# Neuronal Supersensitivity to Acetylcholine Induced by Kindling in the Rat Hippocampus

Abstract. *Kindling is an experimental model of epilepsy in which periodic brain stimulation induces the progressive development of electrical and behavioral seizures. A kindling-induced electrical seizure (afterdischarge) in the rat hippocampus produces prolonged neuronal supersensitivity to microiontophoretically applied acetylcholine after a latency of 40 to 60 minutes. Neuronal acetylcholine supersensitivity is correlated with the further progression of kindling. A larger hippocampal afterdischarge is elicited by a subsequent kindling stimulus delivered in the presence of acetylcholine supersensitivity, but not by one delivered before the onset of the supersensitivity. The results suggest that alteration of synaptic sensitivity to acetylcholine may contribute to kindling and epileptogenesis.*

Periodic stimulation of a number of forebrain structures has been shown to lead to the development and progressive intensification of electrical and behavioral seizure activity in a variety of animal species (*1*). Each stimulus apparently induces a higher level of neural excitability, so that the next stimulus, although equal in intensity to its predecessor, elicits a more intense neural response. An important feature of this kindling phenomenon is that the stimulus-induced increases in neural excitability are quite long-lasting, if not permanent (*1, 2*). Moreover, continued stimulation can lead to a true epileptic state with spontaneously recurring seizures (*3*). These features have attracted attention to kindling as a model for the study of cellular mechanisms of epileptogenesis.

Our hypothesis is that kindling increases excitability by increasing synaptic sensitivity to neurotransmitters. We have studied the effect of kindling on neuronal responsiveness to microiontophoretically applied neurotransmitters in the rat hippocampus. In this report we show that the stimulus-induced electrical seizure (afterdischarge) in the hippocampus induces in pyramidal cells a state of selective and prolonged supersensitivity to microiontophoretically applied acetylcholine (ACh). We further show that a subsequent stimulus continues the kindling progression—that is, elicits a longer afterdischarge—only if it is delivered in the presence of ACh supersensitivity.

Adult male Sprague-Dawley rats (200 to 300 g) were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally). Five-barrel glass micropipettes were used. The central recording barrel contained 2*M* NaCl. Three side barrels contained, respectively, 0.01*M* ACh in 0.2*M* NaCl, *p*H 5.2; 0.2*M* glutamate (Glu) in 0.2*M* NaCl, *p*H 4.5; and 0.1*M* γ-aminobutyric acid (GABA) in 0.2*M* NaCl, *p*H 4.0. The fourth side barrel contained 4*M* NaCl and was used for automatic passage of balancing current. Pyramidal cells were isolated in CA1 of the dorsal hippocampus. Extracellular single-unit activity was amplified and led to a digital counting circuit, which displayed the number of spikes per 10-second time interval.

Baseline dose-response curves were obtained for each neurotransmitter. Response was taken as the average firing rate during a designated 10-second time interval after the onset of the current. After baseline determination, a hippocampal afterdischarge was evoked by electrical stimulation of the ipsilateral fornix via a stereotactically placed bipolar electrode. The stimulus consisted of a 1-second train of biphasic square-wave pulses, 0.1 msec in duration, at a frequency of 100 Hz. At various times following the afterdischarge, the response of the neuron to the neurotransmitters was reassessed. Dose was measured in nanoamperes of microiontophoretic ejecting current.

