Pharmacology Biochemistry & Behavior, Vol. 7, pp. 147–151.

Enhancement of Sexual Behavior in the Female Rat by Nicotine

K. FUXE, B. J. EVERITT*[1] AND T. HOKFELT

Department of Histology, Karolinska Institutet, Stockholm, Sweden and
**Department of Anataomy, University of Cambridge, Cambridge, England*

(Received 10 December 1976)

FUXE, K., B. J. EVERITT AND T. HOKFELT. *Enhancement of sexual behaviour in the female rat by nicotine.* PHARMAC. BIOCHEM. BEHAV. 7(2) 147–151, 1977. – The effects of nicotine alone or in combination with mecamylamine, and the effects of both in combination with DA agonists and antagonists and d-LSD (5-HT agonist) have been studied on the sexual behaviour of castrate, estrogen-treated female rats. The results show that nicotine (as low as 50 μg/kg) significantly increases sexual receptivity. The pharmacological analysis suggests this effect to be mediated by a central, nicotine-like cholinergic receptor whose relationship to DA and 5-HT pathways known to exert inhibitory influences on receptivity in the female rat is also discussed.

Nicotine Mecamylamine Apomorphine Pimozide Sulpiride d-LSD
Sexual receptivity Estrogen and progesterone

IT IS well established that sexual receptivity in the female rat is dependent on the ovarian hormones estrogen and progesterone [3]. During the last ten years, a body of evidence has accumulated [7,19] which demonstrates that the biogenic monoamines may mediate the effects of hormones on sexual behaviour since drugs which modulate aminergic transmission may replace or modify the actions of the hormones (particularly progesterone).

More recently, it has been reported that the cholinegic (muscarinic) compounds pilocarpine, oxotremorine and arecoline can inhibit with short latency lordosis behaviour induced in female rats by estrogen and progesterone [18]. The effects of these compounds appear to be centrally mediated since they were inhibited by atropine [18] (which penetrates into the brain) but not methyl atropine (which does so less readily). On closer examination it was demonstrated that the inhibitory effect of pilocarpine was blocked by the serotonin (5-HT) synthesis inhibitor p-chlorophenylalanine, but not by the catecholamine synthesis inhibitor d-methyl-p-tyrosine [15,16]. This suggests that cholinegic (muscarinic) stimulation inhibits lordosis via a serotoninergic mechanism, it being well known that 5-HT itself exerts marked inhibitory influences on estrous behaviour [6].

In a paper describing the neurochemical effects of nicotine [12], we discuss the possibility that enhancement of sexual receptivity in the female rat caused by this drug might be mediated by 5-HT and perhaps dopamine (DA) neurons, and conclude this to be unlikely. In this communication we present a pharmacological analysis of the effects of nicotine on sexual behaviour which was designed to give additional information on the types of central receptors that may be involved in mediating its actions. Thus, compounds known to enhance sexual activity by actions on either 5-HT neurons (d-LSD, see [9]) or DA neurons (apomorphine, see [8]) have been given in association with nicotine and cholinergic (nicotinic) blocking agents. In this way it was hoped to assess whether the actions of nicotine on sexual receptivity could be modified (enhanced or reduced) by altering 5-HT and DA receptor activity and the degree to which this indicated the mechanisms of action of nicotine itself.

METHOD

Animals and Behavioural Observations

Female Sprague-Dawley rats were used. Ovariectomy was performed at least one month before testing. The rats were housed, 5 per cage, in a room maintained at a temperature of +24°C under reversed lighting (16 hr dark). The females were treated daily at 08.00 hr with 1.0 μg/kg estradiol monobenzoate SC (which induces a low level of receptivity [6]) for at least 5 days before behavioural observations began and for the duration of the experiment.

Behavioural observations were made 4 hr into the dark phase of the reversed lighting schedule by placing each female rat with a cage-adapted, sexually experienced male until she had been mounted 10 times. The proportion of mounts by the male, which elicited a lordosis response (L/M), the female's acceptance ratios (AR) (number of mounts divided by number of mounts plus refused mounting attempts) and soliciting behaviour (proportion of rats displaying hopping and darting movements) were measured. (For further details, see ref. [6]).

