

Effects of ACTH₄₋₁₀ on Self-Stimulation Behavior in the Rat

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Received 18 September 1978

NYAKAS, C., B. BOHUS AND D. DE WIED. *Effects of ACTH₄₋₁₀ on self-stimulation behavior in the rat.* PHYSIOL. BEHAV. 24(4) 759-764, 1980.—The threshold current evoking self-stimulation or multiples of this current was used to investigate the effect of ACTH₄₋₁₀ on response performance for brain stimulation reward in the medial septum (MS) and the medial forebrain bundle (MFB). ACTH₄₋₁₀ in a dose of 50 µg administered SC enhanced bar-pressing for low intensity stimulation but failed to change self-stimulation rates when the base-line rate exceeded 100 responses per 6 min. The peptide lowered the threshold for producing self-stimulation but only in the MS. When an ascending sequence of threshold multiples was used within a session, ACTH₄₋₁₀ treatment resulted in an increase in response rate at 1.2 and 1.5 threshold multiples and a decrease at 3.0 times the threshold but only in the MS. The results indicate that ACTH₄₋₁₀ facilitated response performance at a low response rate by enhancing the rewarding effect of brain stimulation. Furthermore, the peptide changed the response pattern which normally followed reinforcement shift in the MS.

| ACTH ₄₋₁₀ | Self-stimulation | Medial septum | Medial forebrain bundle | Rate-dependent effect |
|----------------------|------------------|---------------|-------------------------|-----------------------|
| Reinforcement shift | | | | |

ACTH and fragments of this hormone affect motivational, learning and memory processes [34]. This statement is primarily based on observations of avoidance behavior. Comparable effects of peptides related to ACTH have been found on behavioral responses based upon reward. Thus, ACTH and fragments such as ACTH₁₋₁₀, ACTH₄₋₁₀ and α -MSH delay extinction of conditioned avoidance responses [1, 4, 18, 31, 32]. These peptides also delay extinction of food- [6, 8, 14, 25] or sexually-rewarded [3] approach responses. Much less is known however about the effect of ACTH-like peptides on behavioral performance in the actual presence of reward. ACTH enhances the acquisition and performance of lever-pressing for water reward as long as the motivation of the rats is stringently controlled [11]. ACTH₄₋₁₀ improves the score for correct performance but only in the first trial of rats in a four-table choice situation for water reward [13].

Intracranial self-stimulation based upon brain-stimulation reward is a widely used approach to study reinforcement processes in the brain [20]. Although this technique provides a well-controlled measure of behavioral performance, no attention has been paid to the question of whether ACTH and related peptides influence self-stimulation behavior. Observations on self-stimulation behavior following adrenalectomy [26] or hypophysectomy [21], which procedures alter endogenous ACTH levels, cannot be easily interpreted as ACTH-like peptide effects on brain functions because they cause multiple alterations in endocrine systems.

The aim of the present experiments was to study the influence of ACTH₄₋₁₀ on behavioral performance using brain stimulation reward. Since a number of the behavioral effects of ACTH and related peptides depend upon motivational variables [2], self-stimulation behavior was investigated by using a wide range of stimulation current intensities to assure a different rate of performance. Furthermore, two distinct neuroanatomical and functional regions in the brain, the medial forebrain bundle (MFB) and the medial septal area, were selected to explore whether ACTH₄₋₁₀ had any differential effect on self-stimulation behavior depending upon the locus. These two areas are probably involved in different neuronal circuits which elaborate self-stimulation behavior [24]. The septal area was chosen since earlier behavioral and neurochemical studies suggested that the septo-hippocampal system is one of the probable sites of action of ACTH-like peptides [29, 30, 35, 36].

METHOD

Animals

Male Wistar rats (Central Breeding Laboratory, TNO, Zeist, The Netherlands) were used which weighed 200-220 g at the time of operation. They were housed in single cages and food and water were available ad lib.

