ENHANCEMENT OF PERFORMANCE FOR BRAIN STIMULATION REWARD AFTER FOOTSHOCK IN RATS

Bogdan SADOWSKI, Przemysław MAREK and Izabela PANOCKA

Department of Behavioral Physiology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences
05-551 Jastrzębiec, Poland

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Abstract. Rats bearing electrodes in the anterior forebrain region (AF), lateral hypothalamus (LH) or dorsal raphe (DR) nucleus were trained to press lever for brain stimulation reward. Ten minutes self-stimulation in all these placements produced a lowering of pain sensitivity as assessed by the hot-plate test. Electric footshock administered on 1 s on/4 s off paradigm for 10 min prior to self-stimulation elevated lever pressing rates in AF rats and in part of LH rats, but not in DR rats. The results are discussed in terms of opiate theory of reinforcement.

INTRODUCTION

An increasing body of evidence points to the role of endogenous opioids in the mechanism of reinforcement produced by electrical stimulation of the brain (25). The following data are usually referred to as accounting for this hypothesis: 1. morphine facilitates self-stimulation (1, 11), 2. this drug will also be self-administered by the animals through various routes (for review see 5), and 3. naloxone, an opiate antagonist, reverses the facilitation of self-stimulation by psychostimulants, e.g.,amphetamine (12), which indicates an involvement of an opioid mechanism in this effect. An additional argument is that naloxone suppresses self-stimulation provided the current and consequently the performance, are below the optimal level (20).
In order to check whether the above findings really discover some physiological process and are not purely pharmacological phenomena it seems appropriate to influence the central opioid circuits in a more natural way. For example, the animals can be faced with environmental factors known to release β-endorphin from the pituitary and/or increase the endorphin level in the brain, and the effect of these factors on self-stimulation can be studied. Some earlier reports show that procedures of this kind, may indeed facilitate self-stimulation. Thus, Deutsch and Howarth (8) described that footshock, or a buzzer previously paired with a footshock, will restore the performance which has been extinguished by non-reinforcement or will delay extinction. Also, Katz and Roth (15) demonstrated that tail pinch increased self-stimulation rates.

It has been known for many years that self-stimulation suppresses the animals' responsiveness to painful or other aversive stimuli (2, 4). This property is probably responsible for the failure to establish conditional emotional response in rats receiving brain stimulation instead of natural rewards (7). The antinociceptive effect of self-stimulation may be linked to the fact that this behavior is accompanied by a strong activation of the pituitary-adrenocortical axis (16–18, 22). Therefore, one should expect a release from the pituitary, equimolar in concentration with ACTH (13), of β-endorphin which would cause analgesia.

In the present study we describe that self-stimulation in three different brain areas produced analgesia of magnitude similar to that following mild electric footshock. The latter facilitated self-stimulation in the anterior forebrain, but not in the dorsal raphe placements.

MATERIALS AND METHODS

Animals. Wistar male rats weighing 200–300 g were used. They were kept five to a cage under natural light conditions, fed with standard laboratory pellets and watered ad libitum.

Surgery and stereotaxy. The animals were anesthetized with Pentobarbital sodium. The rat's head was placed in a stereotaxic instrument (Medicor, Budapest) so that the upper incisor bar was 5 mm above the interaural horizontal plane. The coordinates were taken from De Groot's atlas (10) and from van der Kooy et al.'s (23) paper. A bipolar electrode made of two insulated 0.2 mm stainless steel or 0.254 mm dia nichrome wires bared of insulation at the tips was introduced vertically through a hole trephined in the skull bone. Three standard placements were used: 1. anterior forebrain — 3 mm anterior to the bregma, 1.2 mm lateral to the midline and 6.5 mm deep from the dura, 2. lateral
hypothalamus — 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline and 7 mm below the dura, and 3. dorsal raphe — 5 mm posterior to the bregma and 6.5 mm deep in the midline. Extracranial ends of the electrodes were soldered to pins of a connector. Acrylic cement was used to keep the assembly secure. Antibiotics were administered postoperatively during several days.

