Chronic Hypothalamic Stimulation in Awake Rats Fails to Induce Hypertension

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SUMMARY Daily electrical stimulation of the posterior hypothalamus for 12 weeks in awake rats caused persistent cardioacceleration but barely increased systolic pressure. Subsequent postmortem examination showed extensive fibrosis at sites of electrode implantation in both stimulated and unstimulated rats. Because Folkow and Rubinstein had succeeded in elevating blood pressure progressively by stimulating the “hypothalamic defence area,” chronic stimulation was repeated following their stereotaxic coordinates. Systolic pressure rose but the elevation was significant only on Weeks 8 and 10. To maintain behavioral effects during chronic stimulation, current strengths had to be increased periodically, and stimulated rats gained weight more rapidly than unstimulated controls. Both these findings indicated that electrical stimulation had damaged the brain, but since local fibrosis made histologic verification difficult, additional experiments were done to determine if functional deficits could be detected. Upon further hypothalamic stimulation, electrical thresholds were higher and pressor and sympathetic nerve responses were smaller in rats that had been stimulated chronically than in those that had not. Although our results do not disprove the hypothesis that centrally-induced sympathetic hyperactivity initiates hypertension, they show that blood pressure cannot be elevated by hypothalamic stimulation alone when the brain has been injured.

(Hypertension 1: 498-507, 1979)

UNBIASED testing of the hypothesis that sympathetic hyperactivity caused by repeated emotional or mental stress can initiate hypertension, requires prolonged exposure of animals to some form of stress-induced sympathetic hyperactivity. Most forms like pain,1 aversive operant conditioning,2,3 or psychosocial stimuli4-6 cannot be quantified and consequently, their cardiovascular effects are difficult to assess. On the same basis, Folkow and Rubinstein’s report in 19667 that chronic electrical stimulation of the “hypothalamic defence area” elevates blood pressure progressively in awake rats, constitutes almost irrefutable proof because electrical currents applied to the brain can be regulated precisely. And yet, this crucial observation though often cited, has never been confirmed. The area stimulated was not identified and it has been alluded to variably as the anterior7 or posterolateral8 hypothalamus. Furthermore, because technical difficulties greatly reduced the number of completed experiments, their results can be considered only preliminary. By extrapolating from data in anesthetized rats, they assumed that similar pressor and tachycardic effects also occurred in awake animals, but what was actually happening during chronic stimulation was unknown since tail-cuff pressures were measured only once a week. We recently found that even though pressor responses (recorded directly from indwelling aortic catheters) could be elicited for hours upon repeated stimulation of the posterior hypothalamus, basal pressures remained essentially unaltered.9 Nonetheless, eventual development of hypertension still seemed likely because the original elevation reported was not immediate, but was delayed for more than 8 weeks.

The present studies first intended to determine
whether sustained elevation of blood pressure could be brought about by chronic stimulation of the posterior hypothalamus. After finding that systolic pressures rose only transiently, chronic stimulation was repeated using the same rat strain and stereotaxic coordinates that Folkow and Rubinstein had used. Current strengths had to be increased gradually to elicit the same behavioral effects, thereby suggesting that stimulation sites had been damaged. Since fibrotic changes around the chronically implanted electrodes precluded histologic confirmation of neural damage, electrical thresholds as well as cardiovascular and neural responses to further stimulation were measured in rats that had been stimulated chronically and then compared with similar values from unstimulated controls.

Methods

Female Wistar (Hilltop Lab Animals Inc., Scottsdale, PA) or Sprague-Dawley (Charles River Breeding Laboratories, Wilmington, MA) rats, weighing 200-300 g, were used.

Chronic Hypothalamic Stimulation in Unanesthetized Rats

Two series of chronic stimulations (first on Wistar and then on Sprague-Dawley rats), each lasting for 12 weeks, were done. Rats were anesthetized with amobarbital sodium (7-10 mg/100 g I.P.) and concentric stainless steel electrodes with lateral pole (exposed 0.5 mm length \times 0.5 mm diameter) separated from the tip (exposed 0.5 mm length \times 0.2 mm diameter) by 0.5 mm (NE-100; custom made with chronic connectors by Rhodes Medical Instruments, Woodland Hills, CA), were placed in the hypothalamus following the stereotaxic planes defined by de Groot. Coordinates used for the posterior hypothalamus were: anteroposterior 4.6, lateral 1.0, and dorsoventral -2.5. Those for the area stimulated by Folkow and Rubinstein (hereafter referred to as the anterior hypothalamus) were: anteroposterior 6.0, lateral 1.2, and dorsoventral -2.5. Electrodes implanted 1 week before chronic stimulation started were fixed to the skull with stainless steel screws and dental cement.

