duce cytoskeletal changes in the photoreceptor cells. Indeed, preliminary experiments in which we used microtubule and microfilament inhibitors indicate that the circadian rhythm in retinal sensitivity requires the integrity of cytoskeletal structures (*12*). The regulation of cell motility may therefore play an important role in determining the response characteristics of this sensory system.

In addition to the daily morphological changes described here, the photoreceptors break down and rebuild the rhabdom structure at the first light of day. Such dynamic mechanical effects are not unique to *Limulus* photoreceptors, however. The photosensitive membranes of other invertebrate and vertebrate photoreceptors are periodically broken down and renewed (*13*), although the mechanisms may differ from those in *Limulus* (*3*). Other mechanical effects in the retina include migration of pigment granules (*14*), movement of rod and cone outer segments (*15*), and changes in the synaptic structure (*16*). These mechanical effects, controlled by either light or an endogenous circadian clock, appear to adapt the retina to its photic environment. Thus, in addition to developing neural and biochemical mechanisms of adaptation (*17*), many retinas have evolved mechanical processes for controlling visual sensitivity.

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Classical Conditioning: Induction of Luteinizing Hormone and Testosterone Secretion in Anticipation of Sexual Activity

Abstract. *A classical conditioning paradigm was used to demonstrate that male rats can learn to secrete luteinizing hormone and testosterone in anticipation of sexual activity. Sexually naïve males were exposed to a neutral stimulus and then to a sexually receptive female once daily. After exposure to the paired stimuli for 14 trials, the neutral stimulus was as effective as the female in triggering luteinizing hormone and testosterone secretion. These findings provide two novel perspectives on the control of reproductive hormone secretion in male rats: (i) environmental cues, which males learn to associate with sexual activity, induce the secretion of hormones that regulate pituitary-testis function, and (ii) classical conditioning may be used as a noninvasive method to evoke functional alterations in the secretion of luteinizing hormone and presumably the neuroendocrine pathways that mediate its release.*

Short-term exposure of males to females evokes temporary elevations in the systemic concentration of testosterone in numerous male mammals (*1, 2*). These findings have attracted widespread investigative interest because testosterone supports certain structural and functional aspects of the male reproductive system and plays a pivotal role in the expression of male sexual behavior (*3, 4*). Investigation of the endocrine basis for female-induced increments in circulating testosterone titers has focused on the secretion of luteinizing hormone (LH) since this pituitary gonadotropin is the primary hormone regulating the production of testicular steroids (*5*). Unexpectedly, the outcome of such investigations is equivocal: some studies have demonstrated that LH secretion is elevated after exposing males to females (*6*), but others have failed to detect alterations in LH release despite profound increments in blood testosterone levels (*7*). The controversy surrounding LH-mediated testicular endocrine responses may be due to the lack of conclusive information about stimuli that trigger LH release. Indeed, neither the effects of previous sexual experience nor the relative functional importance of visual, olfactory, auditory, and tactile cues are known in detail for any mammalian species.

Given the ambiguity surrounding the nature of the provocative stimulus, and the suggestion, based on indirect evidence, that the mere anticipation of coitus stimulates testosterone secretion in human and rat males (*8*), we hypothesized that the stimulus that evokes LH secretion during sexual encounters need not originate with a female. Rather, LH secretion could be elicited by ambient cues that males learn to associate with a female during repeated sexual encounters. Our evidence affirms this hypothesis and unequivocally demonstrates that a classically conditioned stimulus can evoke the secretion of hormones from the pituitary gland and testis as effectively as a sexually receptive female.

We studied mature Sprague-Dawley male rats in two experiments. In experiment 1, we determined that the secretion of LH could be altered by a classically conditioned stimulus. We paired a neutral conditioned stimulus (CS) with an unconditioned stimulus (US) known to trigger LH release. The CS consisted of placing a male in a cage containing vapors of methyl salicylate for 7 minutes, and the US consisted of placing a male in another cage with a sexually receptive female for 15 minutes (*9*). Males were assigned randomly to one of five treatments: CS followed immediately by US (CS-US), CS alone (CS), CS followed by a 6-hour delay before exposure to the US (CS/US), US alone (US), or removal from the home cage and being handled for 30 seconds (H). Males were exposed to these treatments once daily for 14 consecutive days at a randomly selected time between 1 and 7 p.m. (*10*). On day 15, males in the CS-US and CS/US groups were exposed to the CS exclusively, while those assigned to groups CS, US, and H were exposed to the CS, US, and handled, respectively. Blood was collected by decapitation 7 minutes after exposure to the indicated stimuli. Males in the US group were killed 7 minutes after being exposed to a female, instead of 15 minutes, to collect their blood at the same time relative to stimulus onset as the other groups. The order in which the animals were killed was alternated among and randomized within groups. The concentration of immunoreactive LH was determined according to a procedure previously validated for use with rat blood serum (*11*). Treatment effects were evaluated by analysis of variance and group means compared by the Newman-Keuls procedure (*12*).