Self-stimulation. Seven days after the surgery the animal were placed in an experimental cage, connected to a stimulator (the ± and the — poles always to the same wires of the bipolar electrode) and trained to press on a lever (5 × 6.5 cm) suspended 2.5 cm above the floor. Each lever press triggered a train of hundred 0.4 ms square pulses at 200 Hz frequency. Pressing during the train or keeping the lever down had no consequences. Electric pulses were delivered to the electrode through a constant current circuit and a 2 μF capacitor. To minimize polarization of the electrodes a flow of reverse current from the capacitor through the brain tissue was facilitated by shortcircuiting the output of the stimulating device during intervals spacing the pulses. The current and the voltage drop across the electrode were monitored on an oscilloscope. The number of lever presses was measured with an electromechanical counter.

As the animals mastered the procedure, the current previously found optimal for training was lowered so as to assure stable responding at approximately 60–70% of the maximum rate. The criterion of stability was the standard error of the mean response rate throughout at least seven consecutive sessions not exceeding 10% of the mean. Animals responding irregularly or in which seizures developed upon brain stimulation were rejected.

Footshocking. In experiments proper the animals self-stimulated for 10 min daily during 5 consecutive days. The number of responses made on the second, third and fourth days served as controls. Performance on the first day was disregarded as occurring after a weekend break. On the fifth day self-stimulation was preceded by 10 min footshock in a cage equipped with a grid floor to which scrambled 1 mA electric pulses were delivered under 1 s on/4 s off paradigm. The duration of footshock was chosen so as to make the subsequent performance measurements at the presumed time of the maximum blood β-endorphin elevation (13). On control days the animals were placed for 10 min before self-stimulation in the same experimental cage, but were not footshocked. To avoid development of conditional fear (which was found to be accompanied by changes in brain dopamine turnover, 14) each animal obtained only one footshock session.
Pain sensitivity. The animals were placed on a copper plate heated with circulating water at 56°C. The latency of a flick of one of the hind legs was taken as a measure of pain sensitivity. The measurements (the number of which was minimized to prevent escape learning) were made by two independent observers before and after self-stimulation or footshock.

Histology. After completion of the experiments the animals were sacrificed, their brains were removed, fixed in formalin and embedded in celloidin. Fifty μm thick frontal sections through the deepest penetration of the electrode in each rat were stained after Weil.

Statistics. Changes in pain sensitivity were evaluated with a three-way analysis of variance taking animal groups (according to electrode placements) and procedures (self-stimulation and footshock) as independent measures, and hot-plate latencies as tests (pre- and post-self-stimulation or footshock) repeated on the same subject. The self-stimulation rates in control sessions were first analyzed with a two-way analysis of variance, the animal groups being independent and the rates on consecutive days repeated measures. As large intersubject difference was revealed, the raw data obtained from each rat during control and experimental sessions were transformed to percentages of control mean, i.e., a mean number of lever pressings during 3 control sessions. The transformed values were then subjected to three-way analysis of variance with a hierarchical design. Animal groups were taken as independent, and the performance before and after footshock as repeated measures. The three control days were regarded as nested under the common control factor. Individual comparisons were based on F ratios for simple effects (24).

RESULTS

Histological examination revealed that anterior forebrain (AF) electrodes were in the nucleus accumbens, the preoptic area and the diagonal band of Broca, lateral hypothalamus (LH) ones in close vicinity of the medial forebrain bundle, and posterior ones ventrally to the periaqueductal gray in the vicinity of or within the dorsal raphe (DR) nucleus. During control sessions the AF rats \(n = 8\) self-stimulated at a mean rate ± SEM of 215 ± 18, the LH rats \(n = 7\) at a rate of 256 ± 31 and the DR rats \(n = 6\) at a rate of 187 ± 29 per 10 min. The currents individually chosen for each rat varied between 100 and 500 μA.

Figure 1 shows hindpaw flick latencies before and after self-stimu-
lation or footshock. Statistical analysis revealed a significant difference between test ($F(1, 15) = 50.10, P < 0.001$) and procedures ($F(1, 15) = 6.94, P < 0.02$), but not procedures x tests interaction ($F(1, 15) = 3.68, P > 0.05$) which makes questionable whether footshock produced significantly more analgesia than self-stimulation. However, upon examination of simple effects the pain thresholds differed between the post-self-stimulation and the post-footshock condition ($t = 3.19, df = 30, P < 0.01$), but not before the two procedures.

