Pharmacology Biochemistry & Behavior, Vol. 11, pp. 11–15. Printed in the U.S.A.

Morphine Withdrawal Induced Behavior in the Syrian Hamster (*Mesocricetus Auratus*)[1]

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Received 24 November 1975

AVIS, H. H. AND H. V. S. PEEKE. *Morphine withdrawal induced aggression in the Syrian hamster* (Mesocricetus auratus). PHARMAC. BIOCHEM. BEHAV. **11**(1) 11–15, 1979.—The effects of morphine withdrawal on a number of behaviors was assessed using a design varying sex, amount of morphine implanted, degree of dependence at the time of testing and amount of morphine antagonist injected. Increases in two types of agonistic behavior were seen and may be related to the aversive interoceptive stimuli associated with withdrawal. Furthermore, correlations for males were found between agonistic behavior and activity, wet shakes, digging and vocalization. Agonistic behavior was correlated only with activity in females.

Morphine withdrawal Aggression Hamster

VIRTUALLY all of the research on the effects of morphine withdrawal on aggression has been done with the rat, and the parameters that mediate this behavior in this species are well documented. Increases in fighting are seen with either shock-elicited aggression [4] or spontaneous aggression [10]. Rearing conditions, lighting, strain differences, opportunity for socialization, sex, age, cage size and season all seem to effect the expression of withdrawal induced aggression in rats [2, 5, 16]. Biochemical and pharmacological studies indicate that dopamine may play a mediating role in withdrawal induced aggression [7, 10, 15].

However, the laboratory rat has been bred for hundreds of generations for ease of handling and docility. This selective process would seem to make the rat a less than optimal subject for studying aggression.

Furthermore, investigators typically have observed only a few withdrawal associated behaviors at a time. Investigators concerned with the pharmacological and biochemical variables mediating withdrawal have similarly sampled only a few behaviors such as withdrawal induced jumping [3,9], vocalization [15], and wet shakes [17]. Individual behavior sequences do not appear in independent packages. There is a stream of behavior [1] and observing any one behavior may produce an arbitrary sample of the animal's behavioral repertoire. Since withdrawal produces a number of physiological and behavioral changes, an examination of the entire repertoire of the animal would seem valuable.

The following research describes the effects of morphine withdrawal on a wide range of behavior of the Syrian hamster (*Mesocricetus auratus*). Although very little is known of the behavior of this species in its native habitat, the agonistic behaviors seen in the laboratory have been extensively studied. These behaviors have been described in detail [8,13].

EXPERIMENT 1: EFFECTS OF PRECIPITATED WITHDRAWAL ON BEHAVIOR

METHOD

Procedure

The animals were 144 golden Syrian hamsters (*Mesocricetus auratus*) half male and half female, purchased from Simonsen Laboratories, Gilroy, California. They were segregated by sex and housed four to a cage for at least two weeks prior to implantation. All animals weighed from 100–160 g at the start of the experiment. One cage of four animals of each sex was used for each condition. The animals were kept on a 12/12 light-dark cycle and all testing was done within the first hour after onset of the dark cycle with the test area illuminated by red light. The animals were maintained on food and water ad lib throughout the experiment.

The animals were made dependent on morphine by the subcutaneous implantation of 75 mg of morphine sulfate. The pellet consisted of morphine sulfate, 75 mg: microcrystalline cellulose, 75 mg: fumed silicone dioxide, 0.75 mg: and calcium stearate, 1.5 mg. The size of the pellet was 3 mm in diameter. All animals were tested in their own home cage and no attempt to determine premorphine social hierarchies was made.

Placebo pellets consisted of lactose. All pellets were formulated by Dr. R. D. Gibson of the University of California Pharmaceutical Technology Laboratory, San Francisco, California. The pellets were implanted with the hamster under light anesthesia (ether) by making a small subcutaneous incision on the back of the animal in the area of the upper vertebrae. The incision was closed with cotton thread. Withdrawal was precipitated by the injection of naloxone hydrochloride donated by Endo Laboratories, Garden City,

[1]This research was supported by United States Army Medical Research and Development Command Contract DADA-17-73-c-142.
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New York. Pellets were left in place during the withdrawal period.

