Carbachol-elicited mouse-killing in the rat: Animals attacked and wound location

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Mouse-killing was elicited in naturally nonkiller rats by bilateral injections of carbachol into the lateral hypothalamus. These carbachol-converted killers also attacked rat pups and adult rats during the period of drug action. The wounds were distributed more widely over the bodies of mice attacked by carbachol-converted killers than by natural killers. These data suggest that carbachol-elicited killing is similar to affective or irritable aggression.

Mouse-killing by rats has been a frequently employed model for aggressive behavior. Natural mouse-killing is a type of predatory aggression characterized by stereotypy and stimulus specificity. Bites are repeatedly directed at the cervical spine and the dead mouse is at least partially eaten. Natural mouse-killers typically do not attack rat pups (Karli, 1956; Moyer, 1968; Myer & White, 1965). However, not all rats are mouse-killers. The percentage of natural killers differs as a function of strain and gender (Lonowski, Levitt, & Larson, 1975).

Mouse-killing can be elicited in natural nonkiller rats by electrical stimulation of the lateral hypothalamus. Two forms of attack have been produced by such stimulation. During affective attack, rats killed mice and attacked other rats; although they did not bite or eat the dead mice. The electrical stimulation was shown to be aversive since the rats escaped stimulation that produced affective attack. During quiet-biting attack, rats killed mice and ate the carcasses, but did not attack other rats. The electrical stimulation producing quiet-biting attack was shown to be rewarding, since the rats self-stimulated at the same stimulus parameters producing attack. The lateral hypothalamus sites producing these two forms of aggression are anatomically differentiable (King & Hoebel, 1968; Panksepp, 1971).

Mouse-killing can also be elicited in natural nonkiller rats by chemical injection of cholinergic drugs into the lateral hypothalamus. Both the cholinomimetic, carbachol, and the cholinesterase inhibitor, neostigmine, in bilateral doses of 50 μ g have been found to convert nonkillers into killers (Smith, King, & Hoebel, 1970). Low doses of carbachol have been found not to produce mouse-killing in natural nonkillers. However, in one study, about 7.0 μ g of carbachol was found to facilitate the killing of natural killer rats (Bandler, 1970), while in a second study, a still lower dose, 2.0 μ g of carbachol, was found to inhibit the killing behavior of natural killers (Lonowski, Levitt, & Larson, 1973). A third study

employing a 20 μ g bilateral dose has replicated the conversion of nonkillers to killers by carbachol injections into the lateral hypothalamus (Lonowski, Levitt, & Dickinson, 1975).

The present experiment further investigated the aggressive behavior of rats converted to mouse killing by lateral hypothalamic injections of carbachol. The specificity of animals attacked was studied by presenting the experimental animals with rat pups and adult rats, as well as mice. The stereotypy of the attacking behavior was evaluated by studying the distribution of the wounds on the bodies of the mice.

METHOD

Subjects

Naive adult female rats of the Long-Evans strain from a Blue Spruce-derived Southern Illinois University strain and weighing 225-300 g at the time of surgery were used as subjects in the experiment. Three groups of animals were used as aggression-eliciting stimuli: adult albino mice of both sexes; Long-Evans-strain rat pups of both sexes, matched to the mice for size; and adult female rats, also of the Long-Evans strain. Adult rats were housed individually with free access to food and water. The mice and rat pups were housed in groups of six with free access to food and water. Rat pups, matched to the size of the mice, varied from about 21 to 25 days of age.

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 8:00 p.m. in the dark under red light. Each rat was first removed from its cage, held gently for 15 sec, then returned to its cage. The handling was performed here because later chemical stimulation required handling prior to behavioral observation.

Upon returning each animal to its home cage, a mouse was placed in the rat's cage. A 12-h observation was then made, noting the incidence of attacks and kills of mice. Only rats that did not kill mice during this 12-h period were included in subsequent testing.

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 to end just above the anterior lateral hypothalamus (AP = 6.5 mm anterior to the interaural line, L = 1.5 mm lateral to the midsagittal line, D = 6.0 mm below the dural surface). The stereotaxic apparatus was adjusted so that the tooth bar and ear bars were at the same level.

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.

Chemical Stimulation

On the night following the postsurgical behavioral test, experimental animals were injected with carbachol beginning at about 8:00 p.m. The animal was removed from its home cage and hand-held by the experimenter while 20 μ g of carbachol was injected into each lateral hypothalamus. The carbachol was dissolved in 1.0 μ l of isotonic sodium chloride solution and injected into the brain via 30-ga needles attached to Hamilton microsyringes. The needle lengths were 2.0 mm longer than the length of the implanted cannulae. The injection procedure took from 1 to 2 min.