Two-way analysis of variance of control self-stimulation rates re-

![Fig. 1. Mean ± SEM hind paw flick latencies after 10 min self-stimulation (Post-self-stim.) and after 10 min footshock (Post-footshock) in rats bearing electrodes in the anterior forebrain (AF), lateral hypothalamus (LH) or dorsal raphe nucleus (DR).](image1)

![Fig. 2. Self-stimulation rates (% of a control mean) in three consecutive control (C) sessions and after 10 min footshock (post-FS). Each column represents mean ± SEM. Abbreviations for electrode placements as in Fig. 1.](image2)
vealed a significant difference between subjects within groups \( F(18, 42) = 10.35, P < 0.001 \), but not within subjects. Control and post-footshock rates expressed as percentages of the control mean in each rat are presented in Fig. 2. Analysis of variance showed significant main effects of animal groups \( F(2, 18) = 4.64, P < 0.05 \) and of footshock \( F(1, 18) = 21.05, P < 0.001 \), and a significant animal groups x footshock interaction \( F(2, 18) = 4.64, P < 0.05 \). Footshock produced an elevation of self-stimulation rates in all AF rats which is reflected by a highly significant simple effect of footshock in this group \( t = 5.10, df = 18, P < 0.001 \). The same simple effect for LH rats was just below statistical significance \( t = 2.00, df = 18, t_{0.05} \) being 2.181). In fact, an increase in the rate of performance after footshock occurred in 5 of 7 rats in this group. No change was seen in DR rats \( t = 0.28, df = 18, \text{non-significant} \).

**DISCUSSION**

The link between the reward and the pain modulatory mechanisms was recently examined by several authors more systematically than in earlier reports quoted in the Introduction. It was found that electric stimulation of some brain structures produces both analgesia and reward, i.e., raises pain threshold in various tests and, in a lever pressing situation, sustains self-stimulation. Dennis et al. (9) described that stimulation of the dorsal raphe and periaqueductal gray self-stimulation points suppressed tail flick responses and increased the formalin test scores. This effect outlasted the duration of the stimulation by several minutes. Similarly, Sandberg and Segal (19) observed an increase in hot-plate latencies in rats stimulated in the locus coeruleus and substantia nigra reward sites. In a thorough exploration of the periaqueductal gray Urca et al. (21) found sites where stimulation delayed tail flick responses and/or produced reward.

The elevation of pain threshold observed in the present study was not due to forced, experimenter — produced stimulation of the reward loci, but followed a conventional lever pressing paradigm, at the rate chosen by the animal. The nature of this self-stimulation — produced analgesia is not known, since opiate antagonists were not used. But relying on the reports that analgesia upon stimulation of reward sites in different structures was partially reversed by naloxone (9, 19), we can propose that self-stimulation causes a release of an endogenous opiate substance which acts both on pain inhibitory and reward circuits (3).

The facilitatory effect of stress on self-stimulation, particularly when it temporally coincides with the stress-induced analgesia, may be due to a sensitization of the reward circuitry by the released endogenous
opiates and thus resemble the stimulatory effect of morphine and other morphine-like drugs of abuse on the central reward mechanisms. The action of morphine is manifested either by an increase in the performance rate (1) or by a lowering of self-stimulation threshold (11). Morphine will be also self-administered by the animals to the brain, and with this route there is a hope for defining the locus of morphine’s action in producing and/or facilitating reward from brain stimulation. The results and the thereof drawn conclusions are conflicting. M. Olds (17) described that rats will self-inject morphine to lateral hypothalamic self-stimulation sites as soon as the current is no longer available. This implies that the reinforcing action of morphine would be directed toward the same neural elements which are excited electrically during self-stimulation. An alternative view derives from the experiments of Bozarth and Wise (6) in which naive rats having no earlier self-stimulatory experience were acquiring a morphine self-administration habit as a new task. The only region where morphine under these conditions produced clear reinforcement was the ventral tegmental area. Microinjections to other placements including the lateral hypothalamus and the periaqueductal gray were not effective (5).

In the light of the above findings the effect of footshock or another stress causing the release of endogenous opioids on self-stimulation may differ depending on whether the brain stimuli activate, directly or transsynaptically, the opioid site(s) of reward or produce reinforcement through another, presumably non-opiate mechanism. The latter possibility concerns the dorsal raphe placements. According to Bozarth (5) the opiate sensitive cells of the periaqueductal gray are essential for the analgesic, but not for the rewarding action of morphine. Self-stimulation in this area, and also in the raphe nuclei, relies on a serotoninergic mechanism, whereas self-stimulation through anterior electrodes activates catecholaminergic circuits where an opiate-dopamine interaction takes place.

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REFERENCES


