

# Copulatory Pelvic Thrusting in the Male Rat is Insensitive to the Perispinal Administration of Glycine and GABA Antagonists

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MORALÍ, G., B. R. KOMISARUK AND C. BEYER. *Copulatory pelvic thrusting in the male rat is insensitive to the perispinal administration of glycine and GABA antagonists.* PHARMACOL BIOCHEM BEHAV 32(1) 169-173, 1989.—The role of the inhibitory neurotransmitters glycine and GABA in the pacing of pelvic thrusting during copulation was assessed in male rats by an accelerometric technique. Either strychnine, an antagonist of glycine (10 µg), bicuculline, an antagonist of GABA (1 µg), or a combination of strychnine (5 µg) plus bicuculline (0.3 µg), and saline as control, were administered intrathecally to sexually active males. Administration of the antagonists either alone or in combination, at these dose levels, produced sensory effects (skin hyperalgesia, scratching or biting the skin) in all rats. Generalized motor seizures occurred in only a few animals. The incidence of ejaculations, but not of mounts, tended to decrease after treatment with the amino acids antagonists. On the other hand, the values of the instantaneous frequency, duration, and rhythmicity of the copulatory thrusting movements performed during mounts, intromissions or ejaculations did not differ significantly from the values obtained under saline treatment. These findings indicate that the duration and rhythmicity of copulatory movements in the male rat are either controlled by synapses that are insensitive or inaccessible to strychnine and bicuculline, or these copulatory components are independent of glycinergic and GABAergic control and are under the control of other neurotransmitter systems.

Copulatory pelvic thrusting      GABA and glycine antagonists      Rat      Intrathecal treatments  
Strychnine and bicuculline

COPULATION in the male rat involves the performance of rhythmic high frequency pelvic thrusting (17 to 22 cycles/sec) against the female's rump (5, 15, 29). This motor pattern indicates that the motoneurons involved in thrusting discharge in a rhythmic manner as the result of alternating periods of excitation and inhibition. Motoneuron inhibition is mediated by a variety of neurotransmitters (11,16), but the two most important at the spinal cord level are glycine and GABA (2,22). Therefore, it appears plausible that these amino acids are involved in the regulation of the neuronal firing underlying pelvic thrusting during copulation.

To test this possibility, strychnine, an antagonist of glycine (1,12), or bicuculline, an antagonist of GABA (1,12), were administered perispinally either alone or in combination, to sexually active males and the frequency, duration, and rhythmicity of pelvic thrusting were assessed by using

the accelerometric technique and power spectrum analysis (4, 5, 23, 24).

## METHOD

Adult, sexually experienced male Wistar rats were used as subjects (Ss). They were maintained under a reversed 14:10 hours light:dark regime. Ss were fed Purina laboratory chow and received tap water ad lib. A catheter (Clay Adams PE-10 tubing, 7.5 cm insertion length) was implanted chronically into the subarachnoid intrathecal space at the lumbosacral level of the spinal cord through an incision in the atlantooccipital membrane following the technique described by Yaksh and Rudy (30). Ss were anesthetized for surgery with Anesthal (Smith Kline and French, sodium pentobarbital, 35 mg/kg b.wt.). At least twenty days of recovery were allowed before testing. Twenty-four rats not showing any

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TABLE 1

EFFECT OF THE INTRATHECAL INFUSION OF STRYCHNINE (10  $\mu\text{g}$ ), BICUCULLINE (1  $\mu\text{g}$ ), OR THEIR COMBINATION (5  $\mu\text{g}$  + 0.3  $\mu\text{g}$ ) ON THE DISPLAY OF THE VARIOUS PARAMETERS OF MALE SEXUAL BEHAVIOR, AND ON THE INCIDENCE OF SENSORY AND MOTOR EFFECTS\*

Treatment	Number of Ss	Percent of Ss Displaying			Percent of Ss Showing	
		Mount	Intromission	Ejaculation	Sensory Effects	Motor Effects
Saline <sup>†</sup>	24	100	96	96	0	0
Strychnine, 10 $\mu\text{g}$	8	100	88	63	100	13
Bicuculline, 1 $\mu\text{g}$	8	88	75	75	100	0
Strychnine, 5 $\mu\text{g}$ + Bicuculline, 0.3 $\mu\text{g}$	8	88	75	63	100	13

\*For a description of sensory and motor effects of the drugs, see the text.

