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Carbachol-elicited mouse killing by rats: Circadian rhythm and dose response

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A dose-response analysis of cholinergically elicited aggression was performed. Bilateral doses of carbachol at 5, 20, 35, and 50 micrograms were injected into the lateral hypothalamus of nonkiller female rats. One group of rats was injected with these doses during the day, while another was stimulated at night. The most effective dose of carbachol eliciting aggression against mice was 20 micrograms bilaterally administered at night (70% of the rats killed or attacked the mice). An injection of 20 micrograms during the day, as well as injections of 5, 35, and 50 micrograms during the day or night, were less effective in eliciting aggressive responses.

Mouse killing by rats is considered an example of a predatory response (Moyer, 1968). The early observations of this behavior pattern indicated that mouse killing is a highly stereotyped and predictable response among killer rats (Karli, 1956). The attack and resultant mouse kill occur quickly with repeated bites directed at the cervical spine, and with subsequent ingestion of the mouse. The phenomenon of mouse killing is somewhat complicated by the observation that not all laboratory rats will kill mice. Among the Long-Evans strain, for example, only 20% of male and female adult rats will attack and kill mice (Bandler & Moyer, 1970; Lonowski, Levitt, & Larson, 1973a).

Many experiments have been directed at altering the frequency of mouse killing by neurological manipulation. These studies have attempted either to decrease the incidence of mouse killing when it occurs naturally, or to increase its frequency, including the elicitation of aggressive behavior where it has not been previously observed. Among the techniques which decrease or abolish the incidence of mouse killing by rats is selective brain lesioning. Specific brain loci, the destruction of which inhibit mouse killing, are the prepyriform cortex (Karli & Vergnes, 1963), the olfactory bulbs (Vergnes & Karli, 1963), and the lateral hypothalamus (Panksepp, 1971).

King and Hoebel (1968) first demonstrated the elicitation of mouse killing in nonkiller rats following electrical stimulation of the anterior lateral hypothalamus. Similar results were obtained by Panksepp and Trowell (1969) and by Panksepp (1971) who differentiated two forms of elicited mouse killing in rats. The first response, referred to as affective attack, is characterized by agitation and escape tendencies preceeding the attack (Panksepp & Trowell, 1969) and by an absence of mouse eating after kills. The second type of attack, quiet-biting attack, is marked by approach tendencies (sniffing), mouse eating, and an absence of the exaggerated arousal seen in affective attack.

Chemical stimulation of the brain has also been shown to alter the incidence of mouse killing in rats. Smith, King, and Hoebel (1970) reported that bilateral injections of crystalline carbachol (carbamyl choline chloride, a cholinergic stimulant) at approximate doses of 50 ± 25 micrograms in the lateral hypothalamus elicited mouse killing in nonkiller female rats. This discovery led Smith, King, and Hoebel (1970) to propose that neurons in the lateral hypothalamus subserve predatory behavior in the rat and that these neurons are cholinergically mediated and are subject to pharmacological manipulation. Since this discovery, however, replications of cholinergically elicited mouse killing have not been forthcoming (Bandler, 1970; Lonowski, Levitt, & Larson, 1973b; Reid, personal communication).

The present study was designed to define more accurately the dose of carbachol which elicits mouse killing in nonkiller rats. A dose-response analysis was performed using four doses of carbachol: 5, 20, 35, and 50 micrograms, bilaterally. Since Smith, King, and Hoebel tested rats in red light during the dark phase of a reversed 12-h day/night cycle, an initial dose-response study was performed in the dark phase of a 12-h day/night cycle. A second dose-response study using the same four doses of carbachol was also performed during the light phase of the cycle.

METHOD

Subjects

Sixty-two female adult Long-Evans rats were used as experimental subjects. Each animal weighed between 200 and 300 g during the experiment. All subjects were housed individually in 7 x 9.5 in. cages, with Purina lab pellets and water continually available. The subjects used in the experiment were constantly maintained on a 12-h day/night cycle (lights on at 8:00 a.m. and off at 8:00 p.m.). Approximately 250 male and female adult albino mice were used as test stimuli. The mice were housed in groups of five with males and females separated, and the entire mouse colony was isolated from the experimental rats.

Behavioral Screening

A preliminary behavioral screening for nonkiller rats was conducted as follows: Female rats were randomly selected from a homogeneous colony and were presented with mice beginning at either 8:00 a.m. in the light (day group) or at 8:00 p.m. in the dark under red light (night group). Each rat was first removed from its cage, held gently for 15 sec, then returned to its cage. The handling of each rat was performed here since later chemical stimulation required handling prior to behavioral observation.

