$[Ca^{2+}]_e$, however, resulted in step increases in $f_{1/2}$ concurrent with increases in force, thus further substantiating the link between Ca^{2+}-dependent force and the intensity fluctuations.

We interpret our results to indicate that the frequency of intensity fluctuations reflects the level of Ca^{2+} activation of the contractile proteins. This suggests that as a result of Ca^{2+} activation the myofilaments are either set in motion or are subject to changes in their refractive indices. However, the magnitude of the fluctuations must not be interpreted as indicative of the precise dynamics of the myofilaments, for although the Ca^{2+}-dependent fluctuations probably originate in myofilaments within the sarcomere, motion between sarcomeres within a given fiber or between the many myofibers composing the muscle probably contributes, to an undetermined extent, to the measurement of the intensity fluctuations. In spite of this potential limitation, measurements of intensity fluctuations adds a new dimension to the study of nonbeating cardiac muscle, permitting the monitoring of the relative level of activation in the nonbeating state and its coupling to subsequent excitation-contraction in cardiac muscle.

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5. The beam of a helium-neon laser (Spectra 147P, λ = 632.8 nm) was focused onto papillary muscle suspended in the chamber. Light scattered by the muscle was collimated by a double pinhole system and detected by a photomultiplier tube (Hamamatsu R928). The photocurrent fluctuations were analyzed by an a-c coupled autocorrelator (Honeywell Saicor 42A).
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Control of the Rhesus Monkey Menstrual Cycle: Permissive Role of Hypothalamic Gonadotropin-Releasing Hormone

Abstract. *In rhesus monkeys with hypothalamic lesions (which appear to abolish the endogenous production of gonadotropin-releasing hormone), normal ovulatory menstrual cycles were reestablished by an unvarying, long-term replacement regimen consisting of one intravenous pulse of synthetic gonadotropic-releasing hormone per hour. This finding is in accord with the hypothesis that the pattern of pituitary gonadotropin secretion throughout the menstrual cycle (basal secretion interrupted, once every 28 days on the average, by a preovulatory surge) is not directed by alterations in hypothalamic gonadotropic-releasing hormone secretion but by the ebb and flow of ovarian estrogens acting directly on the pituitary gland.*

In women and rhesus monkeys, the abrupt discharge of the pituitary gonadotropic hormones that culminates in ovulation seems to be initiated by the rising tide of circulating estradiol secreted by the rapidly developing Graafian follicle near midcycle (*1*). Evidence has recently been advanced in favor of the hypothesis that, in the rhesus monkey, this so-called positive feedback action of estradiol is at the level of the pituitary gland rather than that of the nervous system (*2*, *3*) and that hypothalamic gonadotropic-releasing hormone (GnRH) may, therefore, play only a permissive, albeit obligatory, role in this regard (*4*). We tested and affirmed this hypothesis, which predicts that, in rhesus monkeys devoid of endogenous GnRH, normal ovulatory 28-day menstrual cycles should be subserved by an unvarying GnRH replacement regimen.

In order to abolish endogenous GnRH production, bilateral radio-frequency lesions were placed in the arcuate region of the medial basal hypothalamus of seven adult, intact female rhesus monkeys (*5*). The functional completeness of the lesions was established by the reduction in plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to unmeasurable concentrations and by the loss of responsiveness to the positive feedback action of estradiol when estradiol benzoate (EB) was subcutaneously injected 1 and 3 weeks after the operation (Fig. 1).

The animals were then placed on a GnRH replacement regimen previously found to reestablish gonadotropin secretion in ovariectomized monkeys with similar hypothalamic lesions (*2*, *6*). The GnRH was infused intermittently at a rate of 1 μg/min for 6 minutes once every hour, for 65 to 158 days, through permanently implanted cardiac catheters connected to Harvard infusion pumps by swivel joints; these devices permitted continuous access to the venous circulation without restraining the animals (*2*).

After the GnRH replacement regimen ended, the effectiveness of the hypothalamic lesions was reverified by the continued undetectability of the gonadotropic hormones in plasma and by the unresponsiveness to EB administration, even after bilateral ovariectomy. The time courses of LH, FSH, the ovarian hormones, and prolactin in plasma were assessed by specific radioimmunoassays (*7*).

Within 2 to 3 days after the initiation of the pulsatile GnRH replacement regimen, plasma gonadotropin concentrations began to rise, and, in most animals, estradiol appeared in the circulation shortly thereafter. In four of the seven monkeys in this series (Fig. 1), estradiol then rose rapidly and initiated gonadotropin surges. These surges in turn resulted in ovulation and normal corpus luteum formation, as judged by the resulting time courses in plasma progesterone concentration, which resembled those seen in normal, spontaneous menstrual cycles. When more than one ovulatory menstrual cycle was observed in the course of GnRH administration, the intervals between LH peaks were 28 to 33 days—within the normal range of our rhesus monkey colony.