The behaviors were observed by one of two raters. Inter-rater reliability was .90 based on preliminary observations in which both observers examined animals in the same cage at the same time.

The following variables were examined: sex, number of pellets implanted (1 or 2), amount of naloxone injected (0.5, 2.0 and 4.0 mg/kg) and day after pellet implantation (1, 2 or 3). Observations were recorded on a tally sheet in 30 sec segments for 18 min after the injection of naloxone. Control groups were observed without pellets and injected with naloxone, and with pellets injected with saline. The following behaviors were observed:

(1) Activity. Defined as the continuous movement across at least one quadrant of the cage within 30 sec.

(2) Digging. Defined as the animal scraping at the cage bottom (covered with ground corn cobs) with its front paws. The total number of responses that met this definition per segment was recorded.

(3) Vocalization. Defined as a shrill sound made by any of animals during the 30 sec segment.

(4) Wet shakes. Defined as a rapid movement of the whole body similar to shivering. The total number of wet shakes per 30 sec segment was recorded.

(5) Yawning. Was defined as a rapid wide opening of the mouth so that the canine teeth were exposed. It usually occurred contiguously with an upward thrust of the head.

(6) Defensive/submissive behavior. This class of behavior was defined on the basis of our observations and those of others [8]. Three behaviors were included in this category: full submissive (animal lying flat on its back), upright defensive posture (animal upright with paws covering the ventral marking and head oriented away from opponent), and sideways posture-defensive (animal at right angles to opponent with closest front and rear legs raised and head oriented away from opponent). Only dyadic interchanges were observed and other animals must have been exhibiting defensive-submissive behavior in order to fit this category.

(7) Offensive/dominant behavior. This class was based on the same criteria as above and consisted of the following: upright offensive (animal on hind legs with ventral markings exposed and oriented toward the opponent), sideways offensive (same as defensive but with orientation toward opponent), and any combination of rolling, biting and chasing. An interchange was defined as an offensive dyad when one of the two animals exhibited these behaviors.

RESULTS

Injections of naloxone produced a number of changes in ongoing behavior when given to morphine dependent animals. Since there were no differences between control groups implanted with pellets and injected with saline and control groups implanted with placebo pellets and injected with naloxone these were combined for statistical analysis. These data were collected as frequencies, therefore an appropriate and conservative strategy was to analyze the data by means of a χ^2 test.

Agonistic Behavior

Morphine implanted animals injected with naloxone showed more agonistic behavior than controls ($p<0.001$ χ^2 test). All discussions that follow are comparisons within morphine implanted groups.

