large fluctuations would have arisen in the grand mean SL. Hence a consistent set of scan lines was used throughout the analysis of each film. The resulting grand mean SL was plotted as a function of time from the onset of the contraction.

Two precautions were taken to ensure objectivity of the analysis: each film was processed by a different analyst, and the densitometer tracings were processed in random order so that the analyst had no knowledge of the frame number until the results were plotted. The results for three of the four films are shown in Fig. 3; the fourth result is omitted due to space limitations. Each graph shows SL (mean band spacing) as computed from the film and a photographic record of SL as computed from a single first order. The pauses in the on-line diffraction records are shown as bars in the graph. For all four films, these pauses corresponded to those present in the microdensitometry results, with matching onset times and durations and at similar SL's. This supports the hypothesis that stepwise shortening is a real phenomenon and not an artifact arising from the analysis of a single diffraction order.

One film was also analyzed by optical diffraction; each frame was completely illuminated by an expanded laser beam, and measurements were made of the positions of the resultant diffraction orders. The results (Fig. 3B) are similar to those obtained by microdensitometry. Wedge nonuniformities in film thickness caused frame-specific shifts in the positions of all diffracted orders; this effect was compensated for by measuring the distance between both first orders instead of the position of a single first order.

Minor differences were expected between the results of the on-line diffraction method and the results of the film analysis. Slight movements of the zeroth-order peak may, for example, cause a small change in the band spacing without altering the location of a first-order diffraction peak. Similarly, any augmentation of a diffraction peak by Bragg scattering will affect the film-derived SL. Furthermore, equal weights were attributed to each region of the frame when computing the mean frame SL, whereas for the diffraction process the weights depend on local uniformity of striation spacing.

Some of these effects may have been responsible for the results in plot a of Fig. 3C; here the microdensitometry result shows no clear pause at 17 msec, unlike the on-line diffraction result. On the other hand, plots b and c of Fig. 3C, corresponding to two subregions of the same film, show that the two subsets of the frame shorten differently; plot c shows agreement with the on-line diffraction record but plot b does not. In this case, some local lateral nonuniformity of contraction was apparent when the film was viewed with a movie projector.

Finally, two intriguing features are present in the data. First, the shortening trace in Fig. 3A shows that a majority of time is spent in the pause state and that only short times, perhaps on the order of the minimum time interval that could be resolved, are required to initiate the subsequent full-velocity shortening. Second, any asynochrony of behavior over the sampled domain will always tend to smooth out any discontinuities present in local, small-volume shortening behavior. These features raise the possibility that the wave forms with the longest pauses and highest burst velocities are the most representative of the molecular events.

The implication of these results is that stepwise shortening behavior extends over many sarcomeres in length and probably over at least several myofibrils laterally; no cooperative mechanism of this scope has been identified in muscle. Furthermore, the times required for the transition from pause to burst states are too short to be compatible with diffusion processes. It is perhaps more likely that stepwise shortening is the result of cooperative processes taking place over arrays of contractile proteins. If this is so, the change in SL between successive pauses may be quantized in units of, for example, the actin or myosin repeat distances.

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Diurnal Rhythm of Cytoplasmic Estrogen Receptors in the Rat Brain in the Absence of Circulating Estrogens

Abstract. *The concentration of cytoplasmic estrogen receptors in the brain of ovariectomized female rats varies during the light-dark cycle. There is no variation in the affinity of the receptors for estradiol, and the rhythm is not due to estrogens from nonovarian sources. Pentobarbital reverses the reduction of receptors that occurs in the dark, and melatonin injection in the light partially mimics the action of darkness in reducing receptor levels. The factors that cause this rhythm in brain estrogen receptors may be one means by which light affects reproductive function.*

Light affects reproduction in at least two ways: (i) in a fixed photoperiod many reproductive events are entrained to the light-dark cycle and (ii) alterations of the photoperiod can change reproductive status. An example of the latter is gonadal regression in rodents that are exposed to a shortened period of light (*1*). This effect of light is mediated by the pineal gland, possibly by a pineal product that modulates the feedback actions of gonadal hormones in the brain (*2*). In a fixed photoperiod, events that are entrained to the light-dark cycle in female rats include the luteinizing hormone surge and ovulation (*3*). This rhythmicity is not eliminated by pinealectomy (*4*). However, ovariectomized rats show a circadian rhythm in sensitivity to estradiol administered for the induction of sexual receptivity (*5*); this rhythm is eliminated by lesions of the suprachiasmatic nuclei, which receive input from visual pathways.