[1] Reprint orders to be sent to Dr. Everitt at the Cambridge address.

Pharmacological Experiments

The following drug treatments were superimposed on the daily oestradiol injections; animals (except controls) received only 1 of the treatment regimes.

Nicotine. Nicotine was given as the tartrate or salicylate salt IP in saline 5 min before behavioural observations. All doses are expressed as base. Controls were injected with saline 5 min before observations.

Mecamylamine – nicotine. Mecamylamine (1 mg/kg), a ganglion receptor blocking agent, was given IP 1 hr before nicotine (100 μg/kg base).

Mecamylamine – apomorphine. Mecamylamine – d-LSD. In view of its blocking ability, mecamylamine was also tested against apomorphine- and d-LSD-induced increases in receptivity [6, 9, 11]. Mecamylamine (1 mg/kg) was given IP 1 hr before apomorphine (1 mg/kg IP, a dose known to increase lordosis behaviour). In the same way, mecamylamine was given before d-LSD (10 μg/kg IP, a dose which causes a marked increase in lordosis behaviour).

Sulpiride – nicotine. The antipsychotic drug sulpiride [14] can prevent the apomorphine-induced increase in receptivity [8], probably by blockade of presynaptic DA receptors [25]. It was therefore tested whether sulpiride (10 mg/kg IP) given 15 min before nicotine (100 μg/kg IP) could antagonise the action of the latter to further test its specificity.

Apomorphine – nicotine. D-LSD – nicotine. In order to further analyse the mechanism for the increase in sexual behaviour by nicotine combined treatment with DA and 5-HT receptor agonists and nicotine was performed. Absence of an additional increase of sexual behaviour by combined treatment could suggest that the action of nicotine was in some way linked to the activity in the DA and 5-HT pathways, respectively. The drugs were given simultaneously to the rats and testing was performed 5 min later. The doses were 1 mg/kg of apomorphine, 10 μg/kg of d-LSD and 100 μg/kg of nicotine (see Table 1 and Fig. 1).

Pimozide – nicotine. The possible involvement of DA neurons in the action of nicotine has been further studied by blocking the DA receptors postsynaptically using pimozide, a DA receptor blocking agent (1 mg/kg, IP) 2 hr before nicotine (100 μg/kg) was given. Pimozide is known to increase sexual behaviour by itself by removal of the inhibitory influence of some DA pathways [5]. Absence of an additional effect of nicotine would suggest that the nicotine action in some way is linked to the DA pathways (see Table 1 and Fig. 1).

RESULTS

Nicotine

In the dose range 0.05–0.25 mg/kg, nicotine caused a significant increase in sexual receptivity (Table 2). The 0.10 mg/kg dose was most effective and resulted in high L/M and AR scores as well as soliciting. The 0.25 mg/kg dose, while increasing lordotic responding, was associated with depressed motor activity and, hence, passive acceptance of the male. The 0.01 mg/kg dose was without effect on sexual behaviour (Table 2).

Mecamylamine – Nicotine

As seen in Fig. 2 the increase in sexual behaviour caused by nicotine was effectively blocked by pretreatment with mecamylamine. The difference in L/M ratio between the two groups was highly significant ($p<0.001$; Mann-Whitney U test). AR was not reduced, however, probably because the motor inhibition caused by mecamylamine induces passive acceptance of the male. This side-effect of mecamylamine also explains the increase in AR found with mecamylamine alone.

