Pharmacology Biochemistry & Behavior, Vol. 16, pp. 41–45, 1982. Printed in the U.S.A.

Aggression Induced by Stimulation of the Hypothalamus: Effects of Androgens

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Received 12 June 1981

BERMOND, B., J. MOS, W. MEELIS, A. M. VAN DER POEL AND M. R. KRUK. *Aggression induced by stimulation of the hypothalamus: Effects of androgens.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 41–45, 1982.—Aggressive behavior between male rats induced by electrical stimulation of the hypothalamus (ESH) is stimulated by androgens. This was demonstrated by recording the changes in threshold current intensities (the amount of current needed to induce attack behavior in 50% of the trials), just before castration, after castration, during subsequent treatment with high doses of testosterone propionate, and finally during oil treatment. The results demonstrate that, to induce the same aggressive responses, in absence of androgens more electrical current is needed than when these hormones are present in the general circulation of the ESH stimulated animals.

Hypothalamus Aggression Androgens

AS early as 1943 it was demonstrated that local electrical stimulation of certain brain areas, e.g. the hypothalamus, could induce aggressive reactions in cats [8]. Since then a number of papers have been published which replicated, refined or elaborated these results with regard to the hypothalamus [5, 13, 16, 17, 19, 23].

Although the fact that electrical stimulation of the hypothalamus (ESH) can induce aggressive behavior is no longer an issue of dispute, the interpretation of this phenomenon still is.

Firstly, in rats predatory aggression (mouse-killing) as well as intermale aggression (aggression between two male opponents of the same species) can be induced by ESH from one and the same electrode [1, 13, 16, 19, 25]. Secondly, the aggression observed could be a direct result of the ESH [16] or only a secondary effect. According to some authors [9] ESH induces first pain or distress and only secondarily aggression in response to the pain or distress. The issue therefore remains whether the aggression between two male rats observed during ESH is genuine intermale aggression, or either distress-induced or even displaced predatory aggression, only directed to a conspecific for lack of an appropriate non-conspecific.

It has been demonstrated that spontaneous intermale aggression in full-grown rats is stimulated by androgens. The effects of castration or hormone replacement upon this behavior become apparent within a period of two to four weeks [2,6]. In contradistinction to intermale aggression, distress-induced aggression in these animals is either independent of androgens [7] or it takes more than 50 days before the effect of castration upon this behavior becomes evident [10]. Finally it has been demonstrated that predatory aggression is entirely independent of androgens in the full-grown male rat [3,11]. For these reasons it seems useful to study the short-term effects of castration and hormone replacement treatment upon the ESH-induced aggression in full-grown male rats. Effects of the changes in androgen levels have to be expected if the "intermale" aggressive behavior observed is a direct result of the ESH. They cannot be expected when this behavior is only a secondary result of ESH (aggression in response to pain or distress) or displaced predatory aggression.

METHOD

Subjects

Nine male CPB-WE-zob rats weighing between 300 and 350 g were used as experimental animals. Wistar male rats weighing between 180 and 200 g served as partners in aggression tests. Both types of animals were bred in our own laboratory. However, both strains were derived from strains kept at the Central Institute of Laboratory Animals (CPB-TNO) Zeist, The Netherlands. Experimental animals and their partners were kept at a 12 hour light/12 hour dark cycle, the former singly, the latter in groups of 8 to 10 animals. Food and water were supplied ad lib.

Electrodes

Previously described one hundred and seventy micron external diameter bipolar electrodes, made of teflon-insulated Pt-Ir wires [16] were used.

Surgery

Per animal two stimulation electrodes were bilaterally implanted into the hypothalamus according to a previously described method [16]. The tips were aimed at a point of which the coordinates in the stereotaxic atlas of König and Klippel [12] roughly corresponds to: −2.5 mm D.V.; +/−1.5 mm M.L. and +5.5 mm A.P.

Test Cage

The animals were tested in round Plexiglas cages, 35 cm in diameter and 47 cm high.

Stimulation Techniques

Forty Hz biphasic square-wave pulses with a phase duration of 0.2 msec and a phase interval of 12.5 msec were delivered by two Grass PSIU6 isolated constant-current sources connected to a Grass S88 stimulator. Duration and current intensity of both phases were always equalized. We stimulated with bipolar electrodes, in order to activate only a restricted part of the hypothalamus.

Behavioral Testing

In order to determine from which electrodes aggression could be induced, we used a previously described behavioral testing procedure [16]. According to this method the animals were stimulated, in presence of a naive partner, with at least two trains of 60 sec separated by a 60 sec interval. Current intensity was started at 50 μA and raised by 50 μA steps until attack behavior was shown by the stimulated animal. Behavioral testing was stopped when an upper limit of 400 μA was reached or when motor responses like unilateral circling or excessive jumping precluded further testing.

