Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 82 (2005) 417 – 426
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

# Behavioral differences between subgroups of rats with high and low threshold to clonic convulsions induced by DMCM, a benzodiazepine inverse agonist

Marcos Brandão Contó, José Gilberto Barbosa de Carvalho, Marco Antonio Campana Benedito *

*Departamento de Psicobiologia, Universidade Federal de São Paulo, Escola Paulista de Medicina (UNIFESP/EPM), Rua Botucatu 862, 1° andar, Vila Clementino, São Paulo, SP 04023-900, Brazil*

Received 1 June 2005; received in revised form 15 September 2005; accepted 22 September 2005
Available online 16 November 2005

**Abstract**

In epileptic patients, there is a high incidence of psychiatric comorbidities, such as anxiety. Gamma-aminobutyric acid (GABA) ionotropic receptor $GABA_A$/benzodiazepine allosteric site is involved in both epilepsy and anxiety. This involvement is based on the fact that benzodiazepine allosteric site agonists are anticonvulsant and anxiolytic drugs; on the other hand, benzodiazepine inverse agonists are potent convulsant and anxiogenic drugs. The aim of this work was to determine if subgroups of rats selected according to their susceptibility to clonic convulsions induced by a convulsant dose 50% ($CD_{50}$) of DMCM, a benzodiazepine inverse agonist, would differ in behavioral tests commonly used to measure anxiety (elevated plus-maze, open field) and depression (forced swimming test). In the first experiment, subgroups of adult male Wistar rats were selected after a single dose of DMCM and in the second experiment they were selected after two injections of DMCM given after an interval of 1 week. Those rats presenting full clonic convulsions were termed Low Threshold rats to DMCM-induced clonic convulsions (LTR) and those not having clonic convulsions High Threshold rats to DMCM-induced clonic convulsions (HTR). In both experiments, only those rats presenting full clonic convulsions induced by DMCM and those not showing any signs of motor disturbances were used in the behavioral tests. The results showed that the LTR subgroup selected after two injections of a $CD_{50}$ of DMCM spent a significantly lower time in the open arms of the elevated plus-maze and in the off the walls area of the open field; moreover, this group also presented a higher number of rearings in the open field. There were no significant differences between HTR and LTR subgroups in the forced swimming test. LTR and HTR subgroups selected after only one injection of DMCM did not differ in the three behavioral tests. To verify if the behavioral differences between HTR and LTR subgroups of rats selected after two injections of DMCM were due to the clonic convulsion, another experiment was carried out in which subgroups of rats susceptible and nonsusceptible to clonic convulsions induced by a $CD_{50}$ of picrotoxin, a $GABA_A$ receptor channel blocker, were selected and submitted to the elevated plus-maze and open field tests. The results obtained did not show any significant differences between these two subgroups in the elevated plus-maze and open field tests. In another approach to determine the relation between fear/anxiety and susceptibility to clonic convulsions, subgroups of rats were selected in the elevated plus-maze as more or less fearful/anxious. The $CD_{50}$ for clonic convulsions induced by DMCM was determined for each of these two subgroups. The results showed a significantly lower $CD_{50}$ for the more fearful/anxious subgroup, which means a higher susceptibility to clonic convulsions induced by DMCM. The present findings show a relation between susceptibility to clonic convulsions and fear/anxiety and vice versa which may be due to differences in the assembly of $GABA_A$/allosteric benzodiazepine site receptors in regions of the brain.


*Keywords:* Rats; Clonic convulsions; DMCM; Picrotoxin; Anxiety; Depression; Epilepsy; Elevated plus-maze; Open field; Forced swimming test

## 1. Introduction

Epilepsies are complex neurological syndromes characterized by their recurrence and presenting electrographical and motor disturbances. An important issue regarding the epileptic disorders resides in the fact that some epileptic individuals are

* Corresponding author. Tel.: +55 11 5539 0155; fax: +55 11 5572 5092.
*E-mail address:* ocram@psicobio.epm.br (M.A.C. Benedito).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.09.012

more susceptible to develop certain comorbidities, such as psychiatric syndromes, than others. Anxieties and depressions are such common psychiatric comorbidities occurring in epileptic patients (Marsh and Rao, 2002).

Epilepsies can be considered the result of an imbalance between inhibition and excitation in the brain (Bradford et al., 1995). Therefore, the comorbidities occurring in some of the epileptic individuals could be the result of the imbalance of certain neurotransmitters controlling inhibition/excitation and located in brain regions involved in the generation of symptoms of psychiatric disorders. Currently, the basic research investigating the observed clinical relation between epilepsies/convulsions and psychiatric disorders has been carried out using rats and has been done mostly using the kindling procedure, induced electrically in the amygdala or hippocampus or by systemic injection of convulsants (Adamec and Morgan, 1994; Cannizzaro et al., 1993; Hannesson et al., 2001; Helfer et al., 1996; Kalynchuk et al., 1997, 1998; Nieminen et al., 1992; Wintink et al., 2003).

Gamma-aminobutyric acid (GABA) appears to be involved in several normal or pathological aspects of brain functioning, such as epilepsies, anxieties and depressions (Paredes and Ågmo, 1992; Shiah and Yatham, 1998). Therefore, GABA could be a neurotransmitter candidate to be the link between epilepsies and psychiatric syndromes comorbidity. GABA is considered the main inhibitory neurotransmitter in the central nervous system and its hyperpolarizing effect is achieved through the interaction of the neurotransmitter with two different receptors, the $GABA_A$ ionotropic (Mehta and Ticku, 1999) and the $GABA_B$ metabotropic receptors (Chebib and Johnston, 1999; Enna and Bowery, 2004). Two kinds of GABA inhibitory neurotransmission exist in the brain, a fast and a slow one (Greengard, 2001), and GABA fast inhibitory action is the result of the interaction of GABA with its ionotropic $GABA_A$ receptor (Chebib and Johnston, 1999). The $GABA_A$ receptor has other binding sites besides the GABA one which modulates the synaptic inhibitory GABA action. One of these binding sites is the benzodiazepine allosteric binding site to which different classes of drugs are known to bind (Mehta and Ticku, 1999). One class of such drugs that bind to the allosteric benzodiazepine binding site are the beta-carbolines and some of these drugs, e.g., DMCM (methyl 6,7-dimethoxy-4-ethyl-beta-carboline), have the opposite effect of the benzodiazepine agonists. Instead of inducing an anxiolytic and anticonvulsant effect seen with the benzodiazepine agonists, these drugs are anxiogenic (Cole et al., 1995) and potent convulsants (Braestrup et al., 1982).

