Psicothema 2006. Vol. 18, nº 3, pp. 514-518
www.psicothema.com

ISSN 0214 - 9915 CODEN PSOTEG

Amitriptyline administered after consolidation of inhibitory avoidance does not affect memory retrieval

Andrés Parra, Estrella Everss, M. Carmen Arenas, Concepción Vinader-Caerols and Santiago Monleón
Universidad de Valencia

In Experiment 1, the effect of the administration of the antidepressant amitriptyline (30 mg/kg) for 21 days on the acquisition and consolidation of the inhibitory avoidance task was studied in male and female mice. In Experiment 2, it was evaluated whether amitriptyline administered after the consolidation of this task would block the memory retrieval. Anxiety and spontaneous activity in the elevated plus maze were also assessed. When amitriptyline was given before the training phase of inhibitory avoidance it blocked learning in males and there was a tendency in the same direction in females. However, the drug administered between training and test phases did not affect conditioning. These effects of amitriptyline seem to be independent of its actions on anxiety and locomotor activity. It may be that the effects observed are related to the therapeutic effects of antidepressants.

La amitriptilina administrada después de la consolidación de la evitación inhibitoria no afecta a la recuperación de la memoria. Se estudió el efecto de la administración, durante 21 días, del antidepresivo amitriptilina (30 mg/kg) sobre la adquisición y consolidación de la memoria de una tarea de evitación inhibitoria en ratones machos y hembras (Experimento 1). También se estudió si la amitriptilina, administrada una vez finalizado el período de consolidación, interfería con su recuperación (Experimento 2). En los animales de este experimento se evaluaron la ansiedad y la actividad espontánea en el laberinto en cruz elevado. La amitriptilina, cuando se administró antes de la adquisición, impidió el aprendizaje en los machos y mostró una tendencia en el mismo sentido en las hembras; cuando se administró entre la adquisición y la retención no produjo deterioro. Estos efectos parecen independientes de una acción inespecífica del fármaco sobre la ansiedad o la actividad. Se sugiere que los efectos observados pueden tener relación con los efectos terapéuticos de los antidepresivos.

Antidepressants are prescribed not only for depression but also for a wide range of mental disorders (Baldessarini, 2001). Although they have clinical advantages, the current armamentarium of antidepressants presents an unacceptable lack of efficacy (Gumnick and Nemeroff, 2000). An important limitation in designing better antidepressants is that the mechanism of action responsible for their therapeutic effect is unknown. Although the pharmacodynamics of these drugs at molecular, cellular and system level has been investigated (e.g., Palucha and Pilc, 2002; Shelton, 2000; Shilling and Kelsoe, 2002), at cognitive level studies are less common. The purpose of the present study, and others carried out or being prepared in our laboratory, is to evaluate the effects of amitriptyline on animal cognition to relate them to some characteristics of the therapeutic effects of this and other antidepressants.

It has been suggested that the forced swimming test (FST) shares some characteristics with memory tests, such as the exploration of objects (Ennaceur and Delacour, 1988), and the exploratory behavior in a photo-cell activity cage (Platel and Porsolt, 1982), which are used to determine the effect of drugs on memory.

In this context, it is understood that in the first session of FST the animal should learn to remain immobile, and the second session would be a test of retention of what was learned in the first one (de Pablo, Parra, Segovia and Guillamón, 1989; Martos, Vinader-Caerols, Monleón, Arenas and Parra, 1999; Parra, Vinader-Caerols, Monleón and Simón, 1999).

Since antidepressants deteriorate the execution of FST (i.e., animals swim more than controls in the second session, see Porsolt, Le Pichon and Jalfre, 1977) the following question is raised, do antidepressants deteriorate memory? Animal studies using tests well established in the literature as memory tests could help answer this question. The step-through inhibitory (passive) avoidance, chosen to carry out the present experiments, has been used for decades to test the pharmacological effects of drugs on memory (Gold, 1986). In this task, the animal has to inhibit the crossing to the dark compartment to avoid a footshock (Bureš, Burešová and Huston, 1983).