After returning the experimental subject to its home cage, one of the three aggression-eliciting stimuli (a mouse, a rat pup, or an adult rat) was placed in the cage for a 30-min period. The three stimulus animals were then alternated every 30 min throughout the night (for a total of 12 h) in a counterbalanced order. Incidences of attacks and kills were recorded, as were the locations of wounds on the mice.

Poststimulation Tests

On the third and sixth nights following chemical injection, experimental subjects were retested for mouse-killing, as during the initial behavioral screening described above.

Control Groups

A group of 24 nonkiller adult female Long-Evans rats served as a control group. They received behavioral screening and surgery, just as did the experimental group. They were then injected with 1.0 μ l of isotonic sodium chloride solution in each lateral hypothalamus on the chemical stimulation night. Groups of 15 natural mouse-killer and 15 nonkiller female Long-Evans rats (non-implanted) also served as controls. They were tested in a manner similar to the experimental subjects. After 2 nights of behavioral screening with mice, they were presented with the three aggression-eliciting stimuli in a counterbalanced order on a third night.

Histology

Upon completion of the experiment, each of the implanted rats was sacrificed with an overdose of sodium pentobarbital. Each animal was then perfused with normal saline and a 10% Formalin solution. Forty-micra frozen brain sections were stained with cresyl violet, and studied microscopically to determine the stimulation sites.

RESULTS

Animals Attacked

A total of 95 nonkiller rats were injected with carbachol. Eighteen of these animals displayed aggressive behavior toward the stimulus animals. Fifteen of these 18 experimental animals displayed aggression towards all three stimulus animals. Of the 15 natural mouse-killer rats, 13 displayed aggressive behavior only towards mice on the test night (Table 1). A test for the significance of the

Table 1
A Comparison of the Aggressive Behavior of Carbachol-Converted and Natural Killer Rats

Stimuli Attacked	Carbachol-Converted Killers	Natural Killers
None	77	0
Mice	1	13
Rat Pups	0	1
Adult Rats	0	0
Mice and Rat Pups	1	1
Mice and Adult Pups	1	0
Rat Pups and Adult Rats	0	0
Mice, Rat Pups, and Adult Rats	15	0

difference between two proportions was used to compare the percentage of these animals displaying aggression in each group which attacked only mice, and was found significant (Bruning & Kintz, 1963; $z = 4.77$, $p < .001$).

Fourteen of the 15 natural mouse-killer rats attacked mice. They killed the first mouse presented, and continued to kill mice throughout the night. The one rat that killed rat pups killed only the first two presented. For the 18 carbachol-injected killers, the latency until their first aggressive behavior (towards mice, rat pups, or adult rats) ranged between 10 min and 8 h, with an average of 3 h.

None of the 24 nonkiller rats injected with sodium chloride solution in the lateral hypothalamus displayed aggressive behavior toward the stimulus animals during the test night. The control group of 15 unstimulated nonkiller rats also showed no aggressive behavior on the test night.

On the two poststimulation tests, 3 and 6 nights after the test night, the 18 experimental carbachol-injected animals which showed aggressive behavior following drug injection failed to show aggressive behavior toward mice. The natural mouse-killer rats continued to display mouse-killing. However, the control noninjected nonkiller rats and the control isotonic sodium chloride injected rats (also non-killers) continued not to show mouse-killing behavior.

Wound Location

For the purpose of comparing wound locations on mice attacked by carbachol-stimulated rats to those on mice attacked by natural mouse-killers, the body of the mouse was arbitrarily divided into the nine regions shown in Figure 1. A wound-location analysis was made for only 11 of the 18 converted killers (on 41 mice) and for all 15 natural killers (on 95 mice). Mice attacked by natural killer rats had fewer wounds on their bodies (1.7 vs. 4.5 for mice attacked by carbachol-converted killers; $t(134) = 9.05$, $p < .01$).

Mice attacked by natural killers also showed a greater proportion of wounds on the cervical region

Figure 1. Numbering system for analyzing the distribution of wounds on the bodies of the mice.

(region 1) than did mice attacked by carbachol-injected killers (Table 2; $z = 7.4$, $p < .001$).

Histology

Stimulation sites in the 18 carbachol-converted killers were distributed throughout the lateral hypothalamus area between the optic tract and the mammillothalamic tract. Coordinates ranged from

6.0 to 6.8 mm anterior to the interaural line, 1.3 to 1.8 mm lateral to the midsagittal line, and 7.5 to 8.9 mm below the dura (Pellegrino & Cushman, 1967). Coordinates for the 24 animals injected with sodium chloride that are included in this study were also within these boundaries. A histological analysis was made of the injection sites only in 15 of the 77 carbachol-injected animals which did not convert to aggressive behavior. In only 6 of these 15 animals were both injection sites within the same boundaries, as for the 18 carbachol-converted animals.