Awake rats were stimulated in groups of four (i.e., one group per stimulator) using a timer switch with a single input from the stimulator and four separate outputs, each connected to individual rats (fig. 1A); each rat was then connected for 15 sec/min (e.g., the stimulator was connected from 0 to 15 seconds to the first rat, from 16 to 30 seconds to the second, etc.). Train duration was kept constant at 10 seconds, while unidirectional currents of 35 to 70 \mu A were pulsed into each rat 12-16 hours daily (from 5:00 p.m. until 5:00 or 9:00 a.m. the next day) for 12 weeks. Desired current strengths (frequency 100 cps, pulse duration 1 msec) were delivered by a square-wave stimulator (Grass S-48 or S-88 with constant current units). During stimulation, rats were individually confined in round cages whose tops were covered with copper mesh screen; a harness wrapped around the rat's chest was attached by a steel spring to a slip-ring swivel (Airflyte Electronics, Bayonne, NJ, or BRS/LVE, Beltsville, MD) placed above the cage. Wires inserted in the steel spring (fig. 1B) connected the hypothalamic electrodes through the swivel and timer-switch output, to the stimulator. Control rats were similarly caged and harnessed but electrode cables were not connected to the stimulator. Every morning, at the end of each day's stimulation, harnesses were detached and rats were transferred to regular individual cages kept in the same room; food and water were always available ad libitum during and between daily stimulations.

Systolic pressure and heart rate were recorded once or twice a week with a tail-cuff method previously validated for use in awake rats. Blood flow changes occurring in the tail during cuff deflation were detected with a Doppler ultrasonic flowmeter whose audio output was simultaneously monitored with headphones and superimposed on the cuff pressure tracing for graphic recording. To accustom rats to conditions required for measurement, they were trained for 1-2 weeks before actual measurements began. Daily training included confinement first in a heating box for 10-15 minutes at 39°C and then in a restrainer (baseplate heated electrically to 38°C) for 5-10 minutes while an inflatable cuff and flow probe were attached to the tail. The cuff was automatically inflated and deflated, and after 6-8 training sessions, most rats remained relatively immobile for several inflation-deflation cycles. Daily values for systolic blood pressure were taken once or twice a week.
pressure were averaged from at least three readings. Heart rates were calculated by multiplying the number of tail-cuff pulses in 6 seconds by 10.

On the eighth week of chronic stimulation in Sprague-Dawley rats (second series mentioned in Results), food and water allotted to each rat was measured at the beginning and end of three successive 24-hour periods.

Terminal Experiments on Sprague-Dawley Rats

Stimulated Chronically

After 12 weeks of stimulation, rats were anesthetized with amobarbital and indwelling catheters were implanted in a jugular vein and the lower abdominal aorta. One day later, each awake rat was connected to the harness-and-swivel arrangement for continuous recording of blood pressure by attaching the indwelling aortic catheter through Tygon tubing inside the flexible steel spring to a pressure transducer (Statham P23Gb) located beside the cannular swivel. A correction factor (obtained by dividing the height of the fluid column by the ratio between the densities of mercury and water) was added to the transducer calibration to compensate for hydrostatic pressure resulting from the difference in height between the transducer and the rat. Aside from phasic and mean aortic pressures, heart rates were recorded simultaneously by triggering a biotachometer with the phasic pressure signal from the transducer. Graded currents from a square-wave stimulator were then pulsed through connecting cables to the chronically implanted electrode.

