Some determinants of aggressive behavior induced by morphine withdrawal*

W. MARVIN DAVIS and J. H. KHALSA†

School of Pharmacy, University of Mississippi, University, Miss. 38677

Male and female rats of Sprague-Dawley and Long-Evans strains were given daily intraperitoneal injections of morphine sulfate in increasing dosage over a 15-day period to a terminal dose of 405 mg/kg/day. During withdrawal, fighting behavior occurred equally among older male rats (60 days at start of experiment) of both strains, housed in groups of six, but not at all in younger ones (30 days). A strain difference found earlier in 45-day-old rats was confirmed. Females of either strain or age showed no aggressive behavior during withdrawal. Decreasing available cage space increased the number of aggressive encounters, despite a reduction of group size to four. Saline-injected males of either strain rarely displayed aggression. A circadian rhythmicity of such behavior was not found. There was evidence for a seasonal variation in frequency of morphine-withdrawal-induced aggression, levels being higher in summer than in winter.

Previous investigations have shown that the withdrawal of morphine after chronic treatment induced fighting behavior in male Sprague-Dawley rats and Long-Evans rats (Boshka, Weisman, & Thor, 1966; Thor & Teel, 1968; Thor, Hoats, & Thor, 1970). We have reported a significant variation between three strains of rats (Sprague-Dawley, Long-Evans, and Wistar) in the degree of aggressive response induced by morphine withdrawal (Borgen, Khalsa, King, & Davis, 1970). The present work was conducted to determine the influence of several other factors, such as sex, age, group size, season, and time of day, on aggression associated with morphine withdrawal.

SUBJECTS

Experiment 1

Rats of the Long-Evans (LE) and Sprague-Dawley (SD)¹ strains were used to study the influence of age (plus a suspected interaction of age with strain) and of sex upon withdrawal-associated aggression. An overall total of 288 Ss comprised the sample in this experiment. For each sex, 72 rats were used from each of the strains. These were divided equally between two age levels—30 and 60 days of age at the initial injection of the experimental series.² Among the 36 rats of each sex-strain-age category, 24 received injections of morphine, while 12 received a saline control injection. Ss were housed in groups of six per cage, measuring 35 cm wide x 56 cm deep x 24 cm high. Male and female Ss were housed and observed in

separate but identical rooms to preclude any possible confounding influence from the presence of the other sex.

Experiment 2

To study the influence of group size, 60 male LE rats, 45 days old, were housed under two conditions: Condition A consisted of four Ss per cage (21 x 27 x 20 cm), and Condition B consisted of six per cage (35 x 56 x 24 cm), as in Experiment 1. In each case, there were four morphine groups and two saline placebo groups, as in Experiment 1. An additional 36 SD males under B conditions were

Fig. 1. Comparison of fighting responses during morphine withdrawal in male rats of the Sprague-Dawley (circles) and Long-Evans (squares) strains, 60 days of age at beginning of the experiment.

treated in like fashion to permit an interstrain comparison at this age. Food and water were available ad lib throughout both experiments. All animals were ear-notched and tail-marked for certain identification before being assigned randomly to groups.

Prior to and throughout the experiments, the Ss were maintained on an artificial (fluorescent) illumination schedule of 16 h of light (0600 to 2200) and 8 h of darkness. The rooms were illuminated by 60-W red incandescent bulbs during the dark phase to enable behavioral observation during the withdrawal period. Whereas our earlier study (Borgen, Khalsa, King, & Davis, 1970) was conducted in summer (July-August), the present study was carried out in winter (January-February). Therefore, an evaluation for a potential influence of seasonal variation on this type of aggressive behavior was possible. Equal conditions of lighting, room temperature, and humidity were maintained between the two studies.

PROCEDURE

In both experiments, the Ss were given injections of morphine sulfate intraperitoneally, starting at 15 mg/kg/day, and increasing daily over a 15-day period to a final dosage of 405 mg/kg/day, with equal doses given at 0600, 1400, and 2200 h. Placebo injections were of 0.9% NaCl solution. A constant injection volume of 1.0 ml solution/200 g body weight was used throughout. After the 15-day injection period, the Ss were observed as described earlier (Borgen, Khalsa, King, & Davis, 1970). Briefly, an aggressive response was recorded only when at least two Ss assumed an upright fighting posture, facing each other, and a biting attack with vocalization occurred. Such episodes were recorded, as noted visually by one O, during 15-min periods every 2 h for 6 consecutive days. Statistical comparison of the data from different experimental groups was by means of the Wald-Wolfowitz runs test or the Mann-Whitney test (Siegel, 1956).

RESULTS

Fighting responses of only the older male SD and LE rats (60 days old) are shown in Fig. 1. The younger (30-day-old) males, the females of both ages and strains from the morphine-treated groups did not display any aggression; therefore, they are omitted from the figure. Similarly, placebo-injected Ss of either strain, sex, or age showed no aggressive behavior. It can be seen that the older males of both strains manifested about equal fighting. The only apparent difference was the time of onset of aggression in the two strains, duration being the same. Aggressive behavior

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Table 1
Total Fighting Episodes During Morphine Withdrawal for Male Rats
of Two Strains Under Different Conditions

Strain	Age and Season			
	30 Days Winter	45 Days Winter	45 Days Summer	60 Days Winter
Long-Evans	0 (0)*	153 (0) 304 (3)**	460 (71)	718 (0)
Sprague-Dawley	0 (0)	50 (16)	50 (57)	632 (0)

*Figures in parentheses are numbers of fighting episodes for corresponding saline-injected controls.