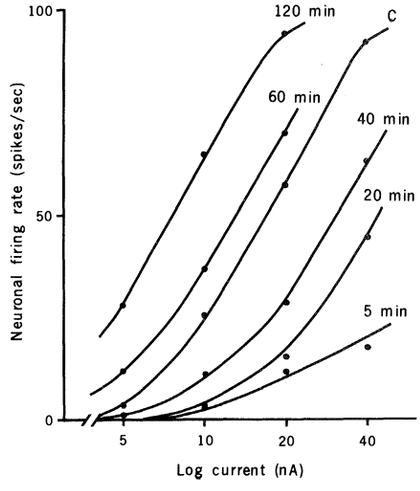


Fig. 1. Effect of a hippocampal afterdischarge on the response of a CA1 pyramidal cell to microiontophoretically applied ACh. Doses of ACh are expressed as logarithmic values of the iontophoretic ejecting current. Neuronal responses are average firing rates during the third 10-second interval after current onset (*9*). The baseline dose-response curve before the afterdischarge is designated by *C*. The other curves were obtained at the designated times after the afterdischarge. Dose-response curves were shifted to the right during the first 40 minutes after the afterdischarge, indicating decreased sensitivity to ACh. At 60 minutes there was a parallel shift of the dose-response curves to the left, indicating a state of ACh supersensitivity. This was still present at 120 minutes.

Fifteen cells in 12 animals were recorded with sufficient stability (at least 1 hour) to define post-afterdischarge responsiveness to the neurotransmitters. We consistently found long-lasting supersensitivity to microiontophoretically applied ACh (Fig. 1). The ACh supersensitivity began after a latency of 40 to 60 minutes and then persisted for the duration of recording or until another afterdischarge was elicited. We never observed a spontaneous return of responsiveness to baseline, pre-afterdischarge levels in any cell studied, including cells followed for more than 4 hours. The supersensitive period was preceded by a period of decreased responsiveness to microiontophoretic application of ACh, beginning immediately after the cessation of the afterdischarge. Typically, cellular responsiveness to ACh reached a minimum within a few minutes after the afterdischarge, then increased, rose above baseline, and entered the period of prolonged supersensitivity.

Neuronal sensitivity to ACh was correlated with the response of the hippocampal population to additional kindling stimuli (Fig. 2). A subsequent kindling stimulus (one equal in intensity to the initial stimulus) elicited a longer afterdischarge if delivered during a period when neuronal ACh supersensitivity was present (Fig. 2A). By contrast, a kindling stimulus delivered before the onset of ACh supersensitivity resulted in a shorter afterdischarge (Fig. 2B). In fact, stimulation during a time of marked subsensitivity to ACh elicited no afterdischarge at all.

This relationship between neuronal ACh sensitivity and kindling-induced afterdischarges was not observed for Glu or GABA. Neurons showed transient fluctuations in sensitivity to Glu following an afterdischarge. Usually, this consisted of an initial period of increased responsiveness followed by a return to approximately baseline levels within 20 to 30 minutes (Fig. 2). Moreover, there was no correlation between the state of post-afterdischarge Glu sensitivity and the length of a subsequent afterdischarge. For example, in Fig. 2B, the second stimulus elicited a longer afterdischarge


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at a time when Glu sensitivity was approximately at control levels, and the third stimulus elicited a shortened afterdischarge when Glu sensitivity was increased. We did not observe any consistent changes in neuronal sensitivity to GABA following a hippocampal afterdischarge. Hippocampal pyramidal cells are extremely sensitive to GABA. Typically, neuronal firing (either spontaneous or Glu-induced) was depressed by merely turning off the retaining current and allowing the drug to diffuse out; virtually complete inhibition was obtained at currents of 5 nA or less. Kindling did not significantly alter this sensitivity.

Our results suggest that increased neuronal sensitivity to ACh contributes to the initial growth of the afterdischarge during kindling. A quantitative relationship, however, cannot be directly established from our data. The afterdischarge is a population event, whereas microiontophoretic responsiveness is assessed in single neurons. We have demonstrated a consistent quantitative association between changes in single-neuron responsiveness to ACh and changes in population responsiveness to a kindling stimulus. This relationship suggests that cholinergic mechanisms are of importance for kindling, but does not exclude contributions from other mechanisms. Our findings, however, are supported by the results of other studies. Vosu and Wise (*4*) showed that intracerebral injection of the cholinergic agonist carbachol was an effective kindling stimulus. Similar results were reported by Wasterlain *et al.* (*5*), who further showed that mixing the injected carbachol with the muscarinic cholinergic antagonist atropine reduced seizures in a dose-dependent manner. Arnold *et al.* (*6*) also found that atropine significantly retarded the growth of electrically kindled seizures when administered intraperitoneally before each stimulation trial. Finally, McNamara (*7*) demonstrated significant reductions in muscarinic cholinergic receptor binding in the amygdala after kindling. Taken together, these results suggest that participation of muscarinic cholinergic receptors may be an important requirement of the kindling process.

