Pharmacology Biochemistry & Behavior, Vol. 30, pp. 337–342. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Flight Behavior Induced by Microinjection of GABA Antagonists Into Periventricular Structures in Detelencephalated Rats[1]

CARLOS TOMAZ,* MARCOS BRANDÃO,† ABDALLAH BAGRI,‡
PASCAL CARRIVE‡ AND PIERRE SCHMITT‡

**Laboratory of Psychobiology, F.F.C.L.R.P., University of São Paulo, 14049 Ribeirão Preto*
†Departament of Physiological Sciences, Biomedical Center
Federal University of Espírito Santo, 29000 Vitoria, E.S., Brasil
‡Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS-5
rue Blaise Pascal, 67084 Strasbourg Cedex, France

Received 4 September 1987

TOMAZ, C., M. BRANDÃO, A. BAGRI, P. CARRIVE AND P. SCHMITT. *Flight behavior induced by microinjection of GABA antagonists into periventricular structures in detelencephalated rats.* PHARMACOL BIOCHEM BEHAV **30**(2) 337–342, 1988.—Behavioral effects of unilateral microinjections into periventricular structures of bicuculline, a classic GABA-A antagonist, and semicarbazide, a glutamic acid decarboxylase blocker, were studied in detelencephalated rats. These drugs produced a behavioral activation together with jumps. However, the characteristics of this behavioral activation differed as the injections were made in dorsal periaqueductal gray matter or medial hypothalamus. These data show close similarities to those observed with intact animals suggesting that GABA-A receptors are involved in the neural control of expression of flight behavior and functions in an intact manner and possibly independent of influences from forebrain structures. At variance with intact animals, these drugs produced contralateral turning behavior when locally injected into MH, pointing to some kind of inhibitory control exerted by telencephalic structures on the expression of circling behavior from diencephalic regions.

GABA Flight Mesencephalic central gray Medial hypothalamus Microinjection Aversion Detelencephalated rats

IT has been proposed that a group of structures in the CNS comprising amygdala, medial hypothalamus (MH), dorsal periaqueductal gray matter (DPAG), deep layers of superior colliculus constitutes, as a whole, a brain aversive system [9], for aversive responses often result when they are electrically [19–21, 24] or chemically stimulated [2,25]. Thus, microinjection of bicuculline methiodide, a traditional GABA-A antagonist, or semicarbazide, a glutamic acid decarboxylase (GAD) blocker, into periventricular structures induces flight behavior. The most important differences between the effects of these drugs occur in both the delay of action and duration of effects [3,16]. While the flight behavior appears almost immediately after the injection of bicuculline into periventricular structures the dealy is much longer for semicarbazide. Qualitatively, however, both drugs produce similar behavioral activation. When injected into DPAG and deep layers of superior colliculus these drugs initially provoke freezing interspersed with turning contralateral to the injection side. This behavior progressively changes to strong locomotor activity together with jumps [2,3]. In the MH these microinjections also produce flight behavior, but there is neither freezing nor turning behavior and the behavioral activation is not as explosive as in the DPAG. Besides, rearing behavior appears as a new component. Interestingly, these drugs, when injected into inferior colliculus, produce an intermediate pattern of responses that may be placed between DPAG and MH [4,6]. Taken together these data indicate that in both DPAG and MH, GABA-A receptors are involved in the control of escape reactions and in the elaboration of underlying aversive effects [2, 10, 25].

The aim of this work is to investigate whether or not the telencephalon is critically involved in flight behavior induced by GABAergic manipulations of periventricular structures. To this end we studied the characteristics of responses elicited by microinjections of either bicuculline methiodide or semicarbazide into DPAG or MH of rats in which forebrain structures were surgically removed [12].

METHOD

Animals and Surgery

Male wistar rats (weighing 290–400 g) served as subjects.

[1]This research was carried out at the Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS-5, Strasbourg, France.

Pre-experimentally the animals were housed individually under standard conditions with free access to food and water and kept on a light/dark cycle (12/12 hr).