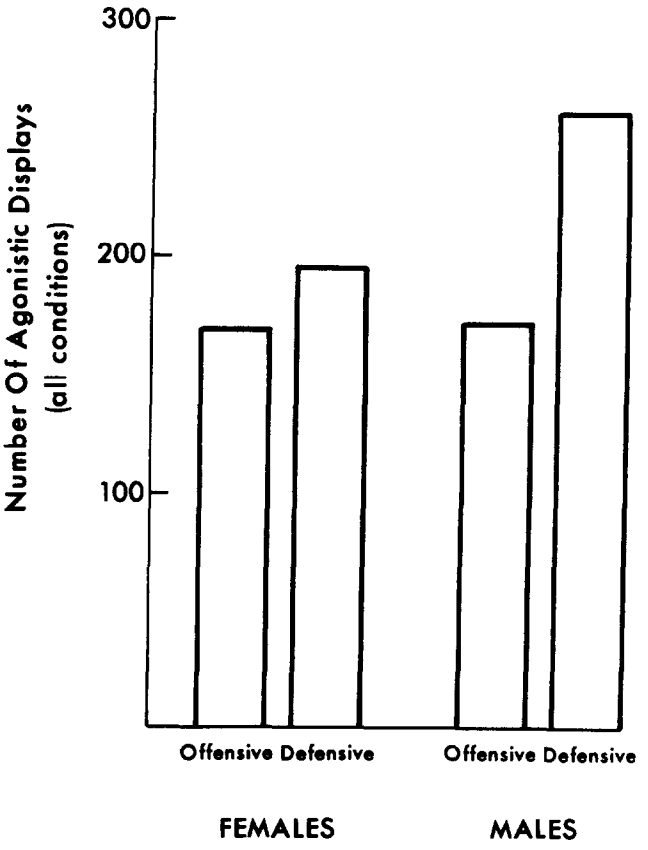

FIG. 1. Number of displays elicited by naloxone injections in morphine dependent animals (offensive and defensive).

Sex

Males and females did not differ in the amount of agonistic behavior shown when both offensive and defensive dyads were combined. There was, however, a significant difference when offensive and defensive dyads were examined separately ($p<0.01$ χ^2 test). This difference was due to the larger number of defensive dyads shown by males (Fig. 1). These data are in contrast to those showing that female hamsters are more "spontaneously" aggressive than males [15] and to the data showing that female rats did not display withdrawal induced aggression [5].

Day After Implantation

Most agonistic behavior occurred on the first or second day after implantation. The difference across days was significant ($p<0.01$ χ^2). Since the χ^2 test is insensitive to order effects, when $k<2$, individual comparisons were made between Day 1 and 2 and 3. Both of these were significant ($p<0.05$ and $p<0.001$ respectively, χ^2 test). As can be seen in Fig. 2, aggressive behavior was greater on Day 1 than Day 2 and less on Day 3 than on either Day 1 or 2. This finding is supported by the observation that after three days encapsulation of the pellet had begun.

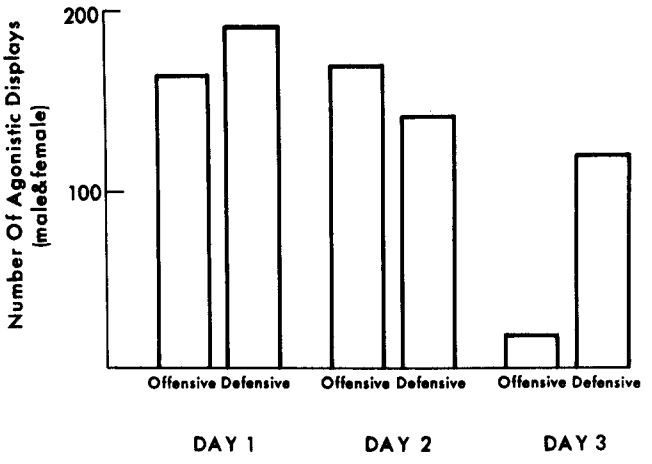

FIG. 2. Number of displays in offensive and defensive behaviors for males and females together as a function of days after pellet implantation.

Number of Pellets

On both Day 1 and Day 2 after implantation, those animals receiving two pellets showed more agonistic behavior than those given only one pellet ($p<0.01$ χ^2 test). By Day 3, perhaps because of the general low level of withdrawal related behavior, there was no effect of the number of pellets implanted ($p>0.10$ χ^2 test).

Dosage of Naloxone

Dosage affected behavior in a complex manner. The precise nature of the relationship is impossible to determine within the design of this experiment and the statistical procedures used.

Other Withdrawal Related Behaviors

For the purpose of statistical analysis groups were combined across naloxone dosage and number of pellets implanted. Thus, only sex and day after implantation are discussed.

Injection of naloxone in dependent animals brought about a number of behavioral changes (wet shakes, digging, vocalizations and yawning and activity) besides an increase in agonistic behavior. The direction of change was the same as for agonistic behavior: all showed an increase over control groups implanted either with lactose pellets and injected with naloxone or implanted with morphine pellets and injected with saline ($p<0.001$ χ^2 test).

