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Behavioural and Endocrine Effects of Naltrexone in Male Talapoin Monkeys

RACHEL E. MELLER, E. B. KEVERNE AND J. HERBERT

Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, England

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MELLER, R. E., E. B. KEVERNE AND J. HERBERT. *Behavioural and endocrine effects of naltrexone in male talapoin monkeys*. PHARMAC. BIOCHEM. BEHAV. 13(5) 663–672, 1980.—The effect of treating captive male talapoin monkeys with naltrexone hydrochloride (500 μg/kg intra-muscular injection twice daily) was studied both in socially living and singly caged animals. The behaviour of the group males and endocrine changes in all treated animals were monitored during the course of treatment and on drug withdrawal. Naltrexone significantly reduced sexual behaviour in previously active males, while increasing grooming interactions. Aggressive behaviour did not change. There was an overall significant elevation in testosterone, LH and cortisol during drug treatment and a significant decrease on withdrawal. Changes in prolactin in response to naltrexone depended upon the pre-treatment level of this hormone: in males in which levels were low, there was a significant elevation in prolactin, while in those with high pre-treatment prolactin, levels were unchanged by the drug. The behavioural changes reported for this primate are in direct contrast to changes reported in rodents, while the hormonal changes, except for prolactin, are comparable to others reported.

Naltrexone Talapoin monkeys Sexual behaviour Grooming Testosterone Cortisol LH Prolactin Dopamine

THE behavioural significance of naturally occurring opiate-like peptides, recently discovered in the mammalian brain and cerebrospinal fluid [4,19], is still open to question, though speculative attempts have already been made to implicate them in mental disorders such as schizophrenia [3, 17, 23]. Numerous experiments, conducted mainly on rats, have shown that both endorphins and enkephalins can produce a variety of motor effects, including catalepsy and immobility (e.g. [3]), while much emphasis has also been placed on their analgesic action, in which respect they resemble morphine or related opiates [1]. Morphine is also known to have more general behavioural actions, including the depression of sexual activity: this raises the possibility that the endogenous opiates may have some role in regulating such behaviour. One purpose of the present study was thus to determine whether blocking the endogenous opiate system with the specific antagonist naltrexone would alter sexual interactions in a non-human primate. No relevant information for experimental primates exists, although it has been reported that both naltrexone and naloxone can enhance sexual activity in male rats [10,27].

It has been suggested that the opiate system is involved in complex psychological states, as well as pain sensitivity, in man [14, 17, 24]. That this might be the case is further suggested by a consideration of the well recognised but little understood relationship between neuroendocrine function and mood. Endocrine changes are now recognised as an important concomitant of affective disorders in humans [5]; the endorphins are known to have marked effects on endocrine state [15, 31, 32]. It is thus possible that the opiates may represent a common link in this relationship.

In captive talapoin monkeys the social structure of a group affects both the endocrine function and the behaviour of individuals within it [22]. The animal's position in the aggressive hierarchy is particularly significant: social subordination, for example, can elevate serum prolactin and cortisol as well as inhibit sexual behaviour in both males and females [21]. The second purpose of this study was therefore to determine whether opiate receptor blockade by naltrexone had any effect on endocrine state as well as behaviour in talapoin monkeys living in a social group, and whether the response of an individual depended on his social position in such a way as to suggest differential activation of the endogenous opiate system according to rank.

METHOD

Animals

The experimental animals were all captive adult talapoin monkeys which had been in the laboratory for at least five years. Details of housing, diet and behavioural observations have been reported elsewhere [22]. Only males were treated with naltrexone: they were housed either in a large group cage (3.5×1.5×1.7 m) or in smaller cages (0.8×0.8×0.5 m) singly or in a pair (see below) in a separate communal room. The dates of drug treatment, blood sampling and behavioural observations are given in Fig. 1 for group and singly caged animals. An interval of one day is shown by consecutive asterisks (marking days of observations).

Group caged males. The group consisted of three or four males and four females. The latter had been ovariectomised and received oestradiol replacement (subcutaneous silastic implants) during each experimental period, a treatment

which renders them sexually attractive to the males. Unlike the females, all the males in the group were intact. The sexes were separated except for the duration of behavioural testing, which took place daily for two 50 min periods in the morning and afternoon (approximately 0900 and 1700). The behaviour of all the group individuals, male and female, was recorded throughout each 50 min test. Behaviour was observed for two 2 day blocks each week, and scored by means of an automated recording technique for subsequent transcription on to a computer. The males' *sexual activity* was measured by scoring the number of times they inspected the females' perineum, mounted or ejaculated. The females' sexual behaviour was measured by the number of times they sexually solicited ("presented to") the males. *Aggressive behaviour* was scored by counting the number of times one animal attacked or threatened another. *Social behaviour* was measured by scoring the number of times an animal invited grooming, or was groomed by another.

Blood Sampling and Hormone Assay

Blood was sampled twice weekly after the second observation period of the second day. All males in the group were bled, whether treated or untreated with naltrexone. Behaviour was not observed on the following day to exclude any adverse effects on the animals from the sampling procedure. This consisted of the withdrawal of 2–3 mls of blood from each male by femoral vein puncture under Ketamine anaesthesia. Blood was left to clot for one hour and then refrigerated overnight, being centrifuged at 4°C the next morning. Serum was stored at −20°C until assayed. Testosterone, LH, cortisol and prolactin were all measured in this laboratory using radioimmunoassay procedures described in detail elswhere [22].