Subsequently, rats were anesthetized with urethane (0.1 g/100 g I.P.) and bipolar electrodes were placed on abdominal sympathetic nerves as described by Takeda and Buñag. Nerves and electrode tips were immersed in mineral oil to reduce tissue drying. Spike potentials were amplified (Grass P15 AC amplifier) and monitored on a storage oscilloscope (Tektronix 5111); to reduce noise, spontaneous respiratory movements were abolished by paralyzing skeletal muscles with decamethionium bromide (Syncurine, 0.2 mg/100 g I.V.) and connecting the rats to a respirator ventilated with a mixture of 50% oxygen and 50% nitrogen. Analog signals for aortic pressure and nerve activity were recorded continuously on magnetic tape while the hypothalamus was stimulated with graded currents. For quantifying nerve activity, original analog signals were played back from tape into an ink-writing recorder and simultaneously fed into an amplitude analyzer (F. Haer and Co., Brunswick, ME) to convert individual spikes into uniform pulses and delete background noise. Since residual activity remaining after ganglion blockade with pentolinium was the same as that obtained after crushing the nerve, the low-level control of the window discriminator was routinely set to filter background noise persisting after pentolinium injection. The number of individual pulses per second were counted with a rate analyzer (F. Haer and Co.) whose output was recorded separately as a histogram, digitized by a computer interface, and the output printed by a programmed calculator (Monroe 1860). Integrated nerve activity before and during hypothalamic stimulation was counted as the number of spikes for 3 seconds. Two drugs were routinely injected through a jugular vein catheter: pentolinium tartrate (Andolysen), 0.5 mg (salt)/100 g; and norepinephrine bitartrate (Levophed), 200 and 400 ng (base)/100 g. Cardiovascular and neural effects of hypothalamic stimulation were both abolished by the dose of pentolinium used.

After each experiment, a perfusion needle was inserted through a thoracotomy into the ascending aorta via the left ventricle and the rats were killed with an overdose of pentobarbital; 10% formalin was then perfused into the brain. Electrodes that had been chronically implanted were removed and checked for electrical continuity; functional electrodes had a resistance of less than 50 Kohm. Excised brains were sectioned transversely at 40-μ intervals with a cryotome and location of electrode tips determined by comparison of unstained sections with the atlas by Pellegrino and Cushman. Brains from Wistar rats in the first series, whether stimulated or not, invariably showed extensive fibrosis at electrode sites which made histologic differentiation between stimulated and unstimulated rats difficult. Because of this, before most rats in the second series were killed, a small DC current was passed to facilitate histological localization of the electrode tip. For posterior hypothalamic placements, most electrode tips were found immediately medial to the mamillothalamic tract; adjacent structures included the posterior hypothalamic nucleus, mamillothalamic and mammillotegmental tracts, dorsal premamillary and medial mamillary nuclei, fornix, medial forebrain bundle, and lateral hypothalamic area. For anterior hypothalamic placements, electrode tips were found lateral to the ventromedial hypothalamic nucleus or anterior hypothalamic area.

Other Acute Experiments

Pressor responses to infused norepinephrine were quantified by simultaneous recording of blood pressure from indwelling carotid catheters and with the tail-cuff method in trained awake rats. A syringe pump was used to infuse appropriate concentrations of norepinephrine intravenously at a rate of 0.18–0.36 ml/min for 2–6 minutes. Tail-cuff measurements were taken intermittently while phasic pressure was recorded continuously by connecting an indwelling carotid cannula with Tygon tubing inserted through a hole on the rat restrainer, to a pressure transducer. With the rat standing upright on its four feet inside the restrainer, the transducer for recording carotid pressure was on the same level as the rat's heart; the horizontal plane for the rat's tail was always about 15 mm lower than that for the heart.

To compare the cardiovascular effects of posterior hypothalamic stimulation with those of anterior hypothalamic stimulation, rats were anesthetized with
sodium pentobarbital (4 mg/100 g I.P.) and aortic pressure was recorded from an indwelling catheter. The same electrode was first used to stimulate one site, then withdrawn and re-inserted for stimulation of the second site in each rat.

Statistics

Data are expressed as averages ± SEM. Correlation coefficients and t tests comparing means of dependent or independent samples were used and differences at a 5% level (p < 0.05) were considered significant.

Results

Chronic Stimulation of the Posterior Hypothalamus in Wistar Rats

After baselines for both systolic pressure and heart rate had become stable, electrodes were implanted chronically into the posterior hypothalamus in eight normotensive Wistar rats. Initial tests were then performed to determine the relationship between stimulus strength and intensity of behavioral responses evoked while the rats were awake. As reported previously, when stimulus strength was graded by increasing either train duration or amount of current applied, four phases of progressively intensifying behavior could be identified. Five-second trains of 35 µA consistently elicited the first two phases of arousal: in Phase I the rats sniffed and looked around without body movement, while in Phase II they ran around in circles and looked up frequently. Doubling train duration of 35 µA to 10 seconds did not alter behavior in three rats, but elicited Phase III responses in the other five; during this phase the rats stood up on both hindlegs with one or both forelegs resting on the sides of the cage. Finally, Phase IV behavior consisting of attempts to escape or climb out of the cage was elicited in all rats by applying 10-second trains of 40 to 50 µA. Four of the five rats that showed Phase III responses to 10-second trains of 35 µA were selected for chronic stimulation, while the other four served as unstimulated controls.

