assessed on six different occasions—once on each of the 3 days before the kindling subjects were first stimulated and once after 24-hour stimulation-free periods at the end of weeks 4, 6, and 8. On each occasion both reactivity to a pencil tap on the base of the tail and resistance to capture were scored on five-point scales from 0 to 4 (*8*) by an experimenter unaware of each animal's experimental history.

To simplify analysis, the means of the three prekindling scores and the three postkindling scores for both measures were determined for each subject (Fig. 1). Kindling of the amygdala or hippocampus produced significant increases in both measures of aggression, whereas implantation and handling (with or without kindling of the caudate) did not (*9*).

In view of the fact that temporal lobe structures have been repeatedly implicated in the control of aggression (*10*), it is not surprising that changes in these structures associated with the development of an epileptic focus should have some effect on aggressive behavior. However, the repeated observation of aggressive behavior in temporal lobe epileptics does not necessarily mean that temporal lobe epilepsy is associated with a change in the neural substrate for aggression. It is difficult in clinical situations to rule out the possibility that these changes in aggressive behavior are a general result of suffering repeated seizures rather than a direct consequence of the underlying neural changes. In our study, however, seizures experienced by the caudate animals were similar in number, form, and duration to those experienced by animals with amygdaloid or hippocampal foci (*6*), yet only the latter two groups of animals displayed increases in aggression.

If the kindling procedure is continued for several months, the subjects eventually develop spontaneous epileptic discharges, which can be recorded from the electrode site; these discharges become associated with spontaneous motor seizures similar to those previously elicited by the stimulations (*11*). Our experiment, however, was concluded before this stage of kindling-produced epileptogenesis was reached; spontaneous electrographic or behavioral seizures were not observed (*12*). Thus, the spontaneous seizure state does not appear to be a necessary condition for the predisposition toward violent behavior observed after hippocampal or amygdaloid kindling.

Many problems complicate the investigation of temporal lobe aggression in clinical populations. For example, it is frequently difficult to determine the exact location of epileptic foci, to quantify the complex and diverse forms of aggression seen in human subjects, and to eliminate the confounding effects of anticonvulsant medication. Thus, although the results of this study can not be applied indiscriminately to human epileptic populations, they confirm and extend clinical observations that by themselves have been unconvincing. Moreover, procedures for producing temporal lobe aggression in laboratory animals should facilitate the experimental investigation of this important clinical syndrome and in so doing provide valuable information concerning the neural substrate of aggressive behavior.

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4. The rats weighed between 285 and 530 g (Canadian Breeding Farm and Laboratories, St. Constant, Quebec). Electrodes were constructed of insulated Nichrome wire (diameter, 0.03 inch) and were implanted according to one of the following stereotaxic coordinates: amygdala, 1.5 mm posterior to bregma, 4.2 mm lateral to the sagittal suture in the right hemisphere, and 8.8 mm ventral to the dura; hippocampus, 4.0 mm posterior to bregma, 4.9 mm to the right of the sagittal suture, and 4.1 mm ventral to the dura; caudate, 1.9 mm anterior to bregma, 3.2 mm to the right of the sagittal suture, and 4.7 mm ventral to the dura. Histological examination confirmed that all electrodes were positioned in the appropriate target structures.
5. The kindling effect does not occur unless stimulations are distributed [G. V. Goddard, D. C. McIntyre, C. K. Leech, *Exp. Neurol.* **25**, 295 (1969)].
6. There were no significant differences in the rates of kindling attributable to the site of stimulation ($P > .10$) in contrast to the observations of G. V. Goddard, D. C. McIntyre, and C. K. Leech (*5*). However, the current intensity used in the Goddard study (50 μa) may not have been high enough to reliably elicit afterdischarges from all sites. In our study, the 400-μa stimulations never failed to elicit an afterdischarge.
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8. Resistance to capture: 0, remains calm when approached and grasped; 1, shys from hand when grasped; 2, avoids hand by running, struggling when captured, or both; 3, leaps to avoid capture and struggles vigorously when captured; 4, leaps and struggles and bites when captured. Response to tail tap: 0, no response; 1, flinches or twists; 2, flinches and moves away rapidly; 3, jumps; 4, jumps at least 6 inches.
9. The mean score of each kindled group was compared to the mean score of its nonstimulated control group both before and after epileptogenesis. None of the three experimental groups was significantly different from its respective controls on either of the two measures before kindling began (all P's $> .05$). However, postkindling means of the hippocampal and amygdaloid groups were significantly greater than those of their respective controls (resistance to capture: amygdala, $t = 3.38$, $P < .003$; hippocampus, $t = 5.04$, $P < .00009$; reactivity to tail tap: amygdala, $t = 3.18$, $P < .005$; hippocampus, $t = 5.84$, $P < .00002$). In contrast, the postkindling means of the caudate group were not significantly greater than those of their controls (both P's $> .05$).
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Suprachiasmatic Nucleus: Use of ^{14}C-Labeled Deoxyglucose Uptake as a Functional Marker