Naltrexone Treatment

Naltrexone hydrochloride (Endo Labs., New York) was dissolved in saline ten minutes before intramuscular injection. The drug was given twice daily at a dose of 500 μg/kg base per injection to three different males in the group, at different times, except in the first experiment where Ff and J5 were treated simultaneously. Figure 1 shows the order of treatment of all the males. Observations were started 10 min after drug (or saline control) administration; treatment continued daily for at least 2 weeks (see Fig. 1).

Individually caged males. In the case of the five males which were studied in the communal monkey room, only changes in hormone levels (and not behaviour) were monitored. One male (C1) had been castrated years previously and during these experiments received no testosterone replacement. This male was paired with, and subordinate to, an intact male; the other males were singly caged and intact. Each animal was injected twice daily (at 0930 and 1630 hours) with either saline or naltrexone hydrochloride (dose as above). Blood was taken three times a week, one hour after the afternoon injection. See Fig. 1 for details.

In the case of the second naltrexone treatment of Ff, and the first of 14 and 340, no withdrawal phase was carried out.

Statistical Analysis

Behavioural data were analysed using the Mann Whitney U Test (two-tailed), and hormonal data by the Wilcoxon matched-pairs test and by *t*-tests (two-tailed). The analysis of behaviour was carried out by testing the changes in an individual's behaviour and not by group analysis. This is considered an appropriate approach to primate behaviour where individual differences, especially in a hierarchically organised group, are well recognised and are in fact an integral part of the study. In the case of hormonal data, where a male was treated more than once, the means of his data were used in the analysis.

RESULTS

Behavioural Changes

The sexual, aggressive and social behaviour of each male in the group is shown before, during and after naltrexone treatment in Figs. 2, 3 and 4. Data collected for male Ff in different experiments—Ff-(1), (2) and (3)—are grouped, while the sets of data for male J5 (rank 3) are separated according to whether or not a fourth (lower ranking) male was present (J5 (1) and (2) respectively). During the course of May 1979 there was a reversal in rank between the first- and second-ranking male, so that when D was treated with naltrexone, he was now the top-ranking male in the group.

Sexual Behaviour

As has been described elsewhere for captive talapoins [7], only the top-ranking male showed overt sexual activity in the presence of other males. Thus Ff mounted and ejaculated with females, while D mounted females but did not ejaculate. The third-ranking male J5 never mounted females (Fig. 2), and this was true for the most subordinate male also.

Treating J5 with naltrexone had no effect on his sexual behaviour, which remained negligible throughout. In contrast, the same treatment of the two top-ranking males (Ff and subsequently D) resulted in a significant decrease in the frequency of their inspections and mounts, and ejaculations in the case of Ff ($p<0.05$ in all cases, see Fig. 2). Sexual solicitations from the females to the formerly active males were also significantly reduced during the time the latter received naltrexone (Fig. 2); the females never solicited the subordinate male during any experimental period.

After withdrawal of the drug the sexual activity of both dominant males remained low. In the case of Ff, his inspections of the females were less frequent than during baseline and he also showed fewer ejaculatory mounts (median per 100 minutes: 2.0 v 4.1; $p<0.05$), although his overall mounting activity returned to former levels (Fig. 2). In the case of D neither his inspections nor mounts returned to baseline levels. These findings could not be explained in terms of the females' solicitations, since these were as high after withdrawal of naltrexone as they had been prior to treatment (Fig. 2).

Aggressive Behaviour

The dominance hierarchy is defined in terms of the direction of aggression between individuals [22]. By definition, therefore, the top-ranking males received no aggression from other males; the little they did receive (Fig. 3) was from the more dominant females in the group. Treatment with naltrexone had no effect on this aggression. As far as the aggressive behaviour shown by the males themselves was concerned, in the case of one (Ff) this remained negligible throughout each experimental period (Fig. 3).

The other dominant male (D) continued to threaten other males at the same rate during naltrexone treatment, but at-