Previous studies have shown that the acute administration of amitriptyline, a mixed serotoninergic and noradrenergic uptake inhibitor with strong anticholinergic and antihistaminergic effects (Richelson, 2003), produces a memory deficit of inhibitory avoidance, apparently not related to its anxiolytic and locomotor effects (Arenas, Vinader-Caerols, Monleón, Martos, Everss,

Fecha recepción: 22-6-05 • Fecha aceptación: 15-11-05
Correspondencia: Andrés Parra
Facultad de Psicología
Universidad de Valencia
46010 Valencia (Spain)
E-mail: andres.parra@uv.es

Ferrer-Añó and Parra, 2006; Parra, Everss, Monleón, Vinader-Caerols and Arenas, 2002). When amitriptyline is chronically administered, this deficit is also observed. Piracetam, a nootropic clinically used in Europe, counteracts the effects of both acute and chronic administration of amitriptyline, although in the latter case only in male mice (Everss, Arenas, Vinader-Caerols, Monleón and Parra, 2005; Parra et al, 2002).

In the present paper, we studied whether the chronic administration of amitriptyline after the consolidation process of inhibitory avoidance had taken place produces a deficit in memory. With this purpose, two independent experiments were carried out, the drug treatment being before the training phase in the one, and 24 hours after this phase in the other. This period of 24 hours is more than sufficient to permit the consolidation of what was learned in the training phase, a process that is considered to need 3 to 4 hours in rats (Izquierdo and Medina, 1997). The long standing use of this drug (it has been in clinical use since the early 1960's) is irrelevant because none of the newer antidepressants have better antidepressant effects, although many have fewer side effects (Barbui and Hotopf, 2001).

Materials and methods

Animals

The experimental subjects were 51 male and 47 female CD1 mice (CRIFFA Leon France) of 42 days of age when arriving at the laboratory. Animals of the same sex were grouped four by four in standard plastic cages in a temperature-controlled room (21 ± 2 ºC) with lights off 07:30-19:30. Food and water were available *ad libitum*. The mice were marked on their backs with indelible ink (Gonzalo Zaragoza, S.L., Callosa de Segura, Alicante, Spain) for individual identification. Experiments were always carried out during the dark phase. Experimental treatment and animal care were always in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

Amitriptyline hydrochloride (Sigma-Aldrich Química, Madrid, Spain) was used diluted in saline solution (0.9 % NaCl). The mice received vehicle or amitriptyline (30 mg/kg) in a volume of 0.01 ml/g body weight. The dose was chosen on the base of its clear effects found in previous studies, both with acute and chronic administration (Arenas et al, 2006; Everss et al, 2005; Parra et al, 2002). This dose of amitriptyline is rather high, nevertheless it can be found in the literature (e.g., Abe, Tabata, Saito, Matsuda, Baba and Egawa, 1996), and its equivalent dose for humans (Food and Drug Administration, 2005) is within the range of normal clinical use (Baldessarini, 2001).

Apparatus

An inhibitory avoidance box for mice (Ugo Basile, Comerio-Varese, Italy) was used in both experiments. The cage, made of Perspex sheets, was divided into two sections (both 15 × 9.5 × 16.5 cm^3) separated by an automatic sliding door. There was a light (24 V, 10 W) in the ceiling of the starting side which was painted in white (light intensity of 290 lx at floor level, measured with the Panlux Electronic2 photometer of GOSSEN, Nürnberg, Germany), whereas the other side was black and always remained dark. The floor was made of 48 stainless steel bars of 0.7 mm in diameter and 8 mm apart.

The elevated plus-maze used in Experiment 2 (Cibertec, S.A., Madrid, Spain) was made up of two open and two closed arms (30 × 5 cm^2 and 30 × 15 × 5 cm^3, respectively) extending from a common central square (5 x 5 cm^2) and elevated 50 cm above floor level on five pedestals. The maze floor was made of black Plexiglas; the open arms had no protective edge while the walls of the closed arms were made from clear Plexiglas with the external sides covered with black paper. The illumination in the experimental room consisted of four neon tubes fixed to the ceiling (light intensity of 110 lx at 50 cm above floor level). The elevated plus-maze task was recorded with a video camera (SONY Handycam CCD-TR401E, Tokyo, Japan).