Although a direct involvement of GABA in the etiology of the epilepsies is yet to be definitively proved, it is well known that a decrease in the inhibitory effect of GABA obtained, for instance, by the administration of a benzodiazepine inverse agonist, triggers convulsions (Braestrup et al., 1982; Chapouthier and Venault, 2001; Clément et al., 1997; De Sarro et al., 1996) and that drugs, such as benzodiazepine agonists, inhibit clonic convulsions (De Sarro et al., 1996) and are anticonvulsant drugs largely used clinically (Kwan et al., 2001). The dual pharmacological and behavioral effects of drugs acting through the allosteric benzodiazepine binding site in the $GABA_A$ receptor has led some authors to raise the possibility of a relation between clonic convulsions and anxieties (Chapouthier and Venault, 2001; Clément et al., 1997; Pellow, 1985).

Open field, elevated plus-maze and forced swimming test are three behavioral procedures largely used with laboratory animals. Open field and elevated plus-maze have been used for animal models of anxiety (Belzung and Griebel, 2001; Pellow et al., 1985; Prut and Belzung, 2003) and the forced swimming test for an animal model of depression (Porsolt et al., 1978). The elevated plus-maze test is used to measure the level of anxiety/fear of an animal, which is based on the time spent and/or the number of entries in the open arms of the apparatus. The higher the time spent and/or the number of entries in the open arms, the lower the anxiety/fear. This effect is seen after the administration of benzodiazepine anxiolytics (Pellow et al., 1985) and the administration of benzodiazepine inverse agonist produces the opposite effects (Cole et al., 1995). In the open field test, the level of anxiety/fear is obtained by measuring the time the animal spends or the number of visits off the walls and to the center of the apparatus . The lower the level of anxiety/fear the higher the time spent or number of visits off the walls or to the center of the open field. This effect is observed after the administration of benzodiazepine agonists (Angrini et al., 1998; Nazar et al., 1999). The forced swimming test is mainly based on the measurement of the time of immobility and struggling, and a higher immobility and a lower struggling has been considered as a "depressive" state in this test. In general, antidepressants decrease the time of immobility and increase the time of struggling (Lahmame and Armario, 1996; Porsolt et al., 1978).

In a normal population, it is possible to detect large behavioural differences between the individuals. Using these individual differences in the population, researchers have been selecting subgroups of individuals presenting large behavioural differences between them (Kabbaj and Akil, 2001; Ho et al., 2002; Pawlak and Schwarting, 2002). In the Wistar rat strain, it was shown that the individuals differ largely in the number of subconvulsive electrical stimuli delivered to the amygdala to develop full clonic convulsions (Sanberg and Ossenkopp, 1978). Based on this data, we sought to separate from a normal population of Wistar rats two subgroups of individuals differing in their threshold to develop full forelimbs clonic convulsions induced by a convulsant dose 50% ($CD_{50}$) of DMCM, an anxiogenic (Cole et al., 1995) and a potent convulsant (Braestrup et al., 1982) benzodiazepine inverse agonist. It is worth mentioning that in mice the sensitivity to convulsions induced by DMCM has a genetic determinant (Seale et al., 1987). Using this approach, we separated two subgroups of rats, injected with the $CD_{50}$ of DMCM after one, in the first experiment, or two administrations of the drug in the second experiment. Those rats developing full forelimbs clonic convulsions induced by DMCM were termed Low Threshold Rats (LTR) and those not showing any signs of motor disturbances were termed High Threshold Rats (HTR).

The aim of this work sought to determine if the rats differing in the threshold to clonic convulsions induced by DMCM

(HTR and LTR subgroups) would also differ in their behavior in the elevated plus-maze, open field and forced swim test.

## 2. Methods

### 2.1. Animals

Subjects were naive adult male outbred Wistar rats, aged 3 months, weighing about 350 g. They were housed in groups of 5–6 per cage (60 × 50 × 22 cm), in a temperature-controlled environment (23 ± 2 °C) with a 12:12 h light–dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) with ad lib food and water. All the experiments were carried out in the afternoon (1:00–5:00 p.m.) to avoid possible influences of circadian variations (Eidman et al., 1990).

This work was approved by our institution Ethics Committee on Animal Research (Proc. 0529/03).

### 2.2. Apparatus

The elevated plus-maze was similar to that described by Pellow et al. (1985). The apparatus was made of wood and consisted of two open arms (50 × 10 × 1 cm) and two closed arms (50 × 10 × 30 cm). Each open arm is divided in three equal sections for the observation of the open arms exploration. The open and closed arms intercross perpendicularly in a region corresponding to the central region (10 × 10 cm) of the apparatus. The apparatus was elevated to a height of 65 cm above the floor level.

The open field apparatus used consisted of a circular arena with a diameter of 80 cm, surrounded by a wall of 36 cm height (Eidman et al., 1990). The apparatus floor is divided in 3 concentric circles, subdivided in 18 equal segments for observation of ambulation. The illumination of the apparatus consisted of 5 lamps of 60 W, positioned 85 cm above the floor of the apparatus.

The forced swimming test apparatus used, similar to that described by Porsolt et al. (1978), consisted of a transparent acrylic cylinder, 50 cm high and 30 cm in diameter. The cylinder was filled with water at 25 °C, until a depth of 20 cm, in a way that the animal was capable of remaining immobile touching the apparatus floor with the tips of its hindlimbs.

### 2.3. Convulsant dose–response curves

In order to separate two subgroups of rats which differ in the threshold to clonic convulsions induced by DMCM, a dose–response curve was obtained to determine the $CD_{50}$ in our Wistar rats line. Clonic convulsions were characterized by the abrupt appearance of bilateral contractions/relaxations of the forelimbs, followed by a loss of postural reflex. This convulsion can be followed by tonic convulsion, depending on the increase in the doses administered and consists of maintaining contractions of the fore and hindlimbs. To obtain the $CD_{50}$, five doses of DMCM (10 rats/dose) were used. The DMCM, purchased from Sigma® (St. Louis, MO), was dissolved in a few drops of 0.1N HCl and diluted in bidistilled water, being injected intraperitoneally at a volume of 0.1 ml/100 g of body weight. The $CD_{50}$ was calculated according to the method of Litchfield and Wilcoxon (1949).

In order to control the possible effect of the clonic convulsion on the behavior of the rats we also selected subgroups of rats according to their susceptibility to clonic convulsions induced by two injections of a $CD_{50}$ of picrotoxin (3.0 mg/kg, intraperitoneally), a $GABA_A$ receptor channel blocker (Johnston, 1996). All the procedures used in this experiment were exactly the same as those described previously to select the subgroups of HT and LT rats. Picrotoxin (purchased from Sigma®, St. Louis, MO) was dissolved in distilled warmed water and injected intraperitoneally.

All behavioral scoring, including convulsions, was done visually by trained personnel and in a way that did not disturb the animals during the behavioural sessions.