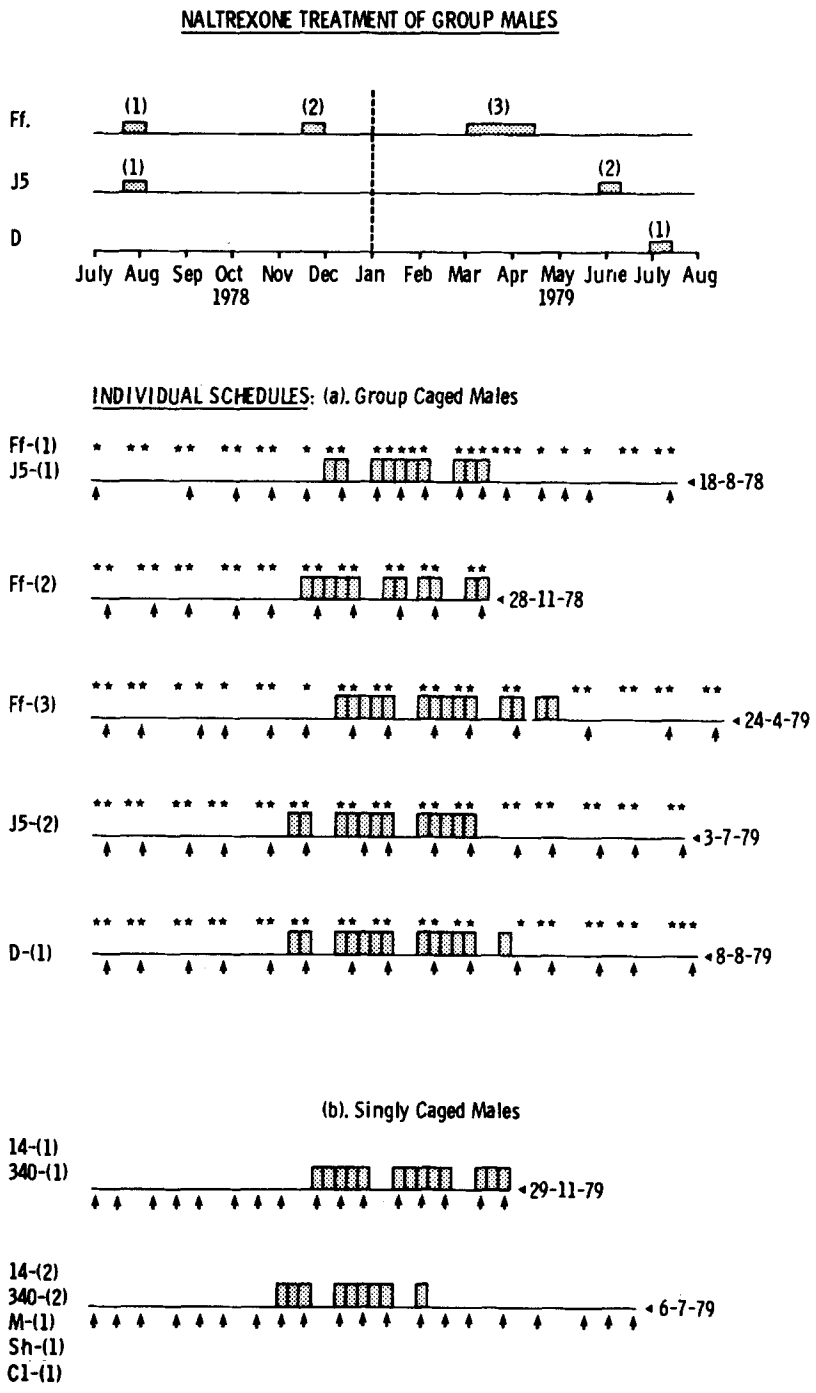

FIG. 1. Plan of experimental design. Upper part—naltrexone treatment (shaded blocks) of group males over 2 years. Lower part—individual treatment schedules of (a) group caged and (b) singly caged males. Each shaded column represents one day of treatment (see text for details of injections). Arrows indicate days on which blood was sampled and stars indicate days on which behaviour was tested.

tacked them significantly less often at this time, an effect which persisted after the drug was withdrawn (Fig. 3).

In the case of the subordinate male (J5), when he was the third-ranking of four males, naltrexone administration altered neither the aggression he received from others nor his own attacks or threats to his subordinate. When the most subordinate of only three males—J5-(2)—, J5 received significantly more aggression from other members of the group than previously, but this level was unchanged during the period he received naltrexone or after its withdrawal (Fig. 3).