Upon returning each animal to its home cage, and after a 5-min interval (used here to approximate a drug-onset period), an albino mouse was placed in the rat's cage. Twelve-hour observations were then made noting the incidence of attacks and kills of mice. Only nonaggressive rats were included in subsequent testing. Behavioral screening of this kind was conducted on 3 consecutive days. After the 3 days of screening, 34 nonkiller rats were obtained for the night group and 28 rats for the day group.

Surgery

Each nonkiller female rat was stereotaxically implanted with two 23-ga. cannulae used for injecting chemicals into localized areas of the brain. Surgical procedures were performed under sodium pentobarbital anesthesia. The tips of the cannulae guide shafts were aimed at (but 2 mm above) the anterior lateral hypothalamus (.4 mm posterior to bregma, 1.5 mm lateral to the midsaggital sinus). Following surgery, each animal was injected with .10 cc streptomycin.

Postsurgical Behavioral Testing

Following surgery by 7 days, each implanted rat was again tested for aggressive behavior with mice in the manner described in the behavioral screening section. These tests were also conducted for 3 consecutive days either at night or during the day for the respective day or night groups. At the end of the third postsurgical test, the body weight of each rat was obtained, and a record of its 12-h water intake during the test was made. Each of the 62 implanted rats was found, following surgery, still not to show aggressive behavior toward the mice.

Chemical Stimulation

On the brain stimulation test day, each rat from the day and night groups was randomly assigned to receive 5-, 20-, 35-, or 50-microgram injections of carbachol. Of the rats in the night group, 8, 10, 8, and 8 rats were selected to receive the various carbachol doses, while in the day group, seven rats were assigned to receive each of the respective carbachol doses.

Each of the doses of carbachol was prepared on the day of stimulation and was dissolved in sterile isotonic saline solution. Stimulation tests were begun at 8:00 a.m. for the day group under normal room lighting and at 8:00 p.m. for the night group in the dark under a red light.

The general testing procedure for chemical stimulation was as follows: Each rat was removed from its home cage and held gently by the experimenter while two 30-ga. hollow needles with attached Hamilton microliter syringes were successively inserted into each implanted cannula. The stimulating needles were lowered to a premeasured depth in the lateral hypothalamus at which point the various doses of carbachol were delivered in a 1-microliter saline vehicle. Upon completion of bilateral hypothalamic injections, each rat was returned to its cage with food and water available ad lib. Following a 5-min interval to allow for drug onset, an albino mouse was placed in the rat's cage.

Observations were continued for 12 h with the noting of the incidence of mouse attacks and kills. Water intake was also monitored during this time. In addition, a record of certain drug-related behaviors was kept, including the latency to behavioral seizures and their duration and the incidence of other autonomic responses previously reported by Smith, King, and Hoebel (1970).

Poststimulation Control Tests

Three and 7 days subsequent to carbachol stimulation, each rat was again tested for aggressive behavior with a mouse. The general procedure was identical to that outlined in the behavioral screening section. Again, however, measures of body weight and 12-h water intake were made.

Histology

Upon completion of the experiment, each of the 62 rats was sacrificed with an overdose of sodium pentobarbital. Each animal was then perfused with normal saline and a 10% formalin solution. Frozen brain sections were taken, stained with cresyl violet, and studied microscopically to determine the stimulation sites.

RESULTS

Night Group

Following carbachol stimulation of the lateral hypothalamus at dose levels of 5, 20, 35, and 50 micrograms bilaterally, rats underwent profound motor seizures. Seizure activity was explosive in nature and was marked by sudden and violent movements. Accompanying the seizures were profuse salivation, piloerection, urination, squealing, and subsequent immobility. The severity of the symptoms and the latency to seizure were not correlated with increasing dose levels of carbachol. The latency to seizure ranged from 15 to 420 sec after stimulation.

The eight rats injected with 50 micrograms of carbachol bilaterally showed spontaneous stimulation-induced seizures (which lasted for several days), aphagia and adipsia, and an average of 21% weight loss 3 days after stimulation; four of these rats died on this 3rd day. The rats injected with 5, 20, and 35 micrograms of carbachol did not show the persistent seizure activity and high mortality rate as evidenced in the 50-microgram group, nor did they show losses in body weight. However, water intake was equally depressed in all groups from an average of 1.9 ml/h on the 3rd postsurgical test day to .7 and .8 ml/h on the 3rd and 7th days following brain stimulation ($F = 347.57$, $p < .001$).