Experimental Procedures

In Experiment 1, mice were randomly assigned by sex to one of two groups (N= 12 - 14), which received saline solution (S) or 30 mg/kg amitriptyline (A) for 21 days. The inhibitory avoidance task was carried out 24 hours after the last injection. Each mouse was individually introduced into the illuminated side of the avoidance box and permitted to explore it for an adaptation period of 90 seconds. The door between the compartments remained closed during this period. The door was then removed and the mouse could stay in the light side for a maximum of 300 seconds. If it did not enter the dark compartment in this time it was discarded, but if it entered, an inescapable footshock of 0.7mA was delivered for 5 seconds and it was immediately returned to its home cage. The inhibitory avoidance test was carried out 24 hours later, using the same procedure except that no shock was delivered. In both sessions, the latencies of crossing were measured in 1/10 seconds. The measure of the inhibitory avoidance was obtained by comparing the performance in the test session with that of the training session.

In Experiment 2, animals were randomly assigned by sex to one of two groups (saline, N= 13 - 16; amitriptyline, N= 8 - 10) using the same denominations as in Experiment 1. They were also subjected to the same treatments as in the previous experiment except that the period between sessions was 22 days and the drug administration began 24 hours after the training session and finished 24 hours before the test session. Immediately after this last phase, the exploration of each animal in the elevated plus-maze was recorded for 5 minutes. Subsequently, the number of entries onto open and closed arms (arm entry is defined as all four paws entering an arm) was scored by a trained observer unaware of the treatment applied. This provided two independent measures of anxiety, i.e. the percentage of time spent in the open arms, and the percentage of open arm entries [(open/open + closed) X 100], as well as a measure of activity, i.e. the number of closed arm entries. The rationale to select these measures is found in File (2001), Lister (1987) and Rodgers and Johnson (1995).

Data analysis

As data from the inhibitory avoidance task did not fulfill the criteria for normality and homogeneity, they were subjected to non-parametric Kruskal-Wallis analysis followed by Mann-Whitney U-tests. The Wilcoxon matched pairs test was carried out to compare training versus test latencies in each group. Mann-Whitney U-tests were used to compare the performance of independent groups. The elevated plus-maze behaviors were

analyzed separately with ANOVA, with Sex and Treatment as factors, and Newman-Keuls-tests were used for *post-hoc* analyses. All analyses were performed with the Statistica software package, version 5.5 for Windows (StatSoft, Inc., 2000).

Results

Experiment 1: Chronic pre-training treatment

Saline males showed inhibitory avoidance, i.e. test latencies higher than training latencies (T= 4.00; p<0.01), but this was not found in the males treated with amitriptyline (T= 39.00; p>0.05). Both saline and amitriptyline-treated females presented inhibitory avoidance (T= 5.00; p<0.01; and T= 10.00; p<0.03, respectively). The treatment did not produce significant differences in the performance of animals, although a tendency in the performance of females in the test session was observed, where the drug-treated mice showed shorter latencies than controls (U= 41.00; p= 0.07). Neither were significant differences found when comparing males and females of the same drug condition (see figure 1).

Experiment 2: Chronic post-training treatment

All groups increased their test latencies in comparison with their training latencies: males S (T= 1.00; p<0.001), males A (T= 1.00; p<0.02), females S (T= 6.00; p<0.01), and females A (T= 2.00; p<0.01). No comparison with Treatment or Sex involved was statistically significant (see figure 2).