<sup>†</sup>In order to simplify this table, data were pooled in a single control group.

sign of motor disturbance after surgery were subjected to two behavioral tests (a control test and an experimental one) separated by ten days.

#### Behavioral Testing

For testing, Ss were placed in cylindrical, Plexiglas observation cages. After a five-min adaptation period, the male was presented with a receptive, estrogen-treated female. Stimulus females received SC 5  $\mu\text{g}$  estradiol benzoate three times per week. The following behavioral variables were measured: (1) *Mount*: mount with pelvic thrusting but without vaginal penetration; (2) *Mount latency*: time from the entrance of the receptive female into the observation cage to the first mount; (3) *Intromission*: mount with behavioral signs of vaginal penetration; (4) *Intromission latency*: time from the entrance of the female into the observation cage to the first intromission; (5) *Ejaculation latency*: time from the first intromission to the ejaculatory pattern.

#### Accelerometric Analysis of Pelvic Movements During Copulation

The copulatory movements of the Ss were recorded by means of an accelerometric technique described elsewhere (4, 5, 9, 23, 24). A cloth harness carrying a strain gauge transducer measuring maximal acceleration in one plane (Grass SPA-1 accelerometer) was adjusted to the rat. The accelerometer was connected to a DC Grass preamplifier coupled to a Grass 7B polygraph, allowing the precise analysis of the following characteristics of the copulatory pattern: a) duration of the pelvic thrusting train in mounts, intromissions, or ejaculations, and b) frequency of pelvic thrusting, i.e., number of pelvic thrusts per second. As described elsewhere (4, 23, 24), a differentiation was made among short and long ejaculatory thrusting trains; these two types of trains can be recognized both by their duration, and by their dynamic organization: short, consisting of a single, regular phase of pelvic thrusting, and long, consisting of two different, extra- and intravaginal thrusting periods. Frequency of pelvic thrusting is similar in both short and long ejaculatory trains. In addition to the analysis of duration and frequency of thrusting, an "on line" analysis of the periodicity of pelvic thrusting during copulation was made in

some cases by using a Nicolet 660B computerized frequency analyzer which provided a frequency spectrum for each train of thrusting (23,24).

#### Drug Treatments

When the male achieved the first intromission, one of the following drugs or combination of drugs (Sigma) was injected through the intrathecal catheter in volumes of 2.5  $\mu\text{l}$  of saline: strychnine sulfate (10  $\mu\text{g}$ ), bicuculline methiodide (1  $\mu\text{g}$ ), or a combination of strychnine (5  $\mu\text{g}$ ) plus bicuculline (0.3  $\mu\text{g}$ ). This volume was pushed into the perispinal space by an additional 6  $\mu\text{l}$  of saline which remained in the catheter, and testing continued thereafter. Control tests were carried out in which Ss were injected with 2.5  $\mu\text{l}$  of saline instead of the drug. The order in which drug and control tests were performed followed a counterbalanced design. Doses were selected from data obtained in a pilot study (25). These doses consistently induce clear sensory changes (see below), but not generalized motor seizures. Convulsions have been found to stop Ss from displaying copulatory activity (25).

#### Observations of Sensory-Motor Effects

The occurrence of signs of either sensory or motor disturbances (see the Results section) following the perispinal (intrathecal) injection, was recorded continuously throughout the testing period. Tests were terminated 30 min after injection or before that time if the Ss had ejaculated and reinitiated copulation. Thirty min is the average duration of the sensory effects induced by the intrathecal administration of strychnine or bicuculline (6,26).

