was administered in amounts up to 50 mg, equivalent to 1000 LD_{50}/kg body weight.

Thus the observations reported earlier (*1*) are now extended to include the chimpanzee, dog, and monkey. It is evident that the action of the murine toxin from *Pasteurella pestis* on mitochondria varies with respect to the species from which the mitochondria are isolated, and that this variation is correlated with the susceptibility and resistance of the animals to the action of the toxin. Our present results also support our earlier hypothesis (*1*) that the susceptibility of animals to the action of the murine toxin of *Pasteurella pestis* is related to the ability of the toxin to inhibit the respiration of heart mitochondria.

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Chemically Induced Epileptiform Seizures in the Cat

Abstract. *Long-term epileptiform seizures were induced in the cat by a single stimulation of the basolateral components of the amygdala with cholinergic agents. These electro-physiological changes were accompanied by persisting alterations of the animal's "emotional responsiveness." It is evident that electroencephalographic seizure activity and overt behavior can be dissociated in this species.*

The role of the amygdaloid complex in "emotional" behavior has been studied theoretically (*1*) as well as experimentally (*2*) by many workers in the past 25 years. Marked changes in "emotional responsiveness" have been observed following complete or partial destruction of the amygdaloid complex, and motor as well as electrophysiological seizures have been induced by electrical stimulation of this portion of the brain. We have now studied the specific effects of stimulating the amygdaloid complex with various cholinergic substances.

Chemical stimulation was applied directly to the basolateral components of the amygdaloid complex of cats. This was accomplished by means of double-walled cannulas insulated with Teflon, which permit repeated applications of minute amounts (0.1 to 2.0 μg) of chemicals in crystalline form to a precisely defined locus in the brain, and allow electroencephalographic recordings to be made from the site of chemical stimulation. The cannulas were implanted stereotaxically into the amygdaloid complex of both hemispheres; details of the chemical stimulation procedure have been described elsewhere (*3*). In addition, recording electrodes were implanted bilaterally into the corticomedial amygdala, postero-ventral hippocampus, ventromedial and lateral hypothalamus, midbrain reticular formation, caudate nucleus, and occipital and motor cortex. A subcutaneous silver wire, or a skull screw, was used as the indifferent electrode. Following recuperation, various neurohumoral agents, as well as control substances, were applied to the basolateral region of the amygdala. The site of stimulation has been verified histologically in five animals.

Within approximately 4 minutes of applying 1 μg acetylcholine chloride (*4*) bilaterally to the basolateral portion of the amygdala, marked electrophysiological seizure activity was recorded from the site of chemical stimulation as well as from the corticomedial aspects of the amygdaloid complex. The high-amplitude, low-frequency, spike activity gradually spread to hypothalamic and hippocampal leads and finally dominated the electrical pattern of all areas of the brain from which electroencephalographic recordings were obtained.

Overt motor seizures appeared within 10 to 15 minutes of chemical stimulation, and were accompanied by an increase in the frequency of spike discharges from all leads. For a period of 10 to 15 minutes, the animals salivated profusely and showed electrophysiological as well as overt motor disturbances which appeared strikingly similar to those seen during epileptic attacks in clinical patients. After the motor seizures the animals appeared exhausted, but were hypersensitive to any form of sensory stimulation. Normal handling was impossible, and the animals attacked other cats as well as the experimenter without apparent provocation. These attack responses were well coordinated and directed, but much more vicious than those normally seen in the cat.

Several epileptiform seizures lasting 2 to 5 minutes were observed during the 24-hour period following cholinergic stimulation. The overt motor disturbances disappeared in the course of the next 24 to 48 hours but the electrical activity of the brain (particularly of the amygdala and hippocampus) remained highly abnormal for many days. Bursts of high-amplitude, low-frequency spikes were recorded as late as 10 to 15 days after a single stimulation with acetylcholine chloride.