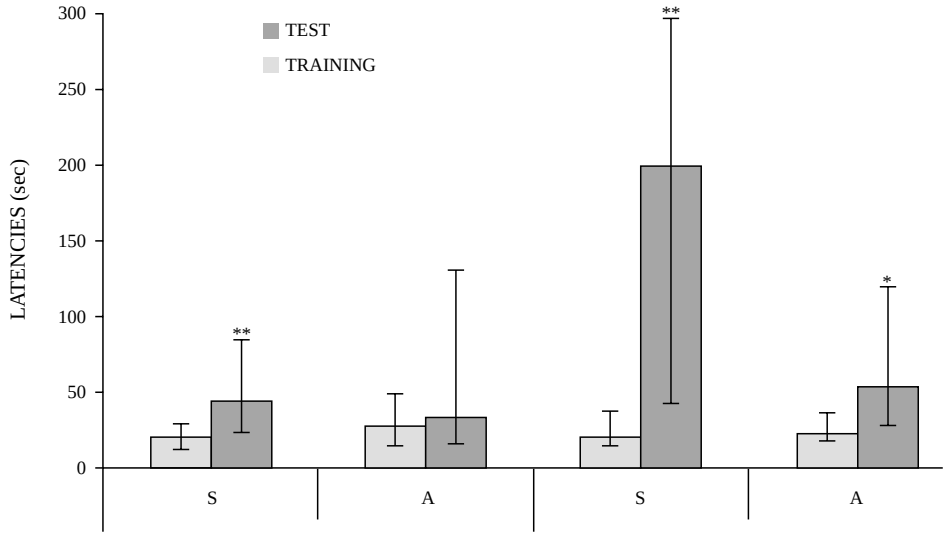

Figure 1. *Effects of chronic administration of amitriptyline (30 mg/kg) before the training phase of an inhibitory avoidance task. Values are expressed as medians (± interquartile range). S: saline, A: amitriptyline. *p<0.03, **p<0.01 vs. training*

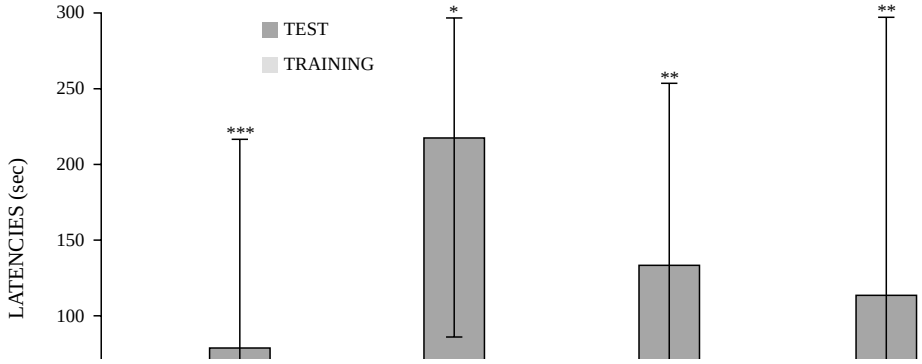

Figure 2. *Effects of chronic administration of amitriptyline (30 mg/kg) between training and test phases of an inhibitory avoidance task. Values are expressed as medians (± interquartile range). S: saline, A: amitriptyline. *p<0.02, **p<0.01, ***p<0.001 vs. training*

In the elevated plus-maze, all female groups remained a lower percentage of time than males on the open arms [$F(1, 42)= 5.37$; $p<0.02$], and neither the factor Treatment nor Sex X Treatment interaction were statistically significant [$F(1, 42)= 0.26$; $p>0.05$, and $F(1, 42)= 1.68$; $p>0.05$, respectively]. The females showed a tendency to enter the open arms less than the males [$F(1, 42)= 3.60$; $p= 0.06$], and again neither the factor Treatment nor Sex X Treatment interaction were statistically significant [$F(1, 42)= 0.27$; $p>0.05$]. When the number of entries in the closed arms were analyzed, factors did not present statistically significant differences either [Sex: $F(1, 42)= 0.13$; $p>0.05$; Treatment: $F(1, 42)= 0.42$; $p>0.05$; and Sex X Treatment: $F(1, 42)= 0.28$; $p>0.05$].

