prisingly low in view of the number of recent studies demonstrating that vestibular-kinesthetic types of stimulation facilitate development in young premature infants (*14*). Thus, when the condition of infants is stable, handling and rocking appear to be beneficial.

Although infants in special care units do not suffer from a lack of visual, auditory, and tactile stimulation, they have relatively little coordinated or integrated sensory experience. Although the long-term effect of these dissociated sensory experiences is unknown, this finding is significant in view of recent evidence showing a deficit in the ability of premature infants up to 1 year of age to integrate tactual-visual sensory information (*15*).

The data also reveal that diurnal rhythmicity across days on physical and social variables was not a characteristic of newborn intensive and convalescent care units. Environmental engineering for special care units may prove necessary for preventing many iatrogenic problems and promoting development of premature infants.

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Hypothyroidism Elicits Electrophysiological Noradrenergic Subsensitivity in Rat Cerebellum

Abstract. *Discharge rates of Purkinje neurons were compared in control and hypothyroid adult rats. Purkinje neurons in hypothyroid rats fired significantly faster and were less sensitive to iontophoretically applied norepinephrine than those in control rats. The subsensitivity of the Purkinje neurons appeared to be primarily due to an alteration in the β-receptor–adenylate cyclase complex, because the sensitivity of these cells to locally applied N^6-monobutyryl adenosine 3',5'-monophosphate (N^6 cyclic AMP) did not change significantly. The sensitivity of the Purkinje neurons to norepinephrine could be restored in hypothyroid rats by administration of triiodothyronine.*

Hypothyroidism elicits a state resembling adrenergic hypoactivity (*1–5*). In hypothyroid rats, a reduction in the norepinephrine-induced accumulation of adenosine 3',5'-monophosphate (cyclic AMP) in the central nervous system (CNS) and a decreased β-receptor density have been reported (*3*). However, few electrophysiological studies have been conducted on identified neurons during hypothyroidism (*6*). The cerebellum offers several advantages as a test system for this type of study. The rate of discharge of the Purkinje neuron (the principal cerebellar neuron) is controlled by a direct norepinephrine-containing pathway from the pontine nucleus locus coeruleus (*7*). Norepinephrine released from the locus coeruleus neurons inhibits the activity of Purkinje neurons in a manner that may involve β-adrenoceptors and the generation of cyclic AMP (*8*). Since the responsiveness of some adrenergically innervated tissues is altered during hypothyroidism, and because the Purkinje neurons are a well-characterized model of central neurons receiving an adrenergic input, we made use of electrical recording methods and iontophoresis to characterize central electrophysiological manifestations of hypothyroidism. We now report that, in adult rats, hypothyroidism elicits an electrophysiological noradrenergic subsensitivity associated with the β-receptor–adenylate cyclase complex, and that this subsensitivity can be restored to normal by administration of the thyroid hormone triiodothyronine (T_3).

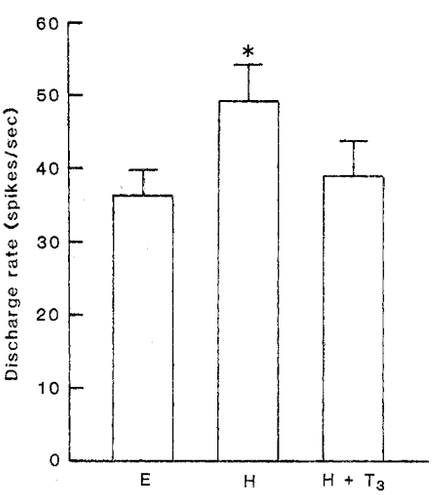

Fig. 1. Histograms of spontaneous discharge rates of cerebellar Purkinje neurons in euthyroid (*E*), hypothyroid (*H*), and hypothyroid rats treated with T_3 ($H + T_3$). Rats were made hypothyroid by feeding them 6-propyl-2-thiouracil (PTU) for 6 weeks as described by Nakashima and Hagino (*17*). The food (Altromin pellets) contained 0.15 percent PTU. During the PTU treatment the animals developed shaggy fur and showed reduced weight gain and diminished motor activity. To restore thyroid activity we injected the hypothyroid rats with 50 μg of T_3 per 100 g of body weight on alternate days for a total of three doses. Hypothyroidism produced a significant (*) increase in the spontaneous discharge of Purkinje neurons. Values (means ± standard error) were significantly different at $P < .05$ (*t*-test).