Surgery was performed under pentobarbital anesthesia (40 mg/kg, IP) with the head of the animal fixed in a stereotaxic apparatus. Pieces of skull (0.5×0.5 cm) were removed on both sides of the sagittal suture by trephining and fine rongeurs, and the telencephalon removed through these openings in one stage by aspiration. To remove the telencephalon, the cerebral cortex and striatum were aspirated first, followed by the ventral forebrain anterior to preoptic area. Finally, the hippocampus was separated from the underlying thalamus. After this ablation one stainless-steel guide-cannula (0.4 mm o.d.; 0.3 mm i.d.) was implanted into either the medial hypothalamus (MH) or the central gray (CG) at the following coordinates, using the lambda point as the reference for each plane:

	PA	ML	DV
MH	4.5 mm	0.3 to 0.5	7.5
DPAG	0.4 to 0.8	1.2	5.6

(with a medio lateral angle of 10°).

The guide-cannula was anchored to the skull by means of an autopolymerizing resin and three stainless-steel screws and sealed with a stainless-steel wire. At the end of surgery, the resulting skull cavity was filled with Gelfoam (Upjohn) soaked in physiologic saline prior to closing the skull with dental cement.

Postoperatively, the animals were fed twice daily intragastrically with a commercial baby food dissolved in saline (10 ml). They were housed in individual plastic cages (38×28×15.5 cm) in a separate room at an ambient temperature between 20 and 23°C, under a 12:12 hr light/dark cycle.

Drugs

Bicuculline methiodide (Sigma) was used at the dose of 35 ng (0.07 nmol) and semicarbazide (Sigma) at 12 μg (110 nmol). Both drugs were dissolved in sterile distilled water and injected in a volume of 0.2 μl at a rate of 0.2 μl/20 sec.

General Procedure

Testing was begun 24 hr after the operation. The experimental apparatus consisted of a circular enclosure 60 cm in diameter and 30 cm high with a floor divided into 12 sections. The experimental procedure was as follows. First, the steel wire was replaced by a stainless-steel injections-needle (0.28 mm o.d.; 0.18 mm i.d.) which protruded 1.0 mm beyond the tip of the guide-cannula and which was linked to a 1 μl Hamilton syringe by means of polyethylene tubing. Each rat was then placed in the center of the circular enclosure and injected with bicuculline methiodide and/or semicarbazide. The injection cannula was gently removed 1 minute following the end of injection. The following behavioral responses were recorded every minute for 25: number of crossings (i.e., number of floor sections traversed), number of rearings either against the wall or in the middle of the cage, number of jumps and rotations. When the rat jumped on the top of the enclosure wall, it was gently taken and replaced at the center of the enclosure.

These rats were assigned to two groups depending on the compound injected. Half of the rats in each group received a MH injection and the other half a DPAG injection.

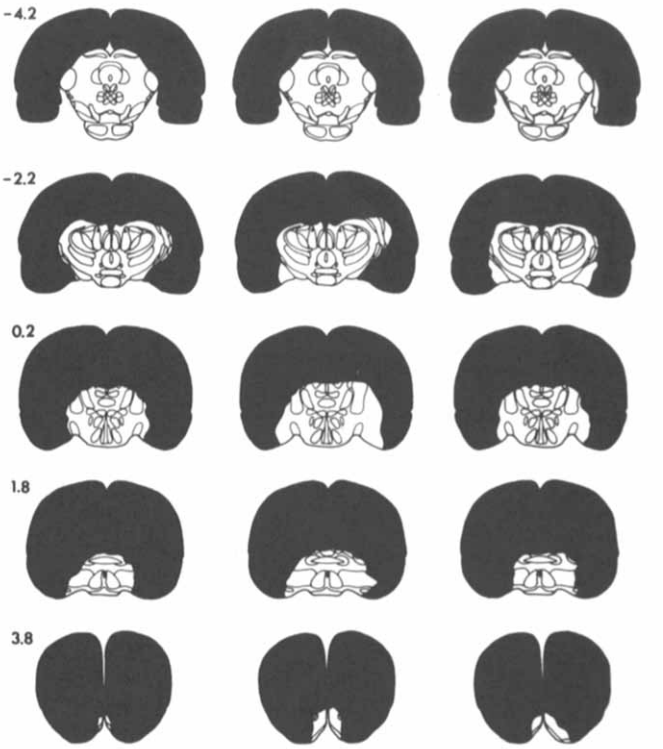

FIG. 1. Reconstruction of brains of three detelencephalated rats on plates from König and Klippel atlas [18].