Exposure to the CS significantly ($P < .05$) elevated serum LH titers in CS-US males (Fig. 1A). The conditioned elevation in LH was similar in magnitude to that achieved by exposing US males to sexually receptive females. In contrast, neither daily exposure to the CS alone nor daily handling enhanced LH secretion in rats exposed to the CS and H treatments. Insertion of a 6-hour delay between exposure to the CS and US during training precluded the formation of a learned association between the two stimuli, as judged by the failure of CS/US males to discharge LH in response to the CS. This finding provides additional evidence that the stimulating properties of the CS depended on the experimental contingency arranged between the CS and US (*13*).

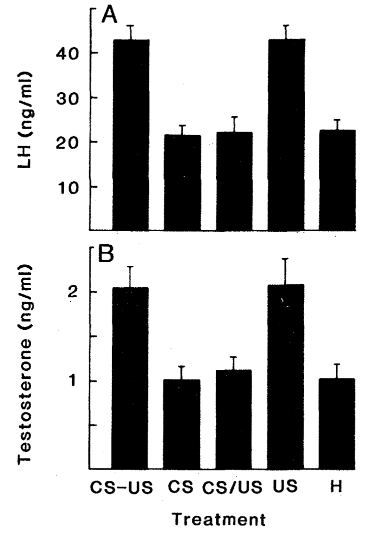

Fig. 1. Concentrations of immunoreactive LH (reference preparation NIAMDD-rat-LH-RP-1) (A) and testosterone (B) in the blood serum of male rats. Each value represents the mean ± standard error of 10 to 12 rats.

We performed experiment 2 to establish that the conditioned rise in LH secretion would cause a conditioned elevation in systemic testosterone titers. Experiment 2 was similar to experiment 1 except that blood was collected 45 minutes, rather than 7 minutes, after males were exposed to the indicated stimuli (*14*). The delay in obtaining blood samples coincided with the time lag between the female-elicited LH surge and increases in systemic testosterone concentrations shown in a preliminary study. The CS-US males exposed to the CS alone on day 15 showed a significant conditioned elevation in serum testosterone levels compared with males in the CS, CS/US, and H treatment groups ($P < .05$) (Fig. 1B). As in experiment 1, the conditioned endocrine response of the CS-US group was indistinguishable from the unconditioned response of the US group to female exposure.

To our knowledge, these results provide the first direct evidence that the secretion of a hormone from the anterior pituitary gland and testis can be altered systematically by conditioned stimuli. This finding has functional and investigative ramifications for the hormonal regulation of male reproduction.

The functional significance of the conditioned change in LH secretion lies principally in the unequivocal demonstration that environmental cues can activate the pituitary-testis axis in a way that mimics, in every respect, the activation achieved by exposure to a female. Thus, pericopulatory endocrine responses need not depend on specific cues emanating from a female, but can be evoked by nonspecific environmental stimuli that a male learns to associate with a female. These findings may explain the failure of earlier studies, including those on human males, to establish a cause-effect relationship between female exposure or sexual stimulation and the secretion of LH, testosterone, or both (*7*). Pericopulatory endocrine responses may have gone undetected in previous experiments with sexually experienced males because the males learned to respond to classically conditioned stimuli while obtaining sexual experience. Under such circumstances, anticipatory endocrine responses could occur well before the overt behavioral events used to time the collection of blood samples and thus obscure the temporal relationship between LH and testosterone secretion since both hormones are rapidly metabolized from the circulatory system of rats and other mammals (*15*).

The activational influence of the conditioned stimulus probably occurred at the level of brain control of LH releasing hormone since this neural hormone directs the secretion of LH from the anterior pituitary gland (*16*). Conditioned changes in LH secretion probably reflect a new functional interaction between association areas of the central nervous system and neuroendocrine pathways that control pituitary LH release. Thus, in addition to the functional importance of the results, our findings illustrate a powerful investigative method for studying the relationship between secretory events at the level of the brain, pituitary, and gonad, since the classical conditioning paradigm allows an investigator to exploit noninvasive measures to manipulate the secretion of hormones. This technique may be of value in studying other neuroendocrine systems as well, since indirect evidence hints that the secretion of adrenocorticotropic hormone and oxytocin may be influenced by classically conditioned stimuli (*17*).