Threshold Current Determination

Threshold current intensities for attack behavior (attack jump or bite attack [16]) were determined by means of an up-and-down design [24]. During threshold determination the current is periodically on for 10 seconds and off for 50 seconds. When a 10 second period of stimulation (trial) does not yield attack, the current intensity for the next trial is increased with a fixed amount of current (step). When a trial does yield an attack the current is decreased by the same step. This procedure is continued until 10 response changes (for attack to non-attack or vice versa) are recorded. The threshold is calculated by taking the mean of the intensities of all response changes. Theoretically, this current intensity will induce attack in 50% of the trials. The step size was always 5 μA, except for one electrode with a relatively high threshold, where a step size of 10 μA was used. All threshold determinations took place during the dark period.

Histology

Upon completion of experimentation, the rats were anesthetized and perfused with physiological saline and 4% formaldehyde. After at least 14 days of storage in formaldehyde the brains were removed from the skulls, sectioned and stained according to the method of Klüver-Barrera.

Experimental Procedure

At least six threshold values were determined for all electrodes. This was done because initial decrease in threshold values for ESH-induced aggression had been reported before [16]. ESH attack threshold decrease occurs mainly between the first and second threshold determination, while a further decline from the second to the third threshold is hardly measurable any more [16]. In order to decide whether the initial threshold decrease had vanished before the experiment was started, an analysis of variance was applied to the last three precastration threshold values of all electrodes.

After these pre-tests the animals were castrated and the thresholds were again determined, two and four weeks after castration. Subsequently the animals were treated with androgens by daily intramuscular injections of testosterone propionate (TP) (500 μg/100 g body weight dissolved in 0.2 ml peanut oil). Thresholds were determined after two, four and five weeks of androgen treatment. To control for possible placebo effects the animals were, after the last-mentioned threshold determination, treated with oil (daily intramuscular injections of 0.2 ml peanut oil). Threshold values were determined after two and four weeks of oil treatment.

Statistics

Threshold changes over the different treatments were analysed using Friedman two-way analysis of variance and Wilcoxon matched-pairs test. Possible changes in the qualitative aspects of ESH induced attacks were tested by means of χ^2 [20].

RESULTS

Behavioral Testing

Aggressive behavior could be induced from 10 of the 18 implanted electrodes. However, in two "aggressive" electrode placements we were unable to determine thresholds of that behavior. The animals carrying these electrodes attacked occasionally at a certain current level, while excessive running and jumping interfered with aggression at higher current levels. Therefore eight electrodes, divided over six animals, were included in the experiment. After the first post-TP treatment test one electrode started to induce severe convulsions, and was therefore excluded from further testing.

Qualitative Description of ESH-induced Attack Behavior

Since the two types of attack behavior (jump attack and bite attack), used as behavioral criteria in threshold determination, have been described in detail elsewhere [16], only a short description will be given here. During jump attack the stimulated animal approaches his opponent with an arched back and marked pilo-erection, often accompanied with tooth-chattering. When the stimulated animal draws near, he extends his forepaws to the opponent and jumps towards him. During this jump the stimulated animal tries to bite the head and kick the belly or chest of its opponent. The bite attack is essentially the same except that the stimulated animal neither jumps nor kicks before he bites. During both ESH-induced types of attack other signs of aggression, like lateral threatening or clinch fight were also observed. These ESH-induced attacks always resulted in marked injuries of the skin of the opponents.

The opponents responded to the ESH-induced attacks by fleeing, boxing (upright defense posture), lying on the back, or freezing. These defense behaviors were never witnessed in the stimulated animals, except for boxing which was seen a few times in the ESH-stimulated animals, but only in response to boxing by the opponent.

Histology

Figure 1 represents a reconstruction of the implantation sites of the various electrode tips. Dots stand for negative electrodes (electrodes from which no aggression could be

