	435	0	273	50	50	0	3

were destroyed in our dogs. Brady and Nauta (*5*), and King (*6*) have pointed out that lesions of the septal region produce dramatic changes in emotionality. However, it appears from some reconstructions of the lesions described by them that the most striking hyperemotionality occurs when the destruction extends anteroventrally, including the regio genualis and parts of the subproreal area in addition.

Within the first postoperative days all animals with genual lesions showed increase in responses motivated by food reward. They appeared to expect food with both the positive and the negative CS, and took the food reward more vigorously than before. It is interesting to note that ablations of the medial precruciate (*7*) or pregenual (*8*) regions also impair the inhibitory food CR's. McCleary (*9*) has reported that subcallosal lesions "disrupt normal performance under circumstances requiring a frightened animal to inhibit responding." However, McCleary's subcallosal lesions included the regio genualis as well.

Recent evidence of Auleytner and Brutkowski (see *1*), indicating that dogs with prefrontal lobectomies temporarily lose their ability to inhibit the classical defensive CR's trained prior to operation, suggests that the medial forebrain areas in the dog (which seem to be homologous to the orbital areas in the monkey) are concerned with suppression of different kinds of motivated and affective responses mediated by hypothalamic mechanisms. Also parts of the amygdala appear to be involved in this system (*10*).

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Toxohormone-like Factor from Microorganisms with Impaired Respiration

Abstract. A substance that significantly depresses liver catalase values when injected into mice has been isolated from biochemical mutants of yeasts and staphylococci with impaired respiration. This is considered as an important argument in support of the Warburg hypothesis on the origin of cancer cells.

Biochemical studies by Warburg (*1*) in the last 30 years led him to the conclusion that transformation of a normal cell into a malignant one may be the result of irreversible damage to the cellular respiratory mechanism. This respiration deficiency, typical of cancer cells, can be induced in microorganisms. If the Warburg hypothesis is true, microbial mutants with impaired respiration would be the "equivalents," among microorganisms, of cancer cells (see *2*), and their biochemical characters would agree with those of malignant cells.

Such a biochemical resemblance seems to be confirmed by studies on some of the most important biochemical features of respiration-deficient microorganisms (*2, 3*).

As a continuation of that line of research, we investigated whether microorganisms with impaired respiration produce certain toxic metabolites like those produced by cancer cells: substances such as the toxohormone of Nakahara and Fukuoka (*4*), a factor which depresses liver catalase.

The production of toxohormone-like substances by respiration-deficient mutants of microorganisms would indicate that these mutants are "equivalents" of cancer cells. Such production would be an important argument in support of the Warburg hypothesis on the origin of cancer cells.

Five different yeast mutants with impaired respiration and two staphylococci mutants were used in the experiments. The yeast mutants were obtained from a parent strain of *Saccharomyces cerevisiae* by treatments with Trypaflavin (*2*) (T_1 and T_4 mutants), manganese (*5*) (Mn_2 and Mn_3 mutants), and methyl violet (*6*) (MV_2 mutant). The mutants were selected by the tetrazolium overlay technique (*7*). They had the following characteristics: Q_{O_2} (N) between 0 and 50; inability to grow on lactate agar; uncolored colonies after overlay with 2,3,5-triphenyltetrazolium chloride; and no alkali production in an acetate medium (*8*).

The staphylococci mutants were obtained from a parent strain of *Staphylococcus aureus* by irradiation with ultraviolet (UV_5 and UV_6 mutants). They had a Q_{O_2} about 45 percent of that of the parent strain, and their respiration was only slightly depressed by 0.02*M* sodium cyanide.

To obtain cell mass, mutants and parent strains were grown for 72 hours in 10-liter fermenters with aeration and stirring. Yeasts were grown in beer wort at 30°C. Staphylococci were grown in peptone-meat extract saline broth at 37°C. Cells were recovered by centrifugation, dried with acetone, and powdered.

One hypothetical toxohormone-like fraction (TH) was obtained from acetone-dried powder of each strain by the Yunoki-Griffin technique (*9*) for preparation of crude toxohormone from malignant tissues; about 1.50 g were obtained from 50 g of acetone-dried powder.

Activity was assayed by injecting preparations of the toxohormone-like fractions into mice and measuring the liver catalase activity. Toxohormone-like fractions were dissolved in distilled water in a concentration of 50 mg/ml. For the assay 0.5 ml of the solution was injected into the peritoneal cavity. After a single injection the mice were deprived of food for 24 hours and then killed. Liver catalase activity was determined by the technique of Bonnichsen *et al.* (*10*) and expressed in terms of the reaction rate per minute, divided by the dry weight of the preparation in grams (*11*).

Table 1 shows the liver catalase activity for mice injected with 25 mg

Table 1. Liver catalase values in mice injected with toxohormone-like fractions (TH) from *Saccharomyces cerevisiae* and *Staphylococcus aureus* and from their mutants with impaired respiration. The liver catalase activity, Kat. f., is the reaction rate per minute, divided by the dry weight of the preparation in grams.

Treatments	No. of mice	Liver catalase activity (Kat. f.)
None; mice fasted for 24 hours	110	75.3 ± 1.7
25 mg of TH from:		
S. cerevisiae		
Parent strain	20	70.0 ± 7.2
Mutant T_1	10	43.9 ± 10.1*
Mutant T_4	10	38.6 ± 5.7*
Mutant Mn_2	10	49.4 ± 3.1*
Mutant Mn_3	10	44.4 ± 3.9*
Mutant MV_2	10	35.6 ± 9.9*
S. aureus		
Parent strain	20	69.8 ± 2.7
Mutant UV_5	10	53.1 ± 3.0*
Mutant UV_6	10	49.8 ± 3.2*

* Significantly different from untreated mice ($P < .01$).