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## Corticotropin-releasing Hormone—induced Seizures in Infant Rats Originate in the Amygdala

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### Abstract

The neuroanatomical substrate of seizures induced by picomolar amounts of corticotropin-releasing hormone in infant rats was investigated. Electrographic and behavioral phenomena were monitored in 42 rat pups aged 5 to 22 days. Rat pups carried bipolar electrodes implanted in subcortical limbic structures, as well as cortical electrodes and intracerebroventricular cannulae. The administration of corticotropin-releasing hormone produced age-specific seizures within minutes, which correlated with rhythmic amygdala discharges. Paroxysmal hippocampal and cortical discharges developed subsequently in some rats. Corticotropin-releasing hormone-induced electrographic and behavioral seizures originate in the amygdala.

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Corticotropin-releasing hormone (CRH) is a 41-amino acid neuropeptide, isolated originally from the mammalian hypothalamus [1]. It has since been shown to be distributed nonrandomly in the central nervous system, and to perform roles other than the control of secretion of ACTH and endorphins from the anterior pituitary [2]. Specifically, central transduction of stress, anxiety, depression, and anorexia have been demonstrated [2, 3].

CRH activates neurons both *in vivo* and *in vitro* [4–9]. The peptide increases both spontaneous and evoked spike discharge from locus ceruleus neurons *in vivo* [4]. CRH induces neuronal depolarization in CA1 and CA3 hippocampal pyramidal cells in the slice preparation *in vitro* [5]. CRH administered into the cerebral ventricles of adult rats causes epileptiform discharges in the amygdala after a 1- to 3-hour delay, which spread to the dorsal hippocampus [6]. These discharges progress over 3 to 7 hours to behavioral and electrographic seizures. The doses needed for frank seizure generation in adult rats are  $1\text{--}5$  to  $3\text{--}75 \times 10^{-9}$  mol ( $0.75\text{--}1.88 \times 10^{-9}$  mol/gm of brain weight) [6–8].

We have previously shown that CRH is a far more rapid and potent convulsant in the neonatal rat [10]. Seizures occur with a latency of as little as 2 minutes and with CRH doses as low as  $7.5 \times 10^{-12}$  mol or  $0.05 \times 10^{-9}$  mol/gm of brain weight [10]. The present study was designed to define the neurobiological matrix of the behavioral and electrographic effects of the neuropeptide. We used infant rats, starting on postnatal day 5.

### Materials and Methods

#### Animals

Timed-pregnancy Sprague–Dawley–derived rats were obtained from Zivic-Miller (Zelionplc, PA). They were housed under a 12-hour light/dark cycle and fed *ad libitum*.

Delivery times were monitored and were accurate to within 12 hours. The day of birth was considered day zero. The pups were kept with the mothers, and litters were culled to 12 pups. Infant rats were subjected to surgery 24 hours before recording and returned to their mothers. CRH was always administered between 9 and 10:30 AM, to minimize diurnal variations in seizure susceptibility [11] and in endogenous CRH levels [12].

### **Surgical Procedure**

Electrodes were implanted under halothane anesthesia, using an infant rat stereotaxic apparatus as previously described [10, 13]. Two or four cortical stainless-steel electrodes were placed over the frontal and posteroparietal cortex. Bipolar twisted wire electrodes, enameled except for the tip, were used. Electrodes were inserted through a burr hole into the amygdala or hippocampus (wire diameter, 0.1–0.15 mm; vertical intertip distance, 0.5–1.0 mm). Electrodes were anchored to the skull with an acrylic cement “cap” attached also to two screws. Age-specific basal-lateral amygdala coordinates, with reference to bregma, were adapted for our strain [14] and are shown in Table 1.

In each age group, several rat pups carried bipolar hippocampal electrodes (Table 2). These were aimed at the dorsal or ventral hippocampus, either simultaneously with those in the amygdala or with a larger array of cortical electrodes. Age-specific coordinates for hippocampal electrodes are shown in Table 2. CRH was injected via a cannula placed stereotaxically in the lateral ventricle [10].

