and other foodstuffs within houses, and the ample harborage for rodents in houses constructed of mud and thatch all contribute to contacts between rodents and humans.

The results of our investigations provide a basis for controlling Lassa fever in West Africa. Because no vaccine is available, rodent control and human quarantine are the only potentially effective means of limiting a community-based Lassa fever outbreak. Rodent populations may be reduced by limiting the availability of food and shelter, by various biological control methods, or, more directly, by trapping and poisoning. *Mastomys natalensis* populations have been successfully reduced by the use of an anticoagulant rodenticide (warfarin) in villages in the Sudan (*22*). Trapping and rodent-proofing of food stores have been used to stop epidemic spread of Machupo virus by *Calomys callosus* in Bolivia (*23*).

The apparent interspecific competition between *Mastomys natalensis* and the more aggressive species *Rattus rattus* demonstrated in Sierra Leone suggests a possible means of biological control but, more importantly, warns against the indiscriminate reduction of rodent populations through trapping and poisoning. Reduction of *Rattus rattus* populations in villages where this species predominates may allow introduction of *Mastomys*, which would then enter a period of logarithmic population growth.

The epizootiologic role of *Mastomys natalensis* in areas other than Sierra Leone remains to be proved. Meanwhile, investigations of high priority for zoologists and public health authorities include studies of the distribution, population dynamics, and behavior of this species in West Africa.

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Thyrotropin-Releasing Hormone: Regional Distribution in Rat Brain

Abstract. *A sensitive and specific radioimmunoassay has been used to measure the distribution of thyrotropin-releasing hormone (TRH) in rat brain. All areas of brain tested, except cerebellum, contained readily measurable amounts of TRH. The hypothalamus contained only 31.2 percent of the total brain content of TRH. These results support recent suggestions of central actions for TRH in addition to its hypophysiotropic functions.*

Hypothalamic thyrotropin-releasing hormone (TRH), whose structure is pyroglutamyl-histidyl-prolineamide (*1*), has been shown to stimulate thyrotropin (*1*), prolactin (*2*), and, at times, growth hormone secretion (*3*) from the pituitary gland. It may have significant effects on behavior and on mood state in addition to its more well established effects on pituitary hormone release (*4–6*). For example, TRH has been shown to potentiate the phenomenon of behavioral excitation induced by L-dopa in mice (*4*). This potentiation was also observed in hypophysectomized animals. Several groups have reported that TRH has mood elevating effects in depressed women (*5*). In one study, normal women were also found to respond to TRH administration with a transient elevation in mood (*6*). In light of these newer observations of the behavioral effects of TRH, a series of experiments was performed to investigate the possibility that TRH is a normal constituent of extrahypothalamic brain tissue. The results indicate that this is indeed the case. Similar results have been presented by other investigators (*7*).

Normal rats (Sprague-Dawley) weighing 200 to 250 g were decapitated and the brains were rapidly removed. By a modification of the technique described by Glowinski and Iversen (*8*), the brains were dissected into six pieces. The hypothalamus was removed first by freeing the sides of its lateral borders. With the optic chiasm as the anterior border, a cut 2 mm deep was made under the hypothalamus, allowing it to be lifted up from the third ventricle. Next an arbitrary cut was made from the anterior border of the hypothalamus straight down through the cortex. This cut yielded a piece, identified here as forebrain, which contained both frontal and some temporal cortex, as well as some anterior diencephalic tissue. The brainstem and cerebellum were separated by blunt dissection. Finally, the remainder of the cortex, called posterior cortex, was peeled back from the remaining posterior diencephalic tissue. In most experiments, pieces of liver, kidney, or

Table 1. Content of TRH in various portions of rat brain. Results are given as mean ± standard error of the mean.

