these membrane particles probably represent proteolipid protein present in the membranes. In addition, it is plausible to presume that some substances of high molecular weight proteins may also be implicated in the constitution of these particles. Freeze-fracture electronmicrographs of the re-formed membranes (figs A and B) showed the 2 types of assemblies of membrane particles. One is the myelin-like arrangement and this type was also recognized on the fracture faces of myelin. The other is a cluster-arrangement and for the occurrence of this type of assembly, the explanation is obscure. However, it seems likely that the intrinsic protein(s) showing hydrophobicity more actively interact with each other under the hydrophilic conditions which were applied in order to prepare the re-formed membranes, and thus the formation of cluster-arrangements is induced. The occurrence of clusters was more frequent than that of the other arrangements.

To obtain more detailed information on the molecular constituents of re-formed membranes, protein composition was analyzed (table 1). An interesting finding was that the protein composition of these membranes closely corresponded to that of myelin. When the re-formed membranes are formed from myelin extracts, it is still more likely that each of the components dissolving in organic solvents may be re-organized according to their mutual biophysical properties. Thus, this evidence supports the idea that the molecules of myelin proteolipids maintain their inherent biophysical characteristics well. Moreover, $^{14}C \cdot 5$-HT binding experiments were performed to examine whether or not the re-formed membranes possess a 5-HT binding capacity. The results (table 2) indicated that these membranes also had the same 5-HT binding capacity as the original myelin extracts. Although studies on the identification of 5-HT binding components from myelin proteolipids are not yet complete, we have reported that other component(s) besides lipids may be implicated in the binding of 5-HT[12,14].

Finally, these results suggest that the organic solvent extraction method retains the inherent biophysical properties of the molecules present in myelin membranes satisfactorily, and thus this technique may provide a tool for studying the nature of receptor or binding components which are strongly associating with lipids in biological membranes.

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Reinforcement with naloxone of N-n-propylnorapomorphine (NPA) capability for stimulating male rat copulatory behavior

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Summary. Naloxone at a dose of 2 mg · kg^{-1}, which per se did not significantly alter the copulatory pattern of sexually active adult male rats, did significantly reduce the intromission frequency as well as latency to ejaculation when administered before a low and inactive dose of N-n-propylnorapomorphine (0.5 µg · kg^{-1}).

Recent studies suggest that naloxone (Nx), widely considered to be an opiate antagonist, may possess a stimulant effect on sexual behavior[1,2]. Moreover the administration of Nx is reported to potentiate certain effects of dopamine-mimicking drugs[3-5].

Previous research in our laboratory established that low N-n-propylnorapomorphine (NPA) doses, i.p. injected into adult male rats, caused a significant increase in the number of spontaneous episodes of penile erection (PE) during a 1-h observation period, presumably through activation of specific DA-receptors in the CNS[6,7]. Nx (0.5–4 mg · kg^{-1}) which per se caused only a modest increase in PE, greatly potentiated the effect of NPA, and the maximum response to NPA 8 µg · kg^{-1} was far in excess of that produced by the most effective NPA dose[7].

The present experiments were carried out to ascertain how the combination of Nx and NPA alters the copulatory behavior of the adult male rat.

Methods and materials. Adult male Wistar rats (S. Morini, S. Polo d'Enza, Reggio Emilia, Italy), initial weight 230 g, were employed. They were housed in groups of 5 and maintained on ad libitum food and water in a quiet climatized (22 ± 1 °C, 60% humidity) room under a reverse light-dark cycle (with light from 23.00 h to 11.00 h).

Female rats (initial b.wt 230 g) of the same strain used as mating stimulus were brought into estrus with a s.c. injection of 0.12 mg estradiol benzoate 48–72 h before use[8]. The tests were performed during the early part of the dark phase. Male copulatory behavior was evaluated as by Dewsbury[9] and the following were recorded: mount and intromission latencies (ML and IL) (time elapsed from the introduction of the female into the cage until the 1st mount and intromission, respectively), mount and intromission frequency (MF and IF) (number of mounts and intromissions preceding ejaculation), ejaculation latency (EL) (interval from the 1st intromission to ejaculation), post-ejaculatory interval (PEi) (time from the 1st ejaculation to the next 1st intromission).