Mecamylamine – Apomorphine. Mecamylamine – d-LSD

As seen in Fig. 1, the increase in sexual behaviour caused

TABLE 1

EFFECTS OF PIMOZIDE, APOMORPHINE AND d-LSD ON THE NICOTINE-INDUCED INCREASE IN SEXUAL BEHAVIOR IN THE OVARIECTOMIZED ESTROGEN-TREATED FEMALE RAT

Treatment	Dose mg/kg	N	L/M	AR
Saline		50	0.20 ± 0.16	0.74 ± 0.10
Nicotine	0.1	20	0.85 ± 0.10^{xx}	0.96 ± 0.13^{xx}
Pimozide + nicotine	1 + 0.1	15	0.75 ± 0.15^{xx}	0.95 ± 0.05^{xx}
Apomorphine + nicotine	1 + 0.1	15	0.70 ± 0.29^{x}	0.90 ± 0.03^{xx}
d-LSD + nicotine	0.01 + 0.1	15	0.95 ± 0.15^{xx}	0.95 ± 0.06^{xx}
Pimozide	1	10	0.70 ± 0.05^{xx}	0.90 ± 0.14^{xx}

Pimozide (1mg/kg, IP) was given 2 hr before nicotine (100 μg/kg) and apomorphine (1 mg/kg) was given at the same time as nicotine (100 μg/kg). Testing was performed 5 min after nicotine treatment. For further details see Table 2, text and [8]. Median and semiquartile deviations are shown. N = number of rats.

No significant differences are found when the experimental groups are compared with the nicotine alone group with the exception of the d-LSD + nicotine group. All groups are, however, significantly different from their respective saline control groups (which have been combined for this table).

^{x}: $p<0.05$; ^{xx}: $p<0.01$; Mann-Whitney U test after a non-parametric one-way analysis of variance.