All rats including unstimulated controls that were not connected to the stimulator were placed individually in round cages and harnessed 16 hours each day for 13 successive weeks. Intensity of behavioral responses diminished spontaneously during stimulation with constant current strengths so that although stimulation began with 35 µA, this was increased by 5-µA increments on Days 4, 13, 24, 33, 42, 69, and 73, until a total of 70 µA was reached during the last 10 days. Systolic pressure occasionally rose in stimulated rats during the 12-week period, but the increases were never sustained; there were no significant differences before (Weeks 1, 2, and 3) and during the first 4 weeks of stimulation, but on Weeks 5, 6, 10, and 13 (Table 1) averages were significantly higher in stimulated than in unstimulated rats. Heart rates, although also almost equal initially, were often higher in stimulated than in unstimulated rats during the stimulation period (all differences were significant except for Weeks 5, 11, and 13 in table 1).

Because rats were removed from the harnesses at 9:00 a.m., by the time pressures were measured in the afternoon, even rats in the stimulated group would have been disconnected from the stimulator for at least 7 hours, and it seemed possible that larger differences may have occurred earlier in the day. Accordingly, similar measurements were taken every morning immediately after the rats were unharnessed. As anticipated, pressures and heart rates of stimulated rats were almost invariably higher in the morning than in the afternoon, but even at the higher morning levels, increases in stimulated rats were significant only on Weeks 5, 7, 8, 9, 10, and 12 for heart rate, and only on weeks 9, 10, and 12 for systolic pressure. Regardless therefore, of when measurements were made, increases in blood pressure occurred sporadically, but were not maintained.

Comparison of Small Carotid and Tail-cuff Pressures During Small Pressor Responses in Awake Rats

Since precise detection of small changes in blood pressure, such as those expected during development of hypertension, would depend entirely on tail-cuff method sensitivity, and although our method could measure increases ranging from 17 to 33 mm Hg accurately, its sensitivity for smaller changes was uncertain. To study this, blood pressures were elevated in nine awake rats whose systolic pressures were raised by infusing norepinephrine (150-200 ng/rat/min I.V.) in two rats whose systolic pressures were

| Table 1. Systolic Pressures and Heart Rates (average ± sem) before and during Posterior Hypothalamic Stimulation in Normotensive Wistar Rats |
|-----------------|-----------------|-----------------|
| Systolic pressure (mm Hg) | Heart rate (per min) |
| Week | Unstimulated | Stimulated | Unstimulated | Stimulated |
| -3 | 123 ± 6 | 124 ± 3 | 349 ± 6 | 351 ± 6 |
| -2 | 130 ± 4 | 123 ± 3 | 343 ± 8 | 349 ± 6 |
| -1 | 130 ± 4 | 132 ± 4 | 335 ± 8 | 339 ± 9 |
| 1 | 129 ± 2 | 128 ± 3 | 359 ± 9 | 367 ± 7* |
| 2 | 121 ± 3 | 122 ± 5 | 352 ± 9 | 388 ± 4* |
| 3 | 128 ± 4 | 120 ± 4 | 352 ± 4 | 368 ± 5* |
| 4 | 128 ± 3 | 126 ± 3 | 355 ± 7 | 370 ± 6* |
| 5 | 121 ± 3 | 130 ± 3* | 359 ± 10 | 376 ± 10 |
| 6 | 119 ± 3 | 132 ± 5* | 342 ± 7 | 364 ± 7* |
| 7 | 126 ± 3 | 132 ± 3 | 339 ± 10 | 369 ± 7* |
| 8 | 119 ± 4 | 128 ± 5 | 324 ± 7 | 360 ± 7* |
| 9 | 119 ± 3 | 129 ± 4 | 332 ± 9 | 367 ± 9* |
| 10 | 120 ± 2 | 132 ± 4* | 335 ± 8 | 367 ± 7* |
| 11 | 119 ± 5 | 122 ± 8 | 346 ± 9 | 360 ± 7 |
| 12 | 123 ± 6 | 125 ± 8 | 322 ± 9 | 350 ± 8* |
| 13 | 128 ± 4 | 138 ± 4* | 333 ± 12 | 362 ± 12 |