Endocrine responses to conditioned stimuli may have an important activational role in shaping a male's subsequent mating behavior by provoking sexual arousal. Indeed, experimental elevations in LH releasing hormone and testosterone independently stimulate several aspects of reproductive behavior in male rats (*18*), but it is not known whether these hormones modify general arousal or induce sexual arousal specifically. Stimuli that induce heightened general arousal, whether through hormonal or neural pathways, facilitate sexual behavior in male rats by reducing the number of intromissions and time required for ejaculation (*19*). Anticipatory endocrine responses, which contribute to general arousal, may prepare the male for sexual encounters and therefore have considerable adaptive value, especially for prey species, by ensuring that a male is adequately aroused to mate effectively and quickly (*20*). In addition to behavioral effects, conditioned systemic elevations in testosterone may participate in the regulation of androgen-dependent functions at several loci, including the accessory reproductive structures (*21*). Thus, anticipatory endocrine responses to conditioned stimuli may be an important functional antecedent of sexual behavior and thereby contribute significantly to the regulation of reproductive behavior and certain physiological aspects of pituitary-testis function.

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Optimal Behavior: Can Foragers Balance Two Conflicting Demands?

Abstract. *According to much current theory, organisms should be able to balance the conflicting demands of the need to feed efficiently and the need to avoid predators while feeding. In an experimental conflict situation, it was possible to evaluate the relative fitnesses associated with the available choices and to compare the observed behaviors with predictions derived from fitness considerations. A backswimmer,* Notonecta hoffmanni, *was capable of balancing these two conflicting factors adaptively.*

In recent years, feeding has been viewed as a behavior molded by natural selection into an optimal state (*1*). In general, optimal foraging theory has taken the maximization of the net rate of energy intake to be the appropriate goal for foragers (*2*). An important assumption of this view is that other factors that can affect an organism's fitness do not affect the optimal foraging strategy. Considerable evidence suggests, however, that at least one such factor, the risk of being eaten while feeding, is important in altering the behavior of foragers (*3*). In particular, either foragers alter their behavior in a way that reduces the risk of predation (*4*) or alterations are greater in response to greater risks of predation (*5*). If the demands of maximizing feeding rate and minimizing the risk of predation conflict (for example, if a forager's predators and prey are found in the same place at the same time), foragers must choose a strategy that is an adaptive (*6*) compromise between these two demands. I attempted to quantify relative fitness in order to examine the hypothesis that feeding behavior represents such an adaptive compromise.

I studied the behavior of an aquatic insect, *Notonecta hoffmanni*, which is commonly found in stream pools and ponds where it feeds on both aquatic prey (such as mosquito larvae) and terrestrial prey trapped on the water's surface (such as adult flies). In the absence of predators, all size classes of *N. hoffmanni* seem to prefer similar sorts of prey and microhabitats (*7*). In addition, the main predators on (and perhaps the main cause of death of) young *N. hoffmanni* are larger conspecifics (*7*). Thus, juvenile *N. hoffmanni* are commonly placed in a situation in which the demands of feeding and avoiding predators conflict. To clarify my terms, predators (adult *N. hoffmanni*) eat foragers (young *N. hoffmanni*) and both eat prey (adult flies).

I conducted experiments in a chamber with a controlled environment at 24°C under a regime of 12 hours light and 12 hours dark. Experimental tubs (38 by 56 cm filled to a depth of 13 cm) were divided into a central region and an edge region through the use of a wooden frame (28 by 46 by 15 cm). The frame rose above the water's surface and constrained surface prey (a strain of wingless adult fruit flies, *Drosophila melanogaster*) to one of the two regions. The sides of the frame were either covered with fiber glass window screening, thereby restricting notonectids (both foragers and predators) to one region or the other, or left open, allowing notonectids to move freely between the two regions.

I experimentally created a conflict situation in which the central region (HH) had high prey density (0.11 fly per square centimeter) but high risk of predation (three adult notonectids), and the edge region (LL) had low prey density (0.03 fly per square centimeter) but low risk of predation (no adult notonectids). In experiment 1, I assessed the relative fitnesses associated with time spent entirely in one region or the other by con-

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