Brain section	Weight of brain section (mg)	TRH content		
		Nanograms	Nanograms per milligram	Percentage of total
Hypothalamus	32.6 ± 1.5	4.1 ± 0.2	0.129 ± 0.007	31.2 ± 2.3
Forebrain	399.0 ± 8.9	3.5 ± 0.3	0.009 ± 0.001	25.6 ± 2.1
Brainstem	185.0 ± 5.5	2.1 ± 0.1	0.012 ± 0.002	16.9 ± 1.8
Posterior diencephalon	213.3 ± 8.7	1.9 ± 0.2	0.009 ± 0.0005	13.8 ± 1.7
Posterior cortex	622.8 ± 11.5	1.3 ± 0.1	0.002 ± 0.0001	10.6 ± 0.7
Cerebellum	244.0 ± 5.1	0.26 ± 0.03	0.001 ± 0.0001	2.1 ± 0.3

lung weighing 200 to 500 mg were also removed. All tissue was rapidly frozen in a Dry Ice–acetone bath immediately after cutting. The samples were then weighed, quickly homogenized in 2 ml of buffer solution (0.01*M* phosphate, 0.15*M* NaCl, *p*H 7.5), and extracted with 10 ml of methanol. After centrifugation for 30 minutes at 2000 rev/min, the supernatants were dried with an airstream overnight at 60°C. The dried supernatants were suspended in buffer solution containing 0.25 percent bovine serum albumin, particulate material was removed by centrifugation, and the supernatant was recovered for assay. The TRH was measured by a sensitive and highly specific radioimmunoassay (*9*). Previous experiments have shown that 89.0 to 103.8 percent of TRH added in vitro to brain tissue is recovered with this methanol extraction technique (*10*).

The results of the TRH assays are shown in Table 1; these are combined results from five separate experiments, each with six male rats. A single experiment was done with six female rats. The results for the male rats show that the hypothalamus contained the most TRH, 4.1 ± 0.2 ng. Other brain sections were found to contain substantial quantities of TRH. Thus, the forebrain contained 3.5 ± 0.3 ng, the posterior diencephalon 1.9 ± 0.2 ng, the brainstem 2.1 ± 0.1 ng, and the posterior cortex 1.3 ± 0.1 ng. In all cases, the extract immunoreactivity gave an assay dose response pattern identical to that of synthetic TRH. The content of the cerebellum, 0.26 ± 0.03 ng, was minimal and only barely detectable. Samples of liver, kidney, and lung contained no detectable TRH. The TRH contents of the various sections of the brain in the one group of female rats studied were similar, although the total brain TRH content, 16.8 ± 0.6 ng, was slightly higher than that in the male rats, which was 13.1 ± 1.2 ng ($P < .01$).

When the values for TRH are expressed as amount per milligram of tissue (wet weight), the hypothalamus is seen to have a tenfold higher concentration of TRH than any other brain section (Table 1). The amount of TRH per milligram of tissue was not evenly distributed throughout the rest of the brain. Rather, concentrations of TRH were five times higher in forebrain, brainstem, and posterior diencephalon than in posterior cortex.

When the results are expressed as the percentage of the total brain TRH found in each brain section (Table 1), the hypothalamus is seen to contain only 31.2 percent of the total brain TRH. The forebrain section contained only a slightly lower percentage of the total brain TRH than did the hypothalamus. The TRH contents of the other sections were considerably smaller proportions of the total. The distribution of TRH in the female rats was virtually identical to that in the male rats.

In the group of female rats an additional dissection was made to separate the frontal cerebral cortex from the underlying forebrain diencephalic tissue at the level of the corpus callosum. Forebrain cerebral cortex contained 1.3 ng of TRH, which was 7.6 percent of the total brain TRH. Anterior (forebrain) diencephalon contained 3.2 ng of TRH, 18.8 percent of the total. When the values were expressed as amount of TRH per milligram of tissue (wet weight), the forebrain cortex had a concentration of 0.005 ng/mg while the anterior diencephalon had a concentration of 0.023 ng/mg. This additional dissection step made it possible for the data to be expressed as total cortex, total diencephalon, hypothalamus, cerebellum, and brainstem. In this group of rats, hypothalamus had the highest concentration of TRH, 0.249 ng/mg. The total diencephalon had a concentration of 0.014 ng/mg, brainstem 0.013 ng/mg, total cortex 0.003 ng/mg, and cerebellum 0.001 ng/mg. This pattern of distribution of TRH is quite similar to that of norepinephrine in comparable brain regions (*8*).