The time course of changes in neuronal ACh sensitivity that we have observed agrees well with the known time course of kindling. The probability of effective kindling is known to decrease if the interstimulus interval is less than about 1 hour (*1*, *8*). This is consistent with our finding that a 40- to 60-minute delay occurs between the stimulus-induced afterdischarge and the onset of ACh supersensitivity. Moreover, Racine *et al.* (*8*) reported that stimuli spaced 30 minutes apart do not produce longer afterdischarges. We observed a similar failure of afterdischarge growth with short interstimulus intervals. This lack of effect on the afterdischarge was correlated with the early period of subnormal responsiveness to ACh. At the other end of the time scale, effective kindling has been demonstrated with interstimulus intervals of several days. We observed no evidence of a return of ACh responsiveness toward baseline even after 4 hours. Therefore, the duration of ACh supersensitivity is at least of the order of several hours. The upper limit is not yet known.

Acetylcholine was the only neurotransmitter for which we could demonstrate a relationship to kindling phenomena. Neuronal sensitivity to Glu or GABA was not consistently related to changes in afterdischarge duration. This implies a selective modification of cholinergic mechanisms. The nature of this modification, however, cannot be established from our present data. Increased ACh responsiveness might arise from one or a combination of the following mechanisms: (i) an increase in the number of postsynaptic cholinergic receptors, (ii) an increase in the sensitivity of individual cholinergic receptors, (iii) a decrease in the inactivation of ACh by acetylcholinesterase, or (iv) an alteration in the function of another neurotransmitter released by ACh. Iontophoretic application of ACh by itself cannot discriminate among these alternatives.

A permanent alteration in neuronal excitability probably underlies the human epileptic state. Our findings show that long-term changes in cellular excitability are associated with increased cellular responsiveness to ACh. This suggests that alteration of synaptic sensitivity to ACh (and possibly other neuroregulators) may contribute significantly to epileptogenesis. This would have important