Abstract. *Glucose consumption of the rat suprachiasmatic nuclei (SCN) was studied under various experimental conditions by means of the [^{14}C]deoxyglucose (DG) technique. The results show that glucose consumption of the SCN, in contrast to other brain structures, is a function of both the time of day and environmental lighting conditions. These data are consistent with the hypothesis that the SCN have an essential role in circadian rhythm regulation and indicate that the DG technique may provide a novel approach for the study of the central neural mechanisms underlying circadian rhythm regulation.*

Mammalian circadian rhythms can be entrained by environmental light and in the absence of light become free-running with a periodicity of approximately 24 hours. The presence of such stable, free-running rhythms without environmental cues has suggested the existence of a central rhythm generator (an endogenous neural clock) (*1*). Recent experiments have strongly suggested that the suprachiasmatic nuclei (SCN) may play an important role in the neural generation and regulation of circadian rhythms. For example, ablation of the SCN results in (i) elimination of the circadian rhythmicity in adrenal corticosterone content (*2*), in pineal *N*-acetyltransferase activity (*3*), drinking behavior and locomotor activity (*4*), and (ii) in alteration of sexual functions (*5*). In addition, the

demonstration of a retino-hypothalamic pathway to and terminating in the SCN (*6*) is consistent with the expectation that there should be a visual input to the endogenous neural oscillator for the entrainment of rhythms.

In view of the lesion studies implicating the SCN as the possible locus of a neural clock, it is important to show that a directly measurable property of the SCN in intact (unlesioned) animals would be affected by environmental light and exhibit circadian rhythmicity. Since regional brain functional activity is closely coupled to regional brain energy utilization (*7*) and since brain is dependent on the continuous provision of glucose for its energy (*8*), we have investigated the possibility of using the rate of SCN glucose consumption as a marker for the level of functional activity in the nuclei. We now present evidence that alterations of the rate of SCN glucose consumption occur in association with experimental alterations of both environmental light and photoperiod.

Glucose consumption was measured by the method (*9*) which allows for simultaneous, in vivo determination of the rates of glucose consumption of individual structures within the central nervous system with the use of tracer amounts of [^{14}C]deoxyglucose (DG). Deoxyglucose and glucose are mutually competitive inhibitors; DG is transported from blood to brain by the same carrier that transports glucose, and it is phosphorylated within cells to [^{14}C]deoxyglucose-6-phosphate (DG-6-P) by brain hexokinase. Forty-five minutes after a single pulse of the tracer is injected intravenously, more than 90 percent of the total tissue ^{14}C-labeled activity is in the form of DG-6-P. The ^{14}C activity is essentially trapped and accumulates as DG-6-P since this substance is not metabolized further and does not leave the brain over the time of measurement. The amount of accumulated DG-6-P is proportional to the tissue's net rate of glucose phosphorylation, which in a steady state is equal to its rate of glucose utilization. The concentration of DG-6-P can be measured quantitatively from autoradiographs, made from x-ray film exposed to sections of a brain from an injected animal. The autoradiographs can be used to calculate the rates of glucose consumption of all the visualized brain structures or, alternatively, to compare pictorially the relative rates of glucose consumption of such structures with one another (Fig. 1, a and c). The greater the optical density (OD) of a particular area on an autoradiograph, the higher is the glucose consumption of that area.

Table 1. Optical densities of DG x-ray film autoradiographs of rat suprachiasmatic nucleus. Lighting during the 45 minutes after injection is indicated by "off" and "on." N.S., not significant at $P = .05$

Group*	Injection time	Lighting after injection	Relative OD†	P
A	0900	On	2.84 ± 0.09	–
B	2100	Off	1.39 ± 0.07	≤.01
C	0900	Off	2.68 ± 0.41	N.S.
D	2100	On	3.64 ± 0.71	N.S.
E‡	0900	Off	2.40 ± 0.41	N.S.