Analysis of Data

The results were analyzed by means of an analysis of variance [27] followed when appropriate by Bonferoni test [7] or nonparametric tests [26].

Histology

The animals which survived and completed the behavioral tests (about 80%) were anesthetized and perfused intracardially with 9% NaCl followed by 10% formalin. The brain was removed, photographed and placed into a 30% sucrose-formalin solution until they sank. Serial 20 μm brain sections were stained with cresyl violet and inspected microscopically in order to localize the injection sites and to verify the brain lesions.

RESULTS

The reconstruction of the brain was based on the gross outline of the remaining structures. Histological examination of the slides showed that the lesions of the detelencephalated rats were similar in shape and size to those previously described for this preparation [13, 14, 22]. Neocortex and hippocampus were gone in all preparations. Residual amygdala tissue as well as fragments of the caudate nucleus-putamen were found unilaterally and/or bilaterally in some animals. In most of the animals the boundaries of the lesion were the preoptic region rostrally and superior colliculus caudally. No relationship, however, was found between amount of tissue removed and the behavioral repertoire evoked by the microinjections. Figure 1 shows the reconstructions of 3 representative brains based on outline histology.

The localization of DPAG and MH microinjection sites

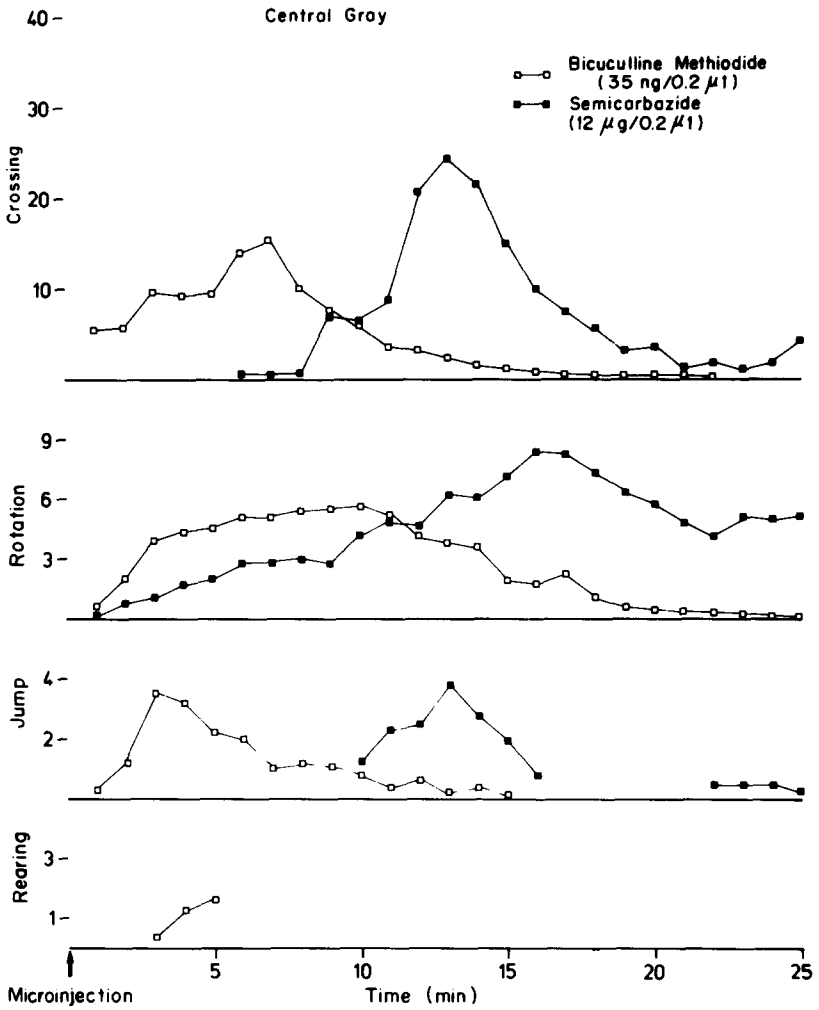

FIG. 2. Time course of the behavioral effects (counts per minute) produced by microinjections into the dorsal periaqueductal gray matter. n=21 and 27 for bicuculline and semicarbazide respectively. The SEM at maximum effects were for crossing: semicarbazide=7.2 and bicuculline=3.7; for rotation: semicarbazide=3.6 and bicuculline=2.1; for jump: semicarbazide=1.5 and bicuculline=1.3; for rearing: bicuculline=0.6.

were similar to those previously described by Schmitt and co-workers [3,25].

Behavior

