*Pharmacology Biochemistry & Behavior*, Vol 24, pp 1065–1070, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

# GABAergic Control of Masculine Sexual Behavior

A. FERNÁNDEZ-GUASTI,* K. LARSSON†[1] AND C. BEYER*

*Department of Psychology, University of Goteborg, Box 14158, S-400 20 Goteborg, Sweden†*
*and Centro de Investigación en Reproduccion Animal, CINVESTAV-UAT, Ap Postal 62*
*Tlaxcala 90-000, México**

Received 2 August 1985

FERNÁNDEZ-GUASTI, A , K LARSSON AND C BEYER *GABAergic control of masculine sexual behavior* PHARMACOL BIOCHEM BEHAV 24(4) 1065–1070, 1986 —Drugs affecting the GABAergic transmission were injected into the medial preoptic anterior hypothalamic area (MPOA) and the masculine sexual behavior analyzed Antagonizing GABAergic neurotransmission by (+) bicuculline methiodide (30 ng/cannula), picrotoxin (50 ng/cannula), or 3-mercaptopropionic acid (10 or 20 μg/cannula) resulted in a drastic shortening of the postejaculatory intervals and a shortening of the ejaculation latency Injection of compounds causing an increase in GABAergic activity, muscimol (25 ng/cannula) or ethanolamine-O-sulphate (80 μg/cannula) depressed masculine sexual behavior Systemic treatment or injection into the nucleus caudatus putamen of compounds affecting the GABAergic transmission did not cause any alteration in the mating pattern. It is suggested that the GABAergic neurotransmission is involved in inhibitory processes underlying the masculine sexual behavior

Medial preoptic area GABA Picrotoxin (+) Bicuculline methiodide 3-Mercaptopropionic acid Muscimol Ethanolamine-O-sulphate Rat masculine sexual behavior

THE amino acid, gamma-aminobutyric acid (GABA), is believed to serve as a major inhibitory neurotransmitter in the mammalian brain (see e.g., [4,29] for references) We recently reported that intracerebral injection of (+) bicuculline methiodide, an agent that antagonizes the action of GABA presumably by interacting with synaptic GABA-receptors [13,14], stimulated the masculine sexual activity of the rat [7]; suggesting that the GABAergic system normally may exert an inhibitory function in the sexual behavior. The current investigation was carried out to further explore this hypothesis. Drugs inducing changes in GABAergic activity were injected into the medial preoptic-anterior hypothalamic area (MPOA), a structure of essential importance for the expression of masculine sexual behavior in rat [12,23]. Elevated GABAergic activity in the MPOA was induced by intracerebral injection of (a) GABA; (b) the potent GABA agonist, muscimol [19]; or (c) ethanolamine-O-sulphate (EOS), an agent that by inhibiting GABA transminase prevents GABAdegradation [1, 8, 16]. A decrease in GABAergic activity was induced by intracerebral administration of (a) the GABA antagonists, (+) bicuculline methiodide or picrotoxin [13,14]; or (b) 3-mercaptopropionic acid (3-MPA), an agent that inhibits gamma-amino decarboxylase, an enzyme involved in the GABA synthesis [15,32]

## METHOD

### *Animals*

Wistar rats (Mollegaard, Veijle), approximately 6 months old at the start of the experiment, were used. They were housed in single cages, under conditions of constant temperature and humidity with food and water available ad lib. The light cycle (12 hr/12 hr) was artificially maintained (light off at 10 a.m.). The animals were selected on the basis of their performance in preliminary tests with receptive females To qualify as an experimental animal, the male was required to achieve at least one ejaculation in three successive 30-min screening tests.

### *Procedure*

All operations were performed under deep anesthesia (Mebumal® 40 mg/kg IP). The rat was mounted in a stereotaxic instrument with the intraaural plane in a horizontal position [18]. Guide cannulae were placed bilaterally on the skull roof and permanently fixed by means of acrylic dental cement The guide cannulae were placed at bregma, 0.6 mm from the midline for injections in the MPOA, and 2.5 mm from the midline at the height of the bregma for injections into the nucleus caudatus putamen. The tip of the guide cannulae reached the level of the dura mater. Three days after the operation, intracerebral injections were made through the guide cannulae by means of an injection cannula (internal diameter 0.20 mm). The injection cannula was lowered 7 mm below the dura for injections into the MPOA and 4 mm below the dura and 2.0 mm below corpus callosum for injections into the nucleus caudatus putamen. The injection cannula was attached to a 5 μl Hamilton syringe. The depth

[1]Requests for reprints should be addressed to. Prof. Knut Larsson, Department of Psychology, Unit of Psychobiology, University of Goteborg, Box 14158, S-400 20 Goteborg, Sweden.

of penetration was controlled by means of a stopper on the injection cannula.