Surgery

Two pairs of stimulation electrodes made of stainless

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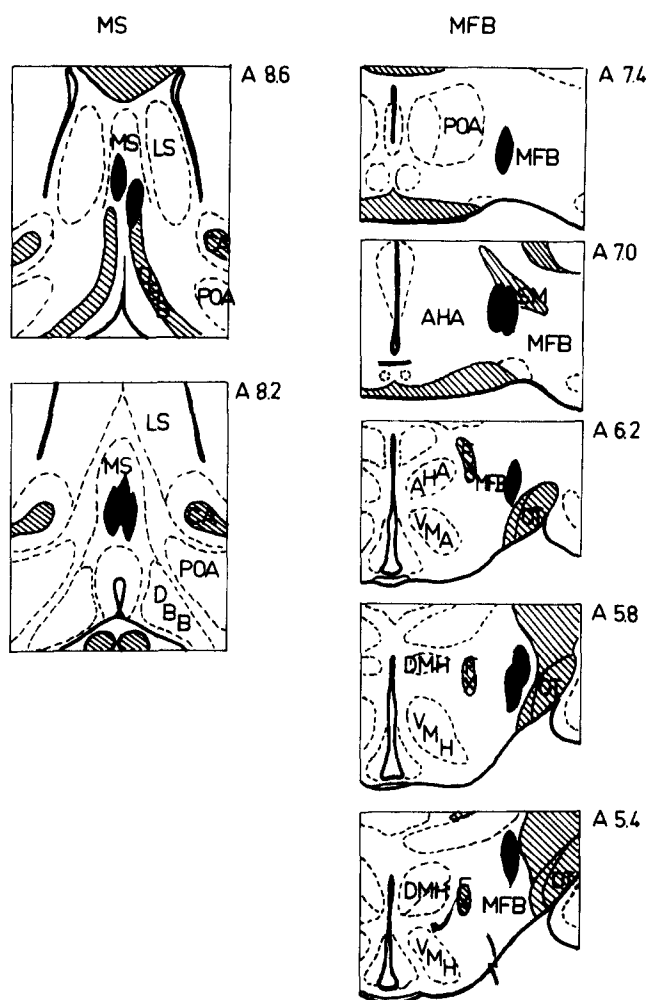


FIG. 1. Localization of the tip of the electrodes (dark areas) in the medial septum (MS) and medial forebrain bundle (MFB). Numbers to the right of the drawings represents the coronal sections according to the atlas of De Groot.

steel wire of diameter $150\ \mu$ were implanted stereotactically under anesthesia (Hypnorm®). The uninsulated tips of the electrodes were aimed into the rostral part of the medial septum (MS) and the medial forebrain bundle according to the atlas of De Groot [11]. The coordinates were as follows: AP=7.4; L=0.2; D=0.7 (MS); AP=6.0; L=1.6; D=-1.1 (MFB). A pair of electrodes was also implanted in one dorsal hippocampus for electro-encephalographic recordings (AP=3.4; L=2.0; D=3.6).

Procedures for self-stimulation

Experiments were carried out in a Skinner-box which had a lever protruding from the wall. Each lever press delivered a train of biphasic current (500 msec; pulse duration 0.1 msec; delay 0.5 msec; frequency 50 Hz). A week after surgery a 6–7 day pretraining period was started. The rats were allowed to self-stimulate for 10 min daily through the MFB and/or MS electrodes at a current level above the expected threshold. Stimulation sites which gave a high rate of self-stimulation were selected for the experiments. The threshold current which evoked self-stimulation was measured on the next 3