#### Statistical Analysis

Proportions of Ss showing mount, intromission, or ejaculatory behavior under each drug treatment were compared to those responding when tested under saline treatment, by the McNemar's test for the significance of changes. For the two parameters analyzed in the motor copulatory pattern (i.e., duration and frequency of pelvic thrusting), individual means were calculated; Student's paired *t*-tests were used to compare individual performances in each of the three drug groups, to their own controls.

TABLE 2  
TEMPORAL CHARACTERISTICS OF THE COPULATORY RESPONSES DISPLAYED BY MALE RATS UNDER THE VARIOUS PERISPINAL TREATMENTS (MEANS OF MEANS  $\pm$  SD)

Treatment	n	Duration (sec)				Frequency of Pelvic Thrusting (thrusts/sec)		
		Mount	Intromission	Ejaculation*		Mount	Intromission	Ejaculation
				Short	Long			
Saline <sup>†</sup>	24	0.47 $\pm$ 0.13 (23)	0.28 $\pm$ 0.09 (23)	0.62 $\pm$ 0.21 (10)	1.16 $\pm$ 0.24 (13)	18.4 $\pm$ 1.5 (19)	19.0 $\pm$ 1.1 (23)	19.2 $\pm$ 1.4 (23)
Strychnine, 10 $\mu$ g	8	0.41 $\pm$ 0.07 (8)	0.28 $\pm$ 0.07 (6)	0.51 $\pm$ 0.13 (3)	1.19 $\pm$ 0.01 (2)	17.7 $\pm$ 0.8 (8)	18.4 $\pm$ 1.0 (6)	18.2 $\pm$ 1.7 (5)
Bicuculline, 1 $\mu$ g	8	0.50 $\pm$ 0.15 (7)	0.24 $\pm$ 0.05 (6)	0.61 $\pm$ 0.10 (3)	1.18 $\pm$ 0.31 (3)	17.2 $\pm$ 1.4 (7)	19.8 $\pm$ 0.8 (6)	18.0 $\pm$ 1.3 <sup>‡</sup> (6)
Strychnine, 5 $\mu$ g + Bicuculline, 0.3 $\mu$ g	8	0.44 $\pm$ 0.07 (7)	0.26 $\pm$ 0.04 (6)	0.81 (2)	1.02 $\pm$ 0.08 (3)	18.8 $\pm$ 1.4 (7)	19.0 $\pm$ 1.5 (6)	19.6 $\pm$ 1.2 (5)

\*Short and long ejaculatory thrusting trains differ both by their duration and by their dynamic organization (see text).

<sup>†</sup>In order to simplify this table, and since no differences were found among the three groups of saline-treated rats, data were pooled in a single control group. Statistical comparisons, however, were made of each drug group versus their own control.

<sup>‡</sup> $p < 0.01$ , paired Student's *t*-test.

Numbers in parentheses correspond to the number of Ss upon which the means were calculated.

## RESULTS

### Sensory-Motor Effects of Amino Acid Antagonists

Administration of either the glycine or the GABA antagonists resulted in distinct sensory and motor effects that were similar, if not identical, for both types of antagonists. Injection of both strychnine and bicuculline also induced a qualitatively similar response to that obtained with either toxin alone. Within the first three minutes postinjection, all Ss started to show bouts of scratching and biting directed at the lower half of the body, actions often accompanied by distress-type vocalizations. During the intervals between spontaneous bouts of scratching, a mild localized tactile stimulus such as a weak jet of air directed to the fur, triggered a bout of scratching and vocalization and often induced strong aversive reactions. These have been considered to be sensory effects of the toxins (6,26). Motor reactions, which appeared later than sensory effects, i.e., around five minutes postinjection, included hopping, fall over with tail whipping, and convulsions (6,26). These effects subsided earlier than the sensory effects. Thus, skin hyperalgesia (vocalization in response to weak tactile stimulation) was usually observable 30 minutes after the injection of the drugs, when all motor effects had disappeared. Convulsions were shown by only two out of the 24 Ss (Table 1).