*Histology*

After completion of the behavior tests, the animals were decapitated and the brain was removed and stored in a freezer. The frozen brain was cut at 50 μm on a microtome and inspection was made for localization of the cannulae track. Only those animals in which the injection cannula track could be followed to the MPOA, according to the boundaries indicated by Konig and Klippel [19], or to the nucleus caudatus putamen, were accepted in the analysis of the results.

*Behavior*

Mating tests were begun 2 hr after onset of darkness. The males were presented with a female brought into sexual receptivity by sequential treatment with estradiol benzoate (12.5 μg/rat), followed 44 hr later by progesterone (0 5 mg/rat) which was injected 6 hr before testing. The following behavioral components were recorded: (a) Intromission latency: time from the entrance of the female into the observation cage to the first intromission; (b) Mount frequency; (c) Intromission frequency: (d) Ejaculation latency: time from the first intromission until ejaculation, (e) Postejaculatory interval (PEI)· time from the ejaculation to the next intromission. In addition, a derived measure was used, the mean inter-intromission interval which was obtained by dividing the ejaculation latency by the intromission frequency in each series of copulations Mount, intromission and ejaculation were recognized because of their particular behavioral characteristics. By series of copulations, is meant the sequence of mounts and intromissions leading up to ejaculation. The mating tests were ended under criteria indicated under the Results section.

*Drugs*

Muscimol, (+) bicuculline, GABA, picrotoxin, 3-MPA and EOS were purchased from Sigma, St Louis; (+) bicuculline methiodide was purchased from Pierce Chemicals. All drugs were dissolved in 0 9% saline. Amount of the compound used and volume injected are indicated below.

TABLE 1

EFFECTS ON MASCULINE SEXUAL BEHAVIOR OF INTRACEREBRAL INJECTION OF PICROTOXIN (50 ng/CANNULA), (+) BICUCULLINE METHIODIDE (30 ng/CANNULA), AND SALINE (0 5 μl)

| | Treatment | | |
|---|---|---|---|
| Behavior Component | Saline (n=9) | Picro-toxin (n=7) | (+) bicucul-line methi-odide (n=10) |
| Series I | | | |
| Intromission latency | 1 4 | 0 1 | 0 5 |
| Mount frequency | 5 | 1 | 1 |
| Intromission frequency | 10 | 7 | 6 |
| Ejaculation latency | 9 7 | 2 1* | 1 6† |
| Inter-intromission interval | 1 4 | 0 3 | 0 5* |
| Postejaculatory interval | 6 5 | 3 2‡ | 0 9‡ |
| Series II | | | |
| Mount frequency | 1 | 3 | 2 |
| Intromission frequency | 5 | 5 | 3 5 |
| Ejaculation latency | 3 0 | 2 1 | 1 2† |
| Inter-intromission interval | 0 7 | 0 4 | 0 4† |
| Postejaculatory interval | 6 9 | 4 4† | 3 1† |
| Series III | | | |
| Mount frequency | 2 | 3 | 6 5 |
| Intromission frequency | 6 | 5 | 3 |
| Ejaculation latency | 3 0 | 2 3 | 1 9 |
| Inter-intromission interval | 0 6 | 0 4 | 0 4 |
| Postejaculatory interval | 8 1 | 5 8† | 3 9† |

The injection was localized to the medial preoptic-anterior hypothalamic area Table shows median values
Mann Whitney U test *$p<0$ 05, †$p<0$ 02, ‡$p<0$ 005 [28]