Electrophysiological data were obtained from 32 adult male Sprague-Dawley rats (200 to 280 g; Charles River). The rats were anesthetized with urethane (1.25 g/kg, intraperitoneally), and allowed to breathe spontaneously. Body temperature was maintained at 37°C with a heating pad. Recordings were made from Purkinje neurons in the vermis, lobules VI and VII, identified by ana-

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tomical location and their characteristic discharge of complex and simple spikes (*9*).

For studies on the spontaneous discharge rate, we used single-barrel glass microelectrodes (3 to 5 megohms), each filled with 3*M* NaCl. For each neuron, the discharges (spikes) per second were calculated with a digital computer. To apply drugs at the site of recording, we used multi-barreled (three or five barrels) micropipettes. Drugs were applied by microiontophoresis or air-pressure ejection. A crystal clock controlled the iontophoresis circuit (*10*) so that pulses of uniform current and duration could be applied at equal intervals. Iontophoretic artifacts of current, drug *p*H, and local anesthesia were controlled as described earlier (*11*). For air-pressure ejection, the barrel containing the drug to be ejected was connected to a regulated air-pressure line. A solenoid valve was used to apply pressure whenever drug ejection was desired. To minimize observer bias and pipette variability, we conducted tests of responsiveness to locally applied drugs using a single-blind, double-animal protocol. For the double-animal protocol, two animals (control and treated) were surgically prepared on the same day in separate stereotaxic frames within the same recording setup. Several Purkinje neurons were recorded first from one animal and then from the second animal. This procedure was repeated in an alternating manner, with the same single- or multi-barreled pipette. Such a protocol minimizes the variability in release which invariably occurs between different electrodes (*12*).

