Lateral Hypothalamic Control of Killing: Evidence for a Cholinoceptive Mechanism

Abstract. *In rats that would not ordinarily kill mice, lateral hypothalamic injection of crystalline carbachol, a cholinomimetic, elicited killing. Norepinephrine, amphetamine, serotonin, and sodium salts were ineffective at the same site. Carbachol was ineffective when injected into the medial, dorsal, or ventral hypothalamus. As additional evidence for a cholinoceptive mechanism, neostigmine elicited killing, and, in spontaneous killers, methyl atropine blocked it. The results indicate that the lateral hypothalamus contains a cholinoceptive component of an innate system that activates killing, and anticholinergic treatment can be used as a means of suppressing killing.*

Electrical stimulation of the lateral hypothalamus will elicit mouse-killing in rats that do not ordinarily kill mice (*1*). Attack and killing elicited electrically from this region of the brain has also been seen in cats (*2*) and opossums (*3*). These experiments suggest that the lateral hypothalamus is normally involved in the control of this behavior. We now report evidence of a lateral hypothalamic neurohumor that mediates killing. By pharmacologically blocking this neurohumor, it is possible to suppress spontaneous killing.

Prior to implanting hypothalamic cannulas for this experiment, it was necessary to distinguish spontaneous killers from nonkillers. An albino mouse was put into the home cage (17 by 17 by 25 cm) of an adult female albino or hooded rat and left for 4 to 24 hours. "Killers" were those that killed a mouse in less than 2 minutes on any 3 days in a row. "Nonkillers" were rats that never killed on any of 17 different days, including 3 days after recovery from implantation of bilateral, 22-gauge cannulas (*4*).

All drugs were administered bilaterally in crystalline form (*5*). Each drug was matched with a sodium salt as a control for the effects of the anion alone. A drug and its control were counterbalanced to reveal possible residual effects of prior treatments. At least 3 days elapsed between successive treatments, which were conducted in red light during the dark phase of the day-night cycle with food and water always available.

In the first experiment either the cholinomimetic, carbamylcholine chloride (carbachol), or sodium chloride was injected into the lateral hypothalamus of nonkillers at the beginning of a 6-hour test when a mouse was put in the rat's home cage. If a rat killed during any 30-minute interval, a new mouse was introduced at the end of the interval. In contrast to sodium chloride, which was never effective, carbachol elicited killing in 12 rats (Fig. 1). The mean latency until the first kill was 45 minutes with a range of 1 minute to 4.25 hours. Prior to killing, the animals were typically active. Piloerection and, occasionally, salivation were also seen. Killing behavior persisted on the average for 2.75 hours. Food intake, but not water intake, was significantly greater with carbachol than with sodium chloride at the end of this 6-hour test ($P < .05$).

Carbachol-induced killing had the appearance of natural killing. The kill was made with a bite through the cervical spinal cord and was not preceded by a series of incomplete or ineffective attacks. Thus, stimulation of the lateral hypothalamus with carbachol was sufficient to trigger the complete behavioral pattern involved in killing, even though these animals had never previously performed or witnessed this response. This implies that a cholinoceptive mechanism for killing is present in functional order, but normally quiescent, in the pacifistic rats.

Carbachol-induced killing showed both neuroanatomical and chemical specificity (Fig. 1). In the first experiment all of the animals with lateral hypothalamic cannulas killed under the influence of carbachol; however, in seven other rats carbachol did not produce killing when injected only 1 mm away in the ventromedial, ventrolateral, or dorsolateral hypothalamus. This makes it unlikely that carbachol-induced killing resulted from diffusion medially toward the ventricle or dorsally into the thalamus. Because carbachol might have produced killing indirectly by the release of adrenergic or serotonergic neurohumors (*6*), we tested L-norepinephrine bitartrate, D-amphetamine sulfate, serotonin creatinine sulfate and DL-5-hydroxytryptophan in varied order on five new rats bearing lateral hypothalamic cannulas. None of the animals killed under the influence of these substances although four of the five subsequently displayed carbachol-induced killing.