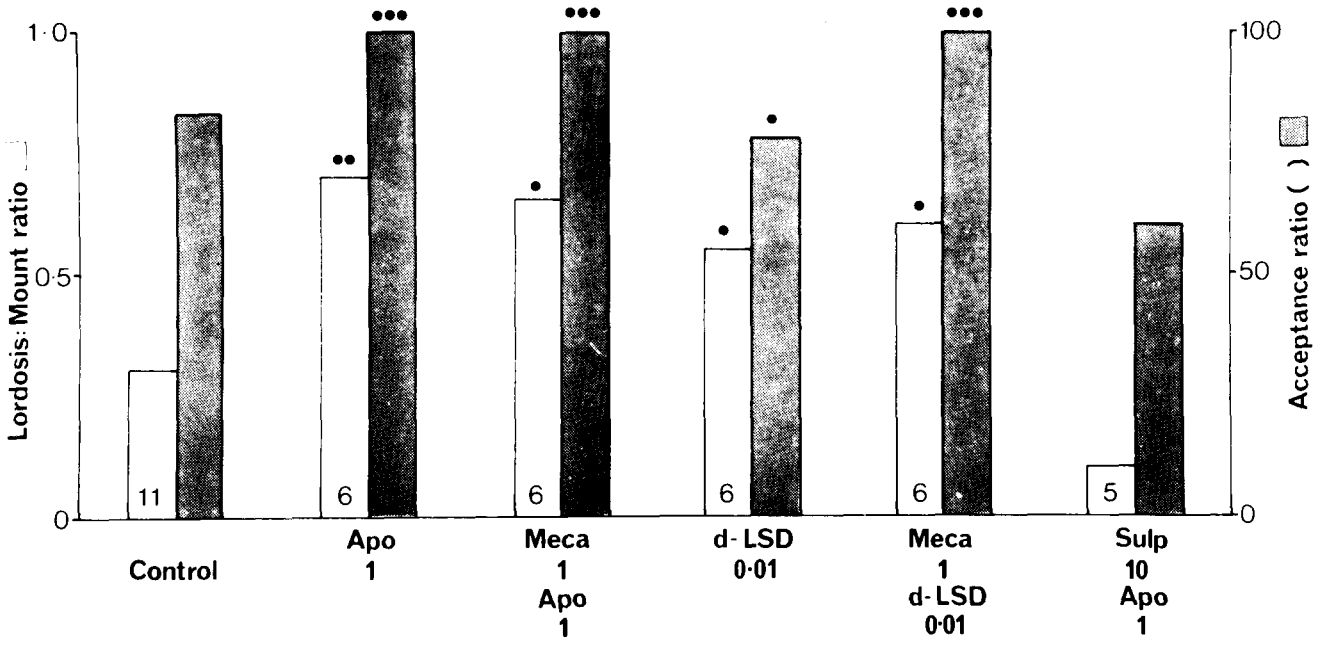

FIG. 1. The effects of mecamylamine and sulpiride on the apomorphine- and d-LSD induced increases in sexual behaviour in the ovariectomized, estrogen-primed female rat. For details on ovariectomy and estrogen treatment, see text to Table 2. Mecamylamine (MECA, 1 mg/kg, IP) (was injected 1 hr IP before apomorphine (APO, 2 mg/kg, IP) and d-LSD (10 μg/kg, IP), which were injected 5 min before testing. Sulpiride (SULP, 10 mg/kg) was injected IP 15 min before apomorphine (1 mg/kg, IP). Values shown are median lordosis:mount and acceptance ratios. Sample sizes are shown in the bars. Mann-Whitney U test. All comparisons have been made with the control group. x: $p<0.05$; xx: $p<0.01$; xxx: $p<0.001$. A non-parametric one-way analysis of variance was used.

TABLE 2

THE EFFECTS OF NICOTINE ON THE SEXUAL BEHAVIOR OF OVARIECTOMIZED, ESTROGEN-TREATED RATS

Treatment	Dose mg/kg	N	L/M	AR	Soliciting
Nicotine	0.25	10	0.80 ± 0.10**	1.00 ± 0.05**	20%^{ns}
Saline	—	10	0.30 ± 0.15	0.57 ± 0.10^{xx}	20%
Nicotine	0.10	10	1.00 ± 0.03**	1.00 ± 0.08**	70%^{xx}
Saline	—	10	0.20 ± 0.12	0.88 ± 0.13	10%
Nicotine	0.05	10	0.80 ± 0.21^{xx}	0.85 ± 0.15^{x}	10%^{ns}
Saline	—	10	0.30 ± 0.09	0.60 ± 0.09	30%
Nicotine	0.01	10	0.20 ± 0.21^{ns}	0.66 ± 0.13^{ns}	0%^{ns}
Saline	—	10	0.30 ± 0.15	0.53 ± 0.08	0%

All rats had been ovariectomized at least one month and received daily injections of 1.0 μg/kg estradiolbenzoate SC. Full details of the testing procedure are given in [8]. Drugs or saline were injected in a volume of 1.0 cc IP. N = number of rats. L/M = No. lordosis responses per No. mounts by male. AR = No. mounts by male per No. mounts + refused mounting attempts, i.e. acceptance ratio, a measure of the female's receptivity. Soliciting = the proportion of females displaying hopping and darting or earwiggling, i.e. behavior characteristic of estrus. Mann-Whitney U-tests (2-tailed). ^{xx}: $p<0.01$; ^{x}: $p<0.05$. ^{ns} = not significant. Values are Median ± semiquartile deviations.

by apomorphine and d-LSD was not in any way blocked by mecamylamine. Sulpiride, on the other hand, effectively blocked the effect of apomorphine (Fig. 1).

Sulpiride – Nicotine

As seen in Fig. 2, sulpiride (10 mg/kg) did not block the increase in sexual behaviour caused by nicotine. Sulpiride (10 mg/kg) alone has no effect.

Apomorphine – Nicotine, d-LSD – Nicotine

As seen in Table 1, apomorphine did not further increase the action of nicotine on sexual behaviour. However, d-LSD