### 2.4. Selection of the subgroups of rats susceptible to clonic convulsions

#### 2.4.1. Selection of subgroups through a single injection of DMCM

In the first experiment, a total of 89 naive rats were used. All rats received a single intraperitoneal injection of the $CD_{50}$ of DMCM. Those rats displaying full clonic convulsions were assigned to the LTR subgroup and those not showing any signs of motor disturbance to the HTR subgroup. After being observed for 30 min for the appearance of clonic convulsions, the rats were returned to their cages and remained in the storage room for the next 20 days without any other disturbance besides the delivery of food and water and cage cleaning. After this interval, they were submitted to the behavioral tests. Although the time interval (20 days) between DMCM injection and behavioral testing was arbitrarily chosen, the reasoning underlying this choice was to avoid any possible influence of the drug on the behavior, since we were looking for phenotype differences belonging to animal's repertoire and not to a long-lasting effect of the drug in the animal's brain.

#### 2.4.2. Selection of subgroups through two injections of DMCM

In the second experiment, a total of 139 naive rats were used. In the separation of HTR and LTR subgroups after two injections of DMCM the procedure for the first dose was exactly the same as already described. After the first injection of DMCM and scoring of the convulsions the rats were returned to their cages where they rested for 1 week in the animal storage room. The time interval between injections was chosen due to the slow degradation of DMCM in the plasma of rats (Schweri et al., 1983). After resting 1 week, the rats received the second injection of the $CD_{50}$ of DMCM. Only the rats developing full forelimbs clonic convulsions (LTR subgroup) and those not showing any signs of motor disturbances after the two DMCM administrations (HTR subgroup) were selected for the behavioral tests. This criterion was used because some of the rats developed full clonic convulsions only after the first injection of DMCM and others

only after the second injection. After resting 20 days from the second injection of DMCM, the subgroups of HTR and LTR rats were submitted to the behavioral tests. Different subgroups of HTR and LTR rats were used in each behavioral test, with the exception of the open field test in which HTR and LTR subgroups tested in the elevated plus-maze were submitted to the open field after a rest of 20 days.

#### *2.4.3. Selection of subgroups of rats through two injections of picrotoxin*

Following the same experimental procedures used to select the subgroups of rats after the DMCM injection, 72 naive male rats were injected with the $CD_{50}$ of picrotoxin (3.0 mg/kg, i.p.). After an interval of 1 week, the rats received the second injection of picrotoxin. Those rats showing clonic convulsions in both drug administrations were assigned to the susceptible subgroup ($N=16$) and those not showing any signs of motor disturbances were assigned to the nonsusceptible subgroup ($N=16$). After a rest of 20 days, the subgroups were tested in the elevated plus-maze. In another experiment, 77 naive male rats were also treated twice with the $CD_{50}$ of picrotoxin following exactly the same protocol as already described. The susceptible and the nonsusceptible subgroups were submitted 20 days later to the elevated plus-maze test and after a rest of 20 days, they were submitted to the open field test.

### *2.5. Elevated plus-maze*

HTR and LTR subgroups selected through one ($N=12$ for each subgroup) or two ($N=15$ for each subgroup) injections of the $CD_{50}$ of DMCM were submitted to the elevated plus-maze. The rats were placed individually in the central portion of the maze and the behaviors were scored for 5 min. The behaviors scored were (1) time (seconds) spent in the open and closed arms; (2) number of entries in the open and closed arms; (3) number of open arms sectors crossed; (4) latency (seconds) for the first entry in the open arms. After returning the rat to its cage, the floor of the maze was wiped clean with diluted ethanol.

The subgroups of rats selected by two injections of picrotoxin were submitted to the elevated plus-maze following the same procedures as those used for the HTR and LTR subgroups.

### *2.6. Open field test*

HTR and LTR subgroups ($N=18$ for each subgroup) selected through a single injection of DMCM were submitted only to the open field test. However, the HTR and LTR subgroups selected after two injections of DMCM and tested in the elevated plus-maze previously were also submitted to the open field after a rest of 20 days. The circular open field used is divided in three concentric circles and we recorded the time spent in the outer (wall circle), in the middle and in the inner circle (off the wall area). This was done because both the middle and the inner circles can be considered unprotected areas for rats. Therefore, they tend to remain, for most of the session time, in the outer circle (wall circle) in close contact to the open field wall. We compared the time spent in the three circles separately and then we added the time spent in the middle to the time spent in the inner circle and compared it with the time spent in the outer circle.

Each rat was placed individually in the center of the apparatus and during 3 min of exposure the following behaviors were scored: (1) time (seconds) spent in the inner, middle and outer circle; (2) ambulation, measured as the number of sections crossed with four paws; (3) frequency of rearings (number of occurrences) at any place in the apparatus and (4) defecation (number of fecal boli). After returning the rat to its cage the open field floor was wiped clean with diluted ethanol.

The subgroups of rats selected by two injections of picrotoxin were submitted to the open field test following the same procedures as those described for the HTR and LTR subgroups.

### *2.7. Forced swimming test*

Different HTR and LTR subgroups selected through one ($N=10$ for each subgroup) or two injections ($N=12$ for each subgroup) of a $CD_{50}$ of DMCM were submitted to the forced swimming test of Porsolt et al. (1978) with a slight modification. Instead of the classical 2 days exposure, the rats were submitted for 3 consecutive days (training, test and retest, respectively), with an interval of 24 h between the exposures. The retest session was included as reported in the literature (Armario and Martí, 1988; Hawkins, 1986; Marti and Armario, 1993). The procedure consisted of putting the rat, individually, in the cylinder containing water and registering the behaviors emitted by the animal during a certain period of time, after which it was removed from the cylinder, dried with a dry cloth and returned to its original cage. The water was changed in the intervals between one rat and the other.

In the first day (training) the rats remained in the cylinder for 15 min, whereas in the two other exposures (test and retest days) the time in the cylinder was 5 min. The behaviors observed in the 3 exposures were (1) immobility time (seconds), in which the animal remains immobile, making only slight movements to keep its head above the water; (2) time of struggling (seconds), which consists of explosive muscular movements against the apparatus wall, in an attempt to escape from the cylinder. The first 5 min of the training session was used for statistical analysis.

### *2.8. Selection of subgroups of rats more or less fearful/anxious in the elevated plus-maze*

One hundred and five naive rats were submitted to a 5-min session in the elevated plus-maze. The selection of more or less fearful/anxious subgroups was based on the time spent and the number of squares crossed in the open arms of the apparatus. Rats above one standard deviation of the population mean were assigned to the less fearful/anxious subgroup and those rats

below one standard deviation of the population mean were assigned to the more fearful/anxious subgroup.

### 2.9. Determination of the $CD_{50}$ of DMCM in subgroups of more and less fearful/anxious rats selected in the elevated plus-maze test

Groups of rats from the more and less fearful/anxious subgroups selected in the elevated plus-maze test were weighed and then injected intraperitoneally with different doses of DMCM (ranging 0.4–0.8 mg/kg). After being injected the rats were kept individually in wire cages and they were observed for 30 min for the appearance of clonic convulsions. The $CD_{50}$ for the subgroups was calculated and statistically compared (Litchfield and Wilcoxon, 1949).

