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7. An analysis of variance for two repeated measures (three choice orders by delay or no delay) (Fig. 1A) revealed a significant effect due to choice order [$F(1, 15) = 8.1$, $P < .01$], delay versus no delay [$F(1, 15) = 19.3$, $P < .001$], and choice order by delay interaction [$F(2, 15) = 3.5$, $P < .05$]. Subsequent Newman-Keuls tests indicated that (i) for the no-delay conditions, performance for the 1-2 and 7-8 choices was significantly better than that for the 4-5 choice ($P < .05$); (ii) for the delay condition, performance for 1-2 choice was significantly better than that for the 4-5 or 7-8 choice ($P < .05$); and (iii) only for the 7-8 choice was there a significant difference between delay and no-delay conditions ($P < .01$).
8. Under Nembutal anesthesia (45 mg per kilogram of body weight, injected intraperitoneally) animals received bilateral electrolytic lesions of the dorsal hippocampus. Insect pins (size 00) insulated with Epoxilite except for 1 mm at the tip were used as electrodes for making the lesions. Direct current (3 mA) was administered for 30 seconds with respect to a cathodal reference electrode in the rectum of the rat. Stereotaxic coordinates, based on a level skull between bregma and lambda and with depths relative to dura, were 4.0 mm posterior, ±1.4 and ±2.8 mm lateral, and 3.6 mm ventral. Animals were allowed 7 to 10 days recovery from surgery before testing was begun.
9. An analysis of variance for two repeated measures (three choice orders by lesion or normal) (Fig. 1B) revealed a significant effect due to choice order [$F(2, 15) = 18.2$, $P < .01$], lesions versus normal [$F(1, 15) = 11.9$, $P < .01$], and choice order by lesion interaction [$F(2, 15) = 9.7$, $P < .01$]. Subsequent Newman-Keuls tests indicated that (i) for the hippocampal lesion condition, performance for the 7-8 choice was significantly better than performance for the 4-5 or 1-2 choice ($P < .01$), and (ii) the hippocampal lesion condition was significantly different from normal only at the 1-2 choice ($P < .01$).
10. An analysis of variance for two repeated measures (three choice orders by delay or no delay) (Fig. 1C) revealed a significant effect due to choice order [$F(1, 15) = 6.5$, $P < .05$], delay versus no delay [$F(1, 15) = 15.8$, $P < .01$], and choice order by delay interaction [$F(2, 15) = 4.0$, $P < .05$]. Subsequent Newman-Keuls tests indicated that (i) for the no-delay conditions, performance for the 7-8 choice was significantly better than that for the 1-2 and 4-5 choice ($P < .01$), (ii) for the delay conditions there were no significant differences, and (iii) only for the 7-8 choice was there a significant difference between delay and no-delay conditions ($P < .01$).
11. At the end of the experiment, the animals were anesthetized with Nembutal, heparinized, and perfused intracardially with 10 percent Formalin in isotonic saline. Brains were excised, frozen, cut in 50-μm sections through the lesion, and stained with cresyl violet.
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Postural Asymmetry and Movement Disorder After Unilateral Microinjection of Adrenocorticotropin 1-24 in Rat Brainstem

Abstract. *A unilateral microinjection of adrenocorticotropin 1-24 in the rat brainstem in the region of the locus ceruleus resulted in postural asymmetry and movement disorder that resembled human dystonia, the severity and duration (2 to 3 days) being dose-dependent. These results show for the first time that neuropeptides in the brainstem may modulate posture and movement, and they suggest that some forms of movement disorder such as dystonia may be due to a disordered regulation of postural and locomotor mechanisms by adrenocorticotropin 1-24.*

The discovery of neuropeptides in the brain suggested the possibility that these substances may exert long-lasting neuromodulatory influences in the brain. However, the precise nature of the neurophysiological functions of these neurohormones remained to be elucidated. We report here for the first time that adrenocorticotropin (ACTH) 1-24 and shorter ACTH fragments exert potent and long-lasting (2 to 3 days) actions on posture and locomotion after direct administration in the rat brainstem. These results suggest that ACTH may participate in the tonic regulation of central programs that control posture and locomotion (*1*). Moreover, certain movement disorders such as dystonia (*2*) may be due to a disturbance in this regulation.

Adult male albino rats (300 to 350 g) were implanted under chloral hydrate (360 mg per kilogram of body weight) anesthesia with a single 30-gauge stainless steel cannula aimed at a site (with half of the rats in the left, the others in the right) 2 mm dorsal to the locus ceruleus (LC) (*3*). After 1 week to allow recovery from the surgery, the rats were microinjected with ACTH 1-24, ACTH 4-10, or α-melanocyte-stimulating hormone [α-MSH or (Ac–Ser^1, Val^{13}–NH_2, ACTH 1-13)] (Table 1); we used a needle prepared from 35-gauge stainless steel tubing [see (*4*) for details] that extended 2 mm beyond the guide tips into the region of the LC. Peptides dissolved in sterile 0.9 percent NaCl solution (and freshly prepared on each day of use) were injected in a volume of 1 μl at the rate of 0.1 μl per 15 seconds. After the microinjection, the rats were placed in individual transparent plastic bins (30 cm in diameter and 60 cm high) for observation.