### **Electrophysiology and Behavior Monitoring**

Recordings were performed in heated, shielded Plexiglas chambers. A Grass 78E Polygraph (Grass Instrument Co, Quincy, MA) was connected via long, flexible wires to freely moving rat pups, which were continuously observed throughout the recording. To ascertain the cerebral origin of recorded discharges, two maneuvers were undertaken. At least 1 rat pup per age was restrained (using medical tape) in an infant-rat stereotaxic apparatus. This prevented all motion of electrodes and wires. Electrographic recordings were compared with those of unrestrained peers, and with those obtained without restraint from the same pup. In addition, a motion-detecting bipolar electrode (“EMG”), was attached to the angle of the jaw or the chest and connected to the Polygraph.

After a 30-minute habituation and baseline recording period, CRH or saline was administered intracerebroventricularly in 1 to 4  $\mu$ l (depending on dose and animal age) using a microinfusion pump. CRH doses used were 0.0375 to 0.3 nmol. These doses have been found to elicit behavioral epileptic phenomena in infant rats [10]. Electrographic recording and observation of behavior were supplemented by intermittent video recording. These procedures were performed for a minimum of 4 hours, or for at least 1 hour subsequent to cessation of abnormal activity. At the end of each experiment, electrolytic lesions were created at the bipolar electrode tips. Rat pups were killed and brains frozen on dry ice. Brains were cut into 20- $\mu$ m sections and stained with cresyl violet for verification of electrode placement.

Seizure definition has been discussed in detail elsewhere [10]. Briefly, behavioral phenomena were considered epileptic if (1) they were not seen before CRH administration [10], and (2) they were recognized as epileptic in infant [15–17] and adult rats [18–20]. Though no formal grading system was used, observed behaviors conformed to the infantile scale of kindling-induced phenomena [15, 17]. The correlation of specific behaviors and electrographic discharges, the goat of this study, is discussed in the following section.

## Results

The ages of rats, doses of CRH administered, and times to onset of CRH-induced seizure are shown in Table 3. Age-specific electrographic and behavioral phenomena are summarized in Table 4. The detailed sequence of age- and dose-dependent CRH-induced behavioral events has been described elsewhere [10].

At the end of the first postnatal week, minimal effective CRH doses (0.038 nm or 38 pm) resulted in stereotyped behaviors (see Table 4). These were accompanied, in most rat pups carrying amygdala electrodes (5 of 6 pups), by abnormal discharges. Semirhythmic spike waves (Fig 1B, D), the appearance of low-amplitude fast (13–15 Hz) activity in the amygdala leads (Fig 1C), or both preceded the onset of jaw myoclonus in 3 of 6 rat pups. In 2 additional pups, amygdala discharges followed the onset of behavioral events. These discharges were confined to the bipolar amygdala leads and to those connected to both amygdala and homologous cortex. No paroxysmal discharges were recorded from either ventral or dorsal hippocampus (not shown).

CRH-induced amygdala discharges in 8- to 10-day-old infant rats are shown in Figure 2. The baseline electroencephalogram (EEG) at this age was more developed than in younger pups (see Fig 2A). Figure 2B, 9 minutes subsequent to administration of CRH, shows rhythmic slow wave discharges in the amygdala leads, which did not correlate with chest motion or with the rhythm of jaw myoclonus (“EMG”). The latency of paroxysmal amygdala discharges and accompanying behaviors (see Table 4) was dose dependent. Using 0.15 nm of CRH, frequent electrographic and behavioral seizures persisted for 4 to 5 hours. The first two panels of Figure 3 illustrate the electrographic correlates of CRH-induced seizures in a pup carrying both amygdala and ventral hippocampus (VH) electrodes. By the end of the second postnatal week (days 11–14), CRH induced repetitive, well-formed sharp wave discharges confined to amygdala leads (Fig 4B–D). No paroxysmal discharges were present in simultaneously recorded dorsal hippocampus and cortex. Rare sharp hippocampal and cortical spikes (see Fig 4D) could not be distinguished with certainty from motion artifacts. In a different rat pup, persistent jaw myoclonus was not associated with abnormal discharges in VH leads (see Fig 3C, D).

During the third postnatal week, larger doses of CRH (0.15 nm or more) were needed to elicit seizures. Behavior was limited to persistent, uncontrollable jaw myoclonus accompanied by grooming, scratching, and so on. Loss of balance was observed in only one 16-day-old pup (given 0.3 nm of CRH), and clonus was not common. CRH (0.15–0.75 × 10<sup>-9</sup> mol) induced spike wave discharges in amygdala leads (Fig 5B). Despite recording periods of up to 9 hours, propagation to hippocampal or conical leads was only rarely observed (not shown). Placement of electrodes in amygdala and dorsal and ventral hippocampus is seen in Figure 6.