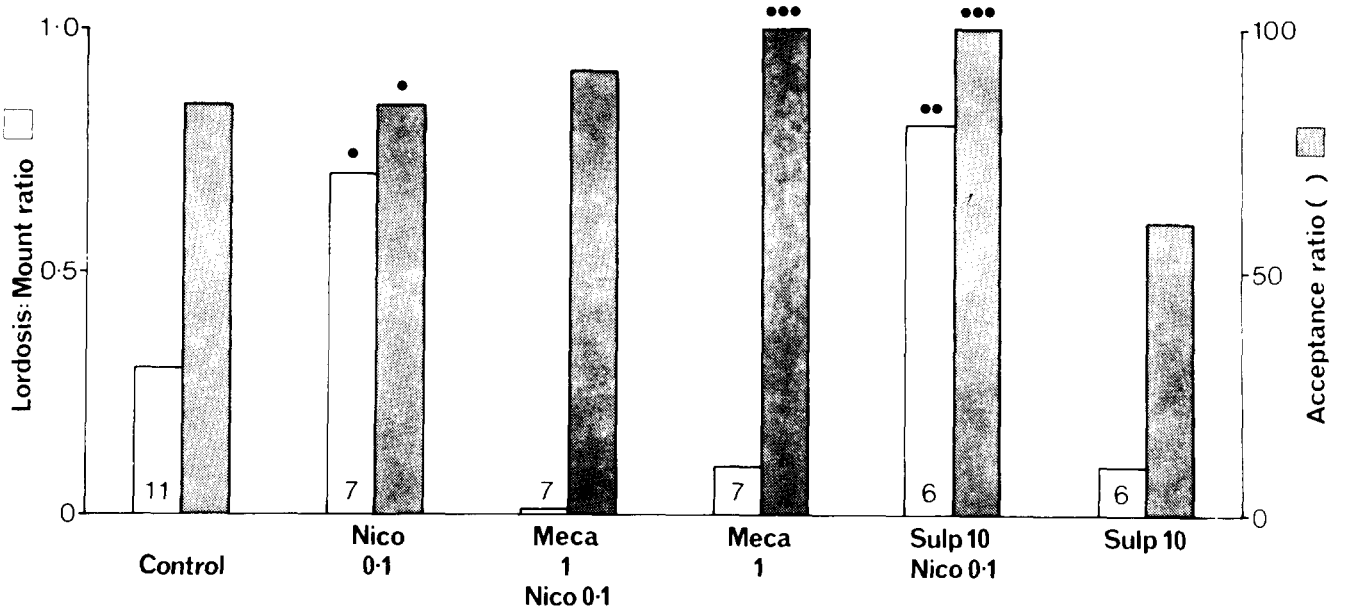

FIG. 2. Effects of mecamylamine and sulpiride on the increase in sexual behaviour caused by nicotine in the ovariectomized, estrogen-primed female rat. For details on ovariectomy and estrogen treatment see text to Table 2. Nicotine (NICO) was used in a dose of 100 μg/kg, injected IP 5 min before testing. Mecamylamine (MECA, 1 mg/kg) was injected IP 1 hr before nicotine. Sulpiride (SULP) was given IP in a dose of 10 mg/kg 15 min before nicotine. Values shown are median lordosis:mount and acceptance ratios. Sample sizes are shown in the bars. Mann-Whitney U test † after a non-parametric one-way analysis of variance. All comparisons have been made with the control group. x: $p<0.05$; xx: $p<0.01$; xxx: $p<0.001$.

slightly increases it ($p<0.01$, Mann-Whitney U test).

Pimozide – Nicotine

As seen in Table 1, pimozide does not increase the action of nicotine on sexual behaviour. Pimozide alone caused the same increase in sexual behaviour as nicotine.

DISCUSSION

The experiments reported herein demonstrate that nicotine, in doses as low as 50 μg/kg, significantly increases all components of sexual receptivity in ovariectomized, estrogen-treated female rats. This may be interpreted either as a replacement of progesterone or an increase in the sensitivity to estrogen by nicotine, since additional treatment of rats receiving low doses of estradiol with either progesterone or more estradiol will cause similar changes in behaviour [3]. The pharmacological analysis indicates that this dramatic behavioural effect of nicotine is due to activation of a central nicotine-like cholinergic receptor, since it was blocked by mecamylamine. This blockade has some degree of specificity since the increases in receptivity induced by low doses of apomorphine and d-LSD (which inhibit activity in DA and 5-HT neurons, respectively [1,13]), were not inhibited by mecamylamine. Nicotine is also seen to cause significant increases in components of sexual behaviour of male rats [2, 23, 24].

Thus it appears that both muscarinic and nicotinic drugs are able to influence sexual activity in appropriately hormone-treated female rats. The fact that muscarinic stimulation is associated with an inhibition of lordosis behaviour emphasizes that the facilitatory effects of nicotine are mediated by a nicotine-like acetylcholine receptor in the brain. The present results, together with other types of behavioural evidence support, therefore, the suggestion that two types of cholinergic receptors exist in the CNS [21,22]. Recent studies using labelled α-bungarotoxin [4,20] and muscarinic blocking agents [22,26] give direct evidence for this view.