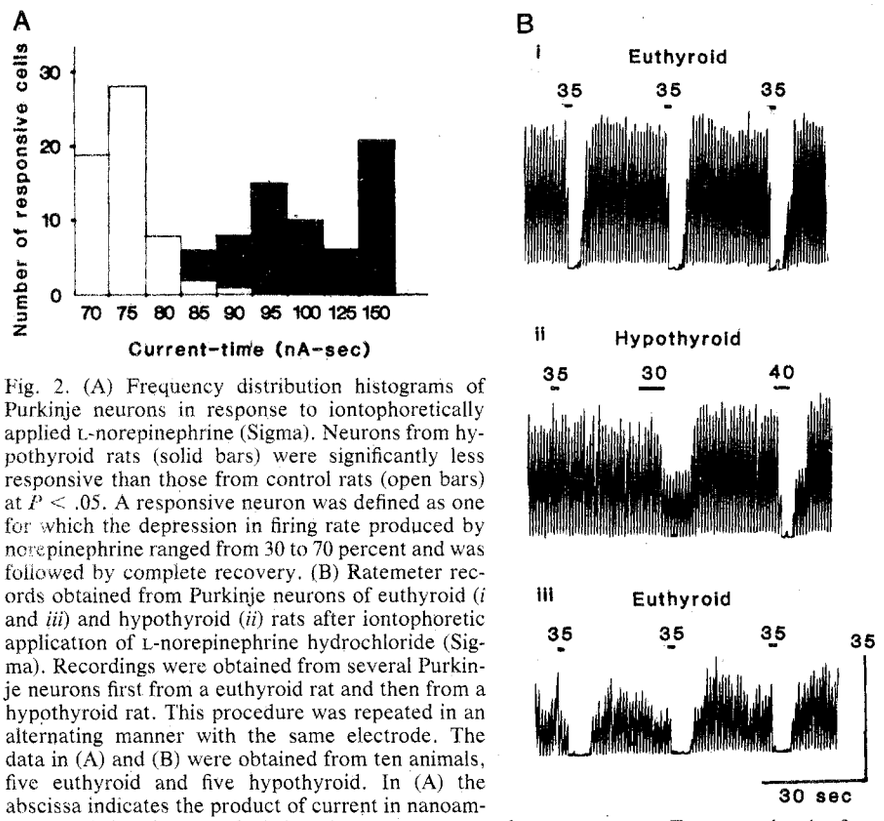

Fig. 2. (A) Frequency distribution histograms of Purkinje neurons in response to iontophoretically applied L-norepinephrine (Sigma). Neurons from hypothyroid rats (solid bars) were significantly less responsive than those from control rats (open bars) at $P < .05$. A responsive neuron was defined as one for which the depression in firing rate produced by norepinephrine ranged from 30 to 70 percent and was followed by complete recovery. (B) Ratemeter records obtained from Purkinje neurons of euthyroid (*i* and *iii*) and hypothyroid (*ii*) rats after iontophoretic application of L-norepinephrine hydrochloride (Sigma). Recordings were obtained from several Purkinje neurons first from a euthyroid rat and then from a hypothyroid rat. This procedure was repeated in an alternating manner with the same electrode. The data in (A) and (B) were obtained from ten animals, five euthyroid and five hypothyroid. In (A) the abscissa indicates the product of current in nanoamperes and time in seconds (nA-sec) necessary to produce a response. For example, the first histogram represents 60 to 70 nA-sec; the second, 71 to 75 nA-sec. The numbers 35, 30, and 40 represent the current, in nanoamperes, of norepinephrine needed to produce inhibition of Purkinje neuron firing. The bars beneath the numbers represent the duration of ejection.

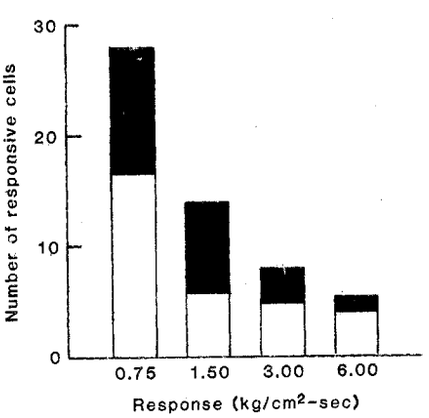

Fig. 3. Frequency distribution histograms of Purkinje neurons from euthyroid (open bars) and hypothyroid (solid bars) rats in response to local application of N^6-monobutyryl adenosine 3′5′-monophosphate (N^6 cyclic AMP; Sigma). There was essentially no difference between the two groups of animals in their response to N^6 cyclic AMP. The data are from ten animals, five euthyroid and five hypothyroid. The abscissa (numbers) represents the product of pressure in kilograms per square centimeter and the time in seconds (kg/cm²-sec) necessary to produce a response. For example, the first histogram represents 0.75 kg/cm²-sec; the second, 1.50 kg/cm²-sec. N^6 Cyclic AMP has the same physiological properties as cyclic AMP (*8*). However, N^6 cyclic AMP is considerably more lipid soluble (thus penetrates cells more easily) and more resistant to degradation by phosphodiesterase than is cyclic AMP (*18*). Pressure ejection, rather than iontophoresis, was used because iontophoresis of cyclic nucleotides requires huge currents (*5*) (> 150 nA) which may produce current artifacts (*11*). Electroosmosis of cyclic nucleotides is not very successful (*8*). The N^6 cyclic AMP was dissolved in normal saline at 0.2*M*. Separate experiments indicated no effects of pressure-applied normal saline alone.

Figure 2A shows frequency distribution histograms of Purkinje neuron responses to locally applied norepinephrine. Norepinephrine was applied, from the same electrode barrel, alternately to Purkinje neurons from cerebelli of euthyroid and hypothyroid rats. Purkinje neurons from hypothyroid rats are significantly less sensitive to norepinephrine application than neurons from euthyroid animals; thus neurons from hypothyroid animals are distributed to the right in the less sensitive area, and those from euthyroid rats to the left in the more sensitive area. There was no change between the two groups in their sensitivity to iontophoretically applied γ-aminobutyric acid (GABA) (not shown). Figure 2B shows ratemeter records obtained when sensitivity to locally applied drugs was being determined. Such records were used to construct the cumulative histogram depicted in Fig. 2A. Administration of T_3 to hypothyroid rats shifted the norepinephrine sensitivity range to that for euthyroid rats (not shown). Figure 3 shows that the Purkinje neuron responses to locally applied N^6-monobutyryl adenosine 3′,5′-monophosphate (N^6 cyclic AMP) were similar in euthyroid and hypothyroid adult rats.