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After the GnRH infusions were discontinued, plasma LH and FSH again fell below detectable limits, as did the ovarian steroids. The gonadotropins remained unmeasurable despite ovariectomy, and EB administration again failed to initiate gonadotropin discharges; these results indicate that functional recovery of the hypothalamic component of the neuroendocrine control system governing ovarian function had not occurred.

The three females in which pulsatile GnRH replacement did not entrain ovulatory menstrual cycles had elevated gonadotropins during the infusion of the decapeptide. In one, however, the ovaries failed to respond to this stimulus, as judged by the absence of measurable plasma estrogens, and in the other two, plasma progesterone concentrations

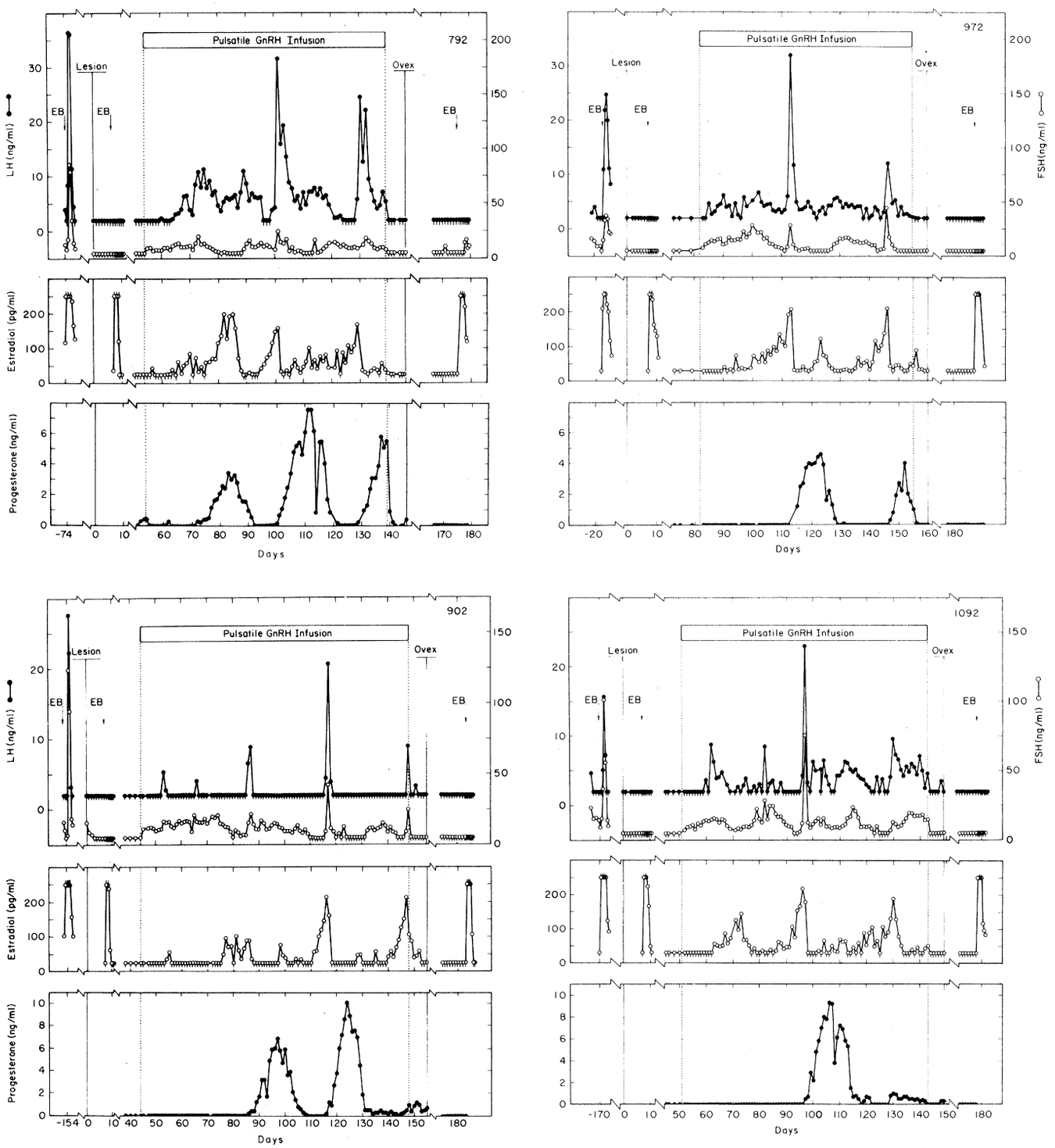

Fig. 1. Induction of ovulatory menstrual cycles in monkeys with hypothalamic lesions by an unvarying GnRH replacement regimen. Estradiol benzoate (*EB*) was given (42 μg per kilogram of body weight) before and after placement of the lesions at time 0. The ineffectiveness of ovariectomy (*ovex*) and EB administration (after discontinuation of the GnRH replacement regimen) in raising LH and FSH to the level of detectability confirms that the lesions continued to be functionally complete. The durations of the GnRH infusions are indicated by horizontal bars. The small vertical lines below some baseline points indicate concentrations below the limits of sensitivity of the assays.

were inappropriately elevated throughout the GnRH replacement period for undetermined reasons.