### 2.10. Statistical analyses

For intergroup comparisons in the open field and in the elevated plus-maze the variables were analysed by the Student's *t*-test or Mann–Whitney *U*-test. For intergroup and intragroup comparisons in the forced swimming test the data was analysed by a two-way ANOVA for repeated measures followed by Duncan's post hoc test. The significance level was set at $P \leq 0.05$.

## 3. Results

### 3.1. Convulsant dose–response curves

In naive rats the value of the $CD_{50}$ of DMCM obtained from the dose–response curve was 0.63 mg/kg (19/20 confidence limits between 0.52 and 0.71 mg/kg) (Fig. 1).

The $CD_{50}$ for tonic convulsions induced by DMCM was also calculated, its value corresponded to 0.80 mg/kg (19/20 confidence limits between 0.75 and 0.85 mg/kg) . In the $CD_{50}$ for clonic convulsions injected to select the subgroups of rats only 20% of the LTR rats presented tonic convulsions.

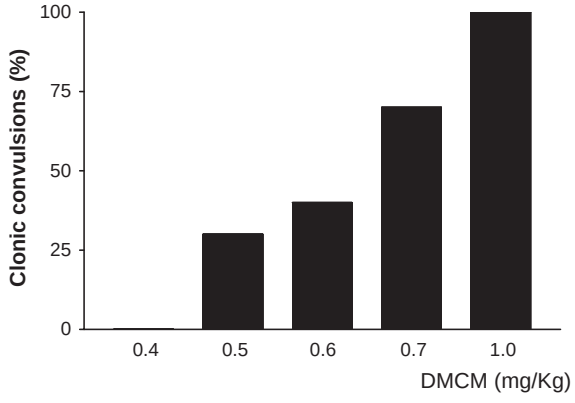


Fig. 1. Dose–response curve of the clonic convulsions induced by DMCM in naive male rats. The convulsant dose 50% ($CD_{50}$) and its upper and lower confidence limits [0.63 mg/kg (0.53–0.72)] were calculated by the method of Litchfield and Wilcoxon (1949). Groups of rats ($N=10$/dose) were injected intraperitoneally with DMCM.

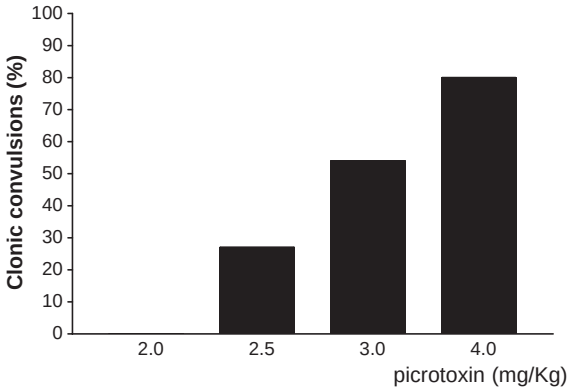


Fig. 2. Dose–response curve for clonic convulsions induced by picrotoxin in naive male rats. Picrotoxin was injected intraperitoneally ($N=10$/dose). The convulsant dose 50% ($CD_{50}$) for clonic convulsions and its upper and lower confidence limits [3.0 mg/kg (2.6–3.5)] were calculated by the method of Litchfield and Wilcoxon (1949).

In naive rats the $CD_{50}$ of picrotoxin obtained from the dose–response curve was 3.0 mg/kg (19/20 confidence limits between 2.6 and 3.5) (Fig. 2).

### 3.2. Selection of HTR and LTR subgroups of rats

In the first experiment 46% of the rats had clonic convulsions (41 rats/$N=89$) after a single dose of a $CD_{50}$ of DMCM. In the second experiment the first injection of DMCM induced clonic convulsions in 37% (51 rats/$N=139$) of the rats and the second injection 39% (54/$N=139$).

### 3.3. Selection of subgroups through two injections of picrotoxin

In the first selection 47% (34 rats/$N=72$) of the rats had clonic convulsions after the first injection of picrotoxin and 53 % (38 rats/$N=72$) had clonic convulsions after the second administration. In the second selection 42% (32 rats/$N=77$) had clonic convulsions after the first administration of picrotoxin and 36% (28 rats/$N=77$) after the second injection.

Table 1
Behavior in the elevated plus-maze of subgroups of rats selected as high (HTR) and low (LTR) threshold to clonic convulsions induced by a single or two injections of the $CD_{50}$ of DMCM

| Subgroup | Latency | Sectors crossed OA | TOA | OAE | TCA | CAE |
|---|---|---|---|---|---|---|
| *Selection through a single injection of DMCM* | | | | | | |
| LTR | 68.5±26.9 | 11.3±2.9 | 37.2±8.2 | 3.2±0.7 | 223±13.3 | 7.2±0.9 |
| HTR | 72.±31.3 | 13.6±2.8 | 45.9±9.4 | 3.7±0.6 | 214±11.8 | 7.3±0.9 |
| *Selection through two injections of DMCM* | | | | | | |
| LTR | 89.3±34.0 | 12.8±3.0 | 29.7±6.5 | 3.4±0.7 | 232±9.5 | 7.7±1.0 |
| HTR | 22.7±6.2 | 19.9±3.9 | 55.9±8.7* | 4.7±0.8 | 206±11 | 8.0±0.9 |

Values are expressed as the means± S.E.M. $N=12$ for each subgroup selected through one injection of DMCM and $N=15$ for each subgroup selected through two injections of DMCM.
LATENCY=time (seconds) for the first open arm entry; OA=open arms; OAE=open arm entries; TOA=time (seconds) spent in the open arms; CAE=closed arm entries; TCA=time (seconds) spent in the closed arms.
* $P<0.05$; unpaired Student's *t*-test.

### 3.4. Elevated plus-maze test

Table 1 shows the results obtained in the elevated plus-maze test. As can be seen in Table 1, there were no significant differences between HTR and LTR subgroups selected after one injection of DMCM in all behaviors scored in the elevated plus-maze ($P>0.05$, Student's $t$-test or Mann–Whitney $U$-test). There was a significant difference between HTR and LTR subgroups selected after two administrations of DMCM, in which the LTR subgroup showed a significantly lower time spent in the open arms of the elevated plus-maze ($t=2.41$; $P<0.05$, Student's $t$-test) (Table 1). There were no significant differences between the subgroups in the other behaviors scored in the test ($P>0.05$, Student's $t$-test or Mann–Whitney $U$-test).

As can be seen in Table 2, there were no significant differences between the subgroups of rats selected after two injections of picrotoxin in the elevated plus-maze test in the two experiments carried out with different subgroups selected and tested at different times (Student's $t$-test or Mann–Whitney $U$-test, $P>0.05$).