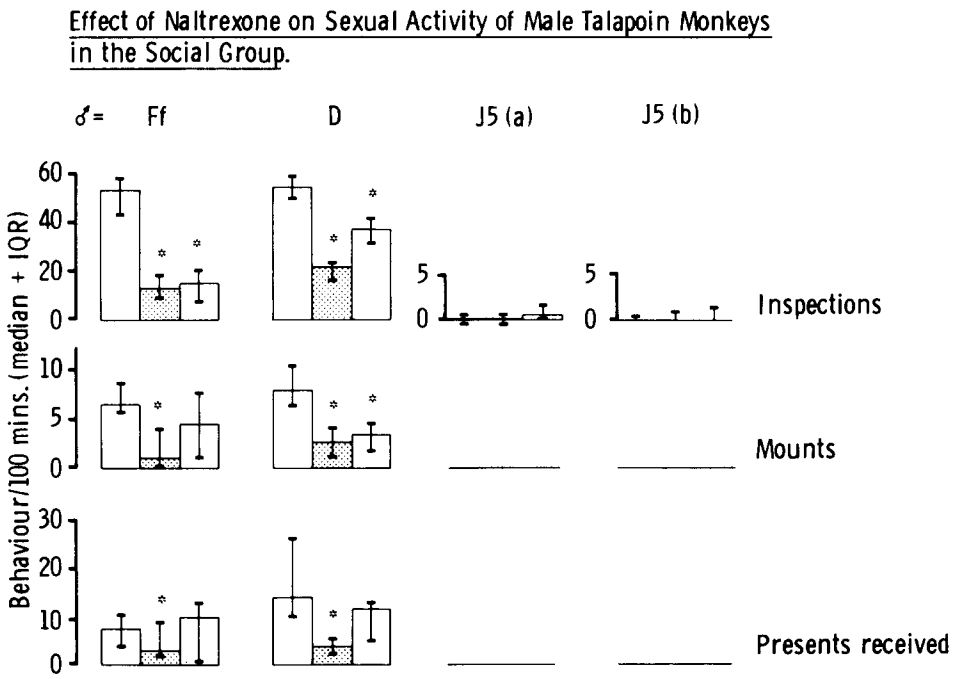

FIG. 2. Effect of naltrexone on sexual activity of male talapoin monkeys in the social group. First open column: saline treatment (baseline, B). Shaded column(naltrexone treatment (N). Second open column: saline (withdrawal, W). *=$p<0.002$ in each case except presents to Ff, where $p<0.05$. In each case N and W compared with B using Mann Whitney U-Test, 2-tailed, based on the following number of behavioural tests for B, N and W resp.: Ff: 20, 45, 12; D, J5 (both cases): 10, 10, 10.

Social Behaviour

There was a significant change in grooming interactions during naltrexone treatment in the case of all the males. Each male invited grooming significantly more often when treated with the drug than during baseline (Fig. 4). In the case of one male (Ff) his grooming invitations remained significantly elevated following withdrawal of naltrexone, while the other males' grooming invitations returned to the very low levels observed before treatment (Fig. 4).

As a result of these changes in invitational behaviour, naltrexone administration was associated with an increase in grooming received by each male, which reached significance in two cases (Fig. 4). Grooming interactions returned to pretreatment levels once naltrexone had been withdrawn.

Hormonal Changes

Figure 5 shows the endocrine response of the group and singly-caged males to naltrexone administration and withdrawal. Naltrexone treatment was associated with an increase in testosterone, LH, cortisol and prolactin. Conversely, withdrawal of the drug led to a decrease in testosterone, cortisol and prolactin for the group males (see Fig. 5). Closer inspection of the endocrine response suggests that the effects of naltrexone were most pronounced at the onset of treatment (particularly with respect to the group males). Since chronic drug treatment might be expected to lead to adaptive changes in receptor sensitivity, it was then considered appropriate to use the mean of the samples taken during the first week under treatment (and likewise for withdrawal) for further statistical analysis.

Figure 6 shows the percentage change in testosterone, LH, cortisol and prolactin in each individual thus calculated during the initial stages of drug administration (upper part) and withdrawal (lower part).

Testosterone, LH and Cortisol

There was a significant increase overall in testosterone, LH and cortisol during the males' treatment with naltrexone (calculated from the means of the samples, see legend to Fig. 6). Withdrawal of naltrexone was conversely associated with a decrease in levels of each hormone (see Fig. 6).

In the case of the untreated males in the group, there were no significant changes in testosterone, LH or cortisol during the periods of administration or withdrawal of naltrexone to the experimental animal. (Data from these males were analysed in each experiment exactly as those of the treated male).

Prolactin

Although there was a tendency for prolactin to increase during naltrexone treatment (Figs. 5 and 6), the overall result was not significant; neither was the apparent decrease on withdrawal of the drug (see legend to Fig. 6). Closer analysis could reveal no relationship between rank and the response of prolactin to naltrexone; indeed, in one individual prolactin levels changed in opposite directions following naltrexone administration on different occasions (Fig. 6). Furthermore, if an animal's level of prolactin was elevated during treatment it did not necessarily decrease on withdrawal of the