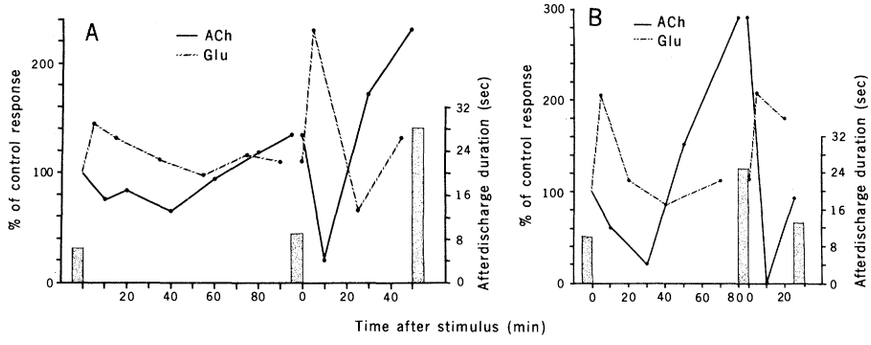


Fig. 2. Time course of post-afterdischarge changes in responsiveness of two hippocampal pyramidal cells to ACh and Glu and relationship between the state of neuronal responsiveness and afterdischarge duration. Afterdischarge duration is represented by the height of the shaded bars. Time between afterdischarges is given on the abscissa. Cellular responsiveness is expressed as the percentage ratio of the post-afterdischarge neuronal firing rate to the baseline rate before the first afterdischarge. Firing rate is the response to one iontophoretic ejection current of each neurotransmitter. Use of single-dose relative response ratios was necessary because of the wide range of post-afterdischarge changes in neuronal sensitivity, particularly to ACh. The finite practical range of iontophoretic current prevented a complete definition of a dose-response curve at all levels of responsiveness. This is illustrated in Fig. 1. During low levels of sensitivity immediately following the afterdischarge (for instance, 5 minutes), sufficient current could not be delivered to obtain high firing rates. During the supersensitive period, however, the lowest current elicited a relatively vigorous response from the cell. No single current level intersects the straight-line portion of each log dose–response curve. This was true for every cell studied; indeed, for most cells the range of responsiveness was greater than that shown in Fig. 1. Therefore, we were unable to quantitatively express changes in sensitivity as "dose ratios"—that is, parallel shifts of the dose-response curves. The response ratios shown are an accurate qualitative representation of post-afterdischarge changes in responsiveness, but the magnitude of these changes will vary as a function of the particular dose (current) chosen. (A) This cell illustrates the typical pattern of post-afterdischarge changes in sensitivity to ACh and Glu. After each of the first two afterdischarges, the response of the cell to ACh was initially below baseline, then rose to enter a period of prolonged supersensitivity. In contrast, sensitivity to Glu rose to a maximum immediately after the afterdischarge and then returned to approximately baseline levels. Subsequent stimuli delivered during the periods of ACh supersensitivity elicited progressively longer afterdischarges, a kindling progression. (B) After the first afterdischarge, this cell also displayed a biphasic subsensitive-supersensitive time course of responses to ACh. Likewise, the second stimulus elicited a longer afterdischarge in the presence of ACh supersensitivity. The third stimulus, however, was delivered only 25 minutes after the second, before the onset of ACh supersensitivity. The resulting afterdischarge was shorter than the preceding one, and thus kindling did not progress. Note that sensitivity to Glu was near baseline when the second stimulus elicited a longer afterdischarge and that the shorter, third afterdischarge occurred in the presence of increased sensitivity to Glu.

implications for the therapy of epilepsy, particularly for the delayed onset forms such as posttraumatic epilepsy, which are most likely to be related to kindling.

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9. This time interval was chosen because of the slow onset of neuronal response to microiontophoretically applied ACh. In agreement with others [T. J. Biscoe and D. W. Straughan, *J. Physiol. (London)* **183**, 341 (1966); K. Krnjevic, *Methods Neurochem.* **1**, 129 (1971)], we found that ACh began to excite neurons only after a latent period of several seconds and then induced a gradual increase in firing frequency over a period of several more seconds. A steady level of induced firing was reached by about 15 seconds.
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## Sleep and Estivation (Shallow Torpor): Continuous Processes of Energy Conservation

Abstract. *Estivation (shallow torpor) in the round-tailed ground squirrel (*Citellus tereticaudus*) is entered through electrophysiologically defined states of sleep. Rapid-eye-movement sleep diminishes as body temperature falls in such a way that, at a body temperature of 26° to 28°C, torpor is characterized by almost continuous slow-wave sleep isomorphic with that observed at euthermic body temperatures.*

Hibernation and estivation are similar physiological processes that conserve energy in homeotherms by the lowering of body temperature ($T_b$) to near ambient temperature ($T_a$) (*1–3*). Conventionally, estivation denotes periods of dormancy or torpor occurring at high $T_a$'s with $T_b$'s above 15°C, whereas hibernation designates dormancy at lower $T_a$'s with $T_b$'s below 15°C (*2*). Hudson recently suggested that "estivation" is a misnomer since there is little evidence that it is directly triggered by either heat or drought (*3*), and he regards estivation as synonymous with hibernation. Perhaps the term "shallow torpor," used here, is more appropriate, since it denotes changes in $T_b$ without bearing causal connotations as does estivation.