It has been shown that synthetic TRH and the TRH in hypothalamic extracts are rapidly destroyed by incubation with normal serum (*10*). To determine if this was also the case for the TRH extracted from extrahypothalamic neural tissue, extracts of brainstem and posterior cortex were incubated for 1 hour at 37°C with normal human serum diluted 1 : 5. Incubation with serum resulted in the loss of 85.5 percent of the TRH in the brainstem extracts and 88.5 percent of the TRH in the posterior cortex extracts. These observations, coupled with the known specificity of the assay and the dose response characteristics of the extracts, suggest that the immunoreactive material in the extracts is indeed TRH.

Some significant questions are raised by these results. The finding that roughly two-thirds of the brain TRH is located outside the hypothalamus suggests that TRH may have other functions in brain besides that of regulating the release of thyrotropin and possibly prolactin. The same conclusion has been reached by other investigators as a result of psychopharmacological studies (*4–6*). Further localization of TRH and determination of its sources and functions in the brain are areas for additional study. The data suggesting that TRH potentiates the L-dopa effects in mice may point to a role for TRH in modifying synaptic transmission or receptor responsiveness in the central nervous system. The localization of significant quantities of TRH in areas of brain where there are high levels of catecholamines and other neurotransmitters may support the notion of a role for TRH in the modification of synaptic transmission. It may also be noted that there are significant concentrations of TRH in areas of brain that are believed to be most involved with behavior and emotional state.

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Thyrotropin-Releasing Hormone in Specific Nuclei of Rat Brain

Abstract. *The regional distribution of thyrotropin-releasing hormone (TRH) in rat brain was studied. The greatest concentration of TRH was found in the median eminence. High concentrations were also found in several hypothalamic nuclei. Outside the hypothalamus, relatively large amounts of TRH were found in the septal and preoptic areas.*

In 1947 Green and Harris (*1*) proposed that neurons located in the hypothalamus might secrete into the portal circulation certain substances which are essential for the normal function of the pituitary (*2*). In the late 1950's and early 1960's hypothalamic extracts were prepared which could stimulate thyrotropin secretion (*3*). The active principle in such extracts, which is called thyrotropin-releasing hormone (TRH), was ultimately isolated, and its structure was determined to be pyroglutamyl-histidyl-prolineamide (*4*). Synthetic TRH has been produced, and a highly sensitive and specific radioimmunoassay has been developed using the synthetic hormone (*5*).

Recently a technique has been described which allows discrete nuclei to be dissected from the brain of the rat (*6*). This technique involves punching small pellets of tissue from frozen sections of the brain under stereomicroscopic control. The method has been used to study the distribution of several putative transmitters among the hypothalamic nuclei of the rat (*7–10*). We report here the localization of TRH in hypothalamic and extrahypothalamic brain nuclei.

Female rats (Osborne-Mendel, NIH strain) (average weight, 150 g) were used. The animals, which were housed in diurnal lighting conditions (light 6 a.m. to 6 p.m., dark 6 p.m. to 6 a.m.) with free access to food and water, were killed by decapitation at 9:00 a.m. Their brains were removed quickly, frozen on a block of Dry Ice, and sectioned with a cryostat-microtome. Sections 300 μm thick were cut through the brain in the frontal plane. In initial experiments relatively large regions of the brain (for example, septum, amygdala, and mammillary body) were cut or punched from the sections. The tissues obtained from two rats were pooled and homogenized in 200 μl of 2.0*N* acetic acid; 5 μl of each homogenate was removed for protein determination according to the method of Lowry *et al.* (*11*). Bovine serum albumin (BSA) served as the standard. The homogenates were centrifuged and the supernatants were lyophilized. Subsequently, the samples were dissolved in a solution consisting of 0.25 percent BSA, 0.01*M* phosphate, and 0.15*M* NaCl (*p*H 7.5), and their TRH contents were determined by radioimmunoassay (*5*). Results were expressed as nanograms of TRH per milligram of protein. Of exogenous TRH (1 ng) added to brain homogenates and carried through the entire assay procedures, 96.2 ± 5.6 percent (mean ± standard error of the mean) was recovered.