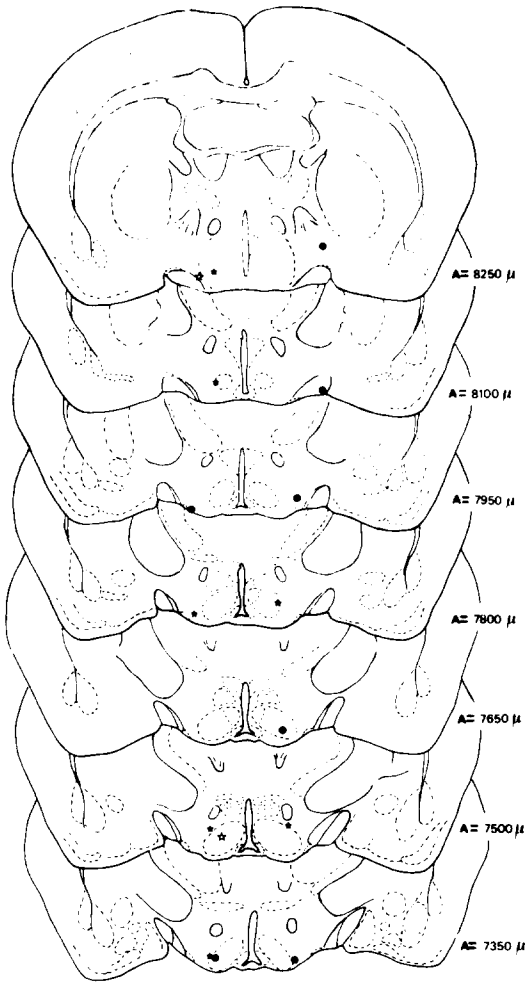

FIG. 1. Localization of electrode tips. A=anterior coordinates; ●=no attack behavior; ☆=attack behavior, no thresholds; ★=attack behavior and thresholds.

induced), stars represent the positive electrode tips (an open star stands for the aggression-inducing electrodes from which no thresholds could be obtained, closed stars stand for those from which aggression thresholds could be obtained). Unfortunately the histology of one animal, carrying one negative and one positive electrode was lost.

Inspection of the locations of the positive electrodes included in the experiment made it clear that they all lay within that subarea of the hypothalamus of which it had been—on base of information from more than 400 electrodes—demonstrated that the probability that electrodes placed in this area will induce aggressive behavior is equal or above 0.8 [14,15]. (The last reference mentioned presents a very precise description of this hypothalamic subarea.)

Pre-testing

Friedman two-way analysis of variance over the last three precastration thresholds yielded no significant result ($\chi^2_r=1.75, df=2, p>0.42$). This shows that prior to castration

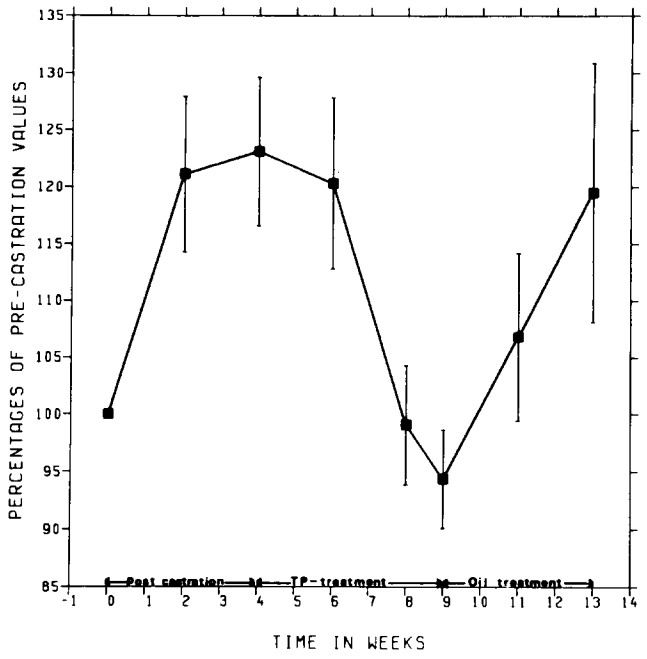

FIG. 2. Average change in threshold values after: castration, TP-, and oil-treatment. Indicated are: the mean values and corresponding standard errors.

electrodes had reached stable base levels. The mean value of the last three pre-castration thresholds was taken as the baseline pre-castration level of each electrode.

Effects of Castration, TP and Oil Treatment

All threshold values increased after castration, decreased during subsequent TP treatment and all but one increased again during the final oil treatment. These changes in threshold value are represented in Fig. 2. Friedman two-way analysis of variance over the mean pre-castration values, the values obtained during: the two post-castration, the three TP and the two oil tests provided a significant result: $\chi^2_r=21.77$, $df=6, p<0.003$. The effects of the various hormonal manipulations were tested separately by means of Wilcoxon matched-pairs test. (A) Both groups of post-castration thresholds (2 and 4 weeks after castration) were significantly higher when compared to the pre-castration values (statistical values for both comparisons: T=0, N=7, $p=0.018$ two-tailed). (B) The threshold values obtained during the first TP treatment test (2 weeks of hormone treatment) did not differ significantly from those obtained during the last castration test (T=10, N=8, $p=0.263$ two-tailed). However, the values obtained during the second and third TP tests (4 and 5 weeks of hormone treatment) were significantly lower than those obtained during the last castration test (respective statistical values: T=0, N=7, $p=0.018$ and T=0, N=6, $p=0.027$, both p values two-tailed). (C) The threshold increase from the last TP determination to the first oil treatment determination (two weeks of oil treatment) did not reach significance, but the threshold increase from the last TP- to the second oil treatment test (four weeks of oil treatment) did yield signifi-

cance (respective statistical values: T=3, N=6, $p=0.116$; T=2, N=6, $p=0.042$, both p values two-tailed).