Three of the withdrawal produced behaviors seem to be related to thermoregulatory mechanisms. Wet shakes have been suggested as a thermoregulatory mechanism in the rat [6], and changes in ambient temperature have been shown to affect digging in hamster pups [1]. Activity increases might also be expected as a means of compensating for decreases in core temperature.

Wet Shakes

There was no difference between males and females when the data were combined ($p<0.01$ χ^2 test). A significant difference across days was seen ($p<0.01$ χ^2 test), with number of shakes being highest on Day 1 and lowest on Day 3.

Digging

Males showed significantly less digging than females ($p<0.01$ χ^2 test) and for both groups there was a significant difference across days ($p<0.01$ for males and females χ^2 test). The relationship between days and digging was different for males and females: females showed most digging on Day 2, while for males Day 1 and 2 were virtually identical. For males, digging was lowest on Day 3, for females on Day 1.

Activity

Females showed more activity in response to morphine withdrawal than males ($p<0.05$ χ^2 test) and the difference was significant across days for females ($p<0.01$ χ^2 test), but not males ($p>0.90$ χ^2 test).

Vocalization and Yawning

In contrast to the effects of withdrawal on digging and activity, males showed more yawning and vocalization than females ($p<0.01$ χ^2 test for both). Both males and females showed more vocalization and yawning than controls, and there were no differences across days.

Relationship Between Agonistic and Other Behaviors

As was discussed above, viewing only one of several withdrawal induced behaviors can be misleading. Therefore, the correlations between agonistic and other behaviors were examined. Table 1 shows the relationships between agonistic behavior and activity, wet shakes, digging and vocalizations for males and females. As indicated, all correlations are significant for males; however, only activity is correlated with agonistic behavior in females and that correlation is quite low.

TABLE 1

SPEARMAN RANK ORDER CORRELATION BETWEEN VARIOUS BEHAVIORS

Agonistic Behavior and:	Males	Females
Activity	0.77*	0.46*
Wet shakes	0.61*	0.32
Digs	0.64*	0.09
Vocalizations	0.78*	0.42

*$p<0.05$

Since three of the withdrawal related behaviors might be linked to thermoregulatory mechanisms, and since, for males at least, these three behaviors are correlated with agonistic behavior, the following experiment was conducted to determine if withdrawal has any direct effect on core temperature in the hamster.

EXPERIMENT 2: EFFECTS OF WITHDRAWAL ON TEMPERATURE IN THE HAMSTER

METHOD

Procedure

Twelve male hamsters were used in Experiment 2. All hamsters were implanted with two pellets of morphine sul-

fate as described in Experiment 1. One hamster died during the operation of implantation. Hamsters had been housed in groups of four at least two weeks prior to implantation and all hamsters weighed at least 100 g at the start of the experiment.

Core temperatues were obtained by inserting a rectal probe approximately 3 cm past the anal sphincter. Because morphine administration results in decreased intestinal motility, any fecal matter that could be palpated was manually expressed prior to insertion of the probe. This procedure was necessary because in preliminary experiments it was determined that when the temperature sensitive tip of the electrode was in contact with a bolus spurious temperatures resulted.

Temperature determinations were made 10 min prior to injection, and 5 min, 15 min, and 30 min after injection. Animals were injected with naloxone (2 mg/kg) or with saline on Days 1, 2 and 3 after implantation. Each of the three groups of four animals received naloxone on two days and saline on one, the order being determined randomly.

RESULTS

Significant changes in core temperature were found on Day 1 ($p<0.01$, Friedman two-way analysis of variance). The median temperature on Day 1 was 37.7°C prior to naloxone injection. The temperature decline was greatest at 30 min (0.4°C). No significant differences were seen on Days 2 or 3. No changes were seen in animals when injected with saline.

