(*9*). Hexokinase has a stereospecificity which could be similar to the observed glucose-stimulated insulin release (*2, 9, 10*), but the Michaelis constant (K_m) for this enzyme in islets (*11*) seems too low to represent the site of the initiating signal. The stereospecificity (at submaximal concentrations) of islet glucokinase is unknown, and, until more is learned of the stereospecificity of this enzyme, an initiating activity limited to this step cannot be excluded. Also, a nonhexose phosphate pathway could be involved. With these reservations, the observed specificity for insulin secretion is consistent with the concept that the initial trigger for glucose-stimulated release is by nonmetabolized glucose at the cell membrane (*12*). Both phases of insulin release seem similarly controlled—an observation consistent with the earlier demonstration that both phases have similar quantitative sensitivity to glucose (*6*). Concurrent experiments show that protection against alloxan toxicity by glucose, presumably a surface membrane effect, also has α-stereospecificity (*13*). Possibly these membrane activities may be distinct from active carrier systems for glucose which, at least in ascites tumor and red blood cells, are β-glucose specific (*14, 15*).

Pure anomers of glucose introduced into the circulation of animals require 7 minutes or longer to reach equilibration (*2*). If an acute endogenous metabolic permutation results in an anomeric specificity for either a glucose production or glucose uptake process, transient changes in the relative concentration of circulating glucose anomers may have to be considered, particularly in regard to insulin secretion and other glucose-regulating endocrine systems. In such a case, errors of interpretation could be compounded by glucose measurements in which β-specific glucose oxidase is used. Although Hill (*16*) found that neither glucagon, insulin, nor epinephrine caused even a transient change in the equilibrium ratio of circulating glucose, comparable and more extensive studies are required in diabetes and other pathologic states.

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Pulsatile Growth Hormone Secretion: Suppression by Hypothalamic Ventromedial Lesions and by Long-Acting Somatostatin

Abstract. *Sequential blood samples, obtained from freely behaving, nonstressed male rats, showed a pulsatile pattern of growth hormone secretion with a mean interval between peaks of 68 minutes. The bursts of secretion were blocked by lesions of the hypothalamic ventromedial nuclei and by administration of a long-acting preparation of synthetic somatostatin.*

Physiologic studies of growth hormone (GH) regulation in the rat have been hindered by the wide variability in plasma GH levels observed (range, between 1 and > 200 ng/ml) (*1*). Recent evidence has indicated that other rat pituitary hormones are secreted in episodic bursts (*2*). We now report that abrupt changes in blood GH occur in freely behaving rats and that these bursts of secretion are dependent on hypothalamic mechanisms.

Male Sprague-Dawley rats (350 to 450 g) were prepared with permanent indwelling jugular cannulas and adapted to small isolation chambers to permit repeated sampling without disturbance to the animal (*3*). Such a sampling procedure is important since minor stress, such as handling, inhibits GH secretion (*4*). Cage adaptation was carried out for a minimum of three periods of 4 to 6 hours over a 7- to 10-day period. After adaptation, individual rats were placed in the isolation box at 0800 hours, and sampling was begun 2 hours later. Blood samples (0.35 ml) were removed every 15 minutes, and the plasma was separated and frozen; red blood cells were resuspended in saline and returned to the animal after the succeeding sample was removed. This technique prevented a fall in the hematocrit reading and permitted removal of multiple samples without hemodynamic disturbance. Plasma GH was measured in duplicate samples by radioimmunoassay; the materials used were supplied by the National Institute of Arthritis and Metabolic Diseases. Plasma corticosterone was determined by a competitive protein binding assay (*5*).

Table 1. Integrated plasma GH levels in control, VMN-lesioned, and PZ-GRIF–treated rats.

Experimental group	*N*	Serum GH (ng/ml per hour)
Intact controls	12	40.2 ± 5.39*
Sham lesions	5	30.5 ± 4.72
Hypothalamic VMN lesions	7	10.3 ± 2.49†
PZ control	6	38.5 ± 4.90
PZ-GRIF	4	9.2 ± 2.02‡

* Mean ± S.E.M. † $P < .01$ compared to sham lesions. ‡ $P < .001$ compared to PZ control.

In the first experiment, 12 normal cage-adapted rats were sampled for periods of 4 to 8 hours. Episodic GH release was identified in each animal. The secretory bursts were characterized by sudden rises in plasma GH from less than 10 ng/ml to concentrations which often exceeded 100 ng/ml (Fig. 1, a and b). The mean peak-to-peak interval of the bursts was 68.0 ± 4.04 (S.E.M.) minutes. The rapid fall in GH from the highest values (Fig. 1, a and b) indicated a half-life of endogenous GH of approximately 5 to 7 minutes,

a value consistent with previously reported rates of plasma GH clearance in this species (*6*). Plasma corticosterone concentrations were in the normal, nonstressed range (11.1 ± 0.98 (S.E.M.) μg/100 ml); there were less frequent bursts with a mean peak of 20.3 ± 2.49 μg/100 ml, which were unrelated to changes in GH. The episodes of GH release could not be correlated with sleep-wake cycles or other behavioral activity. However, the bursts of secretion were inhibited for up to 2 hours by simple procedures, such as opening the cage and handling the rat.