Tests were discontinued when IL or PEi were > 15 min or EL was > 30 min. Of 94 male rats at the start of the

Table 1. Influence of N-n-propylnorapomorphine (NPA), naloxone (Nx) and Nx+NPA on the copulatory behavior of sexually experienced male rats

Experiment	Treatment ($\mu g \cdot kg^{-1}$, i.p.)	ML (sec) (mean ± SEM)	IL (sec)	MF	IF	EL (sec)	PEi (sec)
1 (10)*	Saline	32.12 ± 10.8	35.40 ± 8.20	7.50 ± 2.02	9.67 ± 2.22	385.33 ± 97.03	336.17 ± 25.56
	NPA, 8	30.67 ± 11.16	34.33 ± 13.82	3.77 ± 1.12	4.83 ± 0.70**	152.33 ± 39.19**	316.33 ± 20.54
2 (10)	Saline	20.86 ± 4.11	24.57 ± 11.42	4.71 ± 0.36	10.14 ± 2.05	275.78 ± 65.68	307.29 ± 13.79
	Nx, 2000	19.71 ± 2.46	26.86 ± 13.48	3.86 ± 0.67	7.57 ± 1.07	180.29 ± 41.00	287.14 ± 14.55
3 (10)	Saline	33.44 ± 3.23	40.00 ± 4.03	7.56 ± 2.26	8.89 ± 1.22	328.89 ± 72.65	341.89 ± 12.20
	Nx, 2000 + NPA, 8	24.89 ± 5.31	42.11 ± 9.20	4.22 ± 0.74	5.00 ± 0.47**	127.89 ± 29.66**	313.44 ± 28.80
4 (12)	Saline	21.08 ± 2.49	27.75 ± 3.52	4.83 ± 0.99	8.58 ± 0.66	398.58 ± 67.14	352.75 ± 30.86
	NPA, 0.5	18.50 ± 2.36	26.58 ± 3.11	4.42 ± 1.14	8.25 ± 0.96	337.08 ± 55.33	323.00 ± 13.90
5 (12)	Saline	26.15 ± 8.68	29.85 ± 4.58	7.88 ± 0.80	9.38 ± 0.74	343.77 ± 40.69	345.00 ± 16.84
	Nx, 2000 + NPA, 0.5	28.23 ± 2.00	21.92 ± 5.81	4.92 ± 0.67	6.92 ± 0.68**	210.77 ± 18.12**	331.54 ± 35.73

ML, IL, EL: latency to the 1st mount, 1st intromission and ejaculation, respectively. MF, IF: mount and intromission frequency, respectively. PEi: post-ejaculatory interval. The saline values are the mean of 4th and 5th tests, the constancy of copulatory behavior from one test to the other having previously been ascertained ($p > 0.05$: Student's t-test for paired data). The saline values obtained for the various groups of randomly selected animals do not differ significantly from one another ($p > 0.05$: analysis of variance). * In brackets the number of rats. ** At least $p < 0.05$ with respect to saline (Student's t-test for paired data).

experiments, 54 were considered sexually active, 30 impotent. The remaining 10 were discarded. 'Sexually active' males were those which performed completely at least the last 4 preliminary mating tests out of the 5 conducted at 3-day intervals. 'Impotent' males were those which never responded to any of the 5 preliminary mating tests. The degree of constancy of copulatory behavior in the 4th and 5th pre tests was checked with Student's t-test for paired data, which was also used to compare the values obtained before (mean of 4th and 5th tests) and after administration of the drugs. NPA · HCl (Sterling-Winthrop Research Institute, Rensselaer, N.Y., USA) and Nx · HCl (Endo Labs Inc., Garden City, N.Y., USA) were dissolved in sterile water just before i.p. administration at a constant fluid volume of $1 ml \cdot kg^{-1}$.

Results. Nx ($2 mg \cdot kg^{-1}$, i.p.) administered 25 min before the test (like $4 mg \cdot kg^{-1}$: results not shown) reduced all the mating behavior parameters except IL and ML, but mean values recorded before and after Nx were not significantly different. NPA ($8 \mu g \cdot kg^{-1}$), administered 15 min before the test, behaved similarly, affecting the same parameters, but, in agreement with our previously published results[10], IF and EL were found to be significantly diminished ($p < 0.05$ at least) (table 1).