Microinjection of ACTH 1-24 resulted in an immediate onset of a dose-dependent postural asymmetry and motor disorder (Fig. 1) that resembled the human movement disorder of dystonia. The postural asymmetry was invariably ipsilateral to the microinjection side; if the microinjection was into the left, the animal showed a left-leaning posture, and if into the right, a right-leaning posture. (Bilateral administrations of an equimolar dose of ACTH 1-24 resulted in retrocollis, an arching of the back.) This was accompanied by a disruption in normal locomotion such that when the animal attempted to move, it would wobble, topple over backward, or slowly rotate laterally in a creeping stance. However, when placed on a vertical grid, the animal showed strong grip on all four paws. Moreover, there was no paralysis; all limbs showed brisk withdrawal reflexes when noxious stimuli (pinches) were applied. The fact that the placing and reaching reflexes were unimpaired confirmed that there was no gross sensory deficit. Robust righting reflexes were present, with the animal resisting being placed on its back or being placed on the side contralateral to the microinjection side.

The syndrome we are describing here is distinctly different from the "catatonia" or "waxy flexibility" reported by Jacquet and Marks (*5*) after microinjection of β-endorphin in the rat periaqueductal gray; in these states, the rat could be molded into any position, however awkward, which the animal would then maintain for up to 1 hour. The syndrome reported here also differs from "barrel rotation," that is, brief (10-minute) episodes of rapid rotation along the longitudinal axis that was sometimes observed after intracerebral administrations of various neuropeptides (*6*). In some animals, these dystonic episodes were observed to last longer than 2 to 3 days. Mild stress (handling) appeared to exacerbate these episodes. In the acute phase of this episode, the animal often gave the appearance of being slowly pulled up on one side, with the result in some cases of toppling over backwards. As if to prevent such movements, the animals sometimes adopted the strategy of wedging themselves against the wall of the bin on the ipsilateral side (to the microinjection) so tightly that this resulted in the

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Table 1. Percentage of occurrence and mean duration of postural asymmetry and movement disorder after unilateral microinjection of neuropeptides in the rat brainstem in the locus ceruleus region.

Neuropeptide	Dosage	*N*	Percentage	Mean duration (minutes)
ACTH 1-24	2.9×10^{-9} mole	4*	100	13
ACTH 1-24	8.5×10^{-9} mole	4*	100	25
ACTH 1-24	1.4×10^{-8} mole	4*	100	87
ACTH 1-24	2.9×10^{-8} mole	11	100	>210
α-MSH†	$(1.2 \text{ to } 1.4) \times 10^{-8}$ mole	9	44	31
ACTH 4-10	2.9×10^{-8} mole	9	89	40
Morphine sulfate	2.6×10^{-8} mole	6	0	0
Substance P	$(1.4 \text{ to } 2.9) \times 10^{-9}$ mole	8	0	0
Ethanol	1.0 μl	2	0	0
NaCl, 0.9 percent	1.0 μl	2	0	0

All rats were naïve, except those labeled, which overlapped in an ascending series. †At the limit of solubility.

forced closure of the eye on that side.

Microinjection of the shorter ACTH fragment, ACTH 4-10, resulted in a briefer episode of dystonia, whereas α-MSH also resulted in a brief dystonic action. Control microinjections of vehicle alone (1 μl of 0.9 percent NaCl), ethanol, or substance P (Table 1) had no effect on posture and movement, an indication that the dystonia was not due to the trauma of infusion, lesion, or nonspecific peptide effects. An equimolar microinjection of ACTH 1-24 in Charles River hypophysectomized rats resulted in a more severe and longer-lasting syndrome than in nonhypophysectomized rats. The incidence of toppling over backwards was more frequent and was seen as long as 2 days after the microinjection in two of the rats. Aphagia occurred in two of the five rats, and these animals died in a state of severe emaciation within 4 to 7 days. Thus, the absence of the pituitary appeared to potentiate the action of ACTH 1-24. It has been reported (*7*) that ACTH 1-24 displaces [^{3}H]dihydromorphine from binding sites in the rat brain; this result suggests an interaction with opiate receptors. However, in the present in vivo paradigm, morphine failed to give rise to any dystonic symptoms (Table 1). The opiate antagonist naloxone, given intraperitoneally at 0.1, 1.0, or 10.0 mg/kg immediately before or after the ACTH 1-24, failed to block or reverse the dystonia.