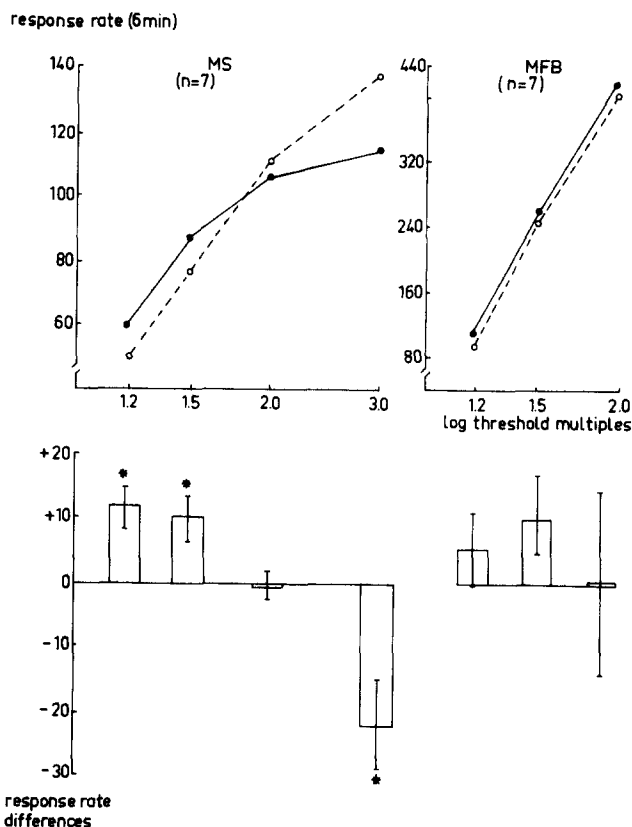


FIG. 2. Changes in the characteristics of self-stimulation behavior in the medial septum (MS) and medial forebrain bundle (MFB) following the administration of $ACTH_{4-10}$ in a dose of $50\ \mu\text{g}$ SC per animal using an ascending sequence of threshold multiples (upper figure). The threshold multiples are given on a logarithmic scale. Mean differences in response rate \pm SEM between peptide and saline sessions are given below. Significance of difference: $*=p<0.05$ (*t*-test). Each value represents observations on 6 (MS) and 7 (MFB) animals with at least 2 replications each.

days. The current intensity was decreased by 2–3 μA steps until the rat stopped regular lever-pressing. The current was then increased and decreased again. Subsequently, the lowest current which evoked self-stimulation was delivered 4–5 times for 2 min with switched-off current intrusion periods. The behavioral criterion of the threshold was a significant difference ($p<0.05$; *t*-test) in the rate of lever-pressing between the current on and off periods.

In the first series of the experiments self-stimulation behavior was studied by using multiples of the threshold current (1.2, 1.5, 2.0 and 3.0 times threshold in the MS and 1.2, 1.5, 2.0 in the MFB) in ascending order. The current was increased every 8 min. The rate of lever-pressing during the last 6 min of each period was recorded. In order to reach a stabilized self-stimulation pattern the rats were trained for 5 days with the ascending multiples of the threshold current before $ACTH_{4-10}$ was applied. At the end of these experiments recordings were made of spike-after-discharges evoked in the dorsal hippocampus by medial septal self-stimulation at a current intensity of 3 times threshold. Hippocampal electrical activity was recorded via impedance transformers and a polygraph. The number of lever presses

until the appearance of the first hippocampal after-discharge was recorded on 2 subsequent days under peptide or saline treatment.

In the second series of experiments self-stimulation behavior was studied by using threshold current and its multiples (1.0, 1.2, 2.0 and 3.0 in the MS; 1.0 and 1.2 in the MFB) but only one current strength was used daily. The rats were allowed to self-stimulate for 8 min and the rate of lever-pressing was recorded for the last 6 min period. The threshold current intensity which evoked self-stimulation was also determined each day under peptide or saline treatment. Self-stimulation after the 8 min session was first extinguished by switching off the current. The current level was then adjusted below the threshold obtained without treatment. The current was increased by 1.0–1.5 μ A steps. Recording of the self-stimulation rate was started when the rat first pressed the pedal at each current and lasted for 2 min. The current at which regular self-stimulation occurred throughout 2 min was accepted as threshold.

Peptide Treatment

ACTH₄₋₁₀ dissolved in saline was injected subcutaneously 1 hr prior to the behavioral test. The dose of the peptide was 50 μ g per rat and administered in 0.5 ml saline. Saline injection was given before the control sessions. The treatments were given in blocks of 5 days during which peptide and saline administrations were alternated. Additionally, the order of tests at the two stimulation sites was also alternated when both MS and MFB electrodes were used in the same rats. The effective dose of ACTH₄₋₁₀ was determined in pilot experiments. These studies also showed that self-stimulation performance of peptide-treated rats returned to the control level on the day after the treatment. Accordingly, a carry-over of the effect of the peptide could be excluded.

Histology

At the conclusion of the experiments the rats were anesthetized with Nembutal and the brain was perfused with 10% Formalin solution via the heart. The localization of the electrodes was determined on 100 μ thick frozen sections.