To obtain additional evidence that carbachol-induced killing is a true cholinergic effect, we tested a reversible anticholinesterase, neostigmine. Killing was elicited by neostigmine methyl sul-

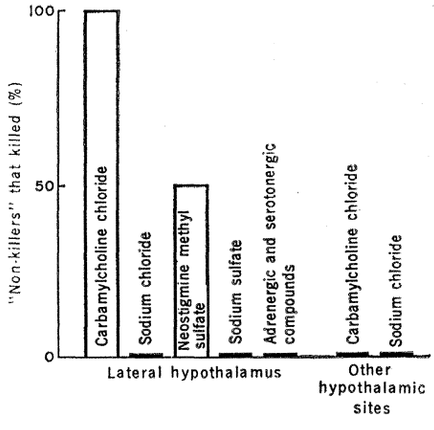

Fig. 1. Killing elicited after lateral hypothalamic injection of carbachol or neostigmine. Each bar represents data from at least five rats.

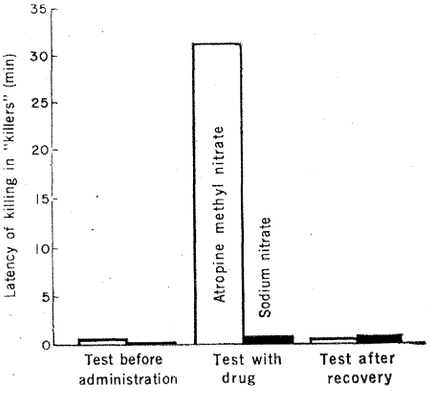

Fig. 2. Killing suppressed in five "killers" following lateral hypothalamic injection of methyl atropine.

Lateral hypothalamic treatment	Result
Cholinomimetic (Carbachol)	Killing in "non-killers"
Cholinesterase inhibitor (Neostigmine)	Killing in "non-killers"
Cholinergic blocker (Methyl atropine)	No killing in "killers"

Fig. 3. Summary of results.

fate, but not by sodium sulfate in five of the ten rats that had displayed carbachol-induced killing in the first experiment (Fig. 1). The average time for the first kill was 2.75 hours with a range of 7 minutes to 5.75 hours. The animals given neostigmine killed at every opportunity between the first kill and the last, a mean of 1.5 hours. Unlike carbachol, neostigmine had no consistent effect on either food or water intake.

The finding that carbachol and neostigmine caused nonkillers to kill mice suggested that it should be possible to suppress killing with a cholinergic blocking agent in animals that kill mice spontaneously. We chose atropine methyl nitrate because the methylated form is less likely to pass from the brain into the blood stream and cerebrospinal fluid (*7*). Identified killers were used. Five minutes after a mouse was killed and removed in a test before drug administration, atropine methyl nitrate or sodium nitrate was injected into the lateral hypothalamus of the rat. After an additional delay of 15 minutes to allow a neurochemical block to form, a second mouse was introduced, and the latency to kill was recorded as a measure of the drug's ability to suppress killing. Fifteen minutes after the second kill, latency was measured a third time when the animal had recovered from the drug's effects. Rats tested with sodium nitrate showed no suppression; they killed within 2 minutes in every test. In five of the same six rats, methyl atropine blocked killing for 12 to 60 minutes. This contrasts with kills in less than 2 minutes in every case during tests before drug administration and during tests after recovery (Fig. 2). There were no partial attacks; thus the block appeared to be complete while it lasted. The rats approached, sniffed, and sometimes followed the mouse but did not kill.

Cannula sites were identified by microscopic examination of frontal sections after cresyl violet staining. Lateral hypothalamic placements were in the medial forebrain bundle in the anterior-posterior plane of the ventromedial nucleus.

In summary, drugs believed to mimic or potentiate acetylcholine elicited killing, and a competitive inhibitor of acetylcholine blocked it (Fig. 3) (*8*). Mouse-killing in rats is motivated (*9*), and, like other motivated behavior, it involves a variety of interacting neural processes such as sensory, motor, reward, and arousal mechanisms. Although the exact nature of the mechanism suppressed with methyl atropine and activated with carbachol and neostigmine is not known, we infer that in the lateral hypothalamus a cholinergic substance, probably acetylcholine, is a neurohumor in part of an innate system for killing. A similar system may exist in other species (*10*). This raises the practical possibility that pharmacological manipulation of such a system could be used in the treatment of pathological aggressive behavior.