### Incidence of Sexual Behavior

The behavioral patterns involved in copulation were differentially influenced by the perispinal administration of the neurotoxins (Table 1). Only two out of the 24 Ss were unable to reassume copulation after treatment with the amino acids antagonists, one after receiving bicuculline, and the other after bicuculline plus strychnine. Excepting these two rats, all other animals continued displaying at least mounts during the period of intoxication. Ejaculatory behavior was inhibited

in some rats, particularly in the groups receiving strychnine either alone or in combination with bicuculline (Table 1), since only five out of the eight Ss treated in each of these two groups showed ejaculation. These proportions, however, did not differ (McNemar's test) from the data obtained when the Ss received saline.

### Motor Copulatory Patterns During Intoxication by Glycine and GABA Antagonists

The characteristics of copulatory pelvic thrusting (duration of the thrusting trains, and frequency of thrusting) in the various copulatory components (mounts, intromissions and ejaculation) displayed under control and experimental conditions are shown in Table 2. The criteria for quantitation of these parameters are shown in Fig. 1. Data from saline-treated animals were pooled in a single control group in order to simplify the table. The characteristics of pelvic thrusting were not affected by the intrathecal administration of saline, since they were very similar to those described elsewhere for intact, nondisturbed male rats (4, 5, 23, 24). The intrathecal administration of the amino acids antagonists either alone or in combination did not significantly affect either the duration or the frequency of copulatory pelvic thrusting (Table 2). Moreover, the various treatments did not alter the rhythmicity of thrusting, as shown in Fig. 1 for a representative mount of a strychnine-treated animal, as compared to a mount displayed under saline treatment.

## DISCUSSION

The intrathecal administration of glycine or GABA antagonists consistently induces clear sensory and motor alterations (6,26). Our present results also show that these neurotoxins tended to decrease the probability of occurrence of ejaculation patterns. The failure to ejaculate shown by