## RESULTS

*Drugs Injected in the MPOA Causing Decreased GABAergic Activity*

*Picrotoxin or (+) bicuculline methiodide* Rats were injected into the MPOA with either picrotoxin (50 ng/cannula), (+) bicuculline methiodide (30 ng/cannula), or saline in a volume of 0.5 μl and the mating behavior was observed for 30 min. Three series of copulations were completed by all participating animals, and the behavior exhibited is indicated in Table 1. The intromission latency, and the mount and intromission frequencies did not show any statistically significant group differences. The ejaculation latency, however, was shortened in the first two series The average inter-intromission interval was significantly shortened in the first two series of copulations in the bicuculline treated animals. The most remarkable behavioral change applied to the postejaculatory intervals which were drastically shortened following the drug treatment Whereas none of the control males resumed copulating before 4 to 5 min after the first ejaculation, all but one of the bicuculline treated rats started to copulate within 1 min after the first ejaculation. As a combined result of shortened ejaculation latencies and postejaculatory intervals, the number of ejaculations increased One of the bicuculline treated animals ejaculated 8 times in 10 min, showing no postejaculatory intervals longer than 1 min

*3-MPA* Rats were injected with 3-MPA (10 or 20 μg/cannula in 0 5 μl saline) and observed through three successive series of copulations Table 2 shows the results of this experiment. The postejaculatory intervals were significantly shortened in the drug treated animals compared to the saline treated controls With exception for an occasional reduction of the mount frequency in the second series of copulations after the administration of 20 μg 3-MPA, no changes were seen in the mating pattern. In pilot experiments (data not reported), animals were injected with either 1 μg or 30 μg/cannula 3-MPA. In the former case, no behavioral effects were seen and in the latter case, convulsions occurred

TABLE 2

EFFECTS ON MASCULINE SEXUAL BEHAVIOR OF INTRACEREBRAL ADMINISTRATION OF 3-MERCAPTOPROPIONIC ACID (10 0 and 20 0 μg/CANNULA) OR SALINE (0 5 μl)

| | Treatment | | |
|---|---|---|---|
| | | 3-mercaptopropionic acid | |
| Behavior Component | Saline (n=7) | 10 0 μg (n=9) | 20 0 μg (n=6) |
| Series I | | | |
| Intromission latency | 1 0 | 0 8 | 2 5 |
| Mount frequency | 5 | 5 | 3 5 |
| Intromission frequency | 20 | 12 | 14 |
| Ejaculation latency | 11 3 | 5 9 | 7 6 |
| Inter-intromission interval | 0 7 | 0 5 | 0 6 |
| Postejaculatory interval | 5 8 | 2 9‡ | 2 0‡ |
| Series II | | | |
| Mount frequency | 6 | 3 | 0 5* |
| Intromission frequency | 6 | 6 | 5 5 |
| Ejaculation latency | 3 5 | 4 1 | 2 4 |
| Inter-intromission interval | 0 5 | 0 7 | 0 4 |
| Postejaculatory interval | 6 7 | 4 2‡ | 2 9† |
| Series III | | | |
| Mount frequency | 5 | 5 | 5 |
| Intromission frequency | 5 | 5 | 5 |
| Ejaculation latency | 2 5 | 2 6 | 5 6 |
| Inter-intromission interval | 0 4 | 0 5 | 0 5 |
| Postejaculatory interval | 7 1 | 5 7† | 2 6† |

The injection was localized to the medial preoptic anterior hypothalamic area Table shows median values
Mann Whitney U test *$p<$ 05, †$p<$0 01, ‡$p<$0 002 [28]

TABLE 3

EFFECTS OF MUSCIMOL (25 ng/CANNULA) OR SALINE (0.5 μl) INJECTED INTO THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMIC AREA ON MASCULINE SEXUAL BEHAVIOR

| | | Animals showing copulatory responses | | |
|---|---|---|---|---|
| Treatment | n | Mount | Intromission | Ejaculation |
| Saline | 8 | 7 | 7 | 7 |
| Muscimol | 10 | 3* | 3* | 1† |

One hour mating tests were performed immediately after the drug injection
Fisher F test *$p<$0 025, †$p<$0 005 [28]

TABLE 4

EFFECT OF ETHANOLAMINE-O-SULPHATE (80.0 μg/CANNULA) OR SALINE (1 0 μl) INJECTED INTO THE MEDIAL PREOPTIC ANTERIOR HYPOTHALAMIC AREA ON MASCULINE SEXUAL BEHAVIOR

| | | Animals showing copulatory responses | | |
|---|---|---|---|---|
| Treatment | n | Mount | Intromission | Ejaculation |
| Saline | 8 | 8 | 8 | 8 |
| Ethanolamine-O-sulphate | 11 | 6 | 4* | 2† |

Animals were observed for 30 min 24 hr after the injection of saline or ethanolamine-O-sulphate
Fisher F test *$p<$0 02, †$p<$0 01 [28]

*Drugs Injected Into the MPOA Causing Elevated GABAergic Activity*

*GABA.* GABA (n=11) or saline (n=9) (GABA: 25 μg/cannula in 0.5 μl saline) was injected in the MPOA and the rats were observed through 3 consecutive series of copulations. With exception for a slight prolongation of the intromission latency (2.8 min in the GABA treated vs. 0.45 min in the saline treated rats, Mann Whitney U test $p<0.05$), no statistically significant differences were seen in any of the behavior components analyzed.