DOUGLAS E. SMITH
MELVYN B. KING
BARTLEY G. HOEBEL
Department of Psychology,
Princeton University,
Princeton, New Jersey 08540

References and Notes

1. M. B. King and B. G. Hoebel, *Commun. Behav. Biol., Part A* **2**, 173 (1968).
2. M. Wasman and J. P. Flynn, *Arch. Neurol.* **6**, 220 (1962).
3. W. W. Roberts, M. L. Steinberg, L. W. Means, *J. Comp. Physiol. Psychol.* **64**, 1 (1967).
4. B. G. Hoebel, *Electroencephalogr. Clin. Neurophysiol.* **16**, 399 (1964). Lateral hypothalamic cannulas were implanted 6.3 to 6.5 mm anterior to the interaural line, 1.5 mm lateral to the midsagittal sinus, and 7.3 to 7.7 mm below the dura, perpendicular to the surface of the skull (A6.5, L1.5, D7.3 through 7.7), and the equivalent of A5.8, L2.0, D7.8 in rats held with skulls tilted.
5. S. P. Grossman, *Amer. J. Physiol.* **202**, 872 (1962). To estimate the amount of the active drugs injected into the brain, the inner barrel of a cannula was weighed, loaded, and reweighed. This procedure was repeated four times: carbachol, 50 ± 25 μg; neostigmine, 50 ± 25 μg; methyl atropine, 20 ± 10 μg. We thank the Mettler Instrument Corporation for the use of a balance.
6. G. B. Koelle, *Fed. Proc.* **28**, 95 (1969).
7. L. S. Goodman and A. Gilman, Eds., *The Pharmacological Basis of Therapeutics* (Macmillan, New York, 1965), pp. 10 and 443–446.
8. Killing was elicited by the muscarinic drug carbamylmethylcholine chloride, although nicotine salicylate was ineffective in the same rats (D. E. Smith, unpublished). These findings, coupled with the fact that the anti-muscarinic, methyl atropine, suppressed killing, suggest that the hypothesized cholinoceptive mechanism is muscarinic, not nicotinic.
9. J. S. Myer and R. T. White, *Anim. Behav.* **13**, 430 (1965).
10. R. D. Myers, *Can. J. Psychol.* **18**, 6 (1964).
11. We thank S. Shrader and S. A. Ahlskog for technical and histological assistance. Supported by a gift from Albert Hendler, PHS grant MH 08493, and NSF grant GB 4586 to B.G.H., NRC grant No. ACDA-0236 and PHS Fellowship 1 F01 MH44514-01 to D.E.S. and by PHS Fellowship 1-F1-MH-41867 to M.B.K.
* Present address: Department of Psychology, State University of New York, Cortland.

12 August 1969; revised 20 November 1969

Cortical Unit Activity in Desynchronized Sleep

Abstract. *Bursts of unit firing associated with surface positive electroencephalogram waves and rapid eye movements account for the mean increase in discharge rate in desynchronized sleep over that of the synchronized phase. Firing rate begins to change toward the value it will assume in desynchronized sleep in the minute before the usual electrographic criteria of desynchronized sleep are present.*

Evidence from experiments in which subjects were deprived of desynchronized sleep (D) (*1*) suggests that the electroencephalograph (EEG) waves of D [or ponto–geniculate–occipital cortex (PGO) spikes, monophasic waves] are important functionally—increase in number of EEG waves after deprivation parallels the duration of the deprivation much more closely than the duration of the D episodes after deprivation. These waves have also been assumed, on the basis of electrographic recording, lesion and pharmacologic studies (*2*), to indicate activity of a brain-stem mechanism which triggers D. Although Calvet *et al.* (*3*) have reported a 50 percent increase in unit firing in segments of D during the EEG waves of D, and Evarts (*4*) and Valleala (*5*) have noted bursts of unit firing following eye movement, unit activity in relation to EEG waves and rapid eye movements has not been determined over the course of a D episode for a group of cells, nor has the time course of the rate change from synchronized sleep (S) to D been examined.

We thus investigated the time of onset of the change in rate of unit firing that accompanies D, whether the rate change was tonic or phasic, and the way in which the rate change was related to electrographic data both within the D period and in transition from S to D. We were especially interested in the relation between unit firing and the phasic electrographic activity of D, rapid eye movements and EEG waves, for two reasons. First, although the precise relation between EEG and events in single units has not been demonstrated, a strong tendency for transcortical EEG waves and bursts of unit firing to occur together with a constant phase relationship would suggest a common cortical process underlying these two phenomena. Second, if the rapid eye movements and the two cortical phenomena also showed a strong tendency to occur together with