### 3.5. Open field test

Table 3 shows the data obtained in the open field test. HRT and LRT subgroups of rats selected after only one injection of DMCM did not differ statistically in all the open field behaviors recorded ($P>0.05$, Student's $t$-test or Mann–Whitney $U$-test). In the subgroups selected after two administrations of DMCM, the LTR subgroup reared significantly more than the HTR subgroup ($t=2.01$; $P<0.05$, Student's $t$-test) (Table 3). A significant difference between HTR and LTR subgroups was obtained when considering the time spent off the wall (the middle plus the inner circle). In this case, the LTR subgroup spent significantly less time off the wall when compared to the HTR subgroup ($t=1.88$; $P<0.05$, Student's $t$-test).

As can be seen in Table 4, there were no significant differences between the subgroups selected after two injections of picrotoxin in the open field test (Student's $t$-test or Mann–Whitney $U$-test, $P>0.05$).

Table 2
Behavior in the elevated plus-maze of subgroups of rats susceptible (S) and nonsusceptible (NS) to clonic convulsions selected after two injections of the $CD_{50}$ (3.0 mg/kg, i.p.) of picrotoxin

| Subgroup | Latency | Sectors crossed OA | TOA | OAE | TCA | CAE |
|---|---|---|---|---|---|---|
| *First experiment* | | | | | | |
| S | 109±38.2 | 6.3±2.6 | 24.2±8.1 | 1.7±0.5 | 252.2±9.8 | 5.4±1.5 |
| NS | 118±39.4 | 7.2±1.7 | 22.2±7.6 | 2.0±0.5 | 251.9±10.7 | 3.9±0.8 |
| *Second experiment* | | | | | | |
| S | 81.3±27.3 | 13.8±0.3 | 30.1±6.0 | 3.0±0.6 | 241.6±8.3 | 6.7±1.1 |
| NS | 132.3±33.8 | 12.0±0.4 | 33.2±9.0 | 3.0±0.8 | 242.4±9.8 | 7.5±0.9 |

The values are expressed as the means±S.E.M. $N=11$ for S and $N=10$ for NS subgroups in the first experiment. $N=16$ for S and NS subgroups in the second experiment.
Latency=time (seconds) for the first open arm entry; OA=open arms; OAE=open arms entries; TOA=time (seconds) spent in the open arms; CAE=closed arms entries; TCA=time (seconds) spent in the closed arms.

Table 3
Behavior in the open field test of subgroups of rats selected as high (HTR) and low (LTR) threshold to clonic convulsions induced by a single or two injections of the $CD_{50}$ of DMCM

| Subgroup | Total AMB | TP | TC | REARS | DEFEC |
|---|---|---|---|---|---|
| *Selection through a single injection of DMCM* | | | | | |
| LTR | 57.9±4.9 | 155.7±3.1 | 23.8±3.1 | 19.7±2.2 | 2.5±0.4 |
| HTR | 57.3±3.4 | 161.3±2.3 | 18.8±2.3 | 17.8±1.4 | 3.0±0.6 |
| *Selection through two injections of DMCM* | | | | | |
| LTR | 62.7±6.8 | 169.5±1.3 | 10.5±1.2 | 14.6±1.8 | 1.4±0.4 |
| HTR | 53.5±6.5 | 164.9±2.0 | 15.1±2.0* | 10.0±1.4* | 1.9±0.4 |

Values are expressed as the means ±S.E.M. $N=18$ for each subgroup selected through a single injection, $N=15$ for each subgroup selected through two injections. Total AMB=total ambulation; DEFEC=defecation; TC=time (seconds) spent off the walls (middle+inner circles); TP=time (seconds) spent in periphery; REARS=rearings.
* $P<0.05$, unpaired Student's $t$-test.

### 3.6. Forced swimming test

As can be seen in Table 5, two-way ANOVA showed that the two subgroups of rats selected either after only one or two injections of DMCM did not differ in the immobility (single DMCM injection: $F_{1,18}=0.24$, $P>0.05$; two DMCM injections: $F_{1,22}=0.01$, $P>0.05$) and struggling (single DMCM injection: $F_{1,18}=2.98$, $P>0.05$; two DMCM injections: $F_{1,22}=3.12$, $P>0.05$) in the forced swimming test. A significant interaction for immobility (one DMCM injection: $F_{2,36}=0.22$, $P>0.05$; two DMCM injections: $F_{2,44}=0.47$, $P>0.05$) and struggling (one DMCM injection: $F_{2,36}=0.07$, $P>0.05$; two DMCM injections: $F_{2,44}=0.06$, $P>0.05$) was also not observed.

As expected, significant differences were detected when intragroup comparisons were made. The two-way ANOVA detected intragroup differences in the behavior of struggling in the subgroups selected through a single ($F_{(2,36)}=58.4$; $P<0.001$) and two injections of DMCM ($F_{(2,44)}=55.1$; $P<0.001$), and of immobility in the subgroups selected through one ($F_{(2,36)}=5.56$; $P<0.05$) and two injections of DMCM ($F_{(2,44)}=11.06$; $P<0.001$).

In the LTR subgroup, the Duncan 's post hoc test detected the following differences: decrease in the time of struggling between the training and the test ($P<0.001$ in both procedures to select the subgroups), between the training and the retest ($P<0.001$ in both procedures to select the subgroups) and between the test and the retest ($P<0.005$ in the single injection selection); increase in the time of immobility between the

Table 4
Behavior in the open field test of subgroups of rats susceptible (S) and nonsusceptible (NS) to clonic convulsions selected after two injections of a $CD_{50}$ (3.0 mg/kg, i.p.) of picrotoxin

| Subgroup total | AMB | TP | TC | REARS | DEFEC |
|---|---|---|---|---|---|
| S | 54.3±4.8 | 159.4±4.2 | 20.5±4.2 | 21.0±1.2 | 2.6±0.4 |
| NS | 56.3±7.5 | 158.7±3.4 | 21.3±3.4 | 23.8±2.8 | 2.7±0.5 |

The values are expressed as the means±S.E.M. $N=10$ for S and NS subgroups. Total AMB=total ambulation; DEFEC=defecation; TC=time (seconds) spent off the wall; TP=time (seconds) spent in periphery; REARS=rearings.

Table 5
Behavior of rats selected as high (HTR) and low (LTR) threshold to clonic convulsions induced by a single or two injections of a $CD_{50}$ of DMCM in the forced swimming test

| Behaviour | LTR subgroup | | | HTR subgroup | | |
|---|---|---|---|---|---|---|
| | Training | Test | Retest | Training | Test | Retest |
| *Selection through one injection of DMCM* | | | | | | |
| Struggling | 78.4±6.2 | 48.0±8.0### | 21.7±6.1**### | 92.7±3.8 | 58.8±6.5### | 36.1±9.5*### |
| Immobility | 43.1±5.3 | 71.0±15.4 | 85.2±13.9# | 55.5±9.4 | 67.8±7.5 | 94.3±20.8# |
| *Selection through two injections of DMCM* | | | | | | |
| Struggling | 62.9±5.3 | 31.2±4.5### | 19.3±3.5### | 57.7±5.8 | 24.0±5.0### | 10.9±2.3### |
| Immobility | 116.2±16.7 | 169.7±12.4## | 163.2±17.6# | 116.4±18.2 | 154.7±16.4# | 171.2±20.2## |

*$P<0.01$; **$P<0.005$ compared to the test of the same group.
Values (time in seconds) are expressed as the means±S.E.M. $N=10$ for each subgroup in the single injection of DMCM. $N=12$ for each subgroup in the two injections of DMCM. #$P<0.05$; ##$P<0.01$; ###$P<0.001$ compared to the training session of the same subgroup. Two-way ANOVA for repeated measures followed by post hoc Duncan's test.

training and the test ($P<0.01$ in the two injections selection) and between the training and the retest ($P<0.05$ in both procedures to select the subgroups).