*Muscimol* Rats were injected with muscimol (25 ng/cannula in 0.5 μl saline) or saline in the MPOA and the behavior was observed for 60 min. Table 3 shows the proportion of animals displaying mounts, intromissions and ejaculations in the mating test. Seven of the 10 drug treated males remained sexually inactive. One of the three active males ejaculated, while the others showed only sporadic mounts and intromissions.

*EOS.* Rats were injected with EOS 80 μg/cannula in 1.0 μl saline and observed in 30 min tests 24 hours after the drug administration [1,16]. Because of the low number of animals showing complete mating pattern after the drug administration, only the proportion of animals showing mounts, intromissions and ejaculations was analyzed. As indicated in Table 4, a statistically significant decrease was found in the number of rats exhibiting intromission and ejaculation behavior. Pilot experiments were undertaken (data not shown) in which the animals were infused with either 40 μg or 100 μg/cannula EOS. In the former case, no behavioral effects were seen while in the latter, the animals showed marked signs of sedation.

*Drugs Injected in the Nucleus Caudatus Putamen*

Animals were injected with saline, muscimol or (+) bicuculline methiodide in the nucleus caudatus putamen and observed through three series of copulations. No statistically significant alterations were observed in the behavior of the drug treated animals with exception for the average inter-intromission interval which in the muscimol treated animals was significantly prolonged during the second series of copulations (Table 5).

*Systemic Drug Administration*

3-MPA (10 or 15 mg/kg), (+) bicuculline (2.5 mg/kg), picrotoxin (0.25 or 0.50 mg/kg) and muscimol (0.5 or 1 0 mg/kg)

TABLE 5

EFFECTS ON THE MASCULINE SEXUAL BEHAVIOR OF INJECTION INTO THE NUCLEUS CAUDATUS PUTAMEN OF SALINE (0 5 μl/CANNULA), MUSCIMOL (25 ng/CANNULA), AND (+) BICUCULLINE METHIODIDE (30 ng/CANNULA)

| | Treatment | | |
|---|---|---|---|
| Behavior Component | Saline (n=7) | Muscimol (n=8) | (+) bicuculline methiodide (n=7) |
| Series I | | | |
| Intromission latency | 2 8 | 6 9 | 8 1 |
| Mount frequency | 13 | 11 | 7 |
| Intromission frequency | 8 | 13 | 12 |
| Ejaculation latency | 13 6 | 16 9 | 12 4 |
| Inter-intromission interval | 1 3 | 1 3 | 1 0 |
| Postejaculatory interval | 6 1 | 8 3 | 7 3 |
| Series II | | | |
| Mount frequency | 3 | 3 | 2 |
| Intromission frequency | 8 | 4 | 5 |
| Ejaculation latency | 6 4 | 4 4 | 2 7 |
| Inter-intromission interval | 0 7 | 1 0* | 0 5 |
| Postejaculatory interval | 8 3 | 8 8 | 7 3 |
| Series III | | | |
| Mount frequency | 4 | 1 | 3 |
| Intromission frequency | 5 | 4 | 4 |
| Ejaculation latency | 3 2 | 2 5 | 4 3 |
| Inter-intromission interval | 0 7 | 0 7 | 0 8 |
| Postejaculatory interval | 8 3 | 8 9 | 7 3 |

Table shows median values
Mann Whitney U test *$p<0.05$ [28]