## Discussion

CRH induces convulsions in adult rats [6–8, 21], though the time course and origin of paroxysmal discharges remain unresolved, Ehlers [7] and Ehlers and colleagues [6] demonstrated the onset of amygdala spikes 1 to 3 hours after the administration of CRH. Behavioral seizures, most commonly jaw myoclonus, developed 3 to 7 hours later. These were accompanied by the spread of paroxysmal discharges to the hippocampus and cortex [6]. Other investigators [8, 21, 22] have described CRH-induced epileptiform discharges localized to the hippocampal leads in both rats and rabbits. Minimal latency to hippocampal spikes was 35 to 39 minutes [22].

We have found CRH to be a more rapid and potent convulsant in the neonatal and infant rat compared with the adult rat [10]. Jaw myoclonus developed in less than 2 minutes, and effective peptide doses were as low as 0.0075 nmol ( $50 \times 10^{-12}$  mol/gm of brain weight). Reported convulsant doses of CRH in the adult were 1.5 to 3.75 nmol, or  $750 \times 10^{-12}$  mol/gm of brain weight [6]. The underlying mechanisms for this age-dependent potency and rapidity of action of CRH remain unclear. CRH gene expression increases markedly at the end of the first postnatal week [23, 24]. That, coupled with a maximal number of CRH receptors present at that time [25], may partially explain this phenomenon.

The neuroanatomical substrate of CRH-induced seizures in the infant rat has not been elucidated. Cortical electrographic correlates of behavioral phenomena have been inconsistent [10], and data regarding adult rodents are in disagreement [6–8, 21, 22]. Jaw myoclonus as an epileptic phenomenon has been extensively described in both adult [6, 20] and infant rats [16, 26, 27]. Some authors ascribe the origin of jaw myoclonus exclusively to the amygdaloid complex [19]. After kainic acid administration, jaw myoclonus has been found as a manifestation of limbic seizures originating in the hippocampus and spreading to the amygdala [16, 18, 20].

Electrographic recording from discrete structures in the neonatal and infant rat is fraught with difficulties due to the small size of brain regions, the low voltage of EEG discharges, and the presence of motion artifacts. Localization was achieved using bipolar amygdala electrodes and validated in smaller animals via amygdala–cortex leads. Not uncommonly, paroxysmal discharges were confined to bipolar amygdala and only one amygdala–cortex lead. Simultaneous recording was obtained from amygdala and either dorsal or ventral hippocampus. Paroxysmal discharges originated from the amygdala (see Figs 3, 4).

Movement artifacts were avoided by several maneuvers. EEGs of completely restrained rat pups were compared with those of free-moving pups. Rat pups were observed constantly, and a motion-detecting electrode was placed at the angle of the jaw or around the chest. The latter detected the increased ventilatory rate and amplitude induced by CRH [28]. Curarization [29] was avoided because it has been reported to alter EEGs in adult rats [30].

The assignment of the amygdala as the origin of CRH-induced paroxysmal discharges is compatible with the observed behavioral phenomena. Furthermore, the amygdala is quite rich in CRH. The central amygdaloid nucleus has the highest concentration of the neuropeptide in the brain [31, 32]. CRH receptors have been demonstrated in the central amygdaloid nucleus and basolateral amygdala [33].

In conclusion, we have demonstrated that age-specific CRH-induced seizures originate in the amygdala. The role of this neuropeptide as an endogenous convulsant in the immature brain, as well as its potential role in the enhanced susceptibility of the neonatal brain to epilepsy, remain speculative [10, 34].