An unilateral central gray or medial hypothalamic microinjection of bicuculline methiodide and/or semicarbazide resulted in a clear behavioral activation in terms of an increase in locomotion, in the number of rearings, turnings and, in some cases, jumps. The occurrence of some or all of these responses was dependent on the brain site of injection.

Microinjections Into the Central Gray

At twenty-one sites the microinjection of 35 ng (0.07 nmol) of the GABA receptor antagonist bicuculline methiodide produced behavioral activation together with jumps. These sites were found to be located in the dorsal and dorsolateral part of the DPAG. Figure 2 shows the time course of these effects. About 1 minute after the end of the injections, the animal began to run rapidly, and this increase in the number of crossings lasted about 12 minutes with a peak during the seventh minute following the injection. Together with the increase in crossings, an increase in the number of contralateral turnings was observed. About one minute after the injection, explosive jumps were observed; some of them led the rats to escape from the enclosure. The period of jumps lasted for 15 min and showed a peak value during the third minute following the injection. Some rearings were also observed between the third and fourth minute after the injection.

Microinjections at twenty-seven sites of 12 μg (110 nmol) semicarbazide, a drug known to block glutamic acid decarboxylase (GAD), also produced behavioral activation accompanied by jumps (see Fig. 2). However, the time course of the behavioral effects was different from that observed after microinjection of bicuculline methiodide. There was a difference in both delay of action and the duration of the induced effects. About 1 min after the end of the injections, the animals began to show rotations that lasted about 30 min with a peak during the 16–17th minute following the injection. Increase in horizontal locomotion (crossings) was observed at about the 6th minute with a peak during the 13th minute. Some jumps were