**These figures are for groups of four per cage; all others represent groups of six per cage.

began between 30 and 40 h of withdrawal for the SD strain and between 60 and 70 h for the LE strain. In each strain, the behavior lasted about 40 h. Data of the earlier study (Borgen, Khalsa, King, & Davis, 1970) and from these experiments bearing on the age and strain factors are combined in Table 1.

Fighting responses of the two drug-treated groups of Experiment 2 are presented in Fig. 2 and Table 1. It is evident that the aggressive behavior among rats housed in smaller but more crowded groups was significantly greater ($p < .01$).

It may be noted, by comparing data of the present and the previous study on 45-day-old LE males (Table 1), that the fighting behavior displayed was less during the winter, total occurrences 153, than that in the summer season, total occurrences 460 ($p < .01$). This is also supported by data from associated controls which showed 71 occurrences in the summer and none during the winter. However, such a difference did not appear in data from the SDs.

Data on aggressive behavior displayed by male Ss of both the present and the previous study were analyzed for a day-night difference in this type of aggression. The totals of all aggressive interactions for each cage group seen during the four intervals of "night" observations were compared to those from the four intervals of "day" observations (the two each immediately preceding and following the dark period). Although there had seemed to be more fighting at night, neither the LE strain nor the combined data of SD and Wistar strains showed a significant difference between night and day.

During morphine withdrawal, another significant behavior observed was the previously described "wet dog shakes" [Martin, Wikler, Eades, & Pescor, 1963]. We again observed a pronounced stretching movement, with apparent hindleg weakness, similar to the "writhing" noted in rats and mice after intraperitoneal administration of irritating and

pain-producing substances, as reported earlier (Borgen, Khalsa, King, & Davis, 1970). Also present, although not previously reported under these conditions, was mounting activity by male Ss toward other males of the group, seen especially in the LE strain. In Experiment 1, mounting behavior was observed 73 times among LEs but only 11 times among SDs. In Experiment 2, there were six such occurrences under the B condition and none in the A condition. This behavior, which has never been noted by us in placebo groups, was observed many times to precipitate severe fights between two morphine-treated rats.

DISCUSSION

It is evident from the present study that the female rats of both strains were not induced to display any aggressive behavior during morphine withdrawal. Lack of fighting among females is not unexpected in view of

various other works on aggression in rats (Valzelli, 1967). However, it is of interest that a new method of inducing fighting by food deprivation plus marijuana treatment was recently reported effective also in females (Carlini & Masur, 1970).

The absence of aggression in the youngest (30-day-old) males and the lesser level in 45-day-old than in 60-day-old rats may be attributable to an incomplete development of those functions of the central nervous system essential for such behavior, because of low levels of testosterone, which has been reported to be critical for the development of brain (Rothballer, 1967).

The lack of a strain difference in the data of Experiment 1 (Fig. 1), in contrast to the results of an earlier study (Borgen, Khalsa, King, & Davis, 1970), may be attributable to a strain-age interaction. Our earlier study (confirmed by additional data here reported), which showed that Long-Evans male rats were more aggressive than Sprague-Dawleys, was conducted with Ss at 45-47 days of age. Because the 60-day-old rats of the present study were about 15 days older, perhaps both strains had by then passed a critical phase of maturation, whereas they were manifesting unequal rates of maturation at the earlier age.

A significant increase in aggressive behavior in Group A of Experiment 2 over that in Group B (Fig. 2) may be explained by the fact that the former Ss were in more crowded conditions (ca 142 cm² per rat) than those in Group B (ca 327 cm² per rat). Our main objective in this experiment was to determine if the group size for such studies could be reduced from six to four. Decreasing the area per rat served effectively to permit such a change in design, not only maintaining, but actually increasing, the level of aggression.

Sofia & Salama (1970) reported circadian rhythms in experimentally induced aggression among mice. Our failure to detect a significant difference in aggressive interaction between the light and dark periods does not provide support for the general occurrence of circadian rhythms in experimentally induced aggressive behavior. However, a direct comparison between the studies is unwarranted because of the many procedural differences.

The latency of onset for morphine withdrawal aggression ranged from about 40 h to more than 60 h. Peak effects were generally between 60 and 80 h. These events are clearly later than the "primary abstinence syndrome" of the rat (Martin, Wikler, Eades, & Pescor, 1963), which

Fig. 2. Comparison of fighting responses during morphine withdrawal in male Long-Evans rats as a function of housing conditions, 142 cm² floor area per rat (squares) and 327 cm² per rat (circles). One saline control group shown by open squares.

appeared by 8-16 h, became fully developed by 24 h, and subsided by about 72 h of withdrawal. Rather, the aggressive behavior more nearly coincides with the "secondary morphine abstinence," which was described as beginning at about 72 h. However, there is presently such a lack of knowledge concerning the factors causing these various postwithdrawal phenomena that it is futile to speculate about their interrelationships.

Lastly, our data for the LE strain indicates a seasonal variation in aggressive behavior, evidenced by a greater and lesser degree of aggressive manifestations during summer and winter seasons, respectively. It may be significant that Fugazza (1963) found higher brain norepinephrine levels for male rats in summer and fall than in the winter. Our results also may be compared with the reports of Valzelli & Garattini (1968), who have detected some behavioral changes in parallel with seasonal variations in brain serotonin levels seen in the laboratory rat. The possibility that there may be a causal relationship between brain

biogenic amine levels and seasonal, strain, and age differences in aggressive behavior seen during morphine withdrawal seems worthy of further investigation.

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NOTES

1. Sources: Sprague-Dawley rats, Holtzman Co., Madison, Wis.; Long-Evans rats, Simonsen Laboratories, Gilroy, Calif.
2. Age-group identification in each case is by age in days upon beginning each experiment rather than age at observation for aggressive behavior.