RESULTS

Typical locations of the stimulation electrodes in the medial septum and the MFB are shown in Fig. 1. Septal electrodes stimulated the main body of the medial septal nuclei but extended anteriorly in the diagonal band of Brocca. Hypothalamic electrodes were located in the anterior part of the MFB.

Figure 2 depicts the characteristics of the self-stimulation behavior when MS and MFB were stimulated during saline treatment. The current was increased stepwise as a function of threshold multiplication. There was a linear relationship between the logarithm of threshold multiples from MFB stimulation sites and the rate of lever pressing. A similar relation was seen at septal self-stimulation but up to twice threshold current. Although the mean response rate at 3.0 times threshold was the highest, it appeared to deviate from linearity. This current level often resulted in temporary arrests of self-stimulation. There was frequent body and head shaking which may indicated spike-after-discharges. Self-stimulation from the MFB resulted in a higher response rate and the slope of the line ($y=25+1204 \log x$; $r=0.823$) was approximately 4 times that of the MS ($y=25+288 \log x$;

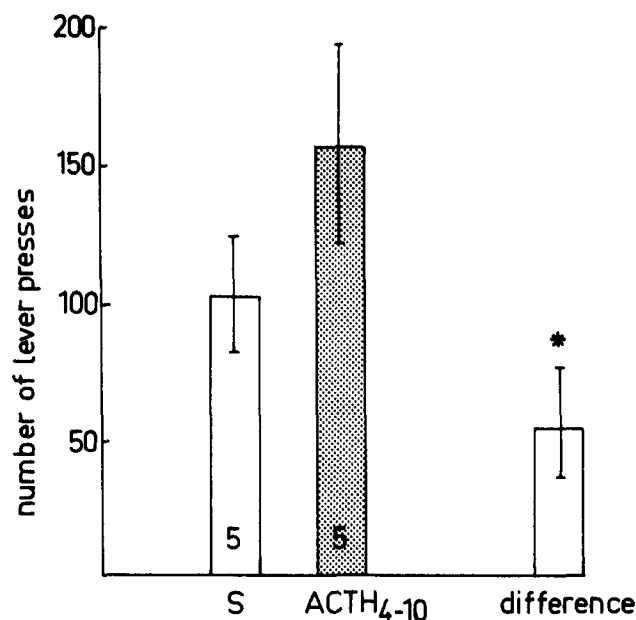


FIG. 3. Effect of ACTH₄₋₁₀ on the appearance of hippocampal spike-after-discharges during medial septal self-stimulation with a current of 3 times threshold. The mean number of lever presses preceding the first hippocampal spike-after-discharge after saline (S) or ACTH₄₋₁₀ administration is depicted (* $p<0.05$).

$r=0.914$). Self-stimulation behavior at 3.0 times threshold in MFB was not studied. Pilot observations showed that this current often induced tonic-clonic seizures.

Administration of ACTH₄₋₁₀ resulted in a marked change in the pattern of self-stimulation behavior with ascending current from MS, but not MFB stimulation sites. The response patterns and the differences in response rates during ACTH₄₋₁₀ and saline treatments are also shown in Fig. 2. ACTH₄₋₁₀ administration significantly increased the rate of MS self-stimulation at low currents but attenuated the rate at higher multiples. This attenuation was significant ($p<0.05$) at 3.0 times multiplication of the threshold current. Self-stimulation behavior was slightly facilitated in the MFB sites but the differences were not significant.

The decrease in the rate of septal self-stimulation as observed after ACTH₄₋₁₀ at 3 times threshold current might have been due to an early appearance of spike-after-discharges in the hippocampus. It was, however, found that administration of the peptide delayed the appearance of spikes in the hippocampus. The appearance of the first spike-after-discharge in the dorsal hippocampus was preceded by a significantly higher number of lever pressings after ACTH₄₋₁₀ than after saline (Fig. 3).

The effect of ACTH₄₋₁₀ on septal and MFB self-stimulation behavior when only one current was used within each session is summarized in Table 1. The number of lever presses appeared to increase significantly during ACTH₄₋₁₀ administration with brain stimulation in the MS with the threshold current and its 1.2 multiple. The response rate was not affected by the peptide at higher stimulation intensities. The rate of self-stimulation was also increased by ACTH₄₋₁₀ when the MFB was stimulated at the threshold level but not at its 1.2 multiple.

