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.

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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

Table 1. Mean firing rates and significance levels (Wilcoxon sign test) for 10 units.

State	Rate (impulse/sec)	Significance of difference in rate from		
		S	D without phasic activity	D	D with phasic activity
S	1.95		N.S.	.01	.001
D without phasic activity	1.53			.001	.001
D	2.88				.001
D with phasic activity	5.26				

a constant phase relationship, this would point to the brainstem as the site of a process common to the generation of all three events, since there is already evidence of brainstem origin of EEG waves and rapid eye movements (*6*).

Single unit activity during sleep and waking (W) was recorded extracellularly in the posterolateral cortex of unrestrained cats (quantitative data is from four cats) by glass insulated platinum-iridium microelectrodes (*7*) mounted on a piston-in-cylinder micromanipulator (*8*). Pentobarbital anesthesia was used during implantation of the cylinder upon which the micromanipulator rested, the electrodes for recording electrooculogram (EOG), parietal EEG, electromyogram (EMG), and a transcortical electrode for recording EEG waves (*9*).

Recording was begun 1 week after implantation procedures. Records were scored for state (W, S, or D) by electrographic criteria (*10*). Unit firing rates and latencies were determined by manual counting of the record of impulses from a Schmitt trigger interposed between an oscilloscope on which unit activity was monitored and a polygraph. The locations of the implant cylinders were verified by histological techniques; the area in which microelectrode recordings were made included the lateral gyrus and the medial and central portions of the suprasylvian gyrus from A 3 to P 8. The sampling period for determination of mean firing rate in each state was 1 minute. Statistical significance was evaluated by nonparametric tests (*11*).

We calculated firing rates during the presence of phasic electrographic activity for a subsample of 10 cells (*12*) (Table 1). The most striking finding is that firing rate in those portions of D without phasic activity is not significantly different from S although the rate for the entire D sample (including both portions with and without phasic activity) is faster than for S. The firing rate in D with phasic activity (this category includes those portions of D with either rapid eye movement or EEG waves, or both) is significantly higher than either S or D without such activity. In fact, 62 percent of all unit firing occurs in conjunction with phasic activity, although the mean duration of phasic activity is only 23 percent of the total duration of D.

In none of the group of 10 cells was the firing rate lower during phasic activity than during periods with no phasic activity. For the whole group

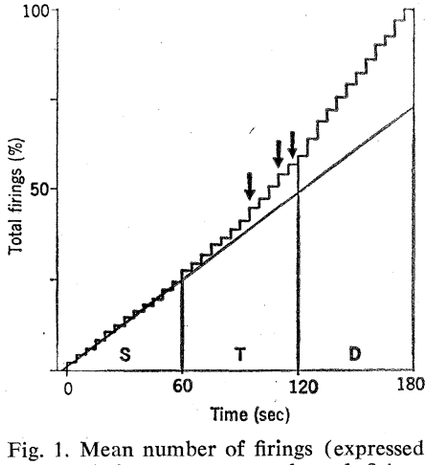

Fig. 1. Mean number of firings (expressed as cumulative percentage of total firings for 180 seconds) per 5-second epoch of 15 units that increased firing rates going from S to D. S, T, and D indicate the 1-minute contiguous segments of synchronized sleep, transition period, and desynchronized sleep, respectively. The slopes of segments of the graph as determined by a linear least squares fit (*16*) are: S, 4.17 (the oblique line on the graph is the fit to the data of S); the first 35 seconds of T, 4.50; the last 25 seconds of T, 6.50; and D, 7.43 (units are percent total firings per 5-second epoch). The concomitance of the average time of occurrence of the first EEG wave is indicated by the first arrow (farthest to the left) and by the increased slope in T. The second and third arrows indicate the average time of onset of the last high voltage slow wave in parietal EEG and of EMG suppression. The first rapid eye movement occurs, by our definition of D, at the onset of D (120 seeconds).

firing rates for those portions of the record with both EEG waves and rapid eye movements are about four times faster than nonphasic portions, and are also higher than with either one present alone. It would thus appear that phasic modulation of unit firing rate is the primary mechanism for the increased mean rate in D.

The temporal relationship between unit firing and phasic activity was also determined in a subsample (*13*). When they occur together, the initial EOG deflection representing the beginning of the rapid eye movement leads the EEG wave by 16 ± 3 msec (standard error of the mean). The burst of unit firing begins 72 ± 3 msec after eye movement starts, and is at a maximum at 134 ± 8 msec. We saw no units that fired before eye movement began. Maximum surface positivity in the EEG waves occurs at 95 ± 6 msec and the maximum deflection of the EOG is at 75 ± 6 msec after the initial EOG deflection. That bursts of unit firing follow the initiation of eye movement is in agreement with Valleala's study (*5*), although our estimate of the latency is higher than his. Increased unit firing and EEG waves are not always associated with rapid eye movements and, when they are, have no relationship to direction, amplitude, or rapidity of the EOG deflection representing the rapid eye movement, which indicates that the cortical responses are nonspecific with respect to these parameters of eye movement. This confirms and extends the findings of Brooks (*14*) that the EEG waves of D are nonspecifically related to eye movement.

