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Opiate Withdrawal Behavior After Focal Brain Stimulation

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WILLIAMS, D. A. AND B. E. THORN. *Opiate withdrawal behavior after focal brain stimulation.* PHARMACOL BIOCHEM BEHAV 21(5)699–703, 1984.—Electrical stimulation of the brainstem abolishes pain, while continued stimulation induces tolerance to the analgesic effect. Analgesic drugs producing tolerance also induce physical dependence, suggesting that the phenomenon of tolerance is associated with addiction. There is evidence that the neural mechanism for stimulation-produced analgesia is related to the release of opiate substances within the brain. We therefore propose that repeated or protracted brain stimulation elicits dependence upon the endorphins released by electrical stimulation of the neurons themselves. To investigate this possibility, rats were given repetitive bursts of analgesic electrical brain stimulation for two hours. Immediately thereafter, they were injected with the opiate antagonist, naloxone. Behaviors associated with low grade opiate withdrawal were observed. These data suggest that prolonged analgesic stimulation can result in naloxone-precipitated behaviors similar to the behaviors exhibited during opiate withdrawal.

Opiate withdrawal Abstinence behaviors Focal brain stimulation Stimulation-produced analgesia Endorphins Naloxone

LIKE analgesic drugs, and the endogenous opiate peptides, focal stimulation of certain loci in the brain relieves pain [15, 26, 21, 23]. Repeated electrical stimulation also elicits behavioral tolerance, the analgesic effect declining after several hours of stimulation [20,24]. The similarity between certain drugs and electrical brain stimulation, insofar as the development of tolerance to the analgesic effect is concerned, raises questions regarding the development of physiological dependence. Repeated administration of opiate analgesic drugs or the endogenous opiate peptides can be followed by physiological dependence and associated abstinence behaviors if the use of the drug is suddenly interrupted, or if a competitive antagonist is administered [3, 29, 31]. Since tolerance to drugs is so often associated with addiction, it is important to inquire whether electrical stimulation of particular brain areas may also result in physiological dependence.

The electrochemical response of the brain to focal stimulation may be far different from the analogous response to analgesic drugs. Although it is difficult to predict a priori the abstinence syndrome associated with focal brain stimulation, a reasonable manifestation would be the appearance of the specific behaviors associated with withdrawal from opiates [30].

The basis for this hypothesis goes beyond the obvious similarity in the capacity of electrical stimulation and the exogenous opiates to relieve pain. There is considerable evidence linking the neural substrate of stimulation-produced analgesia (SPA) to the endogenous opiate system [10]. This work has established that the sites of the brain which support SPA are rich in opiate receptors, and that there is a substantial overlap of neural sites supporting SPA and morphine-induced analgesia [18,31]. It has been additionally demonstrated that periaqueductal gray (PAG) stimulation results in an increased level of the endogenous opiate β-endorphin taken from ventricular fluid [4,14]. Both focal electrical stimulation and narcotic microinjection into the PAG inhibit the firing of spinal cord interneurons [5]. Furthermore, morphine tolerance or morphine dependence inhibits subsequent analgesia by brain stimulation [18,20], indicating a cross tolerance and/or cross dependence between morphine and analgesic brain stimulation. PAG stimulation also delays the development of morphine tolerance [17], perhaps by releasing endogenous opiates. Finally, the opiate antagonist naloxone reverses or attenuates the analgesic effect of electrical brain stimulation [1, 2, 15], further suggesting a link between brain stimulation and the opiates.

In this work, we test the hypothesis that morphine-like withdrawal behaviors can be elicited in rats after prolonged electrical brain stimulation.

METHOD

Experimental protocol was similar to that described in detail in a previous study investigating the phenomenon of tolerance to the analgesic effect of focal brain stimulation [25]. Subjects were male albino rats weighing from 250–350 grams at the time of surgery. Under an anesthetic dose of sodium pentobarbital (8.4 mg/kg body weight, IP), rats were surgically implanted with a bipolar stimulating electrode (wire diameter 0.2 mm), aimed at the ventral aspect of the caudal periaqueductal gray [22].

Animals were tested for pain sensitivity using the tail-flick (TF) test [10, 12, 19]. A standard apparatus constructed in

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this laboratory was used for testing [25]. Tail-flick methodology introduces a noxious stimulus (radiant heat) to a point 5 cm from the tip of the tail; this area of the tail is blackened with a marking pen to provide a uniform surface for heating. Latency to remove (flick) the tail from the source of the stimulus is measured. Baseline TF latencies are established by adjusting the heat source intensity to the point where average latency to flick the tail is between 3.5 and 4.5 sec for four consecutive trials. During the test trials used to determine level of antinociception, heat source intensity remains constant at the level established at baseline for each animal.