GENERAL DISCUSSION

A number of points emerge from the experiments described above. First, two types of agonistic behavior can be elicited by withdrawal—a predominantly defensive pattern and a predominantly offensive pattern. Males show more defensive behavior than females. These two patterns might reflect a continuum of withdrawal induced "aggressiveness" depending on severity of dependence. On the other hand, these two patterns might reflect the relative influence of more than one taxon of agonistic behavior. The defensive dyads may reflect irritable aggression [13], while the offensive dyads might be another taxonomic category. At least part of the agonistic behavior may be the result of aversive internal stimuli produced by withdrawal [2].

The observation that male and female hamsters do not differ in the amount of agonistic behavior is not consistent with other studies showing the females are more aggressive than males, nor with the report showing the female rats do not show withdrawal induced aggression [5]. Species differences could account for the latter findings, but, in addition there are methodological differences between this study and earlier reports. For example, in previous studies the hamsters had been individually housed [14], while the hamsters in the present study were housed in groups of four. Furthermore, spontaneous aggression produced perhaps by isolation, may be different than withdrawal induced agonistic behavior. Another possibility is that withdrawal induced aggression is more severe in males, thus overshadowing the usual greater aggressivity in females.

The significant correlations between activity and agonistic behavior for both sexes suggest the possibility that increased activity may increase the probability of two animals interacting. This increase in activity concomitant with the aversive properties of withdrawal could explain the increase in fighting without requiring a direct effect of aggressiveness and clearly points out the necessity of examining behaviors other than fighting when studying aggression. The lack of correlation in females and relatively high correlation in males for agonistic and other behaviors is curious. Since no attempt was made to ascertain the estrus condition of the females, and since female hamsters do show changes in agonistic behavior related to estrus, it is possible that estrus changes could interact with either fighting or other behaviors to reduce correlations.

The correlation between number of agonistic behaviors and vocalization is consistent with previous findings in rats [16]. However, it should be emphasized that no attempt was made to determine the actual contiguity in the time of these two behaviors. In fact, observation indicated that vocalization was not necessarily related to fighting: vocalizations frequently occurred in the absence of fighting, while the opposite was true as well. However, when totaled within the two minute observation bins, these behaviors appeared to covary.

Since core temperature was reduced only on Day 1, increases in digging, wet shakes and activity cannot be attributed solely to temperature alteration. It is possible that withdrawal produces a dissociation between thermoregulatory mechanisms and the behaviors that are related to them. It is also possible, however, that withdrawal produces rapid fluctuations in core temperatures, and this variability, particularly on Day 2 and 3, obscured any overall changes in core temperature. One would expect that continuous monitoring of temperature would indicate if the latter were the case.

It was reported that naloxone did not produce aggression in morphine dependent rats [6]. In addition to species differences there are a number of methodological differences which may account for this discrepancy. First, we used the pellet implantation method as opposed to daily injections of morphine [6]. Second, we observed the animals immediately after injection of naloxone as opposed to 10 min after naloxone injection [6]. Since in our study examination of the data revealed that most of the aggressive episodes occurred prior to 10 min after injection, the previous failure to observe aggression might be a temporal artifact. Furthermore, although not directly stated, rats in the Gianutsos *et al.* [6] study were apparently individually housed as opposed to being housed in cages of four for our study. Since the laboratory rat is notoriously docile, it may simply be that naloxone induced withdrawal is less potent at eliciting aggression than cessation of daily injections which clearly increases fighting in rats [7]. Thus, the baseline against which aggression is measured may be crucial.

Finally, our data do not support the view that "withdrawal aggression has different mechanism from other withdrawal signs" [6]. Instead, withdrawal reliably elicited aggression, and the agonistic behavior was highly correlated with other withdrawal behaviors (at least for males). The data suggest that the hamster is a useful model for studying withdrawal induced aggression. Differences between the two species should be explored further.

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