## Acknowledgments

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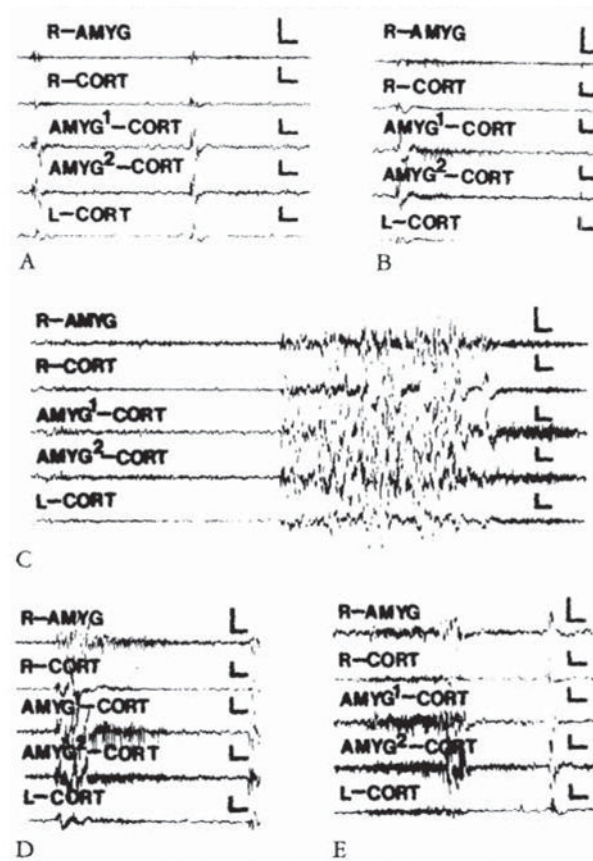
We are thankful to Ms Shana Friedman for her assistance with the manuscript.

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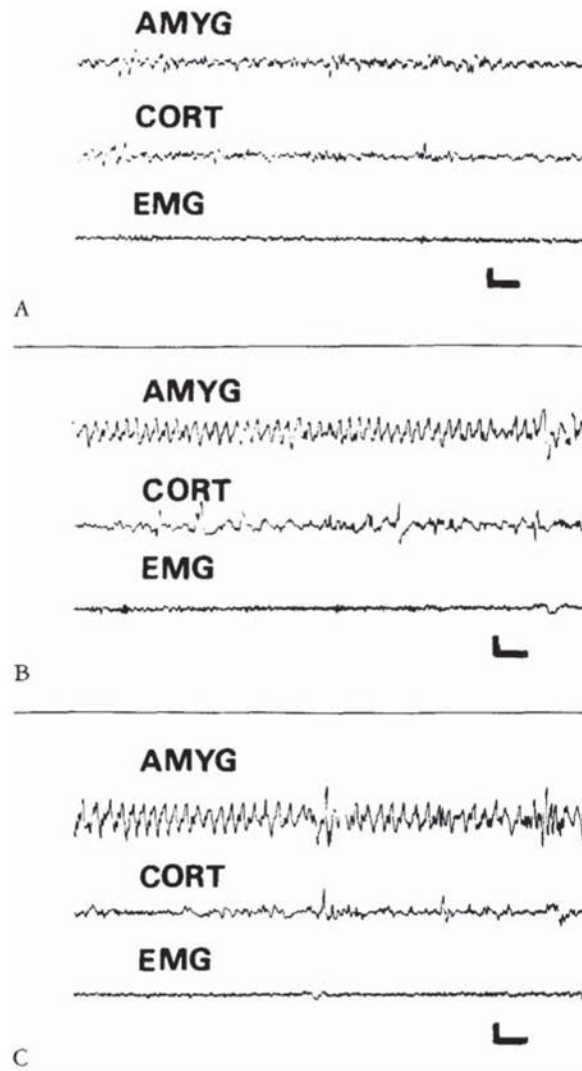
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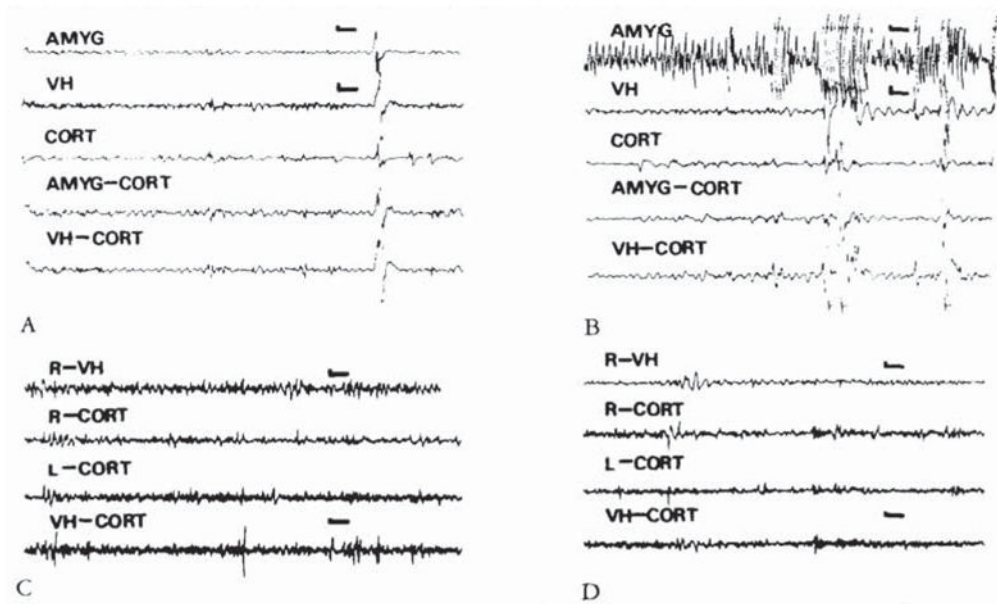