TABLE 6

EFFECT OF SYSTEMIC ADMINISTRATION OF COMPOUNDS AFFECTING GABAERGIC TRANSMISSION ON MASCULINE SEXUAL BEHAVIOR

| | | Behavioral component | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | dose (mg/kg) | N | IL | MF | IF | EL | III | PEI |
| Saline | — | 12 | 0 4 | 2 | 13 | 4 7 | 0 7 | 5 3 |
| (+) bicuculline* | 2 5 | 12 | 0 1 | 3 | 11 | 7 3 | 0 5 | 5 0 |
| Saline | — | 16 | 1 1 | 3 | 12 | 8 1 | 0 7 | 6 8 |
| Picrotoxin* | 0 25 | 16 | 0 2 | 5 | 14 | 9 5 | 0 7 | 6 9 |
| | 0 50 | 16 | 0 2 | 7 | 16 | 10 2 | 0 6 | 5 8 |
| Saline | — | 9 | 0 1 | 4 | 13 | 9 6 | 0 6 | 5 8 |
| 3-mercaptopropionic acid* | 10 0 | 9 | 0 2 | 4 | 8 | 6 5 | 0 5 | 6 6 |
| | 20 0 | 9 | 0 4 | 2 | 8 | 5 5 | 0 6 | 5 5 |
| Saline | — | 6 | 0 2 | 3 | 12 | 6 5 | 0 4 | 5 3 |
| Muscimol† | 0 5 | 8 | 0 2 | 6 | 20 | 11 1 | 0 5 | 5 9 |
| | 1 0 | 7 | 0 1 | 2 | 8 | 4 9 | 0 6 | 6 3 |

Table denotes medial values Observations, for all groups, but for 3-mercaptopropionic acid, were begun 15 min after IP administration Observations for 3-mercaptopropionic acid were begun 10 min after IP injections The sexual behavior was observed through one series of copulation

*Friedman two way ANOVA followed by Wilcoxon T test

†Kruskal Wallis test followed by Mann Whitney U test [28]

IL intromission latency, MF mount frequency, IF intromission frequency, EL ejaculation latency, III inter-intromission interval, PEI postejaculatory interval

were injected IP in a volume of 2 ml/kg saline and tested for sexual behavior through one series of copulations No behavioral alterations were seen in the behavior compared to saline treated controls after any of the drug treatments (Table 6)

## DISCUSSION

Present data show that treatment either by systemic administration or intracerebral injection into the nucleus caudatus putamen of GABA agonists or antagonists was not followed by any alteration in the copulatory behavior. Results also demonstrate that injection into the MPOA of muscimol, a specific GABA agonist, or EOS, an inhibitor of the degradation of GABA, depresses rat masculine sexual behavior, while similar treatment with the GABA antagonists, picrotoxin or (+) bicuculline methiodide, or with 3-MPA, an agent inhibiting the synthesis of GABA, facilitates the expression of the behavior

Common to all three drugs which reduce the GABAergic activity was their capacity to shorten the postejaculatory intervals (PEI) Under normal mating conditions the PEI lasts 4–5 min after the first ejaculation and progressively prolong, in a very regular fashion, by 1–2 min following each successive ejaculation [20,27] Drug treatment produced an abnormal mating pattern characterized by drastically shortened PEIs This effect was particularly striking after treatment with (+) bicuculline, but was obvious as well after injection of picrotoxin or 3-MPA

Treatment with (+) bicuculline and picrotoxin, but not with 3-MPA, also shortened the ejaculation latency and the inter-intromission intervals The reason for these different effects in the copulatory behavior after injection of the different GABA antagonists is not clear One possibility is that their difference in action on the sexual behavior reflects their difference in potency to influence the GABAergic system Pharmacological data show that (+) bicuculline methiodide is a more potent GABAergic antagonist than picrotoxin in several systems [5, 13, 14]. Moreover, systemic administration of 3-MPA reduces central GABA levels only to 20–45% [15] Our finding showing a more marked reduction in the PEI after treatment with (+) bicuculline methiodide than after picrotoxin or 3-MPA injection is in line with this observation Another possible interpretation is that the drugs affect other neurotransmitter systems which are involved in the expression of particular behavior components of mating. Biochemical data show that (+) bicuculline in addition to being a GABA antagonist may influence the cholinergic system [9,30]. We have recently shown that administration of the cholinergic agonist, oxotremorine, causes a highly significant reduction of the ejaculation latency and the number of

intromissions preceding ejaculation, no changes occurring on the length of the PEI [21]. It could be speculated that (+) bicuculline affects the mating pattern by stimulating both the cholinergic system, thereby influencing the ejaculation latency, and the GABAergic system, thereby affecting the PEI Further research, however, is required to test these interpretations