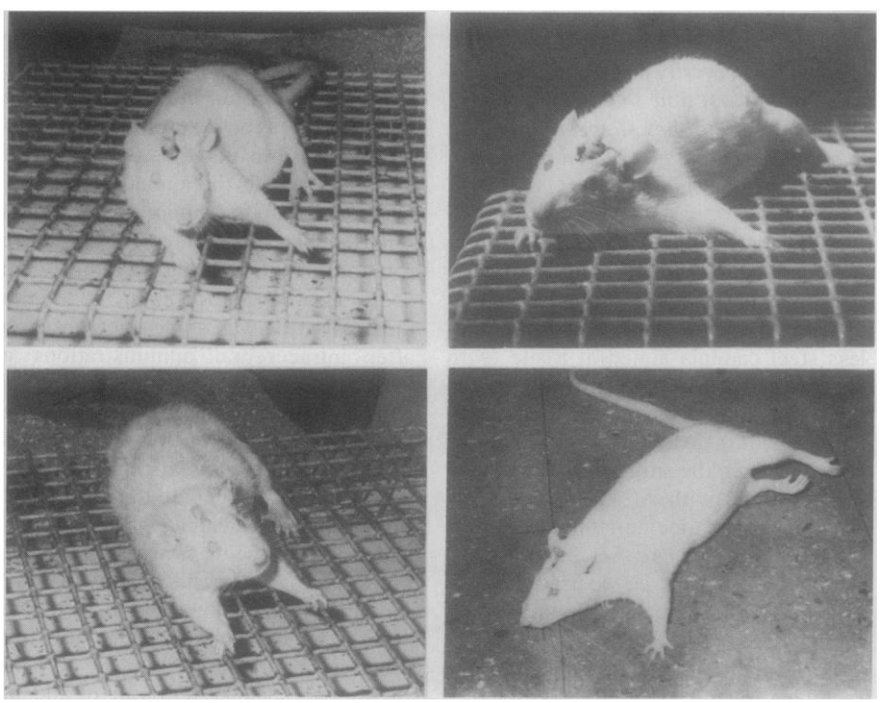

Fig. 1. Postural asymmetry after a unilateral microinjection of 2.9×10^{-8} mole of ACTH 1-24 into the region of the right locus ceruleus.

The dystonic action of ACTH 1-24 is a unique action that, to our knowledge, has never before been reported for any neuropeptide. Other distinctly different actions of ACTH fragments have been reported after intraventricular administrations (for example, stretching and yawning syndrome) (*8*), an indication that the effects reported here are site-specific. Preliminary histological analyses indicated that the microinjection sites were situated in the region of the LC. (Because of its small size, other adjacent sites cannot be ruled out. However, we do not know of any demonstrations of ACTH projections from the arcuate to the vestibular nuclei—an alternative central nervous system site that may possibly mediate the present action.)

In rats and primates, ACTH is found exclusively in cell bodies of the hypothalamic arcuate nucleus that send projections caudally to the brainstem, including the LC (*9*). The LC nucleus, located in the lateral region of the brainstem central gray, is composed primarily of neurons that produce norepinephrine (NE), accounting for almost half of the NE innervation in the mammalian brain (*10*). The LC gives rise to the dorsal NE pathway that innervates the cerebellum, hypothalamus, striatum, and neocortex (*11*). The LC has been suspected of playing a role in Parkinson's disease (*12*) and of regulating basic biological functions. Thus, it is reasonable to surmise that ACTH may participate in modulating posture and movement in this region of the brainstem.

The movement disorder that we report here is characterized by abnormalities in posture and locomotion in the absence of gross motor, sensory, or reflex deficits. The long duration of the effect after administration of ACTH 1-24 may provide insight into the underlying biochemical pathology of certain movement disorders such as human dystonia. The present paradigm provides a useful animal model for the study of these disorders. In addition, these observations point to a novel action of ACTH 1-24 in the central nervous system.

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Focal Cortical Seizures Cause Distant Thalamic Lesions

Abstract. *Topical application of convulsants to the rat sensorimotor cortex in concentrations sufficient to cause repetitive focal motor seizures resulted in acute neuropathology (dark cell neuronal degeneration and spongiform neuropil changes) involving both the cortical seizure focus and certain thalamic nuclei within seizure pathways. Changes in the cortex were localized primarily in layer IV and those in the thalamus in nuclei having reciprocal connections with the cortical focus. The spongiform neuropil changes consisted of massively dilated presynaptic axon terminals in the cortex and postsynaptic dendrites in the thalamus. The dendritic and dark cell changes resemble the excitotoxic damage caused by glutamate and aspartate. Since these putative transmitters may be released locally from recurrent collaterals and remotely from corticothalamic axons, excessive release of glutamate or aspartate may account for the changes in both sites. The abnormal axons in sensory cortex appear to be terminals of thalamocortical neurons. Swelling of these axons may be caused by excessive anti- and orthodromic firing in the course of focal motor seizures.*