Electrical brain stimulation, delivered by a Grass S6C Stimulator, consisted of trains of biphasic, rectangular wave pulse-pairs, 1 msec in duration and separated by 100 μsec pauses. Frequency of the pulses was 50 Hz and intensity of stimulation ranged from 10–100 μA. During brain stimulation and tail-flick tests, animals were confined in cylindrical plastic tubes with small openings at the nose, in the area of the electrode assembly, and at the tail.

During observation for opiate withdrawal signs, rats were placed in a clear Plexiglas observation box (base area 17×22×25 cm). Abstinence behavior was precipitated by the competitive opiate antagonist, naloxone. Naloxone hydrochloride was obtained from Endo Laboratories, dissolved in lactated Ringer's solution and injected intraperitoneally at a constant dose of 1 mg/kg body weight. In an attempt to quantify behaviors associated with opiate withdrawal a checklist format was used, integrating the observations of previous investigators [6, 7, 9, 27, 28]. One distinction made among types of withdrawal signs is that some can be easily quantified and counted as single events while others are more easily checked for their presence or absence during the observation period [6]. Following this distinction, signs were either noted for their presence or absence ("checked") or recorded for their frequency of occurrence ("counted") (see Table 1).

After recovery from implant surgery, subjects were randomly divided into two groups: Animals receiving brain stimulation and animals serving as sham-stimulated controls. All animals, whether receiving real or sham stimulation were subjected to within-subjects control conditions. In the first control condition, before delivery of focal brain stimulation (FBS), all rats were injected with naloxone and observed one hour for opiate withdrawal signs. This condition tested for any possible elicitation of opiate withdrawal behaviors simply from the administration of naloxone. In a second control condition, forty-eight hours later, all rats were confined in the stimulation tubes for two hours, but they did not receive FBS. They were then injected with naloxone and observed one hour for opiate withdrawal signs. This second condition tested for the effect of confinement upon the elicitation of withdrawal-like behaviors. Following another 48 hour interval, the experimental condition was initiated. Baseline tail-flick (TF) latencies were established for all animals and immediately thereafter, brain stimulation commenced. Thirteen animals were given real brain stimulation for 10 seconds, every two minutes, for a total of two hours duration. Six animals were given sham stimulation (electrode lead connected to animal but not to pulse generator). TF tests were given initially after the first burst of FBS and every 10 minutes thereafter in order to monitor the level of antinociception throughout the two-hour stimulation period. At the conclusion of the FBS procedure, Ss were injected with naloxone, and again observed one hour for opiate withdrawal behaviors.

TABLE 1

CHECKLIST OF CHECKED AND COUNTED OPIATE WITHDRAWAL SIGNS

Signs Noted for Their Frequency	
Exploring	Teeth chattering
Flying	Writhing
Wet shaking	Rearing
Signs Noted for Their Presence or Absence	
Scream on touch	Ear blanching
Handling hostility	Abnormal posture
Ptosis	Salivation
Eye-twitching	Swallowing movements
Rhinorrhea	Sedated appearance
Lacrimation	Exopthalmos
Diarrhea	Chromodacryorrhea
Penile Erection or Licking	

RESULTS

Antinociception was analyzed by comparing animals' baseline latencies to the post-FBS latencies. For animals receiving FBS, tail flick latencies rose from 3.98 sec at baseline to 8.55 sec following FBS. Animals maintained their analgesic state for the entire two hours of stimulation, however the mean latency fell somewhat, to 7.09 sec at the 60 minute mark and to 6.69 sec at the 120 minute mark. An analysis of variance comparing TF latency at baseline, after one burst of FBS, after one hour of FBS, and after two hours of FBS, found differences of means which were statistically significant, $F(3,36)=14.73$, $p<0.001$. A post-hoc Newman-Keuls Test revealed the post stimulation TF latencies to be all significantly longer than baseline, $p<0.05$. While the mean latency at the 120 minute mark was still significantly higher than baseline, it was significantly lower than the latencies immediately following the initiation of stimulation and at the 60 minute mark. The difference in latency suggests that although the animals were still analgesic, tolerance was beginning to develop after two hours of stimulation. Animals receiving sham stimulation had a mean baseline TF latency of 4.0 sec, with TF latencies remaining at baseline level during the entire two hour testing period.