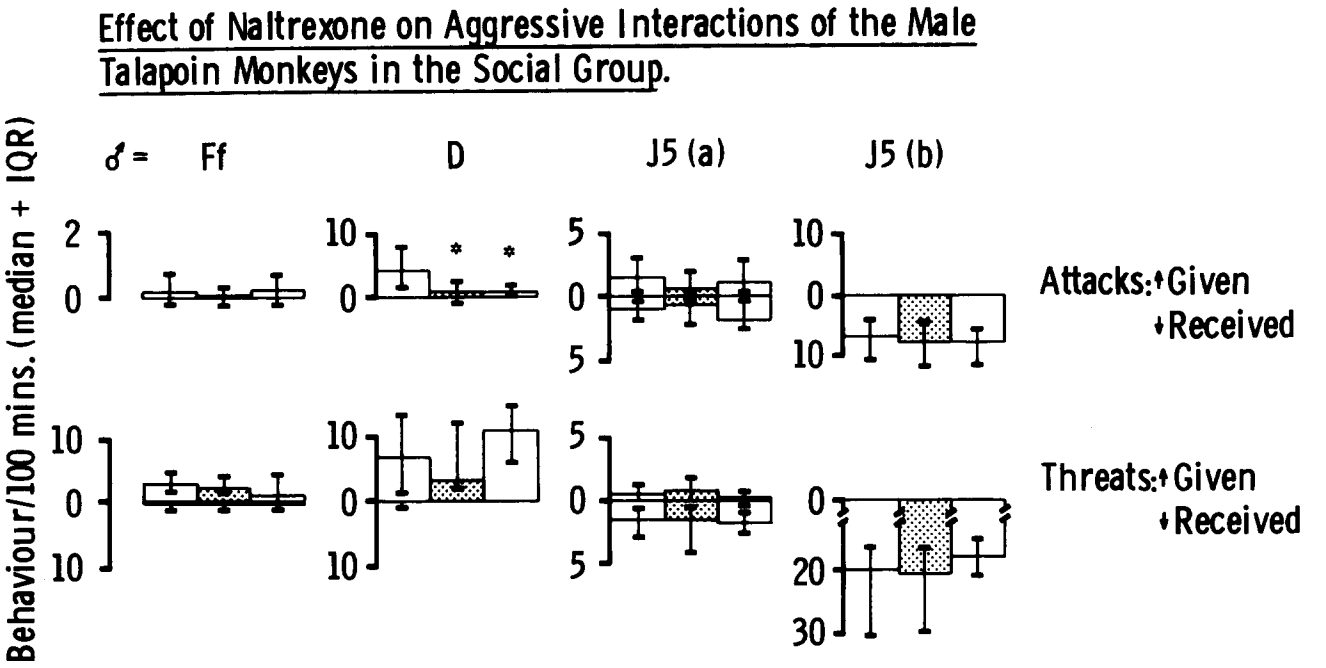

FIG. 3. Effect of naltrexone on aggressive interactions of male talapoin monkeys in the social group. Aggression given is shown above the horizontal, and received is shown below. Shading as Fig. 2. *=$p<0.05$, Mann Whitney U-Test, 2-tailed, based on the same tests as above.

drug. However, there was a significant increase in prolactin in those animals with low pre-treatment values, i.e. the singly caged males, $t(4)=3.11$, $p<0.04$, but not in those in the group with higher baseline levels.

In the case of the untreated males in the group, an unexpected change in prolactin was found during the periods of naltrexone administration to the other male: prolactin levels in these males decreased, $t(3)=3.05$, $p=0.05$, paired t-test at this time. There was no significant change however in prolactin during the period of withdrawal of naltrexone, although there was a tendency for levels to rise in the untreated males at this stage of the experiments.

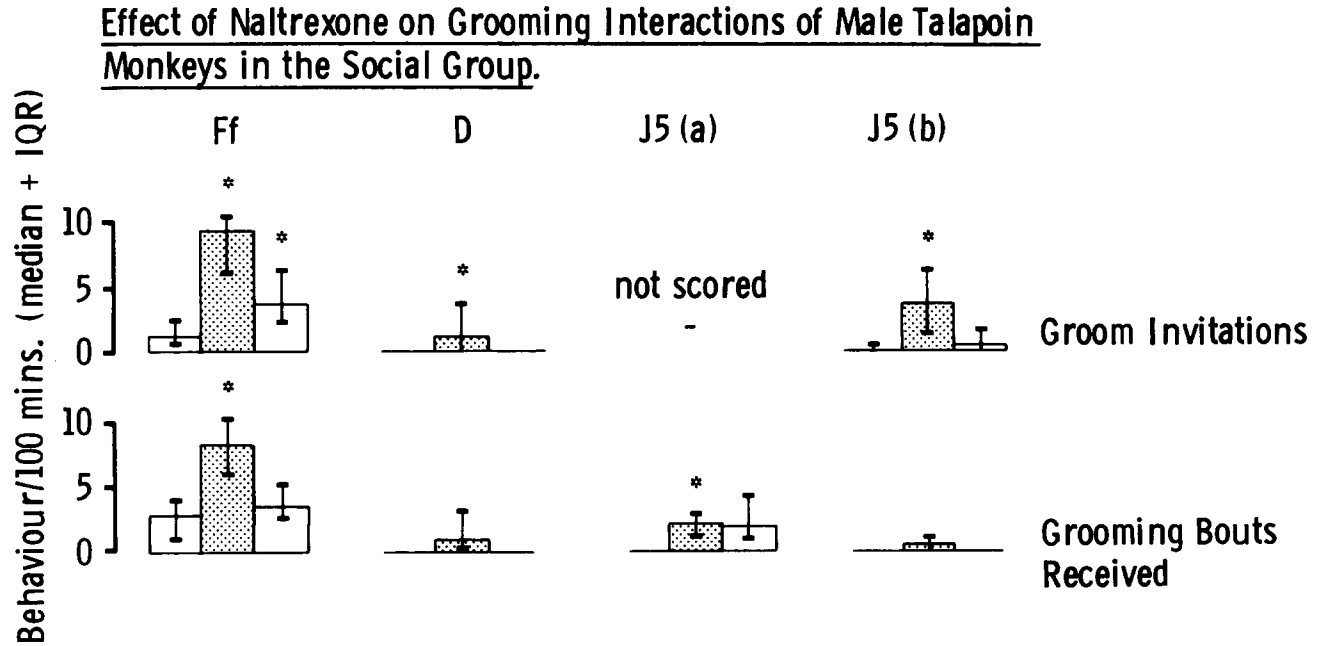

FIG. 4. Effect of naltrexone on grooming interactions of male talapoin monkeys in the social group. Shading as Fig. 2. *=$p<0.05$ or less, Mann-Whitney U-Test, 2-tailed, based on the same tests as above.