In a subsequent series of experiments, performed for another purpose, which differed from the foregoing only in that the post-GnRH infusion ovariectomies were not performed, nine of ten animals ovulated and exhibited normal plasma progesterone patterns during the luteal phase.

Most of the experimentally induced ovulatory menstrual cycles occurred in the presence of elevated plasma prolactin concentrations resulting from the hypothalamic lesions (*5*). These ranged as high as 500 ng/ml, compared with a normal mean of 20 ng/ml. There was no significant difference between the plasma prolactin concentrations of the animals that responded to GnRH with ovulatory menstrual cycles and those that did not, which indicates that a hyperprolactinemia of this magnitude, in the rhesus monkey, does not interfere with ovarian responses to physiologic gonadotropin stimulation nor with the actions of GnRH.

Our findings are consonant with the hypothesis that the ovary is the principal timer of the primate menstrual cycle and that estradiol controls gonadotropin secretion by acting directly on the pituitary gland, the intermittent release of GnRH by the hypothalamus being a permissive, but necessary, component of this control system. This conclusion has recently been reinforced by preliminary studies conducted in collaboration with Ferin and his colleagues, wherein experiments identical to the ones described here but performed on rhesus monkeys with transected pituitary stalks (*8*) rather than hypothalamic lesions yielded essentially identical results.

There thus seems to be a major difference in the hypothalamic control of the monkey menstrual cycle on the one hand and of the rat estrous cycle on the other. In the latter, a bolus of GnRH, discharged into the pituitary portal vasculature in response to the action of estrogen on the central nervous system, is clearly necessary for the induction of the preovulatory gonadotropin surge (*9*). In the rhesus monkey, however, such an increment in hypothalamic activity is not required to initiate this phenomenon.

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Experimental Induction of Puberty in the Infantile Female Rhesus Monkey

Abstract. *Normal ovulatory menstrual cycles were initiated in prepubertal female rhesus monkeys by the infusion of gonadotropin-releasing hormone for 6 minutes once every hour. When this regimen was discontinued, the animals promptly reverted to an immature state. These findings permit the conclusion that neither adenohypophysial nor ovarian competence is limiting in the initiation of puberty and suggest that this process depends on the maturation of the neuroendocrine control system that directs the pulsatile secretion of gonadotropin-releasing hormone from the hypothalamus.*

In all primate species studied, including the human, attainment of reproductive competence is a late maturational event. Although all other aspects of adenohypophysial secretory activity proceed at the adult level long before the initiation of puberty, the hypothalamico-hypophysial system that governs gonadotropin secretion in the adult is nonfunctional during the prepubertal period (*1*, *2*). In this context, the sexually immature female rhesus monkey bears a striking resemblance to adult females with lesions in the arcuate region of the mediobasal hypothalamus (*3*). These lesions, which leave other aspects of adenohypophysial function essentially intact, abolish gonadotropin secretion, presumably by interrupting the normal pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Normal ovulatory 28-day menstrual cycles can be reestablished in such animals by an unvarying physiological GnRH replacement regimen consisting of hourly intravenous infusions of the decapeptide administered by suitably programmed infusion pumps (*4*). We have investigated the question of whether the same hypothalamic hormone replacement regimen that subserves normal ovarian function in adults with hypothalamic lesions can also initiate and maintain normal ovulatory menstrual cycles in immature females.

We used six prepubertal female monkeys that were born in our colony. The monkeys were 11 to 15 months old at the beginning of the study; this is approximately 14 months before menarche and about 20 months before ovulatory menstrual cycles are first observed (*1*, *5*). Each monkey was fitted with a cardiac catheter connected to an infusion withdrawal device that enabled us to administer GnRH and obtain blood samples without having to restrain the animals (*6*). This device, originally used in adult monkeys weighing 5 to 8 kg, was adapted to the infantile animals (1.6 to 2.3 kg) and was well tolerated. It did not interfere with their normal activity, weight gain, or behavior. The GnRH was delivered by Harvard pumps programmed to infuse 1 μg of GnRH per minute for 6 minutes once every hour (*7*). This unvarying infusion regimen was maintained for 93 to 253 days.

Blood samples (1.0 to 2.5 ml, depending on the frequency of sampling) were taken midway between two infusions of GnRH, usually between 0900 and 1200 hours every third day during the first 17 days of GnRH administration and daily

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