ACTH₄₋₁₀ significantly decreased the threshold for self-stimulation from the MS but not from the MFB. Less current was sufficient to evoke self-stimulation for the MS when

TABLE 1
EFFECTS OF ACTH₄₋₁₀ ON THE RESPONSE RATE TO SELF-STIMULATION IN THE MEDIAL SEPTUM (MS) AND THE MEDIAL FOREBRAIN BUNDLE (MFB)

| TMs* | MS | | | | MFB | | | |
|------|-------------------------------|--|-------------|-----|-------------------------------|---|-------------|-----|
| | Response rate/6 min Saline | Response rate/6 min ACTH ₄₋₁₀ [†] | Change in % | | Response rate/6 min Saline | Response rate/6 min ACTH ₄₋₁₀ | Change in % | |
| 1.0 | 27.4 ± 4.5 [‡] | 38.5 ± 5.6 [¶] | 40.4 | (8) | 24.5 ± 4.2 | 38.9 ± 6.9 [§] | 58.9 | (6) |
| 1.2 | 47.7 ± 6.5 | 59.3 ± 5.1 [¶] | 24.5 | (6) | 107.3 ± 19.8 | 116.5 ± 23.3 | 8.5 | (7) |
| 2.0 | 104.9 ± 11.6 | 109.3 ± 10.4 | 4.2 | (7) | — | — | | |
| 3.0 | 138.6 ± 5.2 | 147.1 ± 8.1 | 6.2 | (8) | — | — | | |

*Threshold current multiples; each current was used on separate days.

[†]50 µg/animal SC 1 hr prior to test.

[‡]Mean ± SEM.

[§]*p* < 0.05 (*t*-test).

[¶]*p* < 0.01.

() Number of observations.

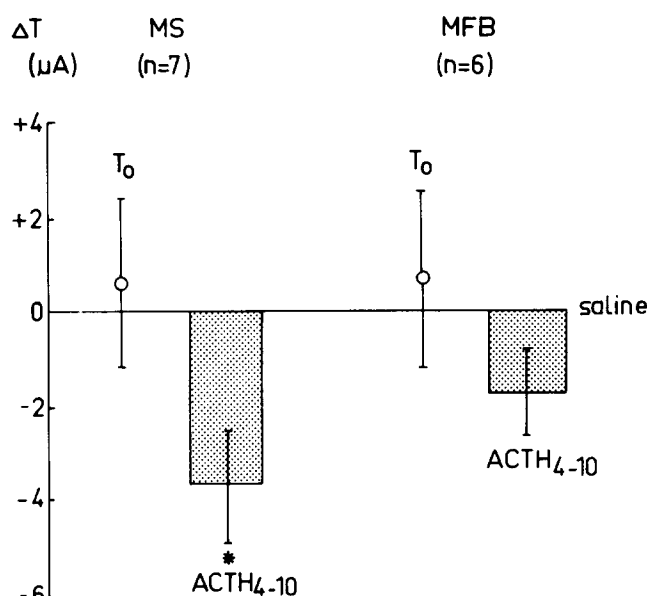


FIG. 4. Effects of ACTH₄₋₁₀ on the threshold current intensity evoking self-stimulation in the medial septum (MS) and medial forebrain bundle (MFB). Differences in threshold (T) between saline and ACTH₄₋₁₀ are presented. T₀ represents the threshold without saline treatment (*=*p* < 0.05; *t*-test).

ACTH₄₋₁₀, rather than saline, was given prior to the session (Fig. 4). Saline did not change the threshold as measured during no-treatment sessions. The thresholds after saline treatments were 43.6 ± 4.2 µA in the medial septum and 37.1 ± 2.5 µA in the MFB.

DISCUSSION

The present experiments showed that ACTH₄₋₁₀ neuropeptide enhanced self-stimulation behavior at low response rates, decreased the threshold to evoke self-stimulation from the MS, interfered with the response pattern that rats normally showed when ascending series of stimulus intensities were used within a session. Furthermore, the appearance of spike-after-discharges in the dorsal

hippocampus during high current intensity septal self-stimulation was delayed by the peptide.