*All rats were maintained in light for 12 hours (0600 to 1800) followed by dark for 12 hours (1800 to 0600) for 7 days before the injection of DG. †The data are expressed as a ratio (mean ± standard deviation) of optical density of suprachiasmatic nucleus (OD SCN) to optical density of ventral hippocampal commissure (OD VHC). Similar data were obtained when OD SCN was expressed relative to the OD of the corpus callosum or the OD of the anterior commissure. Three animals were used for each value. Calculated P values, with the use of Dunnett's multiple comparison procedure for testing several treatments with a control (*14*), compare the relative OD in group A to those for groups B to E. ‡In group E, the lights were not turned on at 0600 of the morning of injection, and the rats remained in continual darkness until their injection and for the 45 minutes afterward.

A total of 15 female Sprague-Dawley albino rats, weighing approximately 150 g each, were divided into five groups of three rats each. All rats were housed in individual cages, given free access to food and water, and maintained in diurnal light (lights on from 0600 to 1800). After 1 week of this light:dark (LD) schedule, rats were transferred to individual covered plexiglass holders and injected intravenously through the tail vein with 50 μc of DG (New England Nuclear; specific activity 53 c/mole) in 0.25 ml of normal saline while they were alert and unanesthetized. As shown in Table 1, the five animal groups were characterized by different injection times and lighting conditions after the injection. For all rats, a small fluorescent desk lamp was turned on briefly (<30 seconds) for positioning the intravenous needle. Animals were restrained in individual holders for 45 minutes after injection and then killed with an overdose of sodium pentobarbital. Brains were removed quickly and frozen in isopentane cooled to −70°C with solid CO_2. Frozen sections (30 μm) of brain were cut on a cryostat, dried quickly, and autoradiographed on x-ray film as described (*10*).

The autoradiographs of the sectioned brains were visually inspected for any changes of OD associated with the experimental conditions. The only changes observed were in the SCN (Fig. 1); in order to compare changes of OD of SCN between experimental groups, the OD of white matter was measured as an internal reference for each rat brain. The OD of each brain structure was determined directly on the autoradiographs by means of a Leitz MPV microscope photometer with an aperture of 0.1 mm. Twenty readings of light transmittance (T) were recorded for each structure in each animal, averaged for each structure, and the average OD for each structure was calculated as $\log_{10}(1/T)$ (*11*). In

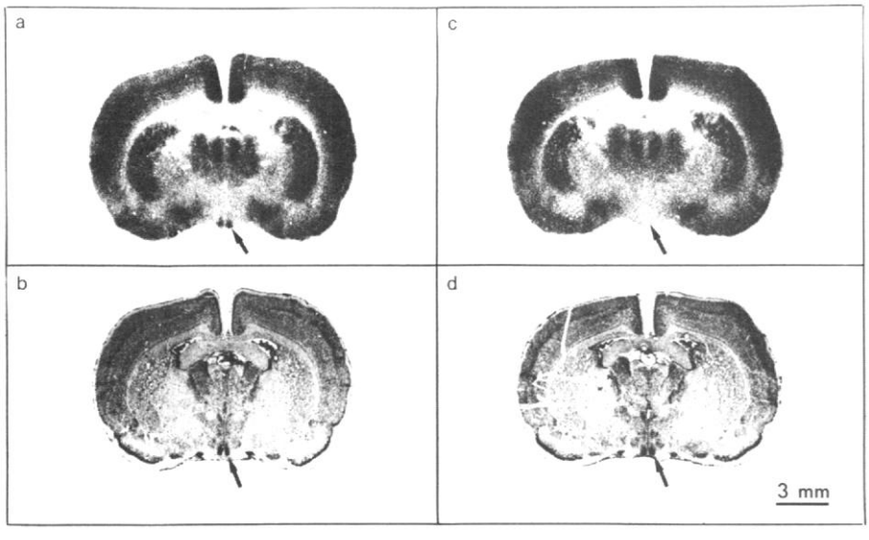

Fig. 1. DG autoradiographs of coronal brain sections from injected rats (a and c). All rats were maintained in diurnal light, 0600 to 1800 for 7 days prior to injection. The autoradiograph in (a) is characteristic of the brains of rats of group A, which were injected during the daytime (0900), and the lights remained on for the 45 minutes after injection. A pair of dark spots [arrow in (a)] corresponds in location to the SCN on an adjacent Nissl section [arrow in (b)]. The autoradiograph in (c) is characteristic of the brains of rats of group B; they were injected during the nighttime (2100), and the lights remained off for the 45 minutes after injection. The SCN are no longer visible [arrow in (c)], even though an adjacent Nissl section demonstrates their presence [arrow in (d)]. Nissl sections were stained with cresylecht violet.

Table 1, the OD for the SCN is expressed relative to that of the ventral hippocampal commissure (VHC). Similar analyses with the OD's of the corpus callosum or anterior commissure as references yielded comparable results (not illustrated). The data in Table 1 show the relative changes of the rate of SCN glucose consumption under these experimental conditions; the data do not represent quantitative determinations of the actual rate of SCN glucose consumption.