Drugs injected in the MPOA stimulating the GABAergic system resulted in a depression of sexual activity. A relationship between the decrease in sexual activity and general motor activity cannot be excluded. However, injection of muscimol in the nucleus caudatus-putamen, a brain structure of essential importance for motor activity, was not followed by any alterations in the sexual behavior, and about 40 percent of the animals treated with compounds increasing GABAergic transmission still showed mounting behavior

Pharmacological data show that muscimol and EOS are more effective in stimulating the GABAergic system than GABA itself [6, 14, 19] Present findings showing a complete abolishment of sexual behavior after injection of either EOS or muscimol, and only a slight prolongation in the intromission latency following GABA administration, are in line with this observation. Furthermore, it is well established from behavioral research [17] that GABA is only effective within a few min after intracerebral administration presumably because of its rapid reuptake and metabolization [4,6].

Behavior alteration resembling those observed after intrapreoptic-area injection of picrotoxin, (+) bicuculline or 3-MPA have been previously demonstrated following electrical stimulation through electrodes implanted in the MPOA [23,31] and after large electrolytic lesions in the medial brainstem at the level of the diencephalic-mesencephalic junction [11]. It appears from these various observations that neurons extended over an area encompassing the MPOA rostrally and the medial mesencephalon caudally constitute part of a neuronal substrate for the regulation of some specific aspects of the sexual behavior and that GABA may be a neurotransmitter in this substrate. Recently electrolytic and neurochemical lesions in the midbrain raphe nuclei and serotonergic pathways [22,24] were reported to cause a shortening of the ejaculation latencies and postejaculatory intervals, suggesting that serotonin may be another transmitter involved in the control of these behavior components. Catecholamines may also have a role in these behavioral features since electrolytic and neurochemical lesions in brain areas containing noradrenaline or dopamine were followed by similar behavior changes [2, 3, 10, 25, 26]. The specific role of these different neurotransmitters in the behavioral alterations cannot be judged on the basis of present data. It should be noted, however, that the effects on the PEI after manipulation of the brain monoamine systems are less marked when compared to those observed after GABA antagonist injection.

It is proposed that a GABAergic system in the MPOA has an inhibitory function in the neural substrates of masculine sexual behavior. It is further suggested that this inhibitory action determines the periods of sexual inactivity following ejaculation. To specify in more detail the neural systems involved in this inhibitory function further studies should be undertaken

ACKNOWLEDGEMENTS

We thank Mrs Elisabeth Wallin for skillful technical assistance and Mrs Claery Persson for typing the manuscript Supported by the Swedish Humanities and Social Sciences Research Council

## REFERENCES

1 Anlezark, G , R W Hortn, B S Meldrum and M C B Sawaya Anticonvulsant action of ethanolamine-O-sulphate and di-n-propylacetate and the metabolism of gamma-aminobutyric acid (GABA) in mice with audiogenic seizures *Biochem Pharmacol* **25**: 413–417, 1976

2 Clark, T K Male rat sexual behavior compared after 6-OHDA and electrolytic lesions in the dorsal NA bundle region of the midbrain *Brain Res* **202**: 429–443, 1980

3 Clark, T., A Caggiula, R. McConnell and S Antelman Sexual inhibition is reduced by rostral midbrain lesions in the male rat *Science* **190**: 169–171, 1975

4 Curtis, D R and G A R Johnston Amino acid transmitters in the mammalian central nervous system *Ergeb Physiol* **69**: 97–188, 1974

5 Dray, A Comparison of bicuculline methochloride with bicuculline and picrotoxin as antagonists of amino acid and monoamine depression of neurons in the rat brain stem *Neuropharmacology* **14**: 887–891, 1974

6 Enna, S J and A Maggi Biochemical pharmacology of GABAergic agonists *Life Sci* **24**: 1727–1738, 1979

7 Fernández-Guasti, A , K Larsson and C Beyer (+) Bicuculline methiodide stimulates masculine rat sexual behavior *Experientia*

8 Fowler, L J and R A John Active-site-directed irreversible inhibition of rat brain 4-aminobutyrate aminotransferase by ethanolamine-O-sulphate in vitro and in vivo *Biochem J* **130**: 569–573, 1972

9 Gardner, C R and R A Webster The effect of some anticonvulsant drugs on leptazol and bicuculline induced acetylcholine efflux from rat cerebral cortex *Br J Pharmacol* **47**: 652, 1973