Essentially the same effects were obtained when choline chloride carbamate (carbachol) (*5*) was applied to the amygdaloid complex, but the electrophysiological as well as behavioral changes appeared to be permanent (see Fig. 1). Very intense motor seizures appeared within minutes of the bilateral application of carbachol and persisted with variable intensity for several hours. Continuous spike discharges were recorded from all leads throughout this period. The uninterrupted motor seizures subsided within 2 to 3 hours of applying carbachol, but the electrical activity of all brain areas continued to show pronounced disturbances in the form of high-amplitude spike discharges. Brief motor seizures (lasting 2 to 4 minutes) occurred at 20- to 30-minute intervals throughout the next 24 hours. The frequency of these motor disturbances decreased gradually over the following weeks to approximately one or two per day, but complete recovery was not observed during a 5-month observation period.

Immediately after stimulation with carbachol, pronounced affective changes became apparent which continued essentially unabated throughout the observation period. Cats which were formerly tame and friendly became extremely vicious and hypersensitive. They remained completely refractory to normal handling and attacked other cats as well as people without provocation or apparent concern for their own safety. Repeated attempts to make them more docile failed to produce any noticeable effects. Following the initial

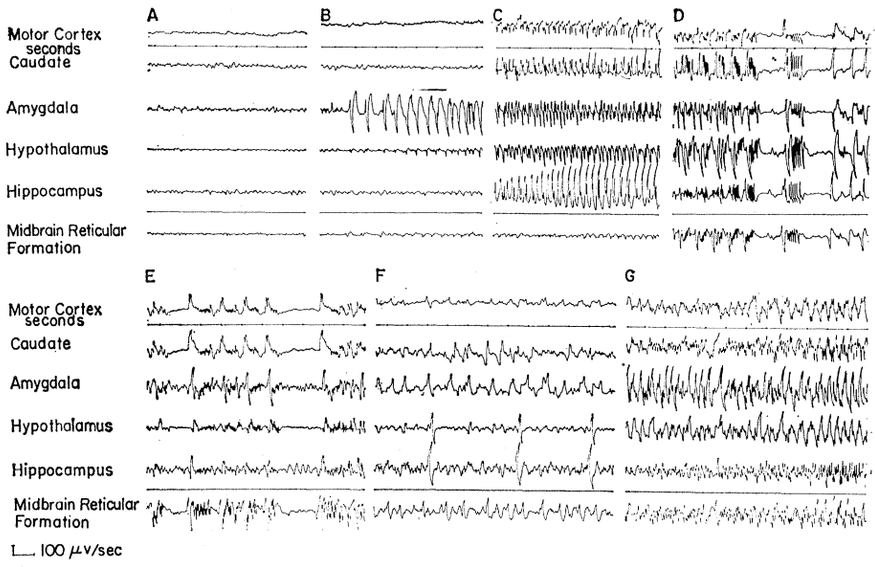

Fig. 1. Electrical activity of the brain following stimulation of the basolateral nuclei of the amygdala with carbachol. *A*, control period immediately preceding central stimulation; *B*, onset of spike discharges in the amygdaloid area, 3 minutes after bilateral administration of carbachol; *C*, records obtained during brief "quiet" period between overt motor seizures, 40 minutes after bilateral stimulation; *D*, electrical activity 4 hours after stimulation (no overt seizure activity); *E*, spike discharges alternating with brief periods of low electrical activity, 24 hours after stimulation (no overt seizure activity); *F*, electroencephalographic pattern 5 months after stimulation (animal vicious but otherwise normal); *G*, spike pattern, recorded within minutes after overt motor seizures, 5 months after amygdaloid stimulation.

period of frequent motor seizures, the animals appeared quite normal in all other respects. The cats fed normally, played with toys and explored the colony if freed from their cages. No persistent sensory or motor deficits were observed.

The electrical activity of all brain areas showed highly abnormal discharge patterns throughout the 5-month observation period. As well as occurring during and following a motor seizure, these electrophysiological disturbances also dominated the resting period at all times. The electroencephalographic records of three animals showed high-amplitude spike-discharge patterns which were often isorhythmic in all brain areas. In two cats, however, there were considerable differences between the electrophysiological abnormalities in the hippocampus and hypothalamus, and those in the amygdala, caudate, cortex and midbrain reticular formation (see Fig. 1). There appeared to be no correlation between these differences in the electrophysiological effects and the behavioral disturbances.