We investigated the temporal changes of unit firing rate in relation to the electrographic criteria of D onset in the following manner. First, we examined the minute of the record before all of the electrographic criteria were present (*10*). For convenience this minute will be called the transition period (T). One minute was chosen as the duration of T because this appeared to be sufficient to encompass the earliest changes of unit activity and because this duration would be comparable to the 1 minute samples used to determine rate in other states. We found that for the sample of 64 units recorded in S, T, and D there was indeed a difference in firing rates between S and T. During S the median firing rate was 2.08 impulse/second; this increased to 3.69 impulse/

second during T ($P < .05$ by sign test). Furthermore, in 49 of the 64 units the direction of the rate change from S to T was in the same direction as that of S to D ($P < .01$ by sign test).

We examined the time course of the change in firing rate from S to D by determining the mean of the cumulative number of firings in 5-second epochs during the minute of S prior to T, during T, and during the first minute of D in a subsample of 15 units (*15*) (Fig. 1). The slope of cumulative counts in D is approximated during the last 25 seconds of T, and even during the early part of T, unit firing is increased over S. For the individual units the time at which the firing rate during T approximated that in D ranged from 60 to 15 seconds before the beginning of D. We also observed a similar approximation to lower D firing rates during T in those cells that decreased firing rates on going from S to D. The curve indicates that a rather long (1 minute) period of acceleration of neuronal firing occurs before the onset of each D period.

Our criterion for the beginning of D (namely, that D begins with the first rapid eye movement that occurs with EMG suppression and low-voltage, fast EEG) was chosen because it seemed to offer the sharpest beginning point. However, no matter which of the conventional electrographic criteria for the beginning of D is used, unit firing will approximate the firing rate of D and change from that of S before the occurrence of this electrographic criterion. We found this to be true by calculating the average time of occurrence (relative to the first rapid eye movement) of the last high-voltage, slow wave in the parietal EEG that was equal in amplitude to those observed in S (this measure gives the earliest estimate of the beginning of low-voltage, fast EEG) and the time at which EMG suppression begins. The mean values of these two measures are 9.97 ± 1.32 seconds and 3.13 ± 1.03 seconds before the first rapid eye movement (Fig. 1). All of these findings show that the change of unit firing rate from S to D does not begin abruptly with the onset of D (as defined electrographically), but rather begins before D. This suggests that activity of the neural mechanisms underlying D is detectable in the absence of the usual electrographic criteria of that state.

There is also a temporal association between the onset of waves in the transcortical EEG and a marked increase in mean unit firing rate (Fig. 1). The first EEG wave in T occurred an average of 25.92 ± 3.15 seconds before the occurrence of the first rapid eye movement of D, and the change in slope of the cumulative firings in T to approximate the slope in D occurred 25 seconds before the first rapid eye movement. This finding parallels the increased rates of unit firing seen in the presence of EEG waves during D.

As there is evidence for brainstem origin of rapid eye movements and EEG waves, we suppose that the bursts of unit firing are also manifestations of brain-stem influence. The consistent increase in unit firing rate during the bursts of firing associated with phasic electrographic activity implies that brainstem activity is phasically excitatory (or disinhibitory) to the units we studied. The finding that all of the mean rate increase of D over S is attributable to increased unit firing during the phasic electrographic activity attests to the strength of this input, and indicates the extent of brainstem control over cortical unit firing during D.

The data from the transition period suggest that cortical unit firing is a more sensitive indicator of brain-stem events responsible for generation of the D episode than are electrographic measures. The electrographic measure that most closely parallels changes in unit firing rate in the transition from S to D is the one that is most sensitive to events generated in brainstem—the transcortical EEG. The data are also consistent with the hypothesis that the EEG waves reflect the activity of the neural elements responsible for triggering a D episode. On the basis of the time course of increases in unit firing rate in widespread brain regions in D, the fully developed D episode would seem to be the result of massive and diffuse recruitment of neurons by a central driving mechanism in the brainstem.

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12. The subsample was obtained by examining a sequence of units from the entire group ($N = 92$) of cells recorded in D until we found 15 that exhibited clearly defined EEG waves (clarity of EEG waves is a function of electrode placement). We then selected 10 cells from this group so that the degree of eye movement related firing as determined by preliminary analysis was comparable to the entire sample of 92 cells. This subsample was also representative with respect to the percentage of cells increasing their mean firing rates from S to D. The duration of a rapid eye movement was defined as the interval beginning with the sharp initial deflection of the EOG and ending after the EOG had peaked and decreased to one-third of its maximal deflection (A.C. coupling with a time constant of 0.45 sec was used for EOG). If another rapid eye movement supervened before this, the same criteria were applied to this eye movement. Duration of EEG waves was defined as the period between the first deflection from base line and the return to base line after the major positive component. With diphasic waves this meant that both the surface negative and surface positive components were included.
13. We examined serially each high speed recording (60 mm/sec) of D until we found 10 with increased firing during eye movements and clearly definable EEG waves. The first 10 eye movements with EEG waves were scored for each cell. The units scored were recorded in widely separated penetrations of the posterolateral cortex. Although this sample is biased in favor of cells that increase firing during eye movements, we have no doubt of the generality of the results with respect to phase relationships.
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