Regarding opiate-like withdrawal behaviors, results indicate that some of the behaviors associated with classic opiate withdrawal are also observed following prolonged focal brain stimulation. Withdrawal sign occurrence was recorded and averaged for six consecutive 10 minute periods following naloxone injection. Data were analyzed separately for the "counted" signs and for the "checked" signs. Analysis was made between subject groups (FBS and sham-stimulated controls) and across conditions (naloxone control, confinement control, and FBS plus naloxone). For the group of animals receiving FBS, analysis of variance for repeated measures revealed differences among the conditions with regard to the withdrawal signs noted for their presence ("checked" signs), $F(2,24)=8.62$, $p<0.01$. The Tukey test for significant differences between means was administered post hoc and revealed a greater frequency of withdrawal signs following 2 hours of FBS than in either of the control conditions without FBS, $p<0.05$. The control conditions

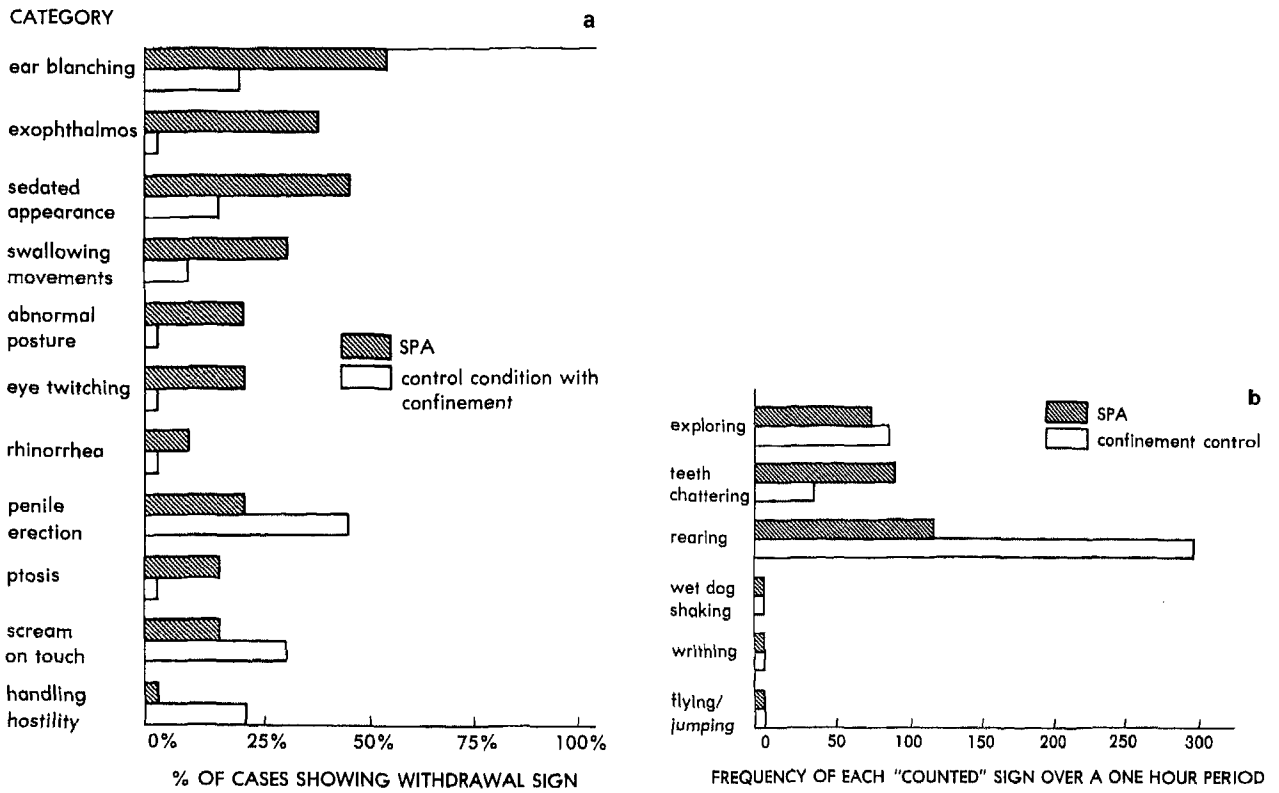

FIG. 1. (a) Percentage of cases showing withdrawal sign category: "checked" signs. (b) Frequency of "counted" signs category. SPA trial vs. 2 hour confinement control condition. Vertical axis refers to the withdrawal sign category. Abscissa refers to the percentage of cases in which the withdrawal sign was observed. Shaded bars represent the trials following focal brain stimulation. Clear bars represent the control condition.

within the experimental group (naloxone alone or naloxone plus confinement) were not significantly different from each other. There were no significant differences in withdrawal sign frequency across conditions from the control group receiving sham stimulation. Differences among the conditions with respect to the signs noted for their frequency ("counted" signs) did not reach significant proportions in either the stimulated or the sham stimulated control group. It appears, therefore, that the occurrence of withdrawal signs noted for their presence or absence (but not "counted" signs) increased in frequency following repeated FBS.