**Fig 1.** Electroencephalograms of a 5-day-old rat. (A) Before corticotropin-releasing hormone (CRH) infusion. (B, C, D, and E) Two, 11.5, 20, 59, and 85 minutes after infusion of 0.15 nmol of CRH into the cerebral ventricle. AMYC = amygdala; CORT = cortex; AMYG<sup>1</sup>/AMYG<sup>2</sup> = one of the wires of the bipolar amygdala electrode. R/L = right / left. Vertical bar = 50  $\mu$ V; horizontal bar = 1 second.



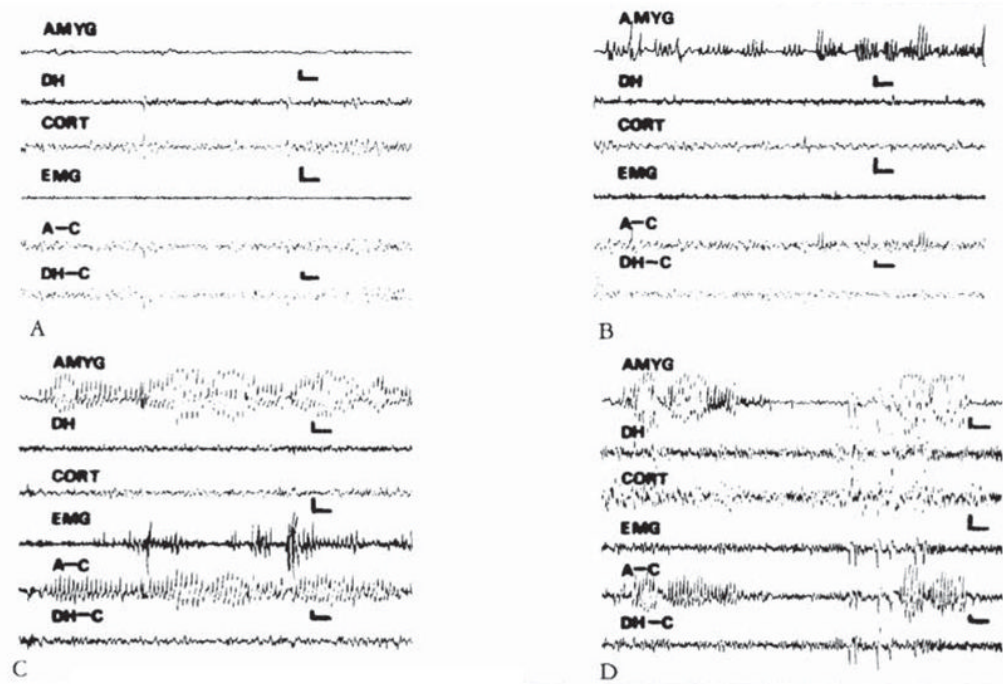


**Fig 2.** Electroencephalograms of a 9-day-old rat before corticotropin-releasing hormone (CRH) administration (A), and 9 and 120 minutes after intracerebroventricular infusion of 015 nm of CRH (B, C). The onset of semirhythmic slow wave discharges, confined to amygdala leads (AMYG), is evident in B. They persisted intermittently for several hours (C). CORT = cortical lead; EMG = motion-detecting electrodes placed over the angle of the jaw. Vertical bar = 50  $\mu$ V; horizontal bar = 1 second.