Current evidence indicates that pituitary GH secretion in the rat is regulated by hypothalamic mechanisms. Lesions of the hypothalamic ventromedial nuclei (VMN) cause growth retardation and a reduction in plasma GH (*7*). Conversely, electrical stimulation of the VMN causes prompt GH release (*8*). Hypothalamic control is believed to be exerted by secretion of hypothalamic peptides [GH-releasing factor (GRF) and GH-release–inhibiting factor (GRIF)] that reach the anterior pituitary via the portal circulation (*9*). Recently, the structure of somatostatin, a peptide with GRIF activity, was determined and synthesized (*10*). Somatostatin has been shown to be active in inhibiting GH release in response to various stimuli in a number of species (*10–12*).

In order to define the role of the hypothalamus in pulsatile GH release, we studied the effect of VMN lesions in seven animals. Bilateral electrolytic VMN lesions were made with a 0.030-gauge, insulated platinum electrode (with 1-mm exposed tip). For each lesion, anodal current of 2.0 ma was passed for 20 to 30 seconds. [The de Groot coordinates were: anterior, (+) 5.8 mm; lateral, (±) 0.5 mm; depth, (−) 2.5 mm.] Placements of the lesions were confirmed by histologic sections. Sham-lesioned animals in which the electrode was lowered into the hypothalamus but in which no current was passed were used as controls.

The lesions, which destroyed approximately 80 percent of the VMN, were effective in completely abolishing pulsatile secretion in five of seven animals and reduced the frequency and amplitude of pulses in the remaining two (Fig. 1c). To evaluate the quantitative aspects of GH release during sampling, the area under each GH secretory curve was calculated by planimetry.

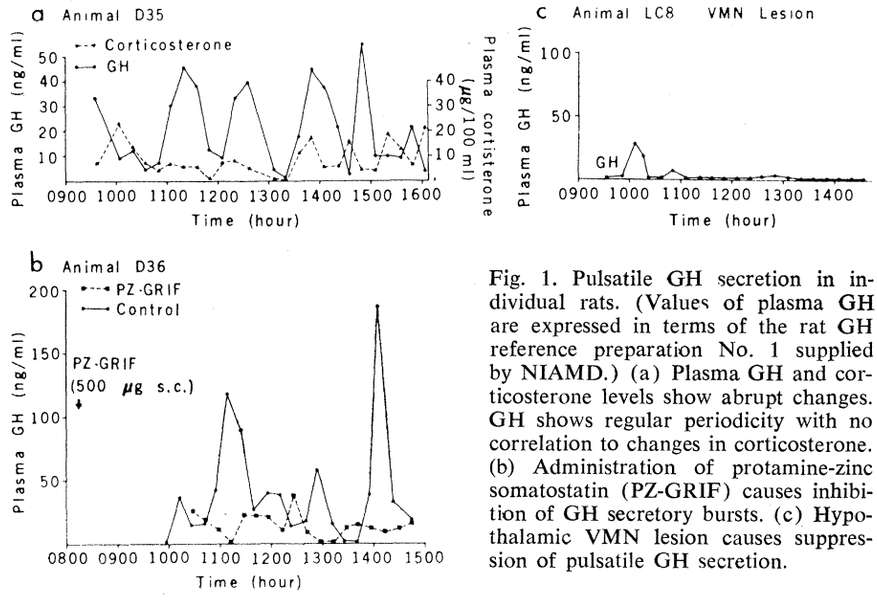

Fig. 1. Pulsatile GH secretion in individual rats. (Values of plasma GH are expressed in terms of the rat GH reference preparation No. 1 supplied by NIAMD.) (a) Plasma GH and corticosterone levels show abrupt changes. GH shows regular periodicity with no correlation to changes in corticosterone. (b) Administration of protamine-zinc somatostatin (PZ-GRIF) causes inhibition of GH secretory bursts. (c) Hypothalamic VMN lesion causes suppression of pulsatile GH secretion.

Animals with hypothalamic VMN lesions showed a significant reduction in mean integrated GH levels compared to those of sham-lesioned animals (10.3 ± 2.49 as compared to 30.5 ± 4.72 ng/ml per hour, respectively; $P < .01$) (Table 1). The value in sham-lesioned animals was not significantly different from that of intact controls.

The effects of synthetic somatostatin on pulsatile GH release were studied in four rats given a subcutaneous injection of 500 μg of linear somatostatin suspended in protamine-zinc (PZ) at 0800 to 0815 hours. Such a preparation is effective in prolonging the duration of action of somatostatin (*11*). All animals treated with PZ somatostatin showed a reduction in pulsatile GH release which persisted for up to 8 hours after administration (Fig. 1b). The result was a significant fall in integrated plasma GH comparable to that found in rats with VMN lesions (Table 1).