The enhancement of self-stimulation rate by ACTH₄₋₁₀ indicates that the peptide facilitated performance of behavior for brain stimulation reward. The facilitation was more obvious in the case of MS than of hypothalamic stimulation. This may suggest that the site of stimulation is of importance for the neuropeptide to affect self-stimulation behavior. Interestingly, corticosteroids affect also septal and MFB self-stimulation differently. Corticosterone and cortisol, whose effects on behaviors are mostly opposite to those of ACTH-like peptides [2], attenuate self-stimulation from the MS but do not affect MFB self-stimulation [5]. Rolls [24] suggested that different neuronal circuits are involved in hypothalamic and septal self-stimulation. The present results seem to agree with Rolls' suggestion and with observations [17,22] that the rate of self-stimulation is considerably lower from the MS at comparable stimulation currents. However, it is more likely that the effect of ACTH₄₋₁₀ depends on the response rate rather than on the site of self-stimulation. ACTH₄₋₁₀ increased response rate both from the MS and MFB whenever baseline responding (control sessions) was below 100 per 6 min. Accordingly, ACTH₄₋₁₀ strengthened the rewarding effect of brain stimulation at low rate responding and thereby facilitated performance. Guth *et al.* [11] reported similar findings with operant responding for water reward. ACTH appeared to facilitate the response rate provided that the control rate was low. It therefore seems that the low response rate, due to low level of incentive motivation, is an essential prerequisite of the modulation of behavioral performance by peptide hormones and their fragments. This character of the behavioral effects of ACTH-related peptides has often been observed in diverse paradigms and it was related to the neuromodulatory function of neuropeptides on brain processes [2]. The low of initial values may be an alternative explanation; to evidence an increase at a higher rate of responding would simply be difficult because of the ceiling effects. That a number of drugs, which interact with neurotransmitter systems, facilitate self-stimulation rate at high base-line level argues against this alternative.

ACTH₄₋₁₀ significantly decreased, in the MS only, the threshold current which evoked self-stimulation behavior. This suggests that the peptide increases the excitability of the septal neuron population. It may be more than coincidence that ACTH₄₋₁₀ affected the septum more profoundly.

Previous observations suggest that the septo-hippocampal system is one of the site(s) of the behavioral action of ACTH-like peptides. Lesions in the rostral-anterior septum or in the dorsal hippocampus prevent the effects of ACTH₄₋₁₀ on the maintenance of conditioned avoidance behavior [35,36]. The excitability of the septo-hippocampal system is increased by ACTH₄₋₁₀ as suggested by electrophysiological observations [28]. The dorsal septal nuclei appeared to be the site of preferential and specific uptake of the behaviorally active ACTH₄₋₉ analogue [29,30]. The stimulation sites in the present experiments were in the medial septum. The extensive connections between the dorsal-lateral and MS nuclei [27] suggest that the peptide probably influenced the function of the MS through intraseptal regulatory connections.

ACTH₄₋₁₀ administration also interfered with characteristics of the response rate of rats which received, in one session, an ascending series of stimulus intensities in a septal location. ACTH₄₋₁₀ enhanced the response rate at lower currents, it was ineffective at twice the threshold and attenuated performance at highest current for self-stimulation. The

simplest explanation may be that the neuropeptide attenuated the contrast between ascending reinforcement shifts [16]. Attenuation of the effects of reinforcement shifts by ACTH₄₋₁₀ on response rate of a food-rewarded operant behavior has been reported earlier [9]. That attenuation of self-stimulation by ACTH₄₋₁₀ was due to an early appearance of spike-after-discharges or convulsions during high intensity septal self-stimulation [12, 19, 33] can be excluded. ACTH₄₋₁₀ markedly delayed the appearance of spike-after-discharges in the hippocampus. Furthermore, the peptide had no effect on performance when only the high current was used during the entire session.

Recent hypotheses on the mode of modulatory action of ACTH and related peptides on behavioral processes emphasize that motivational [33], attentional [15], memory storage [7] and retrieval [23] mechanisms are involved. The findings of the present experiments cannot be easily explained by a single hypothesis. Rather, modulation of various brain processes by ACTH-like peptides results in multiple changes in behavior.

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