The sensitivity of the brain to estrogens may therefore be modulated by factors that are affected by the light-dark cycle. A change in sensitivity to estrogens could be accomplished by influencing cytoplasmic estrogen receptors, since the initial step in the action of the hormone is to bind to such receptors (*6*). We now report that estrogen receptors in the brain exhibit a diurnal variation in the absence of circulating estrogens. Consistent with the idea that the diurnal variation may be related to the pineal gland are our findings that (i) levels of estrogen receptors are lower in the dark when the pineal is active, (ii) the decline in the dark period can be blocked by pentobarbital, and (iii) melatonin injec-

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tions during the light period cause a reduction in estrogen receptor levels that partially mimics the dark-induced decline.

We assayed (*7*) the concentration of cytoplasmic estrogen receptors in the brain, the anterior pituitary, and the uterus of ovariectomized rats at 4-hour intervals during the light-dark cycle. Adult female Long-Evans rats were maintained on a schedule of 12 hours of light and 12 hours of darkness. They were ovariectomized or ovariectomized and adrenalectomized at least 1 week before they were used.

We found a clear diurnal variation in the level of estrogen receptors in the cytosol of the brain ($P < .01$; Fig. 1). The concentrations of receptors in the light were higher than they were in the dark ($P < .02$) and were highest in the afternoon. Levels of receptors declined after the onset of darkness; the low point in the middle of the dark period was 20 percent lower than the afternoon peak ($P < .01$). Receptor levels in the uterus showed a similar diurnal pattern ($P < .01$); a peak at 1500 was followed by a 28 percent reduction after the onset of darkness. The anterior pituitary did not show any consistent pattern. To ascertain whether the light-dark difference was due to a change in the apparent affinity of the receptors for ligand or to a change in their number, we used a Scatchard plot (*8*) to analyze the brain estrogen–binding activity of rats killed in the middle of the light period and in the middle of the dark period. The results (Fig. 2) showed a clear difference in the concentration of receptors, with no change in the apparent affinity (K_d of $0.3 \times 10^{-10} M$ at both time points). Binding activity was 31 percent lower in the dark than it was in the light.

We were concerned that the diurnal variation in brain estrogen receptors might be due to changes in nonovarian sources of estrogens rather than to the modulation of receptor activity by another system. Two previous reports (*9*) had provided suggestions of a diurnal rhythm in estrogen-binding activity, but each had left open the possibility that the rhythm was a reflection of changing plasma levels of estradiol. Since, in our study, the rats were ovariectomized but not treated with estradiol, the only source of circulating estrogens was the adrenal gland. The contribution of the adrenal gland to circulating estrogens in rats is controversial (*10*). We assayed receptor levels in ovariectomized and adrenalectomized rats in the middle of the light period and in the middle of the dark period. A light-dark difference of comparable amplitude

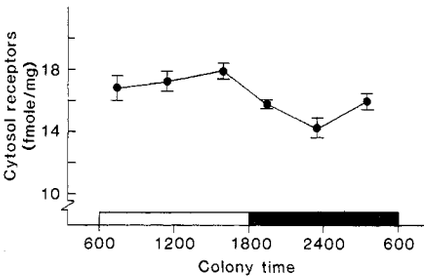

Fig. 1. Diurnal variation in brain estrogen receptors. Cytosol was prepared from the hypothalamus–preoptic area–amygdala of ovariectomized female rats killed at different time points. Receptors were assayed by incubation with [^{3}H]estradiol with or without unlabeled estradiol.