In the preliminary experiments described above, the protein content of each sample was in the range 0.2 to 1 mg. No TRH was detected in samples from the following regions (*12*): pineal gland, habenula, central gray, reticular formation, tegmentum, cerebellar cortex, amygdala, hippocampus, cingulate cortex, parietal cortex, olfactory tubercle, caudate nucleus, and anterior pituitary. When larger samples than those used in this study were assayed, TRH was found in the thalamus, brain-

Table 1. Distribution of TRH in the hypothalamus, preoptic area, and septum. The abbreviations are as used in Fig. 1; *N*, number of samples assayed; ng/mg, nanograms per milligram of protein (mean ± standard error of the mean).

Brain region	Abbreviation	*N*	TRH content	
			ng/mg	Nanograms per nucleus
Hypothalamus			1.3*	
Nucleus periventricularis†	NPE	4	4.25 ± 0.69	0.22
Nucleus suprachiasmaticus	NSC	4	1.79 ± 0.18	0.05
Nucleus supraopticus	NSO	4	0.85 ± 0.17	0.02
Nucleus anterior	NHA	4	0.82 ± 0.28	0.15
Nucleus hypothalamicus lateralis anterior	MFB	4	0.70 ± 0.23	
Nucleus paraventricularis	NPV	4	2.60 ± 0.74	0.15
Nucleus arcuatus	NA	6	3.92 ± 0.90	0.41
Nucleus ventromedialis pars medialis	NVMm	4	9.02 ± 3.3	0.80
Nucleus ventromedialis pars lateralis	NVMl	4	2.95 ± 0.59	0.16
Nucleus dorsomedialis	NDM	4	3.95 ± 0.75	0.29
Nucleus perifornicalis	NPF	4	2.03 ± 0.71	0.06
Area hypothalamica lateralis posterior	MFB	4	1.15 ± 0.49	
Nucleus posterior	NHP	4	1.79 ± 0.20	0.10
Nucleus premammillaris dorsalis	NPMD	4	1.48 ± 0.16	0.03
Nucleus premammillaris ventralis	NPMV	4	1.34 ± 0.27	0.02
Median eminence	ME	4	38.38 ± 8.27	0.89
Mammillary body	MB	6	<0.32‡	
Preoptic area		6	1.09 ± 0.08	
Nucleus preopticus medialis	NPOm	4	1.95 ± 0.13	0.20
Septum		6	0.72 ± 0.09	
Nucleus medialis	Sm	3	0.35 ± 0.07	
Nucleus dorsalis	Sd	3	1.86 ± 0.38	
Nucleus dorsalis pars intermedia	Si	3	0.47 ± 0.11	
Nucleus fimbrialis		3	0.62 ± 0.24	
Nucleus triangularis		3	0.51 ± 0.04	
Nucleus lateralis	Sl	3	2.97 ± 0.34	

* This is based on the data of Winokur and Utiger (*13*), who found 0.129 ± 0.007 ng of TRH per milligram of tissue (wet weight) ($N = 30$) in hypothalamic fragments with an average weight of 32.6 mg. † The periventricular nucleus was removed from brain sections with a small knife. The nucleus consists of a fine line of cells and was contaminated by tissue lying lateral to it in our samples. Therefore the concentration of TRH in the periventricular nucleus may be greater than reported. ‡ TRH could be measured in only three of the six samples assayed. The mean TRH concentration in these samples was 0.32 ng/mg.