With the exception of the 50-microgram-dose group, carbachol-induced seizures subsided within approximately 1 to 5 h after stimulation. When the seizures stopped, it was then that some of the rats were observed to attack and kill mice.

A t test comparison of the proportion of elicited aggressive responses for rats injected with the four doses of carbachol was performed by combining the incidences of mouse attacks and kills associated with each respective dose. However, only those attacks which resulted in multiple wounds on mice were considered appropriate for this analysis.

Table 1 shows the percentage of combined mouse attacks and kills during the 12-h poststimulation test period. Bilateral injections of 20 micrograms of carbachol elicited mouse killing in five rats, and repeated attacks in 2 rats out of 10 stimulated animals (.70). This figure was significantly higher than that obtained at 5 micrograms (two kills out of eight stimulated animals—.25), at 35 micrograms (one kill out of eight stimulated animals—.125), and at

50 micrograms (no attacks or kills))$t = 2.14$, $p < .05$; $t = 2.75$, $p < .05$; $t = 3.33$, $p < .05$). The average latencies to attack or kill mice (including only those animals that showed these behaviors) for the respective dose groups were: 5 micrograms—56.5 min; 20 micrograms–6.3 h; and 35 micrograms–53 min. Mouse eating did not follow killing for any dose of carbachol.

On poststimulation control days, the rats were again tested with mice. Such testing yielded an absence of attacks or kills against mice.

Day Group

The effects of bilateral injections of carbachol during the day at 5, 20, 35, and 50 micrograms of carbachol were quite different from those results obtained at night. However, all 28 stimulated rats exhibited motor seizures and accompanying symptoms as described for the night group; yet it is the distinct impression of the experimenters that the seizures for the day group were less severe than for the night group. An example of this finding is that the day group receiving the 50-microgram dose stopped seizuring within 3 h, and none of these animals exhibited aphagia or adipsia. The severity of seizure activity and latency to seizures were also not related to increasing doses of carbachol for the day group. The range of seizure onset was from 30 to 600 sec following stimulation. Seizures subsided in all animals within 1 to 4 h after stimulation.

There was a significant depression of water intake in all rats from an average of 2.2 ml/h on the third postsurgical test to .7 and .6 ml/h on the two poststimulation control tests ($F = 347.57$, $p < .001$). However, there was no evidence of loss of body weight among the day group subjects. Three days after stimulation, three of the rats died (one each from the 20-, 35-, and 50-microgram groups), but there was no evidence of the aphagia and adipsia which characterized the four deaths in the 50-microgram group at night.

Subsequent to seizure activity offset, a smaller amount of aggressive behavior was observed among the stimulated rats of the day group than had occurred in the night group. The percentage of elicited aggression observed during the post-stimulation test is shown in Table 1.

Following bilateral hypothalamic injections of 5 micrograms of carbachol during the day, two kills were observed out of seven animals (.286); at 20 micrograms, one kill in seven rats (.143) was seen, and no aggressive behavior was produced by injections of 35 micrograms or 50 micrograms of carbachol. A t test for proportions showed that the incidences of aggression for 5 and 20 micrograms were not significantly different from zero, nor were they different from each other; however, the average latencies for kills in those rats that did kill were as follows: 5 micrograms—2 h and 20 micrograms—10 h. In contrast to the findings for the night group, eating of mice followed each observed kill.

Table 1
Percentage of Rats Displaying Aggressive Responses Toward Mice Following Lateral Hypothalamic Injections of Carbachol

Time of Stimulation	Dose of Carbachol (Bilateral in Micrograms) 5	20	35	50
Day Group	29	14	0	0
Night Group	25	70	12	0

Three and 7 days following carbachol stimulation, the rats were again tested with mice. No aggressive behavior was observed.

Histology

A histological examination of the stained brain sections showed that stimulation sites were located between the optic tract and the mammilothalamic tract in the lateral hypothalamus. In addition, lateral hypothalamic lesions approximately .25 mm in diam were confirmed in sections obtained from rats injected with 50 micrograms of carbachol at night. Similar lesions were not observed among the remaining dose groups (including the 50 microgram day group).