Discussion

In Experiment 1, chronic administration of amitriptyline before the training phase blocked the learning of inhibitory avoidance in the male mice but did not have such an effect on the females, in which only a statistical tendency in the reduction of latency was observed in the test phase but without blocking learning. These results are in agreement with those obtained by Everss et al (2005), except with regards the females, which in this case also presented clear effects of amitriptyline. In Experiment 2, in which the pharmacological treatment was administered once the phase of consolidation of the memory was over (Izquierdo and Medina, 1997), no effect was observed either in males or females. This difference in results between Experiments 1 and 2 constitutes the main finding of the present study, i.e. the effect of amitriptyline on inhibitory avoidance seems to be related to the processes of acquisition and consolidation of memory (the procedure used here does not permit the distinction between these two moments in the memory formation) and does not affect the recuperation if treatment begins once the consolidation has ended. This lack of effect on retention is similar to that found, in the same behavioral context, with the antidepressant fluoxetine, a selective inhibitor of serotonin reuptake (Monleón, Urquiza, Arenas, Vinader-Caerols and Parra, 2002).

It is precisely this inhibition of the reuptake of serotonin, resulting in an increase in serotonin in the synapse, which is the mechanism of action that is shared by amitriptyline and fluoxetine. This action could be physiologically responsible for the behavioral effect of the drug observed in Experiment 1. Data which other authors have found with microinfusions of substances (agonists, antagonists or neurotransmitters) in the hippocampus and in structures closely related to it support the idea that serotonin interferes with the formation of the long term memory of inhibitory avoidance (Izquierdo and Medina, 1997; Izquierdo et al, 1998). The other mechanisms of action of the non selective drug, amitriptyline, are not the same or at least not to a degree that is thought important as those of fluoxetine, which is considered to be selective. However, in the present stage of our investigation, it cannot be ruled out that other mechanisms of action of amitriptyline may be implicated in the effects observed, especially its anticholinergic and antihistaminergic actions. There are many references dealing with the impairing effect of anticholinergic drugs on memory (e.g., Gold, 2003), and to the modulating role of histamine, which enhances or worsens the memory depending on factors like the type of task or the brain region implicated (Blandina, Efoudebe, Cenni, Mannaioni and Passani, 2004).

The effect of amitriptyline on inhibitory avoidance is more pronounced in males than in females. In our laboratory, it has repeatedly been found that a drug has a behavioral effect on males but not on females, or if an effect is observed it is less pronounced. This observation has been found in experiments using different drugs and different behavioral tests (Everss et al, 2005; Monleón et al, 2002; Parra et al, 1999; Vinader-Caerols, Ferrer-Añó, Arenas, Monleón and Parra, 2002). Given the variety of neurotransmitters and brain structures involved, it seems simpler to think of a common peripheral reason to explain the differences. This reason could be based on the existence of sex differences in mice in the hepatic enzymes which metabolize the drugs, specifically, the enzymatic activity is 40-100% higher in females than in males (Shapiro, Agrawal and Pampori, 1995), which would give rise to a poorer availability of the drug in the central nervous system of the females in comparison to the males. Whatever the case, our results emphasize the importance of including females in animal studies, as well as keeping in mind the factor of gender when personalizing clinical pharmacological treatment, especially since in humans, gender differences have also been described in the pharmacokinetics of many psychoactive drugs, among them antidepressants (Frackiewicz, Sramek and Cutler, 2000). However, when extrapolating results obtained in mice to humans, not only the sex differences must be taken into account but also the pharmacokinetic differences between species (Lin, 1995).

Sex differences in the behavioral effect of drugs must be analyzed taking into account that there may be a difference in the behavior of control animals, as is the case sometimes found in inhibitory avoidance (e.g., Monleón et al, 2002), and continuously in the Morris water maze (e.g., Cimadevilla, Conejo, Miranda and Arias, 2004; Vinader-Caerols et al, 2002), although in the present study there were no sex differences in control animals.

There was no drug effect on performance in the plus maze, which leads one to believe that after 21 days of administration of amitriptyline, its effects on learning observed in Experiment 1 are not influenced by its effects on anxiety or spontaneous locomotor activity. Parra et al (2002) found that there were no acute effects of amitriptyline on anxiety but there was a dose-dependant reduction in activity. In the present study, the absence of effects on activity could be explained by the possible tolerance developed after chronic administration. Furthermore, the current results replicate those obtained with the same drug and dose, as well as the same apparatus, by Everss et al (2005).