Recent evidence points to a physiological continuity between states of sleep and torpor. Alpine ground squirrels enter hibernation through sleep (*4*). Subsequent periods of wakefulness are associated with a halting or reversal of the hibernation entrance. The progressive lowering of hypothalamic thermosensitivity during the entrance into hibernation appears to be an extension of that which occurs during the transition from wakefulness to slow-wave sleep (SWS) in euthermic mammals (*5, 6*). Thus, sleep and hibernation appear to be homologous physiological processes. In this study we found that shallow torpor, induced by depriving desert round-tailed squirrels of food, is also characterized by almost continuous sleep.

We studied one juvenile (weight, 57 g) and three adult (194, 152, and 143 g) male round-tailed squirrels (*Citellus tereticaudus*), trapped on the Mohave Desert. A reentry tube was implanted 3 mm below the dura in the cortex to measure brain temperature ($T_{br}$), and permanent electrodes were implanted for recording the cortical electroencephalogram (EEG), the hippocampal EEG, the electrooculogram (EOG), and the electromyogram (EMG) (*4*). The electrocardiogram (EKG) could be recorded from EMG leads during periods of reduced activity. Each animal was housed individually in a 25 by 25 by 43 cm wire cage containing a wooden box with cotton nesting material. Throughout the study, animals were maintained in an electrically shielded incubator ($T_a$ = 25° ± 0.5°C) under a photoperiod of 12 hours of light and 12 of darkness (lights on at 8 a.m.).

Shallow torpor was induced by food deprivation during the months of July and August (water was continuously available). Although normal (euthermic) $T_{br}$'s during the day ranged from 34° to 37°C, at night they dropped as low as 32°C (Fig. 1). Therefore, the beginning of an entrance into torpor was defined as the point at which $T_b$ progressively declined below 32°C. Continuous electrophysiological and $T_{br}$ recordings were obtained (Grass model 7 polygraph; paper speeds of 5 or 6 mm/min) over a period of 10 to 26 days, during which time 20 periods of torpor occurred (at least four for each animal). Polygraphic records were scored by 25- or 30-second epochs for wakefulness, SWS, and rapid-eye-movement (REM) sleep (*4*).

The four animals first entered torpor after 2, 3, 5, and 19 days of food deprivation, respectively. Subsequent periods followed at approximately 24- or 48-hour intervals (Fig. 1). A distinct nocturnal pattern of torpor occurring at the usual

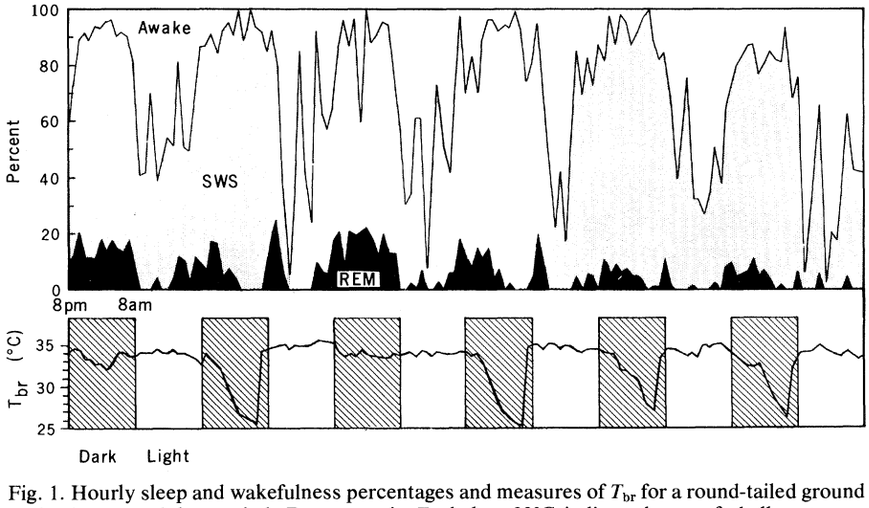


Fig. 1. Hourly sleep and wakefulness percentages and measures of $T_{br}$ for a round-tailed ground squirrel over a 6-day period. Decreases in $T_{br}$ below 32°C indicate bouts of shallow torpor (estivation). Diurnal $T_{br}$'s in other animals ranged as high as 37°C.


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