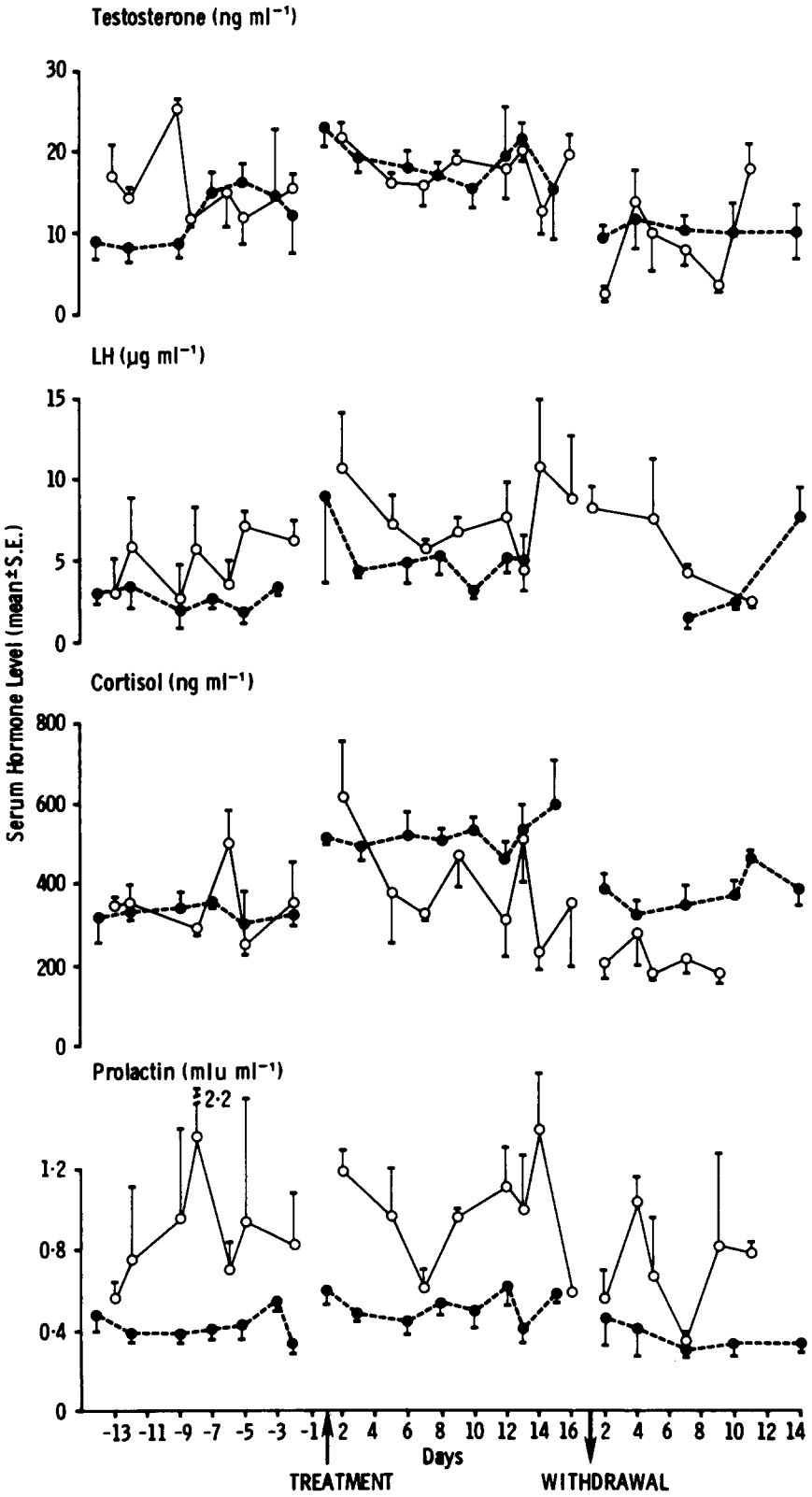

FIG. 5. Effect of naltrexone on endocrine state of group-caged (○——○) and singly-caged (●——●) male talapoin monkeys. Each point represents mean value for males sampled on particular days. ↑ indicates Day 1 of naltrexone administration and ↓ withdrawal of naltrexone.