There are both similarities and differences between withdrawal-like behaviors following FBS and the withdrawal from systemically administered opiates. Animals receiving "low-dose" brain stimulation show ear blanching, swallowing movements and, to some degree, abnormal posture, but do not show wet-shaking, writhing, ptosis, scream-on-touch, or diarrhea. Additionally, animals receiving brain stimulation were observed chattering their teeth, a sign usually associated with high doses of opiates and often masking more recessive signs (see Fig. 1 for a complete breakdown of withdrawal sign categories and their frequency of occurrence). Finally, the time course for withdrawal from brain stimulation is different from that resulting from low dose opiates administered for brief periods and precipitated by 1 mg/kg naloxone [6]. With brain stimulation, the onset of withdrawal-like signs is sometimes later and sometimes earlier than the same signs occurring during opiate withdrawal. Furthermore, with brain stimulation, once the behavior occurs, it seems to persist for the entire observation period, while during withdrawal from systemic opiates, the behavior may last for a shorter duration (see Fig. 2).

DISCUSSION

It is known that the specific behaviors associated with opiate withdrawal depend on many factors, including the dose of the opiate producing the dependence, the length of time the opiate is administered, and the dosage, if any, of a competitive antagonist [6,27]. Abstinence behaviors associated with mild opiate withdrawal (i.e., that induced by low doses of opiates, short administration time, and low doses of antagonists), include diarrhea, ear blanching, swallowing movements, abnormal posture, wet-shaking, ptosis, and scream-on-touch. Some of these behaviors are termed recessive, in that they can be masked or suppressed by dominant behavior associated with severe withdrawal. Severe withdrawal (that precipitated by high opiate dosages, long administration time, and high doses of antagonists) typically induces teeth chattering, flying, jumping, and exploring. These and other motoric behaviors are said to be dominant if they occur in the first fifteen minutes of withdrawal and mask other signs of withdrawal.

The present study used low "doses" of FBS, short administration time, and low dose of antagonist to precipitate withdrawal. The brain stimulation involved the lowest possible current to elicit analgesia, usually not more than 40 μA. Stimulation periods were brief (2 hours) and did not