*p < 0.05 when compared with corresponding average for unstimulated rats.
recorded from carotid catheters and with the tail-cuff method simultaneously. Before infusion, systolic pressures were almost the same, averaging 134 ± 1 in the carotid artery and 128 ± 2 with the tail-cuff method (p > 0.1). Upon infusing norepinephrine, pressure increases measured with both methods (as determined from 107 pairs of concurrent measurements) correlated significantly (r = 0.82; p < 0.01) and were identical (15 ± 1 in the carotid artery vs 15 ± 2 with the tail-cuff method; p > 0.1). However, if only pressor responses of 10 mm Hg or less (n = 35) are compared, average increases in the carotid artery of 6 ± 0.5 were significantly smaller (p < 0.005) than those obtained with the tail-cuff method of 9 ± 1. These smaller responses no longer correlated well (r = 0.21; 5% significance requiring an r of at least 0.325) and their intercept of 6.2 implies that even when carotid pressures were unaltered, increases of as much as 6 mm Hg could be obtained falsely with the tail-cuff method. Thus, in testing the null hypothesis,14 we were more apt to err by seeing nonexistent pressure increases rather than in failing to detect real ones, and because we could measure even small increases in systolic pressure, it is unlikely that any persisting pressor effects of hypothalamic stimulation could have gone undetected.

**Chronic Hypothalamic Stimulation in Sprague-Dawley Rats**

The possibility that neuronal pools in the posterior hypothalamus differ from those in the anterior hypothalamus was first explored by stimulating both hypothalamic areas in rats anesthetized with pentobarbital. In six rats, stimulation of either site invariably increased mean aortic pressure from an average baseline of 99 ± 3 mm Hg. Pressor responses were often biphasic with magnitude of both primary and secondary phases being significantly larger in response to stimulation of the anterior than of the posterior site (table 2). Instead of the tachycardia usually seen in awake rats, heart rates fell from an average baseline of 331 ± 17 beats/min upon stimulation of either site. This reversal was probably caused by anesthesia, since a similar change occurs with amobarbital anesthesia.19 Bradycardia was also more pronounced with anterior site stimulation but because of wide individual variations, only that accompanying the primary pressor response to 50 μA was significant (table 2). These results indicate that the cardiovascular effects of stimulating the anterior site, though qualitatively similar, were stronger than those of the posterior site. Based on this finding the anterior site was stimulated chronically in succeeding experiments.

Electrodes were implanted chronically at the anterior hypothalamic site in 14 Sprague-Dawley rats, of which seven were randomly assigned to the stimulation group while the others were designated as unstimulated controls. After baselines for systolic pressure and heart rate had stabilized, daily stimulation began with 10-second trains of 35 μA, which were increased by 5-μA increments on Days 7, 14, 21, 49, 63, 70, 77, and 84 to a final current strength of 85–95 μA. Whereas averages for heart rate did not differ at any time, those for systolic pressure though initially similar were often higher in stimulated than in unstimulated rats during the latter weeks of stimulation; however, only the differences on Weeks 8 and 10 were statistically significant (table 3).

Body weights in both groups were initially identical (186 ± 2 g for unstimulated and 187 ± 2 for stimulated rats), but starting on the third week and throughout the rest of the 12-week period, stimulated rats were appreciably heavier (323 ± 11 g on Week 12; p < 0.001) than unstimulated ones (267 ± 5 g on Week 12). To determine if this difference was caused by increased food or fluid consumption, food and water intake were measured during the eighth week of stimulation. Three-day averages for drinking did not differ (44 ± 4 ml for unstimulated and 48 ± 3 ml for stimulated rats; p > 0.5), but stimulated rats ate much more (29 ± 2 g/day) than did the unstimulated ones (22 ± 1; p < 0.005). Since this increase would be expected with lesions of the ventromedial hypothalamus,20 it is suggestive of brain damage. Chronically implanted electrodes cause capsules of compact fibrous tissue with scattered lymphocytic infiltrates to form within 18–26 days in both stimulated and control animals,21 and this occurred in the preceding series of Wistar rats after electrodes had been implanted for 13–14 weeks. Under such conditions, neural damage produced by electrical stimulation can be assessed by using specialized stains21 for both light and electron microscopy.22 But even when signs of structural damage are absent, functional deficits may still occur.23,24 Instead of attempting the usual histologic verification, terminal experiments were done to determine if rats that had been stimulated chronically would show any functional deficiencies in response to further hypothalamic stimulation.