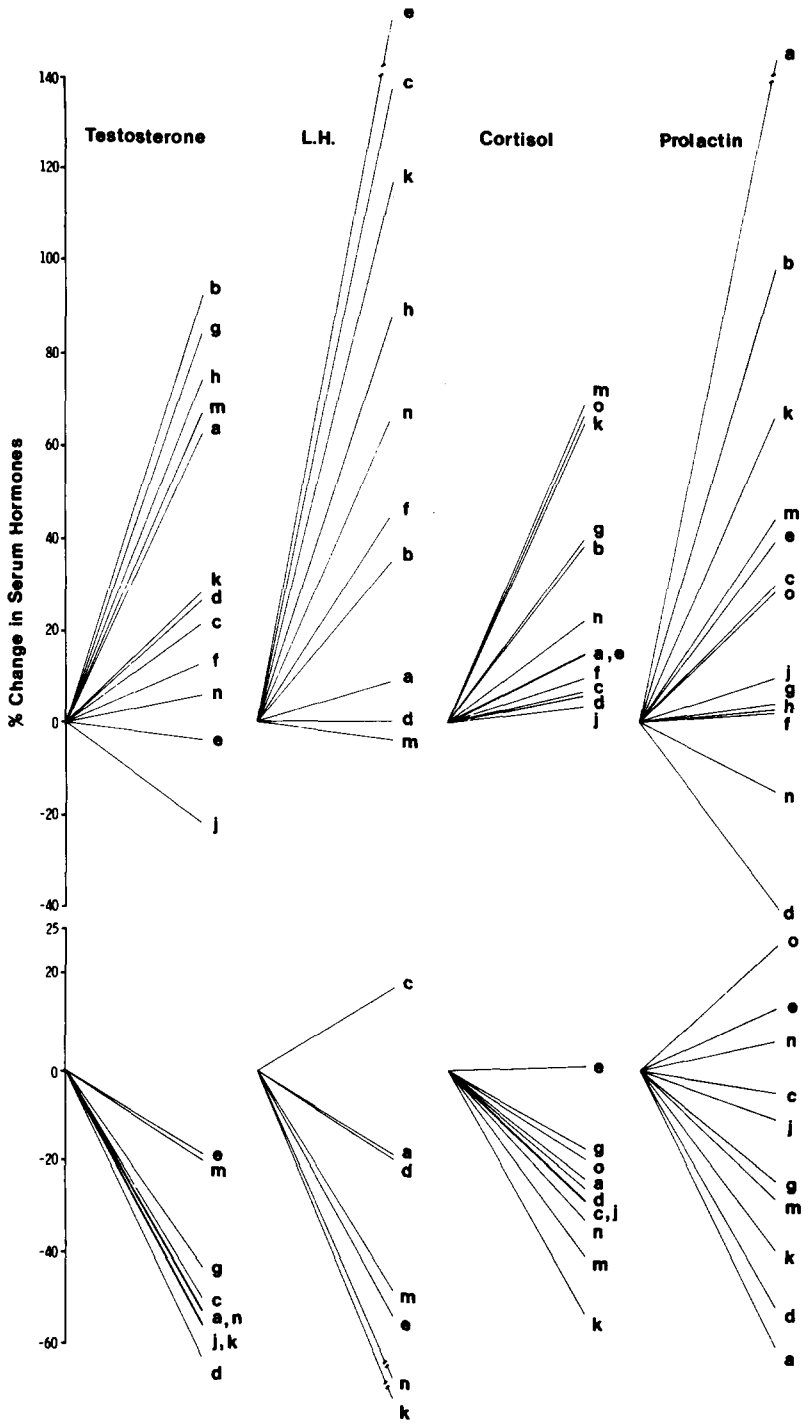

FIG. 6. Percentage change in hormone levels on each occasion of naltrexone administration and withdrawal in male talapoin monkeys. Data are taken from means from samples obtained during first week of treatment or withdrawal (n=3). Group males: Ff-a, b, c; D-n; J5-d, e. Singly-caged males: 14-f, g; 340-h, j; Sh-k; M-m; Cl-o. Order of treatment for males treated more than once given by alphabetical order. Values for such males were meaned and the data analysed for all males using a paired t-test, 2-tailed. Naltrexone administration led to significant increases in testosterone: $t(6)=4.46$, $p<0.005$; LH: $t(6)=3.03$, $p<0.03$; and Cortisol: $t(7)=4.24$, $p<0.005$; but not in prolactin: $t(7)=0.82$. Withdrawal of naltrexone led to significant decreases in testosterone: $t(6)=4.67$, $p<0.002$; LH: $t(4)=3.46$, $p<0.03$ and cortisol: $t(7)=4.67$, $p<0.003$, but not in prolactin: $t(7)=1.51$.

DISCUSSION

The administration of naltrexone to male talapoin monkeys had marked effects on both their behaviour and endocrine state. The reduction of sexual behaviour during naltrexone treatment in those high-ranking males that had previously been sexually active stands in contrast to the results of studies on male rats. In the latter, the evidence suggests that endogenous opiate activity inhibits sexual behaviour, since blockade with naltrexone or naloxone improves performance, either by increasing the number of males copulating or by reducing the ejaculation latency in active males [10,27]. In a study on man in which naloxone was administered, ability to reach orgasm was unaffected but there was an increase in time taken to achieve erection [13]. Once achieved however, there was a decrease in latency to ejaculation, as reported in rats.

The intraventricular administration of β-endorphin to male rats, on the other hand, did not alter their pursuing of females, but apparently blocked mounting once contact had been made [26]. Opiates can also block sexual behaviour in man—heroin abuse being related to loss of libido and sexual activity [34]—but whether this is a direct effect of the drug is not clear.

A number of explanations can be suggested for our findings on primates. Firstly, it is possible that endogenous opiates play no role whatever in the sexual behaviour of male primates, and that naltrexone had adverse side effects which tended to reduce the males' sexual interest. Alternatively, endorphins may actually be necessary for sexual arousal in the talapoin monkey. Thirdly, endorphins may indeed inhibit performance in primates as in rats and the drug may have mixed agonist as well as antagonist properties, or it may differentially block certain classes of opiate receptor. Finally, naltrexone may be having such a marked influence on some behaviours (e.g. grooming) that any possible stimulant effects on sexual activity are overridden and obscured.