DISCUSSION

Eighteen of 95 rats with implants aimed at the lateral hypothalamus displayed mouse-killing behavior following the bilateral injections of $20 \mu\text{g}$ of carbachol. Fifteen of these animals also killed rat pups and attacked adult rats. Latencies to the first appearance of aggressive behavior averaged 3 h. Both stimulation sites in the 18 carbachol-converted animals were distributed within a 1.0-2.0-mm region of the lateral hypothalamus (within 1.0 mm of AP 6.4, L 1.6, D 8.2), while only 6 of 15 non-converted animals had both sites within this same area, suggesting some anatomical specificity. Twenty-four nonkiller animals injected with $1.0 \mu\text{l}$ of isotonic sodium chloride bilaterally into the same area did not convert to killers, suggesting some chemical specificity.

Most carbachol-stimulated killers also killed rat pups and attacked adult rats, while natural killer rats limited their aggressive behavior to mice. Mice killed by natural killer rats also had their wounds more localized at the cervical region than mice killed by carbachol-converted killers. These findings suggest that carbachol-elicited killing is a type of affective or irritable aggressive response, rather than a predatory or quiet-biting response (Moyer, 1968; Panksepp, 1971).

Mouse-killing has also been elicited by systemic injections of the cholinomimetic, pilocarpine. A small percentage (10%-20%) of rats killed after the first pilocarpine injection. With daily injections, more and more animals began to kill until all the nonkillers had converted to killing. Converted killers were also found to kill rat pups. Scopolamine blocked the ability of pilocarpine to elicit killing (Gay & Leaf, 1976; Vogel & Leaf, 1972; Wnek & Leaf, 1973). It may be that the killing behavior elicited by systemic pilocarpine is based on the same mechanism as the central carbachol-elicited killing first discovered by Smith et al. (1970). It may also be the case that daily carbachol injections into the lateral hypothalamus would cause a progressively higher proportion of natural nonkiller rats to convert to mouse-killing.

Table 2
A Comparison of the Wound Location on Mice Attacked by Carbachol-Converted or Natural Mouse Killers

Area on Mouse	Carbachol-Converted Killers		Natural Killers	
	Number of Bites	Percent of Total Bites	Number of Bites	Percent of Total Bites
1	44	24	101	63
2	39	21	11	7
3	8	4	4	2
4	23	12	6	4
5	43	23	17	11
6	11	6	2	1
7	4	2	3	2
8	6	3	4	2
9	9	5	13	8

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.
- BRUNING, J. L., & KINTZ, B. L. *Computational handbook of statistics*. Glenview, Ill: Scott, Foresman, 1963.
- GAY, P. E., & LEAF, R. C. Rat strain differences in pilocarpine-induced mouse killing. *Physiological Psychology*, 1976, **4**, 28-32.
- KARLI, P. The Norway rat's killing response to the white mouse. *Behavior*, 1956, **10**, 81-103.
- 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., & DICKINSON, W. A. Carbachol-elicited mouse killing by rats: Circadian rhythm and dose-response. *Bulletin of the Psychonomic Society*, 1975, **6**, 601-604.
- 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.
- LONOWSKI, D. J., LEVITT, R. A., & LARSON, S. D. Mouse killing and carrying by Maudsley and Long-Evans strain rats. *Bulletin of the Psychonomic Society*, 1975, **6**, 629-631.
- MOYER, K. E. Kinds of aggression and their physiological basis. *Communications in Behavioral Biology*, 1968, **2**, 65-87.
- MYER, J. S., & WHITE, R. T. Aggressive motivation in the rat. *Animal Behaviour*, 1965, **13**, 430-433.
- PANKSEPP, J. Aggression elicited by electrical stimulation of the hypothalamus in the albino rat. *Physiology and Behavior*, 1971, **6**, 321-329.
- PELLEGRINO, L. J., & CUSHMAN, A. J. *A stereotaxic atlas of rat brain*. New York: Appleton-Century-Crofts, 1967.
- SMITH, D. E., KING, M. B., & HOEBEL, B. G. Lateral hypothalamic control of killing: Evidence for a cholinceptive mechanism. *Science*, 1970, **167**, 900-901.
- VOGEL, J. R., & LEAF, R. C. Initiation of mouse killing in non-killer rats by repeated pilocarpine treatment. *Physiology and Behavior*, 1972, **8**, 421-424.
- WNEK, D. J., & LEAF, R. C. Effects of cholinergic drugs on prey-killing by rodents. *Physiology and Behavior*, 1973, **10**, 1107-1113.

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