When Nx ($2 mg \cdot kg^{-1}$) was injected 10 min before NPA ($8 \mu g \cdot kg^{-1}$), however, there was no increase in the stimulant effect relative to that produced by NPA alone.

When the dose of NPA was reduced to $0.5 \mu g \cdot kg^{-1}$, a dose which only mildly influenced PE[7], the copulatory parameters before and after treatment were not modified at all, but pretreatment with Nx altered the effect of this dose of NPA, significantly reducing IF and EL.

Table 2 shows the results obtained with 30 impotent male rats randomly assigned to 5 treatment groups. It must be stressed that our impotent rats not only failed to ejaculate, but never mounted or intromitted in any of the 5 consecutive screening tests.

In the 1st group NPA-treatment ($8 \mu g \cdot kg^{-1}$) induced a complete copulatory pattern in 4 out of 6 impotent rats, though, while ML and IL were considerably higher, IF and EL were lower than those of sexually active rats treated only with saline. These data confirm our previously published results[10]. A similar result was obtained when, in the 2nd group of animals, Nx ($2 mg \cdot kg^{-1}$) was injected 10 min before NPA ($8 \mu g \cdot kg^{-1}$), in fact 3 out of 6 impotent rats successfully performed the copulatory test.

Under our conditions, Nx alone failed to produce any effect in the 3rd group of impotent rats. Further, no effect was observed either with NPA alone ($0.5 \mu g \cdot kg^{-1}$) or with Nx ($2 mg \cdot kg^{-1}$) plus NPA ($0.5 \mu g \cdot kg^{-1}$).

Discussion. The present results support at least 2 main conclusions: 1. In combination with NPA, Nx interferes with sexual behavior, and its influence on spontaneous penile erection in the normal (i.e. sexually active) rat parallels its influence on complete copulatory performance, though this Nx-effect is possible only where there is an intact copulatory mechanism.

2. More specifically, Nx potentiates the effect of a low and otherwise ineffective dose of NPA, a specific stimulant of dopamine receptors in the CNS, this agrees with the quantity of data which at present tend to show that Nx significantly alters the effects of dopaminergic drugs[3-5,11,12]. In an earlier study we demonstrated that Nx-pretreatment caused a dramatic increase in NPA-induced PE, even when the Nx dose was in itself ineffective, and that haloperidol, a typical dopaminergic antagonist, was able to antagonize both the NPA and NPA + Nx effects[7]. In these experiments, Nx-pretreatment significantly diminished IF and EL, which were not modified in a significant manner by a low dose of NPA ($0.5 \mu g \cdot kg^{-1}$). Although interpretation of results in animals is often difficult and controversial[13], we interpret a decrease in these parameters as a facilitation and an increase in sexual activity. This hyperstimulation could result, in man, in ejaculatio praecox: it is not to be

Table 2. Influence of N-n-propylnorapomorphine (NPA) and Nx+NPA on the copulatory behavior of impotent rats

Treatment ($\mu g \cdot kg^{-1}$, i.p.)	ML (sec) (mean ± SEM)	IL (sec)	MF	IF	EL (sec)	PEi (sec)
Saline (6)	-	-	-	-	-	-
NPA, 8 (4)*	251.67 ± 95.48	290.81 ± 65.72	5.00 ± 1.59	5.00 ± 0.58	262.67 ± 69.33	349.37 ± 65.34
Saline (6)	-	-	-	-	-	-
Nx, 2000 + NPA, 8 (3)*	305.40 ± 60.23	358.22 ± 87.31	6.02 ± 2.40	5.62 ± 2.40	203.71 ± 54.88	320.51 ± 75.24

ML, IL, EL: latency to the 1st mount, 1st intromission and ejaculation, respectively. MF, IF: mount and intromission frequency, respectively. PEi: post-ejaculatory interval. *Number of impotent rats which performed the copulatory test after treatment. In brackets the number of rats.

excluded, extrapolating from experiments on animals, that an alteration of the dopaminergic and enkephalinergic systems may be involved in this disorder, though the complexity of the numerous psychological factors involved should always be borne in mind. The precise way in which Nx enhances the sexual stimulant effect of NPA is as yet undetermined. We interpreted our findings as indicating that some Nx-actions are related to the dopaminergic mechanism, which would confirm the existence of a relationship between the action of opiates and dopamine-containing synapses in the brain[12,14-16]. One possible explanation of the influence of Nx on the NPA-effect would involve a Nx blockade of the opiate receptors that have an inhibitory effect upon the dopaminergic system involved in the control of sexual behavior.