**Neural and Cardiovascular Reactivity in Sprague-Dawley Rats Following 12 Weeks of Chronic Stimulation**

Cardiovascular effects of pulsing currents through hypothalamic electrodes implanted 13 weeks earlier were recorded while the rats were awake through aortic catheters implanted 1 day before testing. Some experiments could not be completed due to various technical difficulties and results are therefore summarized from only four rats in the unstimulated and six others in the stimulated group. As in preceding experiments, behavioral and motor responses intensified gradually as strength of stimulating currents was increased. Threshold currents required to elicit a given response differed greatly between groups in that rats which had been stimulated chronically always required stronger currents than unstimulated controls; however, because each group consisted of only a few rats and currents required for stimulation varied widely from rat to rat, differences, although apparently large, were not statistically significant. Baselines for mean aortic pressure averaged 103 ± 4
mm Hg in unstimulated rats and 109 ± 4 in stimulated ones. The strongest current last used for chronic stimulation (90 μA) increased mean aortic pressure by 8 ± 4 mm Hg in the stimulated group, but was too strong for testing in unstimulated controls. Early signs of Phase I arousal, which were unaccompanied by appreciable changes in pressure, required current strengths of 20 ± 9 μA in the unstimulated group as compared with 120 ± 70 μA for the stimulated group. Advanced Phase III behavior required currents of 56 ± 8 and increased aortic pressure by 27 ± 5 mm Hg in unstimulated rats. Induction of the same effects in stimulated rats required currents of 313 ± 194 (averaged from only three of five rats since maximal motor responses could not be elicited at all in the other two) and increased aortic pressure by 35 ± 6 mm Hg.

Diminished pressor responsiveness following prolonged hypothalamic stimulation could be due to loss of either neural or cardiovascular sensitivity. To discriminate between these possibilities, all rats were anesthetized with urethane and in addition to aortic blood pressure, sympathetic nerve activity was recorded. Unstimulated rats had average baselines of 87 ± 5 mm Hg for mean aortic pressure, and 25 ± 2 spikes/sec for sympathetic neural firing; corresponding averages for stimulated rats of 97 ± 5 and 26 ± 2, respectively, were not significantly different. Hypothalamic stimulation with graded currents invariably increased rate of neural firing as well as aortic blood pressure in both unstimulated (fig. 2) and stimulated (fig. 3) rats. Acceleration in rate of neural firing was usually faster in unstimulated than in stimulated rats at all the current strengths tested (fig. 4). Pressor responses to hypothalamic stimulation were also larger; for instance, stimulation with 100 μA increased mean pressure by 25 ± 3 mm Hg in unstimulated and by 7 ± 4 in stimulated rats (p < 0.025). By contrast, pressor responses to injected norepinephrine in the same rats were almost the same: those to doses of 200 ng/100 g averaged 22 ± 4 and 26 ± 3, respectively. Hence, chronically stimulated rats had markedly diminished neural and pressor responses to hypothalamic stimulation even while pressor responsiveness to injected norepinephrine was unchanged.

### Table 2. Cardiovascular Responses to Stimulation of Anterior and Posterior Hypothalamic Sites in the Same Anesthetized Rats

<table>
<thead>
<tr>
<th>Current (μA)</th>
<th>Response</th>
<th>Pressor response (mm Hg)</th>
<th>Bradycardia (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>phase</td>
<td>Anterior (AP 6.0)</td>
<td>Posterior (AP 4.6)</td>
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<tr>
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<td>primary</td>
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<td>100</td>
<td>primary</td>
<td>13 ± 2*</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>secondary</td>
<td>17 ± 5</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

*p <0.025 or less as compared with corresponding value for posterior site; numbers are average ± SEM changes from baselines in six rats as described in the text. AP = anteroposterior coordinate used for stereotaxic placement in the site specified.

### Table 3. Systolic Pressures and Heart Rates (average ± SEM) before and during Anterior Hypothalamic Stimulation in Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Systolic pressure (mm Hg)</th>
<th>Heart rate (per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstimulated</td>
<td>Stimulated</td>
</tr>
<tr>
<td>-3</td>
<td>134 ± 3</td>
<td>133 ± 3</td>
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<tr>
<td>-2</td>
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<td>132 ± 2</td>
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<tr>
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<td>2</td>
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</tr>
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<td>3</td>
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<td>124 ± 4</td>
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<tr>
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<td>8</td>
<td>118 ± 3</td>
<td>129 ± 3*</td>
</tr>
<tr>
<td>9</td>
<td>115 ± 4</td>
<td>125 ± 5</td>
</tr>
<tr>
<td>10</td>
<td>116 ± 3</td>
<td>128 ± 2*</td>
</tr>
<tr>
<td>11</td>
<td>137 ± 4</td>
<td>149 ± 9</td>
</tr>
<tr>
<td>12</td>
<td>133 ± 4</td>
<td>144 ± 5</td>
</tr>
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</table>

*p < 0.05 when compared with corresponding average for unstimulated rats.