The mechanism of action of nicotine on sexual receptivity is, at present, open to question. It is unlikely that it acts indirectly via the pituitary-adrenal axis and release of progesterone since the time course of action of the drug would appear too short (5 min). This is in contrast to the late excitatory effects of pilocarpine and oxotremorine on lordosis in estrogen-treated females which are clearly related to adrenal progesterone release, but which take 3 hrs to develop [17].

The location of this nicotine-like receptor is also unknown at present, but it may be linked to the 5-HT and DA pathways which are well known to exert inhibitory influences on sexual behaviour in the female [7]. Thus, nicotine in high repeated doses has been found to reduce DA turnover in the nucleus caudatus and 5-HT turnover in whole brain as evaluated during a 2 and 3 hr interval, respectively [10,12]. Although the absence of additional excitatory effects of pimozide and apomorphine are consistent with this view, the small but significant enhancement of the effects of nicotine by d-LSD is not. Although it is difficult to assess the importance of this result, further investigation is warranted. A more important argument against the suggestion that the actions of nicotine on sexual receptivity are linked to 5-HT and DA neurons is that although the behavioural effects of nicotine are blocked by mecamylamine, the neurochemical effects on 5-HT and DA turnover (see above) are not [12]. Similarly the facilitatory effects of apomorphine and d-LSD on receptivity are not blocked by mecamylamine. Thus it seems unlikely that the behavioural effects of nicotine are explainable by indirect actions on the 5-HT and DA mechanisms known to subserve sexual behaviour.

It remains to be determined whether nicotinic and

muscarinic cholinergic mechanisms interact to control the onset and offset, respectively, of the receptivity induced by estrogen and progesterone, i.e., are they a primary site of action of these hormones (particularly of progesterone). A perpetual problem of interpretation in studies of drugs and hormone-dependent behaviours is whether the mechanism of action of the former is related to the normal mechanism of action of a hormone or independent of it, and this problem awaits solution in the context of the experiments reported here.

ACKNOWLEDGEMENT

This work was supported by a grant from the Svenska Tobaks Aktiebolaget.