Two types of attack behavior (jump attack and bite attack) were used as criteria in threshold determinations. This was done because, at threshold current intensity, most electrode placements will induce both types of behavior, although generally not in equal proportions. Normally the ratio between these two behaviors remains more or less stable over time. However, since the hormonal manipulations had a clear effect upon the attack thresholds, it is conceivable that these manipulations also had an effect upon the jump/bite ratio. When tested across the four conditions (pre-castration, post-castration, TP-, and oil-treatment) this turned out not to be the case ($\chi^2=4.6$, $df=3$, $p=0.20$).

DISCUSSION

Fighting between male rats induced by ESH is apparently activated by testicular hormones. In absence of androgens more electrical current is needed to induce the same aggressive responses than when these hormones are present in the general circulation of the ESH-stimulated animals.

The changes in attack thresholds following castration, TP- and oil-treatment were consistently observed in all electrode placements, except for one in which a sudden decrease was witnessed during the last oil treatment test. The animal carrying this electrode was run once more through the same hormonal manipulations and test series as before, and provided the following results: a 17.8% increase during the first additional oil treatment, followed by a 24.5% decrease during the subsequent additional TP treatment, followed by a 40% increase during the second additional oil treatment. The decrease in threshold value as registered for this particular electrode during the last oil treatment test of the formal test series may therefore not be ascribed to a deviating reaction to the hormonal manipulation. Evidently, for some other reasons the threshold value for this electrode was shifted to a new base level at the end of the initial test series. Although we cannot explain such sudden shifts, they have been observed before in our laboratory.

As mentioned in the introduction, the literature covering ESH-induced aggressive behavior in rats advances three possible interpretations of this behavior: predatory aggression, pain- or distress-induced aggression and intermale aggression [1, 9, 13, 16, 25]. Only one of these three types of behavior (intermale aggression) can be influenced by androgens within the relatively short periods as used in the present study [2, 3, 6, 7, 10, 11]. The results of the present study demonstrate the stimulating effect of androgens upon ESH-induced aggression. We therefore conclude that ESH-induced aggressive behavior between two male opponents is not a secondary response in reaction to primarily induced pain or distress, nor is it displaced predatory aggression only directed to a conspecific for lack of an appropriate prey. It most probably represents genuine intermale aggression.

The idea that ESH primarily induces intermale aggression and not predatory aggression, is in line with previous observations [16], demonstrating that the amount of current needed to induce mouse-killing is about two to three times as high as that needed to induce intermale aggression. Furthermore it has been suggested that the morphology of ESH-induced mouse-kill behavior has features of intermale aggression, which are not seen during spontaneously occurring mouse-kill responses [16], a suggestion which is in line with observations of Woodworth [25]. Yet the above presented idea seems to be in conflict with other observations of Woodworth [25], demonstrating that the ESH attack latencies in rats towards mice are shorter than those towards male opponents. Other authors [1], however, claim opposite results (shorter latency times for attacks towards male opponents compared to those towards mice). It should be stressed, however, that the intensity of ESH induced attack varies depending upon the sex, weight, and strength of the opponent [13,16]. Observations in our own laboratory have shown that just adult male opponents (180 to 200 g) are more rapidly attacked than older and heavier ones. The male opponents used by Woodworth were rather heavy (average weight 461 g) and this could very well explain the relatively long attack latencies.

According to descriptions in the literature [4] *the behavior of winners of intermale aggressive interactions* is characterized by high levels of piloerection, biting and lateral display, *that of losers* by high levels of boxing, lying on the back and freezing, while *pain-induced aggression* is characterized by high levels of freezing and boxing and (compared to that of winners and losers) by intermediate levels of lying on the back. The ESH-induced aggressive behavior as observed in the present study or as described by others [16] clearly differs from that of losers of intermale aggressive interactions and also from that of pain-induced aggression. It is however very much alike the behavior of winners of intermale aggressive interactions. This suggests that ESH induces intermale aggression and not aggression in response to primarily induced distress or pain, a suggestion which is in concordance with the conclusion of this study, based upon hormonal data.

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