The absence of tolerance in the effect of amitriptyline on inhibitory avoidance is interesting in the search for the mechanism of therapeutic action of antidepressants. This action does not present tolerance, while the side effects tend to disappear or diminish their intensity (Baldessarini, 2001). The animal models of the action of antidepressants in which tolerance is observed should be avoided.

In summary, chronic administration of amitriptyline has a deteriorating effect on inhibitory avoidance in male mice, while the effect is slighter in females. Amitriptyline exerts its effect when administered before the acquisition and consolidation of the memory but not when these have concluded. It does not present tolerance and it seems to be independent of the unspecific effects on anxiety and activity.

Acknowledgements

The authors wish to thank Arancha Ferrer Añó for her help in the experiments. The investigation has been carried out with the financial support of (a) the «Generalitat Valenciana», Valencia,

Spain (Ayuda: GV97-SH-22-89) and (b) jointly, el Ministerio de Educación y Ciencia (Spain), and the European Union, through the funds FEDER (Ayuda: BSO2003-07163). Estrella Everss received a grant from «Generalitat Valenciana» from 1999 to 2003. We also wish to thank Ms Miriam Phillips for the English translation of the manuscript.

References

Abe, M., Tabata, R., Saito, K., Matsuda, T., Baba, A. and Egawa, M. (1996). Novel benzodioxan derivative, 5-[3-[((2S)-1,4-benzodioxan-2-ylmethyl) amino]propoxy]-1,3-benzodioxole HCl (MKC-242), with anxiolytic-like and antidepressant-like effects in animal models. *The Journal of Pharmacology and Experimental Therapeutics, 278*, 898-905.

Arenas, M.C., Vinader-Caerols, C., Monleón, S., Martos, A.J., Everss, E., Ferrer-Añó, A. and Parra, A. (2006). Are the effects of the antidepressants amitriptyline, maprotiline and fluoxetine on inhibitory avoidance state-dependent? *Behavioural Brain Research, 166*, 150-158.

Baldessarini, R.J. (2001). Drugs and the treatment of psychiatric disorders: depression and anxiety disorders. In J.G. Hardman, L.E. Limbird and A.G. Gilman (comps.): *Goodman & Gilman's The pharmacological basis of therapeutics* (pp. 447-483). Nueva York: McGraw-Hill.

Barbui, C. and Hotopf, M. (2001). Amitriptyline v. the rest: still the leading antidepressant after 40 years of randomised controlled trials. *British Journal of Psychiatry, 178*, 129-144.

Blandina, P., Efoudebe, M., Cenni, G., Mannaioni, P. and Passani, M.B. (2004). Acetylcholine, histamine and cognition: two sides of the same coin. *Learning and Memory, 11*, 1-8.

Bureš, J., Burešová, O. and Huston, J.P. (1983). *Techniques and basic experiments for the study of brain and behaviour*. Amsterdam: Elsevier.

Cimadevilla, J.M., Conejo, N.M., Miranda, R. and Arias, J.L. (2004). Sex differences in the Morris water maze in young rats: temporal dimensions. *Psicothema, 16*, 611-614.

de Pablo, J.M., Parra, A., Segovia, S. and Guillamón, A. (1989). Learned immobility explains the behaviour of rats in the forced swimming test. *Physiology and Behavior, 46*, 229-237.

Ennaceur, A. and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. I: Behavioral data. *Behavioural Brain Research, 31*, 47-59.

Everss, E., Arenas, M.C., Vinader-Caerols, C., Monleón, S. and Parra, A. (2005). Piracetam counteracts the effects of amitriptyline on inhibitory avoidance in CD1 mice. *Behavioural Brain Research, 159*, 235-242.

File, S.E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioural Brain Research, 125*, 151-157.