The non-specific effects of naltrexone include the possibility that opiate blockade might lower the animal's pain threshold and so lead to general dysphoria. However, the sexual interest of these males decreased immediately following treatment, when there was no evidence for general ill-effects: indeed, during the course of drug administration neither food intake nor weight declined. It would seem therefore that the drug effect on behaviour is an immediate one, and indeed this seems more likely than a hormonally mediated effect in view of the stimulation of testosterone (see below). It did, however, become clear that the actual naltrexone injections were strongly aversive: animals showed a reluctance to be caught or handled for the drug injection which had never been seen during the saline control period. After the injection however, the monkeys no longer looked disturbed, and could not easily be distinguished from untreated males.

The possibility that endogenous opiate activity may be necessary for sexual interest in primates cannot be ruled out by our results but there is no evidence from the present or other studies to support it.

A possible explanation seems to be in terms of a competing behaviour. Naltrexone had a striking effect on the grooming interactions of each male, irrespective of rank, with the animals apparently seeking out close social contact, a type of "comfort" behaviour which is clearly incompatible with sexual activity. Changes in this category of behaviour are particularly interesting in view of the hypothesis put forward by Panksepp and his colleagues that the positive affect arising from social bonds may be mediated by cerebral endorphin-containing systems [28]. When such systems are blocked, the individual might be expected to seek extra social comfort to compensate. Further preliminary studies that we have carried out on male and female pairs of talapoin monkeys have also shown naltrexone (and naloxone) to provoke a striking increase in "huddling" together and invitations to be groomed: in some cases the animals seem to seek the closest physical contact possible (unpublished data). Evidence from these studies shows that the increased wish to be groomed is not associated with any increase in self-grooming or scratching, suggesting it is not related to any local discomfort in the fur.

Most of the hormonal data are in agreement with other published findings on naloxone or naltrexone activity, suggesting that the drug is effectively blocking opiate receptor function in this context at least. Opiates are known to depress testosterone in both rats and man [6,25]: in our studies naltrexone administration was associated with elevated levels, and withdrawal with a marked decrease, possibly caused by rebound opiate activity or increased sensitivity to endogenous opiates. Since LH also increased during naltrexone treatment, a finding also reported in man [24], it seems that testosterone was raised because of pituitary stimulation, rather than by a direct action on the testis—though there is evidence in the rat for opiates inhibiting steroido-genesis in the adrenal by interfering with LH-induced cAMP activity similar to that seen in the testis [18].

Stimulation of LH by naltrexone is in line with an inhibition of this hormone by opiates in rat [6], and is also compatible with the suggested inhibitory influence of endorphins on dopamine release [29], dopamine having been found to promote LHRH secretion in the median eminence [2,30]. This postulated interaction between endorphins and dopamine is not however consistent with the increases in prolactin seen in some animals during naltrexone treatment, since increased dopamine activity would be predicted to inhibit prolactin release. The present findings also conflict with evidence in the rat that prolactin is raised by endorphins and suppressed by naltrexone [15]. The effect of naloxone or naltrexone on prolactin in primates and man is not clear, with one report of a suppression of prolactin [12] and others showing no effect on the hormone [9,20]. The observed increase in prolactin in our study may relate to a nonpharmacological effect of the drug on the animals' endocrine state, an effect that is probably secondary to changes in the animals' affective state during opiate blockade. This interpretation receives some support from our finding that individuals with comparatively high pre-treatment levels of prolactin showed no marked changes, whereas those with low levels showed elevated prolactin during the time they received the drug.

The decrease in prolactin in the untreated males during the period of naltrexone treatment of other males present was an unexpected result, but it perhaps reflects the behavioural effect of the drug on the treated individual. In each case where a dominant male received naltrexone, prolactin fell in the subordinates. Since naltrexone tended to decrease aggressive as well as sexual interactions in the dominant male, his rank was less strongly asserted at this time. This probably represented a less stressful situation for the lower-ranking males, and hence their plasma prolactin fell.

The effect of naltrexone on cortisol in these experiments was in line with data from humans, in which naloxone significantly increased plasma levels of ACTH and cortisol [33]. Since β-endorphins are known to be secreted in equimolecular

amounts with ACTH from the anterior pituitary [16], it is possible that inhibition of opiate activity by naltrexone leads to an increase in the output of both as the result of reduced negative feedback—the converse interaction, of corticosteroids inhibiting pituitary endorphin secretion, having been reported [11]. If endorphins are elevated by chronic social stress (e.g. subordinacy), an interesting function of this might be to limit the individual's exposure to high levels of his own corticoids.

The administration of naltrexone to male talapoin monkeys had marked effects not only on social behaviour and sexual activity, but also on hormonal state. It is perhaps surprising that whereas testosterone was elevated sexual behaviour was reduced, since in untreated males (in which behaviour and endocrine state can be manipulated by the social environment [21,22]) relatively high testosterone levels and an active sexual life tend to occur together [8]. It may be that exogenously administered naltrexone is more effective in one part of the cerebral opiate system than another, although this remains to be established. The contrasting effects of opiate blockade on the sexual behaviour of rodents and primates also needs explanation, but none is apparent at present. Whether it is significant that rats are typically treated with much higher doses than are primates is not yet clear. Finally, consistently marked changes in grooming interactions following naltrexone administration suggest that an opiate system may be more concerned with "bonding" and "affiliative" behaviour than with sexual activity.

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