DISCUSSION

The results of the present study may be regarded as a replication of the cholinergically elicited mouse killing phenomenon reported by Smith, King, and Hoebel (1970). Bilateral hypothalamic injections of 20 micrograms of carbachol, administered at night, was the most effective dose eliciting attacks and kills against mice in nonkiller female rats. A similar injection of 20 micrograms of carbachol during the day was without effect, as were doses of 5, 35, and 50 micrograms, administered both during the day and at night. Since an absence of behavior was confirmed on pretest and posttest control days, it would appear that 20 micrograms of carbachol exerts a specific pharmacological action and does not permanently convert natural nonkiller rats to killers. We might mention that we have also succeeded in a preliminary study, in eliciting mouse killing at night, in male Long-Evans rats. Four of eight males killed within 6 h of bilateral injections of 20 micrograms of carbachol.

Since a higher incidence of cholinergically elicited aggression was obtained during the night phase of a 12 h light/dark cycle, there is reason to suspect a diurnal rhythm associated with the phenomenon. A similar circadian rhythm has been reported for alpha-adrenergic stimulation of the hypothalamus which elicited feeding in rats (Margules, Lewis, Dragovich, & Margules, 1972).

Smith, King, and Hoebel (1970) interpreted their data as evidence for a cholinoceptive component in the lateral hypothalamus which controls predatory aggression (mouse killing) in the rat. For the following reasons, results of the present study seriously question such an interpretation: Several behaviors suggestive of aversive effects were observed in animals following injections of 20 micrograms of carbachol at night. Each of the response patterns is not reflective of natural mouse killing behavior. Shortly after carbachol brain stimulation at this dose, rats exhibited prolonged motor seizures. During the extent of these seizures, rats showed piloerection, profuse salivation, uncontrolled urination, and vocalization. In addition, following mouse kills, mice were not eaten (by the night group). An experiment in this laboratory has indicated that 90% of natural killer Long-Evans rats eat mice following kills.

Panksepp (1971) described two forms of mouse killing elicited by electrical brain stimulation of the lateral hypothalamus: quiet-biting attack and affective attack. While quiet-biting attack shares many of the response components seen in natural mouse killing, affective attack resembles pain-induced or defensive aggression. The response components seen in both affective attack and cholinergically elicited attack are autonomic arousal, vocalization, and an absence of mouse eating. The similarities between cholinergic and affective attacks do not support the interpretation of a cholinergic substrate for predatory aggression in the rat.

REFERENCES

BANDLER, R. J., JR. Cholinergic synapses in the lateral hypothalamus for the control of predatory aggression in the rat. *Brain Research*, 1970, **20**, 409-424.

BANDLER, R. J., JR., & MOYER, K. E. Animals spontaneously killed by rats. *Communications in Behavioral Biology*, 1970, **5**, 177-182.

KARLI, P. The Norway rat's killing response to the white mouse. *Behavior*, 1956, **10**, 81-103.

KARLI, P., & VERGNES, M. Role du rhinencephale dans le controle du comportement interspecifique rat-souris. *Journal of Physiology*, 1963, **55**, 272-273.

KING, M. B., & HOEBEL, B. G. Killing elicited by brain stimulation in rats. *Communications in Behavioral Biology*, 1968, **2**, 173-177.

LONOWSKI, D. J., LEVITT, R. A., & LARSON, S. D. Mouse killing or carrying by male and female Long-Evans hooded rats. *Bulletin of the Psychonomic Society*, 1973, **1**, 349-351. a

LONOWSKI, D. J., LEVITT, R. A., & LARSON, S. D. Effects of cholinergic brain injections on mouse killing or carrying by rats. *Physiological Psychology*, 1973, **1**, 341-345.. b

MARGULES, D. L., LEWIS, M. J., DRAGOVICH, J. A., & MARGULES, A. S. Hypothalamic norepinephrine: Circadian rhythms and the control of feeding behavior. *Science*, 1972, **178**, 640-642.

MOYER, K. E. Kinds of aggression and their physiological basis. *Communications in Behavioral Biology*, 1968, **2**, 66-87.

PANKSEPP, J. Aggression elicited by electrical stimulation of the hypothalamus in albino rats. *Physiology and Behavior*, 1971, **6**, 321-329.

PANKSEPP, J., & TROWELL, J. Electrically induced affective attack from the hypothalamus of the albino rat. *Psychonomic Science*, 1969, **16**, 118-119.

SMITH, D. E., KING, M. B., & HOEBEL, B. G. Lateral hypothalamic control of killing: Evidence for a cholinoceptive mechanism. *Science*, 1970, **167**, 900-901.

VERGNES, M., & KARLI, P. Declenchement du comportment d'aggression interspecifique rat-souris part ablation bilaterale des bulbes olfactif. Action de l'hydroxyzine sur aggressivete provoquee. *Compt. Rend. Soc. Biol.*, 1963, **157**, 1061.

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