was found in ovariectomized-adrenalectomized rats (19.6 ± 0.8 compared to 16.4 ± 0.8 fmole per milligram of protein; $P < .02$). As a further check that the light-dark difference we observed was not simply due to estrogens from an unknown source binding to receptors, we used an exchange assay (*11*) to measure levels of brain nuclear estrogen receptors in ovariectomized rats in the middle of the light and dark periods. If estrogens were binding to cytosol receptors, then the receptors would be expected to appear in the nuclear fraction. Cell nuclei from the hypothalamus–preoptic area–amygdala of animals killed in the light and dark periods showed identical

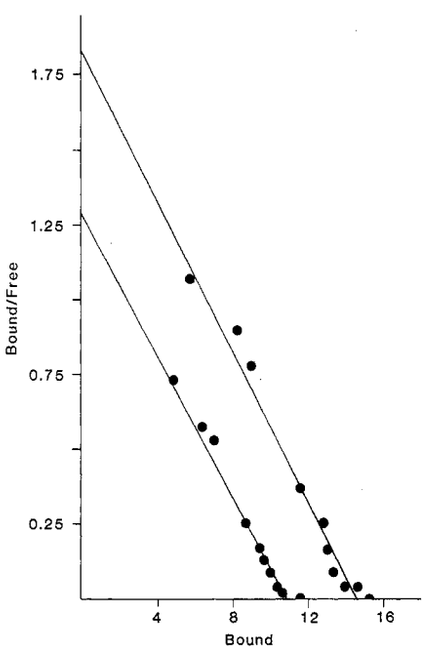

Fig. 2. Scatchard plot of brain estrogen receptor binding from animals killed in the middle of the light or the middle of the dark period. Cytosol was incubated with a range of concentrations of [^{3}H]estradiol. Parallel lines indicate equivalence of affinity for the steroid but a difference in the concentration of receptors.

low levels of nuclear estrogen receptors. The values for both were 9 ± 1 fmole per milligram of DNA; the saturation capacity in this tissue is 250 to 300 fmole per milligram of DNA when a large pulse of estradiol translocates cytosol receptors to nuclei (*12*). We conclude that the observed variation in cytosol receptors is probably not due to variations in estrogens from nonovarian sources.

The diurnal variation in estrogen receptors disappears in animals that are anesthetized for 30 to 60 minutes before being killed. After we discovered this phenomenon, we systematically examined the effects of anesthesia. Sodium pentobarbital (Nembutal, 40 mg/kg) or saline was injected intraperitoneally 1 hour before decapitation at 0300 or 1500. A significant interaction of the photoperiod and anesthesia was found ($P < .05$). Pentobarbital reversed the decline in receptors during the dark period (19.3 ± 0.5 compared to 15.9 ± 0.5 fmole per milligram of protein; $P < .01$), but had no significant effect in the light (19.3 ± 0.4 compared to 17.9 ± 0.5).

The cause of the observed diurnal variation in brain estrogen receptors is undetermined. The pattern is consistent with the hypothesis that a pineal product interacts with the receptor system; pineal activity increases shortly after the onset of darkness in rats on a 12:12 light-dark photoperiod (*13*), and we found declining receptor levels with the onset of darkness. The reversal of the decline by pentobarbital is consistent with this hypothesis, since one action of pentobarbital is to block neural transmission in the superior cervical ganglion (*14*), the source of the adrenergic stimulation of the pineal. Since melatonin is a pineal product with neuroendocrine actions (*15*), we examined the effects of melatonin injections on estrogen receptors. Melatonin (100 μg in a 10 percent mixture of ethanol in saline) or vehicle was injected twice, at 30 and 60 minutes before the animal was killed at 1500. Melatonin caused a significant decline in brain estrogen receptor levels ($P < .02$). The effect, although small (an 8 percent decline from 18.5 ± 0.4 to 17.1 ± 0.2 fmole per milligram of protein), was consistent in three separate experiments. Pituitary and uterine estrogen receptors did not show a consistent response to melatonin. Melatonin therefore acts indirectly to modulate estrogen receptors of the hypothalamus–preoptic area–amygdala in vivo, possibly by interacting with its own receptors in the brain (*16*).