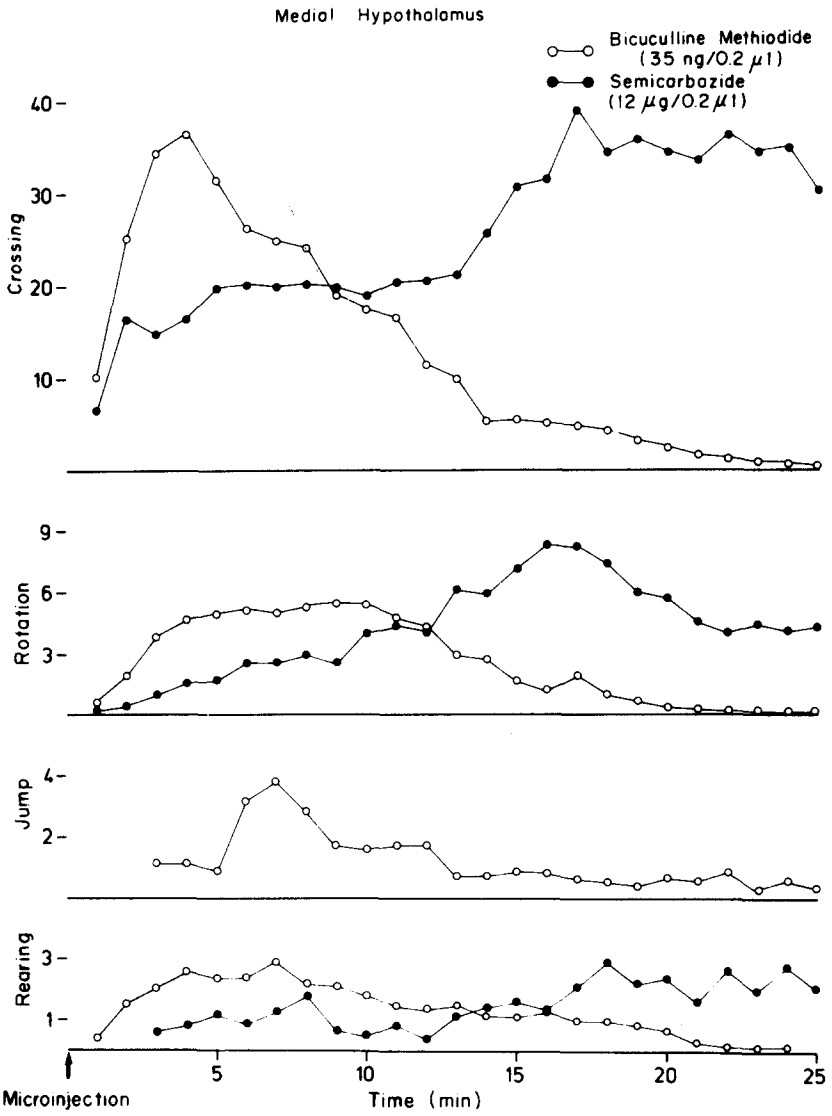

FIG. 3. Time course of the behavioral effects (counts per minute) produced by microinjections into medial hypothalamus of either bicuculline or semicarbazide. n=26 and 16 for bicuculline and semicarbazide, respectively. The SEM at maximum effects were for crossing: semicarbazide=9.1 and bicuculline=8.9; for rotation: semicarbazide=3.2 and bicuculline=2.1; for jump: bicuculline=1.9; for rearing: semicarbazide=1.4 and bicuculline=1.2.

also observed 10 minutes after the injection. No rearing was elicited. The total number of crossings and rotations (contralateral turning) after semicarbazide was significantly higher than that recorded after bicuculline (Bonferoni test, $t=5.58$ and 5.74; for crossings and rotation respectively, $p<0.05$ in both). The number of jumps did not differ from that recorded after bicuculline injection ($t=2.26$, $p>0.05$).

Microinjections Into the Medial Hypothalamus

At twenty-six sites, a 35 ng bicuculline microinjection produced a clear behavior activation. Figure 3 shows the time course of the effects in this group. About one minute after the microinjection, the animals started to run in the open-field, often rearing along the wall and sometimes jumping out of the enclosure. The number of crossings increased rapidly also, reaching a peak value at the seventh minute. Jumps were observed from the third to the 25th minute with a peak value at the 7th minute. This behavioral activation was less explosive, more oriented/coordinated than that observed after a DPAG injection.

Microinjections of 12 μg of semicarbazide at sixteen sites also produced behavioral activation but without jumps. During the first minutes following the microinjection, the animals started to run in the enclosure, frequently changing their direction. This increase in the number of crossings reached a peak value at the 17th minute following the microinjection. A similar time course of effects was observed for the contralateral turnings. Rearings were observed from the third to the 24th minute with a peak value also at the 17th

minute following the microinjection. As can be seen in Fig. 3, the time course of the effects after semicarbazide differed from that observed after bicuculline injection. Thus, the number of crossings recorded following semicarbazide was significantly lower for the first four minutes (Wilcoxon, $t=2.53, p<0.05$) and significantly higher from the 13th to the 25th minute (Wilcoxon, $t=2.59, p<0.05$). The same tendency was observed for the number of rotations recorded, lower for the first ten minutes (Wilcoxon, $t=2.48, p<0.05$) than those recorded in response to bicuculline. No such difference was observed for the number of rearings.