In the HTR subgroup, the Duncan's post hoc test detected the following differences: decrease in the time of struggling between the training and the test ($P<0.001$ in both procedures to select the subgroups) and between the training and the retest ($P<0.001$ in both procedures to select the subgroups); increase in the time of immobility between the training and the test ($P<0.05$ in the two injections selection) and between the training and the retest ($P<0.05$ in the single injection selection and $P<0.01$ in the two injections selection).

### *3.7. Susceptibility to clonic convulsions induced by DMCM in more or less fearful/anxious subgroups selected in the elevated plus-maze*

Table 6 shows the data obtained in the selection of the subgroups of more or less fearful/anxious rats in the elevated plus-maze. As can be seen in Fig. 3 the more fearful/anxious subgroup of rats had a significantly lower $CD_{50}$ for the clonic convulsions induced by DMCM [Potency ratio 1.58(1.0–2.4), $P=0.05$] (Litchfield and Wilcoxon, 1949).

## 4. Discussion

The present findings show that subgroups of rats selected only through the administration of two injections of a $CD_{50}$ of DMCM differed significantly regarding the fear/anxiety measures in the elevated plus-maze and open field tests. The lower time spent by LTR subgroup in the open arms of the elevated plus-maze and in the off the wall area of the open field is an indication of their higher fear/anxiety. Our results also show that more fearful/anxious rats selected in the elevated plus-maze are more susceptible to clonic convulsions induced by DMCM. Finally, our results showed that the differences obtained in our study are not due to the induction of clonic convulsions, since subgroups selected after two injections of picrotoxin did not differ significantly in the elevated plus-maze and open field tests.

Kindling, the experimental procedure in which initially subconvulsant stimulus given repeatedly becomes convulsant (Racine, 1972) could be involved in the procedure of injecting rats twice with DMCM; however, this seems unlikely since kindling is obtained after several daily stimulations, chemical or electrical. Moreover, there was not an increase in the susceptibility to clonic convulsions between the first and the second administration of DMCM (see Results) that is seen during the kindling procedure. The lack of behavioral differences in the subgroups of rats selected after two injections of picrotoxin also does not favor a kindling effect induced by DMCM.

In our study DMCM was shown to be a potent convulsant drug yielding a steep dose–response curve for clonic convulsions. The $CD_{50}$ of DMCM for clonic convulsions obtained in our study using outbred male Wistar rats is similar to that reported in the literature obtained in a Wistar line/strain selectively bred for the occurrence of spontaneous absence-like seizures but never showing any spontaneous motor convulsion (Vergnes et al., 2001).

The data obtained in our experiments showed that HRT and LTR subgroups did not differ in the elevated plus-maze and open field tests when they were selected through a single injection of a $CD_{50}$ of DMCM. Although the number of rats

Table 6
Selection of subgroups of more (MF) or less (LF) fearful/anxious rats in the elevated plus-maze

| Subgroup | Latency | Sectors Crossed OA | TOA | OAE | TCA | CAE |
|---|---|---|---|---|---|---|
| MF | 172.6±21.9 | 1.4±0.3 | 4.7±1.0 | 0.8±0.2 | 270.0±2.4 | 6.5±0.6 |
| LF | 12.3±2.6* | 34.0±1.8* | 85.4±3.6* | 9.2±0.5* | 174.3±6.3* | 8.9±0.3* |

The values are expressed as the means±S.E.M. $N=35$/MF subgroup and $N=31$/LF subgroup. Latency=time (seconds) for the first open arm entry; OA=open arms; OAE=open arms entries; TOA=time (seconds) spent in the open arms; CAE=closed arms entries; TCA=time (seconds) spent in the closed arms.
* $P<0.0001$.

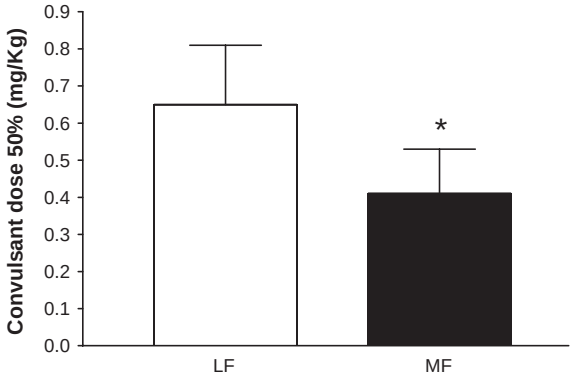


Fig. 3. The convulsant doses 50% ($CD_{50}$ ± 19/20 confidence limits) for clonic convulsions induced by DMCM (i.p.) in subgroups of less (LF) and more (MF) fearful/anxious rats selected in the elevated plus-maze test. *$P$=0.05, unpaired Student's $t$-test.

showing full clonic convulsions after the two injections of DMCM did not differ (see Results), in this procedure it was observed that some rats presented full clonic convulsions only after the first and other rats only after the second injection of DMCM. Contrary to the selection by a single injection of DMCM, the selection of HTR and LTR subgroups of rats through two injections of DMCM selected individuals whose threshold to clonic convulsions did not vary.

The subgroups selected through two injections of DMCM also showed differences in the rearing number in the open field, the LTR subgroup reared more than the HTR subgroup. Rearing has been considered an exploratory behavior triggered by the novelty of an unexplored environment (Crusio, 2001). The higher rearing in the LTR subgroup may indicate a higher brain stimulation caused by the stimuli present in the new environment. This supposed higher sensitivity of LTR subgroup to environmental stimuli might be in agreement with their lower threshold to clonic convulsions induced by DMCM. Data in literature seems to support our assumption since it was shown that rats kindled to the dorsal hippocampus presented a higher number of rearings in the open field test (Hannesson et al., 2001).