### Discussion

The hypothalamic defense area, as commonly studied in cats, consists of circumscribed neuronal pools extending from the telencephalon to the mesencephalon, from which expressions of rage or aggression can be elicited by electrical stimulation, with the lowest thresholds concentrated in the perifornical region. Whether rats similarly manifest rage or aggression, or have the same anatomical boundaries for the hypothalamic defense area as cats, is debatable. Assuming that it does exist in rats, both the sites we stimulated are probably included because similar behavioral and cardiovascular effects could be elicited from either site. More frequent induction of tachycardia from the posterior hypothalamus, and conversely, the larger pressor responses from the
anterior hypothalamus, suggests the involvement of different neuronal pools. Despite these acute cardiovascular effects, however, because increases in systolic pressure produced by chronic stimulation at either site were slight and unsustained, we must conclude that hypertension did not occur in any of our experiments.

In light of Folkow and Rubinstein's previous success, an important question, therefore, is why we failed to induce a sustained elevation in blood pressure. Whether any procedure intended to induce hypertension in rats succeeds or fails depends almost entirely on tail-cuff method sensitivity inasmuch as pressure changes during the early development phase would be very small. Recognizing the inherent fallacy of testing such methods in anesthetized rats, our tail-cuff method was validated in awake rats under the same conditions existing during chronic measurements. We already knew its accuracy for pressor responses ranging in magnitude from 17 to 33 mm.
Hg,\textsuperscript{19} but since even smaller changes were initially expected to occur during chronic hypothalamic stimulation, further tests were done to establish its maximum sensitivity. If pressor responses to chronic hypothalamic stimulation resemble those produced by intravenously infused norepinephrine, our tail-cuff method would be capable of measuring pressure increases of 15 mm Hg accurately; however, for pressor responses of 10 mm Hg or less, it was likely to register falsely positive changes. On this basis, our failure to induce hypertension could not have been due to an inability to detect small changes in systolic pressure.

A more plausible explanation for the divergent results may be gleaned from methodological differences in rat selection or brain stimulation. Folkow and Rubinstein used rats of either sex and those showing stereotyped motor movements or behavior patterns incongruous with the defense reaction were excluded. By contrast, we used females exclusively and while only the most behaviorally responsive rats were stimulated in our first series, those in the second series were grouped randomly. Based on the direct relationship between intensity of behavioral responses and of corresponding cardiovascular effects elicited by posterior hypothalamic stimulation,\textsuperscript{9} it is conceivable that the rats they stimulated may have been more responsive than ours (i.e., larger pressor effects were produced during chronic stimulation in their rats) and this could account for some differences. Alternatively, although stainless steel electrodes were used in both studies, other electrode characteristics may have changed stimulus parameters considerably. Whereas Folkow and Rubinstein used a single electrode with a 0.5-mm bare tip, we used a concentric electrode whose two poles were 0.5 mm apart. Since neural lesions caused by ions diffusing from iron electrodes can be avoided by using cathodal currents\textsuperscript{28} (if we assume they pulsed mild cathodal currents into the brain by using the implanted monopolar electrode together with an indifferent electrode attached elsewhere) then it may explain how brain damage may have been minimized in their experiments.

The usual proof for neural damage is histological demonstration of abnormal structural changes, but since this was neither practical nor feasible here, we opted for functional evidence instead. Among our findings, those indicating that neural injury had been produced are the following: 1) to elicit equal behavioral responses, current strengths for chronic stimulation had to be increased periodically; 2) progressive weight gain and hyperphagia (indicative of damage to the ventromedial hypothalamus\textsuperscript{20}) occurred during stimulation of the anterior hypothalamus; 3) after 12 weeks of chronic stimulation electrical thresholds for eliciting behavioral and pressor effects in awake rats were much higher in the stimulated than in the unstimulated group; and 4) when the same rats were later anesthetized, the magnitude of pressor and sympathetic nerve responses to further stimulation was smaller in the stimulated than in the unstimulated group. Reduction of pressor responsiveness in chronically stimulated rats apparently does not involve a loss in cardiovascular sensitivity, since pressor responses to injected norepinephrine were unaltered. Nonetheless, if chronic hypothalamic stimulation is to be effective in inducing hypertension, aside from trying to minimize brain injury (i.e., by using biphasic currents or platinum electrodes\textsuperscript{33, 24, 30}) other pressor mechanisms may also need to be present. As proposed by the "mosaic theory,"\textsuperscript{190} sympathetic hyperactivity is probably just one of many factors that initiate hypertension, and in order to become an effective trigger it needs to be combined with other predisposing factors.