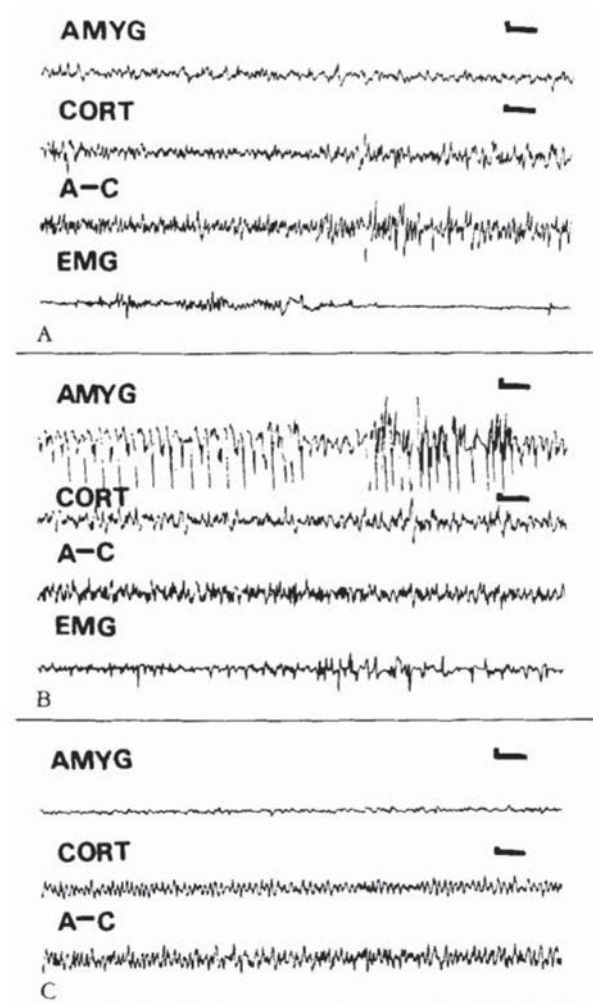


**Fig 3.**

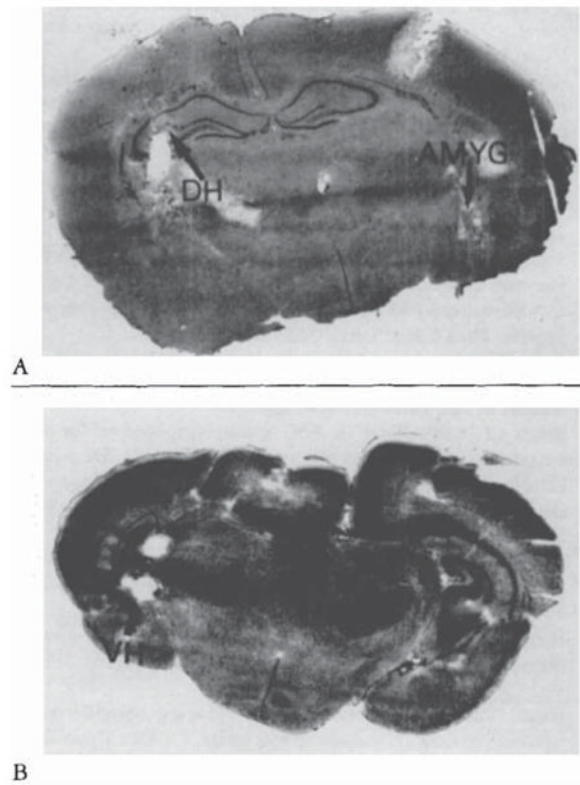
(A, B) Electroencephalograms (EEGs) of an 8-day-old infant rat, carrying both bipolar amygdala (AMYG) and ventral hippocampus (VH) electrodes, before administration of corticotropin-releasing hormone (CRH) (A) and 8 minutes after administration of 0.15 nm of the peptide (B). Paroxysmal discharges originate in the amygdala leads. Movement artifacts are later seen in all leads. (C, D) EEGs of an 11-day-old rat before (C) and 26 minutes subsequent to the administration of 0.15 nm of CRH. Though the pup displayed jaw myoclonus, only attenuation of the theta rhythm is seen in the ventral hippocampus (VH). CORT = cortex; AMYG-CORT/VH-CORT = a lead combining one of the subcortical electrodes to the cortical one. Vertical bar = 50  $\mu$ V; horizontal bar = 1 second.



**Fig 4.** Electroencephalograms of a 13-day-old rat. (A) Baseline. (B, C, and D) Two, 35, and 174 minutes after intracerebro-ventricular administration of 0.15 nm of corticotropin-releasing hormone. High-voltage, repetitive discharges are present exclusively in bipolar amygdala (AMYG) and amygdala-cortex (A-C) leads, and not in bipolar hippocampal (DH), DH-cortex (DH-C), or the motion-detecting (EMG) leads. Vertical bar = 50  $\mu$ V; horizontal bar = 1 second.