On the other hand, the failure of Nx-pretreatment to potentiate the effect of a relatively high dose of NPA ($8\ \mu g \cdot kg^{-1}$) on mating behavior in sexually active rats may be because this NPA dose was already maximally active in these experiments. The findings in the present study on impotent rats would appear to contradict the observation by Gessa[2] that Nx induced a complete copulatory pattern in sexually inactive male rats. This discrepancy may well result from the different experimental conditions, however. In impotent rats, NPA ($8\ \mu g \cdot kg^{-1}$) was able to induce copulatory behavior in about 50% of the animals, confirming our previous results[10]. Nx-pretreatment did not alter the percentage of rats displaying the copulatory pattern, nor did it influence IF and EL, which were very low after NPA-treatment compared with the values for saline-treated sexually active rats. In conclusion, the ability of Nx to potentiate the NPA-effect on copulatory behavior can be observed only in a normally functioning physiological system, that is, in sexually active rats. In a situation of complete sexual inadequancy, that is in impotent males, while a strong dopaminergic agonist is still capable of activating sexual behavior in about 50% of rats, no stimulant effect of Nx can be shown.

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Mineralocorticoid treatment and the adrenalectomy-induced increase in monoamine oxidase activity[1]

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Summary. Bilateral adrenalectomy in the rat results in an increase in heart monoamine oxidase activity in animals drinking water and in animals drinking 0.9% saline. Daily administration of deoxycorticosterone acetate prevented the increased monoamine oxidase activity in the animals drinking saline but not in those drinking water.

As early as 1936, Richter[2] reported that normal rats drank similar amounts of sodium chloride solution and water when given a choice. In contrast, adrenalectomized animals were found to drink more of the saline solution. In another study, Richter[3] reported that administration of the synthetic mineralocorticoid, deoxycorticosterone acetate (DOCA), reduced sodium chloride intake to normal levels in adrenalectomized rats. Later, Fregly and Waters[4] schowed that administration of graded doses of either aldosterone or DOCA to adrenalectomized rats produced a 'U-shaped dose-reponse relationship' between percent change in intake of NaCl solution and dose of the drugs. That is, at low doses the sodium chloride intake was not decreased by the drugs and at high doses the appetite was actually increased while at intermediate doses the sodium chloride intake was decreased to control levels.

Bilateral adrenalectomy in the rat produces a number of changes other than alterations in sodium chloride appetite. Included among these are a decrease in blood pressure and increases in norepinephrine turnover, and monoamine oxidase (MAO) activity[5-10]. Each of these changes can be prevented by administration of appropriate doses of adrenal steroids.

In a previous study from this laboratory, it was shown that having the animals drink saline and administering daily doses of DOCA prevented the changes in blood pressure and in heart norepinephrine turnover and MAO activity[7]. In this same study the decrease in blood pressure was prevented by saline and low doses of glucocorticoids, either cortisol or corticosterone, but neither of these latter dosage schedules prevented the increase in heart norepinephrine turnover and MAO activity. Other authors have found that administration of larger doses of glucocorticoids among with saline to drink did prevent the adrenalectomy-induced increases in urinary catecholamine excretion and in tissue MAO activity[6,8,9]. Thus, many of the changes brought about by adrenalectomy can be prevented by administration of saline along with either a mineralocorticoid or a glucocorticoid provided that the steroid dosage is sufficient. The purpose of this work was to determine if the effects of DOCA on the adrenalectomy-induced increase in MAO activity are related to its effects on sodium metabolism.

Materials and methods. In these experiments, 200-250 g male Sprague-Dawley derived rats were adrenalectomized through a midline dorsal incision under pentobarbital anesthesia. Controls were sham-operated. All animals were