Clonic convulsions have been shown to be generated in the forebrain (Gale, 1988) and forebrain regions, such as the limbic structures amygdala and hippocampus, are sensitive to clonic convulsions induced electrically (Adamec and Morgan, 1994; Hannesson et al., 2001; Helfer et al., 1996; Kalynchuk et al., 1997, 1998; Nieminen et al., 1992; Racine, 1972; Sanberg and Ossenkopp, 1978; Wintink et al., 2003) or by a direct delivery of a convulsant drug into the brain structure (Sierra-Paredes and Sierra-Marcuño, 1996a,b). These limbic brain regions are clearly involved in emotional behavior and their susceptibility to develop clonic motor convulsion upon stimulation indicates that they are also part of the brain circuits involved in the generation/triggering of clonic convulsions. Benzodiazepine agonists were shown to increase the time spent in the open arms of the elevated plus-maze (Grahn et al., 1995) and inhibit convulsions (De Sarro et al., 1996), whereas DMCM decreases the time spent in the open arms when injected in an anxiogenic/subconvulsant dose (Cole et al., 1995) and induces convulsions (Braestrup et al., 1982). This dual effect of these drugs together with our present data may favor the earlier assumption of an association between susceptibility to clonic convulsions and anxiety/fear (Chapouthier and Venault, 2001; Clément et al., 1997; Pellow et al., 1985) and may also indicate that the common brain circuit controlled by $GABA_A$/benzodiazepine allosteric receptor is different between the HTR and LTR subgroups.

DMCM was shown to bind to $GABA_A$/benzodiazepine receptors in the rat brain with high affinity and these binding sites showed a heterogeneous distribution throughout different brain structures, higher specific binding was observed in the frontal cortex and hippocampus (Braestrup et al., 1983). Benzodiazepine agonists, antagonists and inverse agonists bind to the interface of alpha/gamma subunits of the $GABA_A$ receptor (Mehta and Ticku, 1999). DMCM has been shown to bind with different affinities to $GABA_A$ receptors containing different alpha subunits, the order of affinity is alpha1 > alpha2 = alpha3 > alpha5 > alpha6 (Luddens and Wisden, 1991). $GABA_A$ receptors containing the alpha 1 or 2 subunits have been shown to be involved in seizure protection and those containing the alpha2, alpha3 or alpha5 subunits in the anxiolysis (Rudolph et al., 1999). Therefore, one possible explanation for the differences between HRT and LRT subgroups of rats could be a difference in the assembly of $GABA_A$/benzodiazepine receptors containing alpha subunits in those structures generating/controlling the expression of convulsions and fear/anxiety.

A difference between HTR and LTR subgroups in the pharmacokinetics of DMCM could be responsible for the difference in the susceptibility to the clonic convulsions induced by the $CD_{50}$ of the drug. However, this possibility seems to be unlikely because it does not explain the differences in behavior, which is dependent on the brain functioning, observed 20 days after the second dose of the drug. Moreover, at the time of behavioral testing the rats were drug-free.

There is a paucity of data regarding a possible relation between susceptibility to clonic convulsions and the behavior in the forced swimming test. The available data is still controversial; for instance, rats chemically kindled with pentylenetetrazol or picrotoxin did not differ in the immobility time in the forced swimming test (Cannizzaro et al., 1993); however, rats electrically kindled in the amygdala showed decreased immobility (Wintink et al., 2003) or no changes (Helfer et al., 1996), and increased immobility was observed in rats partially kindled through corneal stimulation (Sattin et al., 1994). Ho et al. (2002) submitted two subgroups of male Wistar rats to the forced swimming test, selected in the elevated plus-maze with either low or high fear/anxiety according to the time spent in the open arms. The results obtained in their experiment did not show a significant difference in the immobility time between the subgroups. Our data showed that HTR and LTR subgroups in the two experiments (selection through one or two administrations of DMCM) showed the typical differences expected in the forced swimming test, such as increased immobility and decreased struggling in the test/retest days; however, there were no statistically significant

differences between the HTR and LRT subgroups in the two experiments. Therefore, considering that the forced swimming test has been used as a model of depression (Lahmame and Armario, 1996; Porsolt et al., 1978), the data obtained in our study indicates that differences in the threshold to clonic convulsions induced by DMCM does not influence the "depressive" behavior induced by the forced swimming test.

In conclusion, the data obtained in the present work shows that rats presenting a lower threshold to clonic convulsions induced by DMCM, a benzodiazepine inverse agonist, showed an increased fear/anxiety when exposed to the elevated plus-maze and the open field. On the other hand, rats more fearful/anxious in the elevated plus-maze are more susceptible to clonic convulsions induced by DMCM. This relation between clonic convulsions and fear/anxiety may be due to differences in the assembly of $GABA_A$/allosteric benzodiazepine site receptor subunits in the brains of the individuals. The higher rearing number in the open field observed in the LTR subgroup suggests a relation between exploratory behavior and clonic convulsions. Our data indicate that a difference in the threshold to clonic convulsions involving the allosteric benzodiazepine site is not involved in the behavior measured in the forced swimming test.

## Acknowledgements

The authors express their sincere gratitude to Marcela I.F. Venditti and Glen Norman Young for their help in the preparation of this manuscript. We also thank Ricardo Marques for his technical assistance. This research was supported by Associação Fundo de Incentivo à Psicofarmacologia (AFIP). M.B. Contó was the recipient of a fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

## References

Adamec RE, Morgan HD. The effect of kindling of different nuclei in the left and right amygdala on anxiety in the rat. Physiol Behav 1994;55:1–12.

Angrini M, Leslie JC, Shephard RA. Effects of propranolol, buspirone, pCPA, reserpine, and chlordiazepoxide on open-field behavior. Pharmacol Biochem Behav 1998;59:387–97.

Armario A, Martí AGO. Forced swimming test in rats:effects od desipramine administration and the period of exposure to the tes on struggling behavior, swimming, immobility and defecation rate. Eur J Pharmacol 1988;158:207–12.

Belzung C, Griebel G. Measuring normal and pathological anxiety-like behaviour in mice: a review. Behav Brain Res 2001;125:141–9.

Bradford HF, Belinda JC, Hillmann M, Seidelmann D, Klewer M, Jones GH. Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. Psychopharmacology 1995;121:118–26.

Braestrup C, Schmiechen R, Neef G, Nielsen M, Petersen EN. Interaction of convulsive ligands with benzodiazepine receptors. Science 1982;216:1241–3.

Braestrup C, Nielsen M, Honoré T. Binding of [$^3$H]DMCM, a convulsive benzodiazepine ligand, to rat brain membranes: preliminary studies. J Neurochem 1983;41:454–65.

Cannizzaro G, Flugy A, Cannizzaro C, Gagliano M, Sabatino M. Effects of desipramine and alprazolam in the forced swim test in rats after long-lasting termination of chronic exposure to picrotoxin and pentylenetetrazol. Eur Neuropsychopharmacol 1993;3:477–84.

Chapouthier G, Venault P. A pharmacological link between epilepsy and anxiety? TIPS 2001;22:491–3.

Chebib M, Johnston GAR. The 'ABC' of GABA receptors: a brief review. Clin Exp Pharmacol Physiol 1999;26:937–40.