Our initial finding was that the autoradiographs of the brains from those rats injected with DG during the daytime (0900) and kept in the light (group A in Table 1) showed a pair of dark spots (arrow, Fig. 1a) which corresponded in location to the SCN on an adjacent Nissl section (arrow, Fig. 1b). The relative OD of the SCN in group A rats is shown in Table 1. In contrast, the autoradiographs from those rats injected during the nighttime (2100) and kept in the dark (group B in Table 1) showed barely visible SCN (arrow, Fig. 1, c and d), and their relative OD was significantly less than that of the nuclei in rats of group A (Table 1). Thus, comparing day and night values (group A compared to group B, respectively), there was a twofold increase in relative OD of the SCN during the daytime. The SCN's appear to have been selectively influenced by the light schedule, since no other brain structures appeared to exhibit this dramatic contrast in relative OD (Fig. 1).

Since the experiments in daytime (group A) and nighttime (group B) were performed in the presence and absence of environmental light, respectively, the possible effects of light during the experimental period were examined in experiments represented by groups C and D. Group C rats were injected at 0900. As usual, the lights had been on since 0600 of the morning of injection, but (unlike the experiment at 0900 with group A rats) the lights were turned off immediately after injection and remained off for the next 45 minutes. In these rats, the SCN were still clearly visible on the autoradiographs (not illustrated), and their relative OD was not significantly different from that of the nuclei in rats of group A (Table 1). Group D rats, on the other hand, were injected at 2100. As usual, the lights had been off since 1800 of the evening of injection, but (unlike the experiment at 2100 with group B rats) the lights were turned on immediately after injection and remained on for the next 45 minutes. In these rats, the SCN became very dark on the autoradiographs (not illustrated), and their relative OD was in fact higher than that of nuclei in rats of group A (Table 1). Therefore, turning off the lights after injection during the day had no significant effect on the relative OD of the SCN (group A compared to group C on Table 1), whereas turning on the lights after injection during the night produced a 2.6-fold increase in the relative OD of the SCN (group B compared to group D in Table 1). The latter effect of environmental light is consistent with the activation of a retino-hypothalamic pathway to the SCN (*6*) and with the report of the rapid effect of light exposure during the night on *N*-acetyltransferase activity in the pineal gland (*12*).

The above finding that there was no effect of turning off the lights after injection in daytime-injected animals (group C in Table 1) led us to investigate the possibility that the SCN were already active metabolically at this time (0900) independent of environmental light cues. That is, the SCN may have increased their glucose consumption at 0900 as a result of an entrained endogenous rhythm. The experiment represented by group E rats addressed this possibility. In this experiment, after the usual 7-day LD schedule, the lights were not turned on at 0600 of the morning of injection. The rats remained in the dark until their injection (0900) and for the 45 minutes afterward. In these rats, the SCN were clearly visible on the autoradiographs (not illustrated), and their relative OD was not significantly different from that of the nuclei in rats of group A (Table 1). Thus, this experiment shows that the increased daytime SCN glucose consumption occurs even in the absence of environmental light cues.

We have presented the results of experiments in which SCN glucose consumption has been used as a marker for SCN functional activity. We have found not only that the SCN increase their glucose consumption during exposure to light but also some evidence compatible with an endogenous rhythm of SCN glucose consumption even in the absence of environmental light cues. We do not know what proportion of SCN glucose consumption occurs in each of the compartments of the nuclei (such as glia, perikarya, and unmyelinated fibers). We also do not know what proportion of SCN glucose consumption is accounted for by each of the many energy-demanding processes involved with increased SCN functional activity (transmitter release and uptake, ion pumping, intracellular transport, macromolecular synthesis, and the like). Since direct electrical and photic stimulation of the optic nerve produces both excitatory and inhibitory electrophysiological activity in the SCN (*13*), we are unable to determine whether excitation or inhibition is responsible for the increased SCN glucose consumption during exposure to light.

Many more experiments are necessary before the existence of an endogenous circadian rhythm of SCN glucose consumption is proved—for example, DG injection at various clock times and under specific entrainment schedules (*1*). However, we do believe that the DG technique may offer an opportunity in intact, unlesioned animals to test the hypothesis that the SCN play an essential role in the neural control of endogenous rhythms (*5, 6*). The evidence for this hypothesis thus far has come from brain lesion studies (*2–6*), and the DG technique may provide a useful alternative for future work on the neural structures responsible for the generation and maintenance of circadian rhythms.

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