The finding of pulsatile GH release explains the wide variability in plasma GH levels observed in the rat and indicates the importance of longitudinal studies for the assessment of the effects of a given experimental procedure on regulation in this species. The normal concentrations of corticosterone and the absence of any correlation between the rise and fall in GH and in corticosterone argue against any role of stress in the mediation of the observed changes in plasma GH.

Episodic or pulsatile release of anterior pituitary hormones appears to be a general secretory pattern in many species, including man (*2, 13*). In the rat, pulsatile secretion of luteinizing hormone and follicle-stimulating hormone is observed after castration and after hypothalamic deafferentation, which suggests that the cyclicity is controlled by intrinsic hypothalamic mechanisms (*2*). In the case of pulsatile GH release, our experiments with VMN lesions indicate that the hypothalamus is also essential for this response. The bursts of GH secretion could be attributed to either intermittent release of GRF or to periods of somatostatin-induced inhibition, since the latter has a very short (approximately 5 to 10 minutes) duration of inhibitory action after intravenous administration, even when administered in large doses (*11*).

Such a short biologic duration of action, which is characteristic of all the hypothalamic regulatory hormones discovered to date, has greatly limited their therapeutic efficacy. Our findings show that a PZ suspension of somatostatin is effective in blocking pulsatile secretion for several hours after administration. This demonstration of inhibition of physiologic GH release has important implications for future studies of the therapeutic use of this agent for suppression of GH secretion.

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Sex-Dependent Behavioral Effects of Cerebral Cortical Lesions in the Developing Rhesus Monkey

Abstract. *Male rhesus monkeys with orbital prefrontal lesions were impaired on behavioral tests at 2½ months of age whereas similar deficits were not detected in females with comparable lesions until 15 to 18 months of age. The results suggest that the maturation of a cortical region in the primate brain proceeds at different tempos in males and females.*

The literature of comparative and physiological psychology contains abundant evidence of behavioral differences between the sexes, not only with regard to the more obvious reproductive activities of the species, but in such nonreproductive behaviors as food preferences, emotionality, aggression, and play (*1, 2*). Since differences between the sexes in behaviors such as aggression and play may be construed as intimately bound up with reproductive activities, it is not surprising that these, as well as the more plainly evident sexual behaviors, can be modified by gonadal hormones at critical periods in development (*1*). Further, since gonadal hormones are thought to affect behavior by influencing brain mechanisms (*3*), it would also not be surprising if neural structures subserving sex-typical behaviors were to differ in males and females. Indeed, anatomical differences between male and female rats have recently come to light in studies of the preoptic area, a region of the brain that has been implicated in reproductive function (*4*).

In addition to evidence for differences between males and females in reproductive and related activities, substantial evidence exists from studies of human aptitudes for sex differences in performance on intelligence tests (*5*). However, it is not known to what degree these differences are rooted in cultural patterns and to what degree, if any, they can be attributed to genetically determined dimorphism in neural structures subserving cognitive functions. We have discovered a sex difference in the learning performance of monkeys whose orbital prefrontal cortex had been removed in infancy. Our findings suggest that regions of the neocortex may be sexually dimorphic in nonhuman primates at certain stages of development.

The evidence emerged unexpectedly in the course of experiments concerned with the effects of early cortical and subcortical lesions on cognitive behavior (*6*). The monkeys of relevance to the present report are those that (i) had been given bilateral orbital prefrontal lesions in infancy (1 to 8 weeks) or as juveniles (18 to 24 months) or were age-equivalent unoperated controls; and (ii) had been tested at various ages on an object discrimination reversal task or on spatial delayed-response problems, tests that are interchangeable as measures of the integrity of orbital prefrontal functions (*7*).

In all cases, bilateral lesions were made in one stage under aseptic conditions. Infants were anesthetized with ether or methoxyflurane; juveniles, with sodium pentobarbital (40 mg per kilogram of body weight). The ablation involved removal of cortex on the inferior convexity of the frontal lobe and all of the cortical tissue on the ventral surface of the lobe rostral to the Sylvian fissure and lateral to the olfactory stria (Fig. 1a).

The object reversal test involved training monkeys to discriminate between two objects differing in color, size, and shape. After the animals reached criterion (two successive 30-trial sessions with 90 percent correct in each session), the reward contingencies were reversed so that the previously positive object became negative. The monkey's score for this task was the total number of errors to criterion made over six reversals. In the delayed-response task, the monkey was trained to observe the experimenter conceal a bait in the left or right of two food wells located on a test board in front of the animal. The position of the baited well on successive trials was governed by a modified random order. On any given trial, the monkey could select the baited food well only after an opaque screen had been interposed between the monkey and the test board for up to 5 seconds. In a related task, delayed alternation, the monkey was required to alternate between the left and right food wells on successive trials separated by 5-second intervals. The monkey's score for each of these tasks was the number of trials required to achieve a performance criterion of 90 correct responses in 100 consecutive trials. Detailed procedures for behavioral testing have been described (*6*).

When testing was completed, the brains of the operated monkeys were perfused and processed for histological and anatomical analysis. To date, anatomical results are available for 21 of the operated cases. The remaining monkeys are still being studied.