Food and Drug Administration. Guidance for industry and reviewers. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. Available at: http://www.fda.gov/cber/gdlns/dose.htm. Accessed September 06, 2005.

Frackiewicz, E.J., Sramek, J.J. and Cutler, N.R. (2000). Gender differences in depression and antidepressant pharmacokinetics and adverse events. *The Annals of Pharmacotherapy, 34*, 80-88.

Gold, P.E. (1986). The use of avoidance training in studies of modulation of memory storage. *Behavioral and Neural Biology, 46*, 87-98.

Gold, P.E. (2003). Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiology of Learning and Memory, 80*, 194-210.

Gumnick, J.F. and Nemeroff, C.B. (2000). Problems with currently available antidepressants. *Journal of Clinical Psychiatry, 61(Suppl 10)*, 5-15.

Izquierdo, I. and Medina, J.H. (1997). Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory, 68*, 285-316.

Izquierdo, I., Medina, J.H., Izquierdo, L.A., Barros, D.M., de Souza, M.M. and Mello e Souza, T. (1998). Short- and long-term memory are differentially regulated by monoaminergic systems in the rat brain. *Neurobiology of Learning and Memory, 69*, 219-224.

Lin, J.H. (1995). Species similarities and differences in pharmacokinetics. *Drug Metabolism and Disposition, 23*, 1.008-1.021.

Lister, R.G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology, 92*, 180-185.

Martos, A.J., Vinader-Caerols, C., Monleón, S., Arenas, M.C. and Parra, A. (1999). Efectos de la fisostigmina y de la nicotina sobre la inmovilidad aprendida en la prueba de natación forzada. *Psicothema, 11*, 631-639.

Monleón, S., Urquiza, A., Arenas, M.C., Vinader-Caerols, C. and Parra, A. (2002). Chronic administration of fluoxetine impairs inhibitory avoidance in male but not female mice. *Behavioural Brain Research, 136*, 483-488.

Palucha, A. and Pilc, A. (2002). On the role of metabotropic glutamate receptors in the mechanisms of action of antidepressants. *Polish Journal of Pharmacology, 54*, 581-586.

Parra, A., Everss, E., Monleón, S., Vinader-Caerols, C. and Arenas, M.C. (2002). Effects of acute amitriptyline administration on memory, anxiety and activity in male and female mice. *Neuroscience Research Communications, 31*, 135-144.

Parra, A., Vinader-Caerols, C., Monleón, S. and Simón, V.M. (1999). Learned immobility is also involved in the forced swimming test in mice. *Psicothema, 11*, 239-246.

Platel, A. and Porsolt, R.D. (1982). Habituation of exploratory activity in mice: a screening test for memory enhancing drugs. *Psychopharmacology, 78*, 346-352.

Porsolt, R.D., Le Pichon, M. and Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature, 266*, 730-732.

Richelson, E. (2003). Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. *Journal of Clinical Psychiatry, 64(Suppl 13)*, 5-12.

Rodgers, R.J. and Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neuroscience and Biobehavioral Reviews, 21*, 801-810.

Rodgers, R.J. and Johnson, N.J. (1995). Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacology Biochemistry and Behavior, 52*, 297-303.

Shapiro, B.H., Agrawal, A.K. and Pampori, N.A. (1995). Gender differences in drug metabolism regulated by growth hormone. *The International Journal of Biochemistry and Cell Biology, 27*, 9-20.

Shelton, R.C. (2000). Cellular mechanisms in the vulnerability to depression and response to antidepressants. *The Psychiatric Clinics of North America, 23*, 713-729.

Shilling, P.D. and Kelsoe, J.R. (2002). Functional genomics approaches to understanding brain disorders. *Pharmacogenomics, 3*, 31-45.

StatSoft, Inc. (2000). *Statistica for Windows* (Computer program manual). Tulsa, OK: StatSoft Inc.

Vinader-Caerols, C., Ferrer-Añó, A., Arenas, M.C., Monleón, S. and Parra, A. (2002). La maprotilina anula las diferencias entre ratones machos y hembras en el laberinto de agua de Morris. *Psicothema, 14*, 823-827.