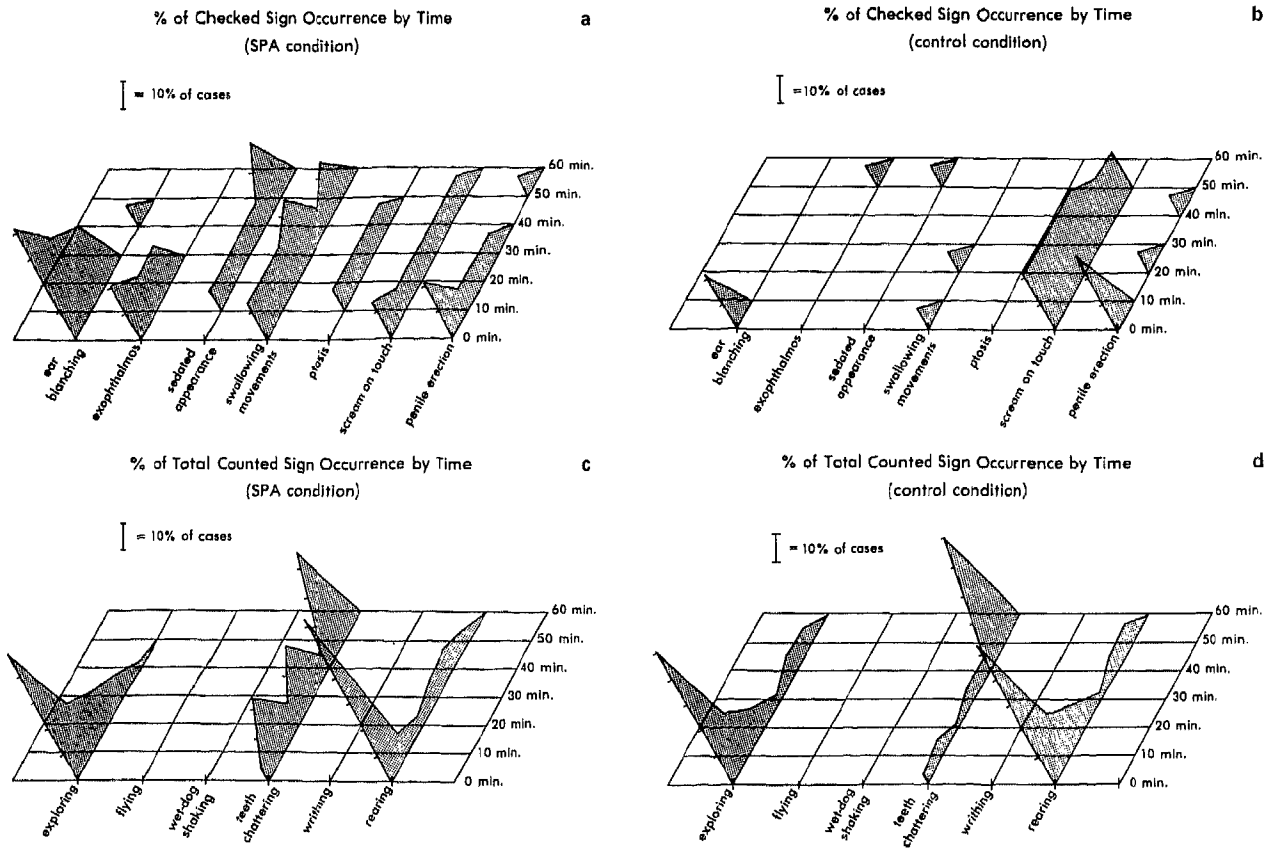

FIG. 2. Time course of withdrawal sign occurrence after naloxone injection. Ordinate: subdivisions of six 10 minute observation periods; abscissa: a categorical listing of the withdrawal signs. Elevation refers to the incidence of each sign. One mm of elevation represents 10% of the cases. (a) "checked" signs, post-stimulation; (b) "checked" signs, confinement control; (c) "counted" signs, post-stimulation; (d) "counted" signs, confinement control.

result in total reversal of antinociception. Finally, the dose of naloxone (1 mg/kg) is on the lower end of the concentration used to precipitate opiate withdrawal [6]. In this sense, therefore, the electrical stimulation used in this experiment is analogous to a modest opiate administration—that sufficient to precipitate low-level withdrawal behavior without the symptoms of severe high-dose withdrawal.

It is interesting that no motor behaviors associated with low dose opiate withdrawal occurred, and that most abstinence behaviors seen were associated with autonomic changes. This suggests some degree of neuroanatomical specificity in terms of the substrate for opiate-like withdrawal behaviors. However, not all of the autonomic signs associated with low dose dependence were observed. For example, diarrhea, one very reliable low-grade opiate withdrawal sign associated with autonomic changes, did not occur. This may suggest a further specificity in neuroanatomical substrates for opiate withdrawal behavior. On the other hand, it is possible that some abstinence signs are peripherally mediated. Because the stimulus causing the withdrawal-like behaviors was centrally produced (i.e., focal brain stimulation), if a certain abstinence sign is peripherally mediated, this sign should not be expected. There is some suggestion that diarrhea is in fact peripherally mediated [31], and therefore, this particular sign should not be expected.

Teeth chattering, the single high-dose dominant sign, was evident throughout the entire sixty-minute observation period. Because of its dominant characteristics, it may have masked other low-dose withdrawal behaviors. It is also speculated that the teeth chattering was related to stress-induced hypothermia, as evidenced by the observance (to a lower degree) of teeth chattering in the confinement control condition.

It was expected that withdrawal-like behaviors associated with FBS would differ behaviorally and temporally from the classical opiate withdrawal syndrome. Two groups of behaviors not associated with opiate withdrawal but observed in about half of the stimulated animals were absence of sleep and a vigilant stance (characterized by prolonged rocking of a tense, hyperalert posture). Possibly these are signs associated with some type of rebound effect from the stimulation. Interestingly, the other half of the stimulated animals demonstrated a sedated appearance, rather than a vigilant appearance (a low-grade opiate withdrawal sign).

This study suggests that focal stimulation of areas of the brain which are rich in endogenous opiate receptors can result in behaviors associated with those signs observed during opiate withdrawal. Nevertheless, the pattern of observed behaviors following FBS is quite different from patterns seen after low grade opiate withdrawal. That any behaviors simi-

lar to opiate withdrawal can be elicited from brain stimulation alone suggests a powerful demonstration of the link between neural mechanism for stimulation-produced analgesia and for administered opiate substances.

It is hypothesized that stimulation of longer duration and/or an increase in the dose of naloxone used to precipitate withdrawal would produce a dose-response effect similar to that seen when increasing doses of morphine are used to study classic opiate withdrawal. Future research will focus on such experimental manipulations.

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