DISCUSSION

The present data demonstrate that unilateral microinjections of GABA inhibitors such as bicuculline or semicarbazide into MH or DPAG induced flight behavior in detelencephalated rats. This behavior showed close similarities with that previously described in intact animals [3,25]. In fact bicuculline produced a behavioral activation together with jumps independently of the structures stimulated. As in intact animals, the jumps were more coordinated after bicuculline injection in MH than in DPAG and differently from MH, rearing behavior was not significant in DPAG. Semicarbazide produced similar results with the exception of jumps which were not produced by MH microinjections of the drug. Thus, these results indicate that flight behavior is under a tonic inhibitory control through a GABAergic system in MH and DPAG which is not influenced by telencephalic structures.

Several evidences support the hypothesis that GABAergic projections from substantia nigra pars reticulata (SN) to deep layers of superior colliculus and DPAG play a major role in the expression of turning behavior [1, 8, 11]. Thus, microinjections of GABA agonists like muscimol into substantia nigra, or GABA blockers like bicuculline or picrotoxin into deep layers of superior colliculus, produce similar locomotor asymmetries [5, 15, 17, 23]. In this experiment we obtained contralateral turning behavior in detelencephalated rats following microinjections of bicuculline or semicarbazide injected into DPAG in the same manner as it happens in intact animals [3] suggesting that a mesencephalic GABAergic system is able to control the emergence of turning behavior without the influences of forebrain structures. These findings were in agreement with previous results [22] showing that removal of the whole telencephalon changed neither the direction nor the magnitude of the circling responses induced by microinjections of GABA agonists or antagonists into SN.

A surprising finding was that bicuculline and semicarbazide also induced turning behavior when injected into MH of detelencephalated rat, this effect being more intense with semicarbazide. In intact animals this response is rarely seen following microinjections of GABA blockers into this structure [3]. It is worth noting that this behavior was already observed even during the first minutes after local application of semicarbazide into MH; i.e., during the latency period of the drug action and that rotation is a salient feature of the hyperexcitability manifested in detelencephalated rats [22]. Therefore, it is possible that the behavioral activation induced by this drug was mainly manifested through intensification of preexistent rotations. These responses may have prevented the appearance of semicarbazide-induced coordinated jumps which are the main expression of flight behavior at the MH level. Anyway, these results suggest that a GABAergic system may exist at level of MH which inhibits the emergence of turning behavior.

Taken together, our findings suggest that an intrinsic diencephalic and/or mesencephalic GABAergic system tonically inhibits the generation of flight behavior and which may be independent of control by telencephalic structures.

ACKNOWLEDGEMENTS

Carlos Tomaz was supported by a fellowship from Brazilian National Research Council (CNPq) and the Deutscher Akademischer Austauschdienst (DAAD). Marcus Brandão was supported by the Federal University of Espírito Santo (UFES) and CNPq. The authors are grateful to Mrs. M. J. Angst and G. Rudolf for their skillful technical assistance.