**Fig 5.** Electroencephalograms (EEGs) of an 18-day-old rat. (A) Baseline. (B, C) Nine and 143 minutes after intracerebroventricular administration of corticotropin-releasing hormone (0.3 nm). High-voltage spike and slow wave discharges confined to the amygdala (latency, 4 minutes) (B). Two hours later, amygdala EEG has normalized (C). AMYG = amygdala; CORT = cortex; A-C = amygdala-cortex lead; EMG = motion-detecting electrode (see text). Vertical bar = 50  $\mu$ V; horizontal bar = 1 second.



**Fig 6.** Histological verification of electrode placement in subcortical structures of the infant rat, Cresyl blue–stained, 20- $\mu$ m-thick coronal sections. (A) Amygdala (AMYG) and dorsal hippocampus (DH) (B) Ventral hippocampus (VH).

**Table 1**

## Age-Specific Basal-Lateral Amygdala Coordinates

Age (days)	AP	Lat	V
4-5	-1.5	3.5	6.0
7-9	-1.5	3.6	8.0
10-12	-1.5	3.6	8.0
15-17	-1.5	3.6	8.5

AP = anteroposterior; Lat = lateral; V = ventral.

**Table 2**

Age-Specific Coordinates for Hippocampal Electrodes

Age (days)	Dorsal Hippocampus		Ventral Hippocampus	
	AP	Lat	V	Lat
5-7	-1.5	1.6	2.8	3.5
9-12	-1.5	1.7	3.0	4.2
13-18	-2.0	2.0	3.0	4.5

AP = anteroposterior; Lat = lateral; V = ventral.

Table 3

CRH-induced Seizures: Latency, Peptide Doses, and Age of Rat Pups

Age (days)	n	CRH (nmol)	n	Latency (min)	Duration (hr)	Electrode Location
5-6	10	0.038	2	9.0 ± 1.0 <sup>a</sup>	>5;2	A:A
		0.075	2	5.5 ± 0.7 <sup>a</sup>	5;>5	A:A
		0.15	6	4.9 ± 1.5 <sup>a</sup>	2 to >5	2A;2DH;2VH
8-10	13	0.15	11	5.9 ± 1.5 <sup>a</sup>	all >3	6A;2VH;1DH;2 (A + VH)
		0.30	2	3;5	>4	A + VH;A
11-15	13	0.08	1	N/A <sup>b</sup>	3	A
		0.15	10	3.8 ± 0.5 <sup>a</sup>	1 to >4	4A;2(A + DH); 1VH;3(A + VH)
		0.22	1	N/A <sup>b</sup>	>4	A
		0.30	1	<2	3	A
16-22	6	0.15	1	16	2	A
		0.22	1	10	<2	A + VH
		0.3	3	4.0 ± 1.7 <sup>a</sup>	2;2;3	DH;A:A
		0.75	1	2	2.7	DH

<sup>a</sup>The mean plus the standard error of the mean.<sup>b</sup>Not available, animal restrained to control for motion artifact (see Materials and Methods)

CRH = corticotropin-releasing hormone; n = number of rats; A = amygdala; DH = dorsal hippocampus; VH = ventral hippocampus; A + VH, A + DH = simultaneous amygdala and hippocampal electrodes.



**Table 4****Behavioral and EEG Characteristics of CRH-induced Seizures: Age Dependence**

Age (days)	Behavior	Electrographic Correlates
5–6	JM; forepaw grooming and clonus; tonus; LOB	Amygdala; semirhythmic spikes (4/6); fast activity (2/6)
8–10	JM; semirhythmic fore paw clonus (run); LOB	Rhythmic slow wave or spike wave; rare cortical discharge
11–14	JM; grooming, scratching; “swimming”; LOB	Amygdala rhythmic spike wave; rare H or cortex sharp waves
16–22	JM; grooming; rare LOB or “major” seizures	Similar to 11- to 14-day-old group; higher voltage

EEG = electroencephalography, CRH = corticotropin-releasing hormone, JM = jaw myoclonus manifesting as vigorous, persistent, and unstoppable licking and chewing; LOB = loss of balance; H = hippocampus.