REFERENCES

1. Aghajanian, G. K. and B. S. Bunney. Pre- and postsynaptic feedback mechanisms in central dopaminergic neurons. In: *Frontiers of Neurology and Neuroscience Research*, edited by P. Seeman and G. M. Brown. Toronto: University of Toronto Press, 1974, pp. 4–11.
2. Bignami, G. Pharmacologic influences on mating behaviour in the male rat. Effects of d-amphetamine, LSD-25, strychnine, nicotine and various anticholinergic drugs. *Psychopharmacologia* **10:** 44–58, 1966.
3. Davidson, J. M. Hormones and reproductive behaviour. In: *Reproductive Biology*, edited by H. Balin and S. Glasser. Amsterdam: Excerpta Medica, 1972.
4. Eterovic, V. A. and E. L. Bennett. Nicotinic cholinergic receptor in brain detected by binding of a-(^{3}H)bungarotoxin. *Biochim. biophys. Acta* **362:** 346–355, 1974.
5. Everitt, B. J., K. Fuxe and T. Hökfelt. Inhibitory role of dopamine and 5-hydroxytryptamine in the sexual behaviour of female rats. *Eur. J. Pharmac.* **29:** 187–191, 1974.
6. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Role of monoamines in the control by hormones of sexual receptivity in the female rat. *J. comp. physiol. Psychol.* **89:** 556–572, 1975.
7. Everitt, B. J. Cerebral monoamines and sexual behaviour. In: *Textbook of Histology*, edited by J. Money and H. Musaph. Holland: Elsevier, 1976.
8. Everitt, B. J. and K. Fuxe. Serotonin and sexual behaviour in female rats. Effects of hallucinogenic indolealkylamines and phenylethylamines. *Neuroscience Letters* **4:** 215–220, 1977.
9. Everitt, B. J. and K. Fuxe. Dopamine and sexual behaviour in female rats. Effects of dopamine receptor agonists and sulpiride. *Neuroscience Letters* **4:** 209–313, 1977.
10. Fuxe, K., L. Agnati, P. Enneroth, J.-Å. Hustavsson, T. Hökfelt, A. Löfström and P. Skett. The effect of nicotine on central catecholamine neurons and gonadotrophin secretion in the male rat. *Med. Biol.* (in press).
11. Fuxe, K., L. Agnati, B. J. Everitt, T. Hökfelt, A. Ljungdahl and M. Perez de la Mora. On the action of B(4-chlorphenyl) GABA, γ-hydroxy-butyrolactone and apomorphine on central dopamine neurons. In: *Advances in Biochemical Psychopharmacology*, edited by E. Costa and G. Gessa. New York: Raven Press, 1977, pp. 489–494.
12. Fuxe, K., T. Hökfelt and B. J. Everitt. On the action of nicotine on central 5-HT neurons. *Pharmac. Biochem. Behav.* **555**
13. Haigler, H. J. and G. K. Aghajanian. Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. *J. Pharmac. exp. Ther.* **188:** 688–699, 1974.
14. Laville, C. L. and J. Margarit. Sur les effets neurologiques centraux de sulpiride. *Path. Biol.* **17:** 71, 1969.
15. Lindström, L. H. The effect of pilocarpine in combination with monoamine oxidase inhibitors; imipramine or desmethylimipramine on oestrous behaviour in female rats. *Psychopharmacologia* **17:** 160–168, 1970.
16. Lindström, L. H. The effect of pilocarpine and oxotremorine on oestrous behaviour in female rats after treatment with monoamine depletors or monoamine synthesis inhibitors. *Eur. J. Pharmac.* **15:** 60–65, 1971.
17. Lindström, L. H. Further studies on cholinergic mechanisms and hormone-activated copulatory behaviour in the female rat. *J. Endocr.* **56:** 275–283, 1973.
18. Lindström, L. H. and B. J. Meyerson. The effect of pilocarpine, oxotremorine and arecoline in combination with methylatropine or atropine on hormone-activated oestrous behaviour in ovariectomized rats. *Psychopharmacologia* **11:** 405–413, 1967.
19. Meyerson, B. J. and C.-O. Malmnäs. Brain monoamines and sexual behaviour. In: *Determinants of Sexual Behaviour*, edited by J. Hutchison.
20. Polz-Tejera, G., J. Schmidt and H. J. Karten. Autoradiographic localization of α-bungarotoxin-binding sites in the central nervous system. *Nature* **258:** 349–351, 1975.
21. Shlechter, M. D. and J. A. Rosecrans. Behavioural evidence for two types of cholinergic receptors in the CNS. *Eur. J. Pharmac.* **15:** 375–378, 1971.
22. Schleiffer, L. S. and M. E. Eldefrawi. Identification of the nicotinic and muscarinic acetylcholine receptors in subcellular fractions of mouse brain. *Neuropharmacology* **13:** 53–63, 1974.
23. Soulairac, M.-L. and A. Soulairac. Action de la nicotine sur le comportement sexuel du rat male. *C.r. Séanc. Soc. Biol.* **166:** 798–802, 1972.
24. Soulairac, M.-L. and A. Soulairac. Monoaminergic and cholinergic control of sexual behaviour in the male rat. In: *Sexual Behaviour: Pharmacology and Biochemistry*, edited by M. Sandler and G.-L. Gessa. New York: Raven, 1975, pp. 99–116.
25. Tagliamonte, A., G. de Montis, M. Olianas, L. Vargin, G. V. Corsini and G. L. Gessa. Selective increase of brain dopamine synthesis by sulpiride. *J. Neurochem.* **24:** 707–710, 1975.
26. Yamamura, H. I., M. J. Kuhar, D. Greenberg and S. H. Snyder. Muscarinic cholinergic receptor binding: Regional distribution in monkey brain. *Brain Res.* **66:** 541–546, 1974.