Clément Y, Bondoux D, Launay JM, Chapouthier G. Convulsive effects of a benzodiazepine receptor inverse agonist: are they related to anxiogenic processes? J Physiol (Paris) 1997;91:21–9.

Cole JC, Hillmann M, Seidelmann D, Klewer M, Jones GH. Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. Psychopharmacology 1995;121:118–26.

Crusio WE. Genetic dissection of mouse exploratory behaviour. Behav Brain Res 2001;125:127–32.

De Sarro G, Chimirri A, Zappala M, Guisti P, Lipartiti M, De Sarro A. Azirinol [1,2-*d*][1,4]benzodiazepine derivates and related 1,4-benzodiazepines as anticonvulsant agents in DBA/2 mice. Gen Pharmacol 1996;27:1155–62.

Eidman DS, Benedito MAC, Leite JR. Daily changes in pentylenetetrazol-induced convulsions and open-field behavior in rats. Physiol Behav 1990;47:853–6.

Enna SJ, Bowery NG. $GABA_B$ receptor alterations as indicators of physiological and pharmacological function. Biochem Pharmacol 2004;68:1541–8.

Gale K. Progression and generalization of seizure discharge: anatomical and neurochemical substrates. Epilepsia 1988;29:S15–34.

Grahn RE, Kalman BA, Brennan FX, Watkins LR, Maier SF. The elevated plus-maze is not sensitive to the effect of stressor controllability in rats. Pharmacol Biochem Behav 1995;52:565–70.

Greengard P. The neurobiology of slow synaptic transmission. Science 2001;294:1024–30.

Hannesson DK, Howland J, Pollock M, Mohapel P, Wallace AE, Corcoran ME. Dorsal hippocampal kindling produces a selective and enduring disruption of hippocampally mediated behavior. J Neurosci 2001;21:4443–50.

Hawkins J. Inbreeding considerations in a REM sleep model for rat swimming activity. Experientia 1986;42:134–6.

Helfer V, Deransart C, Marescaux C, Depaulis A. Amygdala kindling in the rat: anxiogenic-like consequences. Neuroscience 1996;73:971–8.

Ho YJ, Eichendorff J, Schwarting RKW. Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behav Brain Res 2002;136:1–12.

Johnston GAR. $GABA_A$ receptor pharmacology. Pharmacol Ther 1996;69:173–98.

Kabbaj M, Akil H. Individual differences in novelty-seeking behavior in rats: a c-fos study. Neuroscience 2001;106:535–45.

Kalynchuk LE, Pinel JPJ, Treit D, Kippin TE. Changes in emotional behavior produced by long-term amygdale kindling in rats. Biol Psychiatry 1997;41:438–51.

Kalynchuk LE, Pinel JPJ, Treit D. Long-term kindling and interictal emotionality in rats: effect of stimulation site. Brain Res 1998;779:149–57.

Kwan P, Sills GJ, Brodie MJ. The mechanisms of action of commonly used antiepileptic drug. Pharmacol Ther 2001;90:21–34.

Lahmame A, Armario A. Differential responsiveness of inbred strains of rats to antidepressants in the forced swimming test: are Wistar Kyoto rats an animal model of subsensitivity to antidepressants? Psychopharmacology 1996;123:191–8.

Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;96:99–113.

Luddens H, Wisden W. Function and pharmacology of multiple $GABA_A$ receptor subunits. TIPS 1991;12:49–51.

Marsh L, Rao V. Psychiatric complications in patients with epilepsy: a review. Epilepsy Res 2002;49:11–33.

Marti J, Armario A. Effects of diazepam and desipramine in the forced swimming test: influence of previous experience with the situation. Eur J Pharmacol 1993;236:295–9.

Mehta AK, Ticku MK. An update on $GABA_A$ receptors. Brain Res Rev 1999;29:196–217.

Nazar M, Siemiatkowski M, Czlonkowska A, Sienkiewiz-Jarosz H, Plasnik A. The role of the hippocampus and 5-HT/GABA interaction in the central effects of benzodiazepine receptor ligands. J Neural Transm 1999;106:369–81.

Nieminen SA, Sirviö J, Teittinen K, Pitkanen A, Airaksinen MM, Riekkinen P. Amygdala kindling increased fear-response, but did not impair spatial memory in rats. Physiol Behav 1992;51:845–9.

Paredes RG, ?gmo A. GABA and behavior: the role of receptors subtypes. Neurosci Biobehav Rev 1992;16:145–70.

Pawlak CR, Schwarting RKW. Object preference and nicotine consumption in rats with high vs low rearing activity in a novel open field. Pharmacol Biochem Behav 2002;73:679–87.

Pellow S. Can drug effects on anxiety and convulsions be separated. Neurosci Biobehav Rev 1985;9:55–73.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47: 379–91.

Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3–33.

Racine RJ. Modification of seizure activity by electrical stimulation: Motor seizure. Electroenceph Clin Neurophysiol 1972;32:281–94.

Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acid A receptor subtypes. Nature 1999;401:796–800.

Sanberg PR, Ossenkopp K. Kindling rates in Wistar rats: an analysis of individual differences. Physiol Behav 1978;20:205–7.

Sattin A, Pekary AE, Lloyd RL. TRH gene products are implicated in the antidepressant mechanisms of seizures. Ann N Y Acad Sci 1994;739: 135–53.

Schweri MM, Martin JV, Mendelson WB, Barrett JE, Paul SM, Skolnick P. Pharmacokinetic and pharmacodynamic factors contributing to the convulsant action of beta-carboline-3-carboxylic acid esters. Life Sci 1983;33: 1505–10.

Seale TW, Abla KA, Roderick TH, Rennert OM, Carney JM. Different genes specify hyporesponsiveness to seizures induced by caffeine and the benzodiazepine inverse agonist, DMCM. Pharmacol Biochem Behav 1987;27:451–6.

Shiah I, Yatham LN. GABA function in mood disorders: an update and critical review. Life Sci 1998;63:1289–303.

Sierra-Paredes G, Sierra-Marcuño G. Effects of NMDA antagonists on seizure thresholds induced by intrahippocampal microdialysis of picrotoxin in freely moving rats. Neurosci Lett 1996;218:62–6.

Sierra-Paredes G, Sierra-Marcuño G. Microperfusion of picrotoxin in the hippocampus of chronic freely moving rats through microdialysis probes: a new method of induce partial and secondary generalized seizures. J Neurosci Methods 1996;67:113–20.

Vergnes M, Boehrer A, He X, Greney H, Dontenwill M, Cook J, et al. Differential sensitivity to inverse agonists of $GABA_A$/benzodiazepine receptors in rats with genetic absence–epilepsy. Epilepsy Res 2001;47: 43–53.

Wintink AJ, Young NA, Davis AC, Gregus A, Kalynchuk LE. Kindling-induced emotional behavior in male and female rats. Behav Neurosci 2003; 117:632–40.