Focal epilepsy in man is the clinical expression of a pathological lesion of neocortex causing excessive neuronal firing through local and long circuits. Early studies in which depth electrodes were used to trace seizure spread have recently been extended with [^{14}C]deoxyglucose autoradiography (*1*). During convulsions, there is a marked increase in metabolism in seizure pathways in subcortical sites. In thalamus, in particular, a neocortical focus can cause both orthodromic and antidromic activation of thalamocortical relay (TCR) cells (*2*). Changes in neuronal firing rate, increases in extracellular potassium, and changes in glucose and amino acid metabolism can be as intense in the thalamus as in the neocortical focus (*1*, *3*).

Pathological studies of experimental focal epilepsy have emphasized changes in cortex, particularly the swelling of processes identified as dendrites (*4*). Experimental seizures induced in the limbic system by convulsants or repetitive electrical stimulation are associated with acute lesions distant from the focus (*5*). Distant lesions, or "double pathology" occur in human temporal lobe epilepsy and probably play a role in the expression of clinical convulsions (*6*). We undertook to assess neuropathological changes associated with focal sensorimotor seizures in local and distant seizure pathways.

Sprague-Dawley rats were anesthetized with halothane, and an epidural well was prepared over the forelimb sensorimotor cortex (*7*). After the animal recovered from anesthesia, one of the following convulsants was infused into the well: 18 m*M* folic acid, 100 m*M* sodium penicillin, 2.5 m*M* bicuculline methiodide, or 0.3 m*M* picrotoxin. The concentrations were chosen to cause an equivalent state of repetitive focal seizures. In control rats, the well was infused with vehicle. At the end of a seizure period (2 hours or more), animals were killed with barbiturate, and brains were fixed in situ by perfusion with 1.5 percent glutaraldehyde and 1 percent paraformaldehyde buffered with phosphate (*p*H 7.3 to 7.4) for light and electron microscopy (*8*).

Animals were alert and freely mobile during focal cortical discharges (Fig. 1a). Concomitant jerking of the contralateral forelimb did not interrupt ventilation. Animals displaying brief recurrent seizures for longer than 2 hours had characteristic lesions in the cortex and thalamus (Figs. 1b and 2).

Histological examination of the cortex revealed two patterns of damage. Superficial laminae (layers I to III) of the motor cortex and the sensory cortex beneath the well exhibited signs of injury, including swelling of cell bodies and processes of both glia and neurons, scattered dark neurons, and edema of layer I. Similar, but milder, changes were observed in controls, suggesting an artifact resulting from surgical trauma. The other pattern occurred only in animals with recurrent seizures and consisted of conspicuous dark cell and spongiform changes mainly in layer IV of the adjacent forelimb sensory cortex (Fig. 2a),

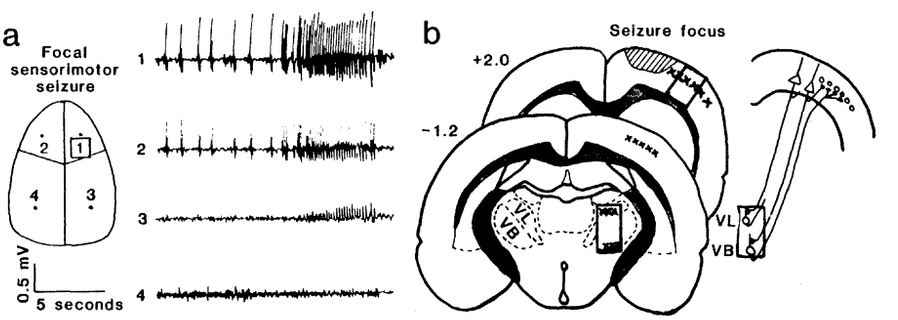

Fig. 1. (a) The site and relative size of the epidural well (1) over the forelimb sensorimotor cortex is indicated on a dorsal view of the rat skull. Wire electrodes were inserted into the epidural space at the focus, contralateral homotopic cortex (2), and bilateral occipital cortices (3 and 4). A characteristic electroencephalogram shows spikes and short seizures (one every 1 to 5 minutes) with 2.5 m*M* bicuculline methiodide in the epidural well. This general type of pattern occurred with each convulsant. (b) Neuropathological changes (xxx) occurred principally in middle cortical layers within the posterolateral extension of the focus and in subcortical terminal fields having a reciprocal relation with the focus. Numbers indicate approximate anteroposterior coordinates relative to bregma. Abbreviations: *VB*, ventral-basal complex; *VL*, ventralis lateralis.

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