Our results suggest that the sensitivity of Purkinje neurons to norepinephrine is

diminished during hypothyroidism in adult rats. The spontaneous discharge rate of Purkinje neurons is determined to a large extent by the tonic inhibitory noradrenergic afferents originating in the nucleus locus coeruleus (*13*). Thus, an increased spontaneous discharge in the Purkinje neurons of hypothyroid rats is consistent with the diminished responsiveness of such neurons to norepinephrine. A hypoactive afferent projection from the locus coeruleus is ruled out by several studies on the peripheral and central nervous systems (*5, 14, 15*) demonstrating an increased amount of norepinephrine discharged at the sympathetic nerve endings during hypothyroidism. Thus, alterations in postsynaptic function could conceivably lead to the electrophysiological changes reported here (*6, 15*). β-Receptors might be altered in number or in their affinity or functional coupling with adenylate cyclase. In fact, diminished β-receptor binding (with no change in affinity) during hypothyroidism has been demonstrated (*3, 15*). In peripheral tissues, parallel changes in adenylate cyclase activity have been reported (*16*). Our electrophysiological studies agree well with previous observations (*3, 6, 15, 16*). In another study (*2*), the activity of cyclic AMP phosphodiesterase (E.C. 3.1.4.17) did not change in the cortices of hypothyroid rats. The studies we report here suggest that the subsensitivity occurring during hypothyroidism resides in the β-receptor–adenylate cyclase complex. Furthermore, our results and those of others (*3, 6, 15*) suggest that T_3 may decrease the number of β-receptors which might thus account for the subsensitivity of Purkinje neurons to norepinephrine.

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Bat Predation and the Evolution of Frog Vocalizations in the Neotropics

Abstract. *Bat predation has probably had an important influence on the evolution of frog vocalizations in the Neotropics. The rate at which fringe-lipped bats capture frogs is significantly higher when the frogs are calling. These bats respond to a wide variety of calls from edible frogs, and, when simultaneously presented with a choice, choose the recorded call of a palatable species over that of a poisonous species and the call of a small species over that of one too large to capture. Thus the selective advantages of loud, rapid mating calls in anurans are balanced by an increased risk of predation.*

Many animals use conspicuous vocalization to attract mates. The benefits are obvious, but biologists have long suspected that this also leads to increased vulnerability to sound-responsive predators (*1, 2*). Although such counterselection is believed to influence the evolution of vocal advertisement (*3*), documentation is rare (*2*) and is entirely lacking for vertebrates. In this report we show that the fringe-lipped bat (*Trachops cirrhosus*) uses acoustic cues to capture calling frogs, and we consider the possible role of call-responsive predators in the evolution of anuran calling and courtship behavior.

On 35 nights from January to June 1980 we visited 14 frog breeding sites on Barro Colorado Island, Panama (*4*). *Trachops cirrhosus* was observed hunting on each night. Seven of the nights were spent at a breeding pond of the frog

Table 1. Responses of *T. cirrhosus* to playbacks of the advertisement calls of four anuran species.

Species	In cage		In field	
	Bats tested	Responses	Sites visited	Responses
Hyla boulengeri	5	35	6	66
Bufo typhonius	5	5	6	3
χ^2	42.81 (10), $P < .005$		70.63 (12), $P < .005$	
Physalaemus pustulosus	5	36	3	26
Leptodactylus pentadactylus	5	1	3	2
χ^2	47.15 (10), $P < .005$		31.52 (6), $P < .005$	

Table 2. Responses of *T. cirrhosus* to the recorded advertisement calls of two anuran species played at different repetition rates and volumes.

Species "calling"	Bats tested	Responses
Physalaemus pustulosus		
1.6-second interval between calls	3	22
3.2-second interval between calls	3	2
χ^2		22.62 (6), $P < .005$
Centrolenella fleischmanni		
1.6-second interval between calls	3	18
6.4-second interval between calls	3	1
χ^2		22.19 (6), $P < .005$
Physalaemus pustulosus		
78 dB SPL	2	13
74 dB SPL	2	2
χ^2		14.14 (4), $P < .01$

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