An alternative to the hypothesis of a pineal role in this rhythm is offered by a report that noradrenergic activity can

affect brain receptors for progesterone (*17*). Noradrenergic systems display diurnal variations in activity (*18*), and a similar interaction of neurotransmitter activity and steroid binding could occur with estrogens.

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Myelinated Nociceptive Afferents Account for the Hyperalgesia That Follows a Burn to the Hand

Abstract. *Monkeys and human subjects were exposed to a series of thermal stimuli before and after a 53°C, 30-second burn to the glabrous skin of the hand. The responses of C- and A-fiber nociceptive afferents in the monkeys and subjective responses by the humans were compared. The burn resulted in increased sensitivity of the A fibers, decreased sensitivity of the C fibers, and increased pain sensibility (hyperalgesia) in the human subjects.*

Tissue injury, inflammation, and certain nerve injuries may lead to hyperalgesia, which is characterized by spontaneous pain and a decrease in the pain threshold. The neural mechanism of hyperalgesia is probably based on sensitization of the receptors of primary nociceptive afferents. Much attention has been given to the sensitization that may occur in C-fiber nociceptive afferents (*1*) sensitive to mechanical and heat stimuli (CMH), but little attention has been given to the sensitization that may occur in A-fiber nociceptive afferents (*2, 3*) responsive to mechanical and heat stimuli (AMH). In this study we present evidence that AMH's, not CMH's, mediate the hyperalgesia that results from a 53°C, 30-second thermal injury to the hand.

The responses of single nociceptive afferents in anesthetized monkeys were compared with subjective judgments of pain in human subjects. The receptive field of the nociceptive afferents and the human subjects were exposed to an identical sequence of thermal stimuli before and after a 53°C, 30-second burn to the glabrous skin of the hand (*4*). A laser thermal stimulator under radiometer feedback control (*5*) provided stepped increases in skin temperature superimposed on a 38°C base temperature; the area stimulated was 7.5 mm in diameter. The test sequence consisted of ten 3-second stimuli. The stimuli were delivered every 30 seconds. The first stimulus was always 45°C, and the remaining nine stimuli were presented in random order and ranged from 41° to 49°C in 1°C increments. Stimuli in both psychophysical and neurophysiological studies were delivered on the following schedule: test, 10-minute rest, test, 5-minute rest, burn, 10-minute rest, test, 10-minute rest, test. Subjective judgments of pain were measured with the magnitude estimation technique (*6*). Subjects assigned an arbitrary number (the modulus) to the magnitude of the pain evoked by the first stimulus (45°C) of the first test sequence. This stimulus was considered painful by all the subjects. Subjects then judged the painfulness of subsequent stimuli in that run and subsequent runs relative to the modulus. Nonpainful stimuli were assigned a value of zero.

Standard techniques (*2, 7*) were used to record from single fibers of the median and ulnar nerves that innervate the hand in monkeys (*Macaca fasicularis* and *M. mulatta*), which were first anesthetized with pentobarbital. Firm squeezing of the skin was used to search for nociceptive afferents, which could be readily differentiated from low-threshold mechanoceptive units by the vigorous response of the latter to light touching. Threshold for mechanical sensitivity was determined with calibrated nylon monofilaments. Conduction velocity of particular fibers was determined at the end of the experiment by measuring the latency of responses to suprathreshold electrical stimuli applied to the receptive field with intradermal electrodes.

Only fibers whose receptive fields were restricted to the glabrous skin were considered. Fifteen CMH's and 14 AMH's were studied with the protocol described here. An additional 23 CMH's

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