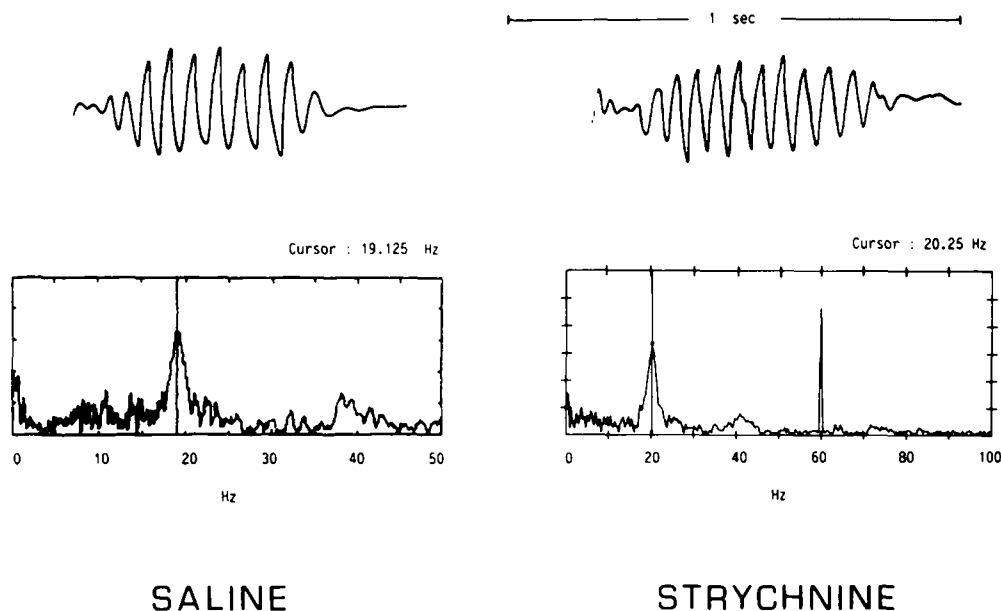


FIG. 1. Representative accelerometric (upper) and power spectra (lower) tracings of a mount performed under saline, and a mount performed three minutes after the perispinal administration of  $10 \mu\text{g}$  strychnine, when already sensory effects (skin hyperalgesia and scratching) occurred. Note that the duration of the mount, the frequency of thrusting movements (number of pelvic thrusts per second), the amplitude (area under each cycle), and the dynamic organization of the mounting trains, can be accurately determined from the accelerometric records, while the rhythmicity of thrusting can be assessed by the power spectrum analysis. Rhythmicity is related to the width of the prevailing peak in the spectrum frequency analysis. Note that the frequency values and the rhythmicity of both mounting trains were very similar. The frequency spectrum of the mount performed under strychnine was recorded at the 100 Hz mode, so that the sharp peak resulting from an unfiltered 60 Hz signal appeared for comparison purposes.

some Ss was not due to motor incoordination or deterioration of the animal's condition, since they displayed normal mounting. However, since most of the nonejaculating Ss only showed a few intromission patterns, it is possible that these alterations were related either to interference with normal penile erection or with the identification of sensory cues involved in vaginal penetration.

Models designed to explain rhythmic alternating motor patterns such as scratching, swimming, and walking include spinal inhibitory interneurons as important modulators of these behaviors (8,21). Moreover, inhibitory interneurons including Renshaw cells have been found to discharge in complicated but rhythmic patterns during rhythmic alternating behaviors, i.e., scratching and walking (3,13). Analysis of the pelvic movements performed during copulation, i.e., rhythmic alternation of contraction of flexor and extensor muscles during thrusting, strongly suggests the participation of both Ia inhibitory interneurons and Renshaw cells (14, 17, 19) in this motor pattern. Both inhibitory interneurons are known to use glycine (11,28) and in some cases also GABA (10) as neurotransmitters.

Our data show that the neuronal processes underlying the organization of the motor copulatory pattern (i.e., duration, frequency and rhythmicity of thrusting in mounts, intromissions, and ejaculations) were not disrupted by the glycine and GABA antagonists even when administered in combination. These treatments, nevertheless, induced dramatic sensory effects indicating occupation of glycine or GABA recep-

tors. Therefore, the results could be interpreted as evidence that neither glycine nor GABA participate in the control of the intratrain characteristics of the motor copulatory pattern in the male rat. It is, however, possible that these inhibitory amino acids do indeed control the intratrain characteristics of pelvic thrusting via synapses that are insensitive to these antagonists. Thus, glycine synapses insensitive to strychnine have been described in the CNS including the spinal cord (7, 27, 28). Particularly relevant to these results is the observation that the pause in Renshaw cells discharge normally following their activation is resistant to strychnine as well as to bicuculline and picrotoxin (28). This pause is due to increased inhibitory input from other inhibitory glycinergic interneurons (mutual inhibition). Therefore, if the mutual inhibition of Renshaw cells is related to the patterning of motoneuron discharge during copulatory pelvic thrusting, this could help to explain our failure to influence this motor pattern by strychnine. Moreover, bicuculline is known to antagonize GABA effects at GABA-A receptors but not at GABA-B sites (1), and therefore, GABA could play a role in the pacing of copulatory movements by acting at the latter receptors.

It is also possible that neurotransmitters other than glycine and GABA produced by intra- and/or supraspinal neurons regulate motoneurons discharge during pelvic thrusting. Thus, brainstem neurons may impose a rhythmic firing pattern to the motoneurons involved in copulatory movements. Direct descending inhibitory influences on

motoneurons have been traced from noradrenergic locus coeruleus neurons as well as from raphe serotonergic neurons (20). Lesions of descending monoaminergic pathways by perispinal administration of 6-hydroxydopamine alters some parameters of male rat copulatory behavior (i.e., the postejaculatory interval) but not the motor copulatory

pattern itself (18). Yet, subtle changes in pelvic thrusting may have been undetected by the pure visual observation used in this study. Therefore, the possibility that these monoaminergic or other inhibitory systems to the spinal cord are involved in the temporal patterning of thrusting movements should be explored.

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