Previous studies have shown that the effective spread of small quantities of crystalline carbachol is less than 1.0 mm from the site of administration. This suggests that the generalized electrophysiological disturbances recorded in the present experiments represent a propagation of epileptiform discharges from the amygdaloid area to distant regions of the brain. It is possible that the disturbances occur in all areas of the brain because of volume conduction rather than neural transmission, but several observations do not support this interpretation.

1) Within minutes after the administration of carbachol, very high-amplitude spike discharges appeared in the amygdala (Fig. 1, panel *B*) but the electrical activity of other brain areas which subsequently showed pronounced disturbances appeared unaffected.

2) A notable absence of high-amplitude spiking in the midbrain reticular formation was observed during the first hour after stimulation (panel *C*) but not during later stages of the stimulation effect (panels *D* through *G*).

3) Although some animals frequently showed essentially isorhythmic spiking in all areas of the brain, two or more distinct discharge patterns were seen in the recordings of others (see panel *F*).

These observations also suggest that the spike discharges could not have originated in the vicinity of the indifferent electrode; this conclusion is supported by the fact that recordings obtained exclusively from electrodes that were deeply inserted (in the right and left hypothalamus, or adjacent points in the reticular formation) were essentially indistinguishable from those obtained by using the indifferent electrode as the reference point.

The persistence of the effects of a single carbachol administration is difficult to interpret. Comparable injections of carbachol into the hypothalamus, midbrain reticular formation, thalamus and amygdaloid regions, other than the baso-lateral area (*6*) produced behavioral changes which did not persist longer than 30 to 60 minutes. General electrophysiological disturbances were not observed. Localized spiking has been recorded following hypothalamic stimulation but this was short-lived and did not spread to other areas of the brain.

Microscopic examination of the histological material from our experiments on the effects of chemical stimulation of the hypothalamus, amygdala, thalamus and midbrain reticular formation has not revealed evidence of tissue damage greater than that produced by the cannula implant itself. Some chemical deposits have been noted in most cases and these may be responsible for the persisting seizure activity, but this does not explain the selective sensitivity of the basolateral region of the amygdala or the apparent specificity of the cholinergic agents. The administration of carbachol to other areas of the brain produces similar deposits but no persisting behavioral or electrophysiological effects, and the injection of various centrally active agents (such as serotonin, norepinephrine, or gamma-aminobutyric acid) and control substances (sodium chloride, sodium nitrite, barium chloride, posterior pituitary extract) into the basolateral amygdaloid area failed to duplicate the effects of cholinergic stimulation. Chemical specificity is further indicated by a comparison of the *p*H and osmotic effects of the various substances. Although the side-effects of carbachol and acetylcholine are difficult to assess and may not have been duplicated precisely by any of the control agents, such a wide range of *p*H and osmotic properties was covered in our control tests that it appears improbable that these factors should account for the observed results (*7*).

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4. This was preceded by application of a fraction of 1 μg of physostigmine, which did not, by itself, elicit comparable effects.
5. Choline chloride carbamate (carbachol) is a parasympathomimetic which is not hydrolized by cholinesterase and therefore produces a much more prolonged cholinergic stimulation.
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7. Supported by grant No. MY 5331 from the National Institutes of Health. The chemicals used in these experiments were kindly provided by Merck, Sharp and Dohme, Rahway, N.J. Some of the initial observations were made in collaboration with F. P. Gault, Yale University.

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Handling of Pregnant Rats: Effects on Emotionality of Their Offspring

Abstract. *Pregnant rats were either unmanipulated or were handled for 10 minutes three times daily throughout pregnancy. Offspring remained with their natural mothers or were cross-fostered within and between experimental and control groups. When tested at 45 and 100 days of age, the offspring of handled mothers were found to be generally less emotional than the controls.*

By using conditioning techniques, it has been shown that prenatal maternal "anxiety" increases offspring emotionality in the rat (*1, 2*). The effects which might obtain from other types of behavioral treatment of a pregnant animal are not known. Various manipulations, notably "handling," decrease emotionality in the rat when administered postnatally. The present study, then, was designed to determine the effects of prenatal handling, that is, handling of the pregnant animal, on emotionality of the offspring.