References

Editors’ Note:

The results reported in this manuscript are provocative in that they appear to be at variance with those reported by Folkow and Rubinstein in 1966. Accordingly, we asked Dr. Folkow to comment on the observations of Buňag and Riley. Because we feel that these comments are particularly pertinent in the interpretation of the data of Buňag and Riley, we are publishing them below.

The Editors

Comment

Having been asked by the Editors of Hypertension to comment upon the interesting paper by Buňag and Riley, the obvious key question is why their animals showed far more signs of hypothalamic lesions, and consequently far less sustained neurohormonal pressor responses to chronically repeated topical stimulations, than in the earlier study by Folkow and Rubinstein,¹ which Buňag and Riley tried to reproduce.

At first glance nearly identical procedures seem to have been used, but in reality the stimulation techniques differ in at least one point, which is probably crucial and which Buňag and Riley briefly discuss in their paper. We used “monopolar” stimulations with one, very thin (diameter 0.1-0.15 mm) stainless steel electrode used for “specific” cathodal stimulation in the hypothalamic defence (pressor) area and with an “unspecific,” large anode placed outside the skull. Of course, some brain damage always results from this approach, but evidently the local damage was far less prominent in our results than those by Buňag and Riley. For example, we never noted any disturbances in the food-intake control in our stimulated animals, but this was evidently common in Buňag and Riley’s rats, clearly showing gradually more extensive hypothalamic lesions afflicting also the nearby appetite-regulating centers. Buňag and Riley’s use of much thicker (0.5 mm diameter) concentric electrodes for topical bipolar stimulations was probably the major cause of these lesions. First, thicker electrodes placed in the delicate hypothalamic structures of rats are likely to produce more lesions on insertion. Second, local bipolar stimulation gives a more concentrated

local current field close to the tips, which is likely to produce more lesions. Also, increased current strength then becomes necessary, with further lesions, and so on. From the tiny cathode tip used in our experiments, current spreads diffusely to surrounding neurons and soon becomes “subthreshold” farther out, and a less harmful activation of a bigger group of neurons is likely to ensue. Both groups used stainless steel, but there are many variants of “stainless” steel and the type we used by chance might have been less detrimental for the tissues in the long term. Also, other seemingly minor differences in technique may have contributed to put the balance between effective stimulation and secondary neuronal lesions in a more favorable situation in Folkow and Rubinstein’s experiments — and experimental series are never exactly equal even when one attempts a precise repetition. It is quite obvious that there are today much better methods to further minimize topical lesions in chronic central nervous system (CNS) stimulations, as indicated by the fact that chronic topical stimulations have been used with success even in man. Dr. Rubinstein had, furthermore, a great deal of experience of long-term stimulations in the brain when our experiments were started: first, during a long collaboration with Dr. Delgado at Yale; and second, during 1 year of studies of behavior in cats with chronically implanted hypothalamic electrodes in this department.

In addition, there should by now be little doubt that hypothalamic excitatory neurohormonal discharge patterns (note that it is here not a matter of only direct nervous influences but important hormonal links are nearly always associated) can considerably influence the development of hypertension, notably also the classical defence reaction here employed. For instance, accentuated “mental stress” in spontaneously hypertensive rats greatly aggravates their variant of primary hypertension, while a chronic reduction of environmental stimuli by means of social deprivation clearly attenuates and delays the development. Beyond all reasonable doubt such “psychogenic” influences express themselves via limbic-hypothalamic neurohormonal control stations. Furthermore, the prolonged environmental “stress” techniques, successfully utilized in mice and monkeys to produce mild chronic hypertension in genetically normotensive animals, also inevitably operate by physiological activations of appropriate limbic-hypothalamic structures. Here the so-called hypothalamic defense area is the most likely candidate to convey such psychogenic influences on the cardiovascular system. Accordingly, a direct topical activation of such structures, if appropriately patterned and carried out, should give largely the same result, as indicated by our results. Undoubtedly, however, it calls for a minimum of secondary CNS lesions and it can only be concluded that Folkow and Rubinstein were somewhat more lucky in avoiding this, compared with Buñag and Riley.

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References
Chronic hypothalamic stimulation in awake rats fails to induce hypertension.
R D Buñag and E Riley

Hypertension. 1979;1:498-507
doi: 10.1161/01.HYP.1.5.498

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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