REFERENCES

1. Beckstead, R. M., V. B. Domesick and W. J. H. Nauta. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res* **175:** 191–217, 1979.
2. Brandão, M. L., J. L. De Aguiar and F. G. Graeff. GABA mediation of the antiaversive action of minor tranquilizers. *Pharmacol Biochem Behav* **16:** 397–402, 1982.
3. Brandão, M. L., G. Di Scala, M. J. Bouchet and P. Schmitt. Escape behavior produced by blockade of glutamic acid decarboxylase (GAD) in mesencephalic central gray or medial hypothalamus. *Pharmacol Biochem Behav* **24:** 497–501, 1986.
4. Brandão, M. L. and A. Fin. Is the inferior colliculus part of the brain aversive system? *Braz J Med Biol Res* **19:** 481A, 1987.
5. Brandão, M. L. and P. Schmitt. Role of nigrocollicular GABAergic fibers in the genesis of aversive behavior. In: *Neurosciences and Behavior,* edited by M. L. Brandão. Vitória: UFES, 1987, pp. 31–44.
6. Breese, G. R., G. D. Frye, T. J. McCown and R. A. Mueller. Comparison of the CNS effects induced by TRH and bicuculline after microinjection into medial septum, substantia nigra and inferior colliculus: Absence of support for a GABA antagonist action for TRH. *Pharmacol Biochem Behav* **21:** 145–149, 1984.
7. Dixon, W. J. *BMDP Statistical Software.* Los Angeles: University of California Press, 1981.
8. Faull, R. L. M. and W. R. Mehler. The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. *Neuroscience* **3:** 989–1002, 1978.
9. Graeff, F. G. Minor tranquilizers and brain defense system. *Braz J Med Biol Res* **14:** 239–267, 1981.
10. Graeff, F. G., M. Brandão, E. A. Audi and M. T. B. Schutz. Modulation of the brain aversive system by GABAergic and serotonergic mechanisms. *Behav Brain Res* **21:** 65–72, 1986.
11. Hopkins, D. A. and L. W. Niessen. Substantia nigra projections to the reticular formation, superior colliculus and central gray in the rat, cat and monkey. *Neurosci Lett* **2:** 253–259, 1979.
12. Huston, J. P. and A. A. Borbely. The thalamic rat: General behavior, operant learning with rewarding hypothalamic stimulation and effects of amphetamine. *Physiol Behav* **12:** 433–448, 1974.
13. Huston, J. P., C. Tomaz and I. Fix. Avoidance learning in rats devoid of telencephalon plus thalamus. *Behav Brain Res* **17:** 87–95, 1985.
14. Huston, J. P., M. Joosten and C. Tomaz. Reversal learning of an avoidance response in detelencephalated rats. *Exp Neural* **91:** 147–153, 1986.

15. Imperato, A. and G. DiChiara. Behavioural effects of GABA agonists and antagonists infused in the mesencephalic reticular formation-deep layers of superior colliculus. *Brain Res* **224:** 185–193, 1981.
16. Killam, K. F. and J. A. Bain. Convulsant hydrazides. I. In vitro and vivo inhibition of vitamin B6 enzymes by convulsant hydrazides. *J Pharmacol Exp Ther* **119:** 225–262, 1957.
17. Kilpatrick, I. C., G. L. Collingridge and M. S. Starr. Evidence for the participation of nigrotectal γ-aminobutyrate-containing neurones in striatal and nigral derived circling in the rat. *Neuroscience* **7:** 207–222, 1982.
18. König, J. F. R. and R. H. Klippel. *The Rat Brain in Stereotaxic Coordinates*. Baltimore: Williams and Wilkins, 1963.
19. Milani, H. and F. G. Graeff. GABA-benzodiazepine modulation of aversion in the medial hypothalamus of the rat. *Pharmacol Biochem Behav* **28:** 21–27, 1987.
20. Nashold, B. S., Jr., N. P. Wilson and G. S. Slaughter. Sensations evoked by stimulation in the midbrain of man. *J Neurosurg* **30:** 14–24, 1969.
21. Olds, M. E. and J. Olds. Approach-escape interactions in rat brain. *Am J Physiol* **203:** 803–810, 1962.
22. Papadopoulos, G. and J. P. Huston. Removal of the telencephalon spares turning induced by injection of GABA agonists and antagonists into substantia nigra. *Behav Brain Res* **1:** 25–38, 1980.
23. Scheel-Kruger, J., J. Arnt and C. Magelund. Behavioral stimulation induced by muscimol and other agonists injected into the substantia nigra. *Neurosci Lett* **4:** 351–356, 1977.
24. Schenberg, L. C. and F. G. Graeff. Role of the periaqueductal gray substance in the anti-anxiety action of benzodiazepines. *Pharmacol Biochem Behav* **9:** 287–295, 1978.
25. Schmitt, P., G. DiScala, M. L. Brandão and P. Karli. Behavioral effects of microinjection of SR95103, a new GABA-A antagonist, into the medial hypothalamus or the mesencephalic central gray. *Eur J Pharmacol* **117:** 149–158, 1985.
26. Siegel, S. *Non-Parametric Statistics for the Behavioral Sciences*, Kogakusha International student edition. New York: McGraw Hill, 1956.
27. Winer, E. J. *Statistical Principles in Experimental Design*, 2nd edition. New York: McGraw Hill, 1971.