10 Hansen, S , C Kohler and S B Ross On the role of the dorsal mesencephalic tegmentum in the control of masculine sexual behavior in the rat Effect of electrolytic lesions, ibotenic acid and DSP4 *Brain Res* **240**: 311–320, 1982

11 Heimer, L and K Larsson Drastic changes in the mating behavior of male rats following lesions in the junction of diencephalon and mesencephalon *Experientia* **20**: 460, 1964

12 Heimer, L and K Larsson Impairment of mating behavior in male rats following lesions in the preoptic anterior hypothalamic continuum *Brain Res* **3**: 248–263, 1966/67

13 Johnston, G A. R Physiological pharmacology of GABA and its antagonists in the vertebrate nervous system In: *GABA in Nervous System Function*, edited by E Roberts, T N Chase and D B Tower New York Raven Press, 1976, pp 395–411

14 Johnston, G A R Neuropharmacology of amino acid inhibitory transmitters *Annu Rev Pharmacol Toxicol* **18**: 269–289, 1978

15 Karlsson, A , F Fonnum, D Matthe Sorenssen and J Strom-Mathisen Effect of the convulsive agent 3-mercaptopropionic acid on the levels of GABA, other aminoacids and glutamate decarboxylase in different regions of rat brain *Biochem Pharmacol* **23**: 3053–3061, 1974

16 Kilpatrick, I C , M S Starr, A Fletcher, T A James and N K MacLeod Evidence for a GABAergic nigrothalamic pathway in the rat *Exp Brain Res* **40**: 45–54, 1980

17 Koella, W P GABA systems and behavior In: *Amino Acid Neurotransmitters*, edited by F V DeFeudis and P Mandel New York Raven Press, 1981, pp 11–21

18 Konig, F R and F R Klippel *The Rat Brain* Huntington, NY Krieger Publishing Co , 1967

19 Krogsgaard-Larsen, P , G A R Johnston, D Lodge and D R Curtis A new class of GABA agonist *Nature* **268**: 53–55, 1977
20 Larsson, K Features of the neuroendocrine regulation of masculine sexual behavior In *Endocrine Control of Sexual Behavior*, edited by C Beyer New York Raven Press, 1979, pp 77–163
21 Larsson, K and S Ahlenius Facilitation of male rat sexual behavior by stimulation of central cholinergic receptors In *Proc 14th Coll Int Neuro-psychopharmacologicum Congr* New York Raven Press, 1984, pp 659 (Abstr )
22 Larsson, K , K Fuxe, B J Everitt, M Holmgren and P Sodersten Sexual behavior in male rats after intracerebral injection of 5,7-dihydroxytryptamine *Brain Res* **141**: 293–303, 1978
23 Malsbury, C W Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area *Physiol Behav* **7**: 797–805, 1971
24 McIntosh, T K and R J Barfield Brain monoaminergic control of male reproductive behavior I Serotonin and the post-ejaculatory refractory period *Behav Brain Res* **12**: 255–265, 1984
25 McIntosh, T K and R J Barfield Brain monoaminergic control of male reproductive behavior II Dopamine and the post-ejaculatory refractory period *Behav Brain Res* **12**: 267–273, 1984
26 McIntosh, T K and R J Barfield Brain monoaminergic control of male reproductive behavior III Norepinephrine and the post-ejaculatory refractory period *Behav Brain Res* **12**: 275–281, 1984
27 Sachs, B D and R J Barfield Temporal patterning of sexual behavior in the male rat *J Comp Physiol Psychol* **73**: 359–364, 1970
28 Siegel, S *Non Parametric Statistics for the Behavioral Science* New York Mc Graw Hill, 1956
29 Snyder, S H The glycine synaptic receptor in the mammalian central nervous system *Br J Pharmacol* **53**: 473–484, 1975
30 Svenneby, G and E Roberts Bicuculline and n-methylbicuculline competitive inhibitors of brain acetylcholinesterase *J Neurochem* **21**: 1025–1026, 1973
31 Van Dis, H and K Larsson Induction of sexual arousal in the castrated male rat by intracranial stimulation *Physiol Behav* **6**: 85–86, 1971
32 Wu, J -W and E Roberts Properties of brain l-glutamate decarboxylase inhibition studies *J Neurochem* **23**: 759–767, 1974