Data were obtained from a total of 138 offspring of primiparous Sprague-Dawley rats. These females were placed with males each evening and pregnancy was determined by vaginal smears taken the following morning. By random selection, half the pregnant animals remained unmanipulated and half were handled for 10 minutes three times daily (once each morning, afternoon, and evening) throughout the period of gestation. Pregnant animals were group-housed until approximately 1 week before delivery when they were individually placed into nesting cages. Handling consisted of picking up the animal and holding it loosely in one hand.

Litters were culled to seven or eight pups within 48 hours of birth and cross-fostering was also accomplished within this time. An equal number of litters remained with their natural mothers, were cross-fostered to mothers of that same group, or were cross-fostered to mothers of the opposing group. After this time the nesting cages in which the animals were housed were not cleaned and the pups were not manipulated in any way until weaning at 21 days. After weaning, animals were segregated by sex and treatment, and group-housed in standard laboratory cages. Food and water were available at all times.

Approximately half the animals were tested for emotionality at 45 days and 120 animals were tested at 100 days in an open-field situation. The field was 5 ft (1.5 m) in diameter and marked off into 7.5-inch (19-cm) squares and four concentric circles. Behaviors recorded were squares traversed and entries into the inner concentric circles (inversely related to emotionality), and defecation (directly related to emotionality). At 100 days all animals were also observed in an emergence-from-cage test in which the time required by animals to emerge from their open home cage (directly related to emotionality or "timidity") was recorded up to a maximum of 900 seconds.

Beginning at weaning a biweekly record of body weight was kept for 13 weeks. These data indicated no difference in the absolute weight or rate of growth between the prenatally handled and control animals.

Taken together, the emotionality data did not reveal any consistent tendency for animals fostered to handled mothers to differ from those fostered to control mothers. The data obtained from the open-field are given in Table 1. An analysis of variance applied to squares traversed revealed no differences as a function of group, sex, fostering, previous experience in the field, or any interaction of these. Inspection of the data on the percentage of animals entering inner circles did not suggest the presence of any interactions within either the 45- or 100-day tests. Chi-square analyses indicated that a somewhat larger percentage of the combined offspring of handled mothers approached the center of the field at 100 days, but not on the earlier test.

Table 1. Open-field behavior in prenatally handled (H) and control (C) offspring.

Group	Squares traversed (mean No.)	Animals entering inner circles (%)	Animals defecating (%)
	Test age 45 days		
H (N = 28)	25.6	14.3	17.9
C (N = 30)	29.5	16.7	63.3
p	> .10	> .10	< .01
	Test age 100 days		
H (N = 59)	25.2	45.8	20.3
C (N = 61)	23.2	27.9	45.9
p	> .10	< .10	< .01

Defecation in the open-field also showed no interaction effects. Within each subgroup of handled and control offspring at both 45 and 100 days, an equal or greater number of control offspring relative to handled offspring defecated. Chi-square analyses indicated that the number of prenatally handled animals defecating in the field was significantly lower than the number of control offspring on both tests ($\chi^2 = 10.54$; $\chi^2 = 7.71$).

The F test showed a significant Group × Fostering interaction on the

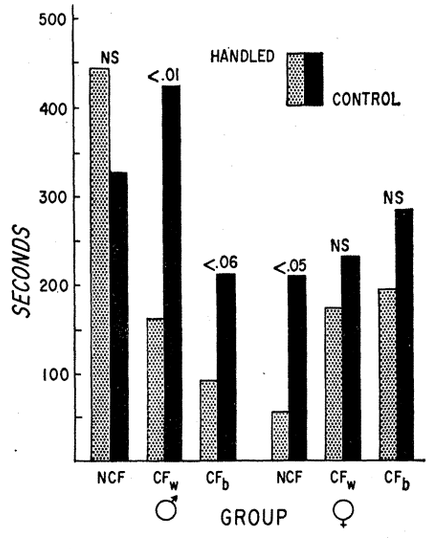

Fig. 1. Mean time required by prenatally handled and control offspring to emerge from their home cage (NCF = non-cross-fostered; CF_w = cross-fostered within group; CF_b = cross-fostered between groups).