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Research report

# Piracetam counteracts the effects of amitriptyline on inhibitory avoidance in CD1 mice

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**Abstract**

The purpose of the present work was to study the effects of amitriptyline on animal cognition in relation to some characteristics of its therapeutic effects. The modulation of acute and chronic effects of amitriptyline on inhibitory avoidance in male and female mice by piracetam was investigated. In Experiment 1, mice were subjected to the training phase of inhibitory avoidance conditioning 60 min after acute piracetam (100 mg/kg) or physiological saline administration. Immediately after the behavioural task, they received a single injection of the tricyclic antidepressant amitriptyline (30 mg/kg) or physiological saline. Twenty-four hours later, subjects were tested for avoidance. In Experiment 2, the same doses of amitriptyline and piracetam were chronically administered. Mice were subjected to the training phase of inhibitory avoidance on the 22nd day, and to the test phase 24 h later. Forty-five minutes after test, subjects explored the elevated plus-maze for 5 min in order to assess whether the effects of amitriptyline on avoidance performance may reflect general behavioural changes. Results obtained were that: (a) acute and chronic amitriptyline impaired inhibitory avoidance of male and female mice, (b) piracetam counteracted the effect of acutely administered amitriptyline on inhibitory avoidance, and (c) piracetam counteracted the effects of chronically administered amitriptyline in males but not females in the same learning task. These effects do not seem to be mediated by non-specific drug effects on spontaneous motor activity or anxiety.


*Keywords:* Amitriptyline; Piracetam; Inhibitory avoidance; Elevated plus-maze; Nootropics; Antidepressants; Sex differences; Mice

## 1. Introduction

Antidepressants are prescribed for a variety of mental disorders, in addition to depression [3]. They have clinical benefits; but the current armamentarium of antidepressants has an unacceptable lack of efficacy [20]. One of the limitations for designing better antidepressants is a consequence of the fact that their mechanism of therapeutic action is unknown. The pharmacodynamics of antidepressants at molecular, cellular and system levels has been investigated by many authors, e.g. [34,51,52]. Studies on the effects of antidepressants at the cognitive level are less common but likely to be beneficial in improving our understanding of these drugs. At this level, the purpose of the present work was to study the effects of amitriptyline on animal cognition in relation to some characteristics of the therapeutic effects of this drug in human beings.

Previous studies carried out with the forced swimming test (FST; [8,26,39]) suggested that this test shares some characteristics of the short tests used to assess the effects of drugs on memory such as those employed by Ennaceur et al. [9–11], and Platel and Porsolt [41]. FST can be considered as a memory test in which animals learn to be immobile in the first session such that the second session can be used as a retention test [39]. Given that antidepressants impair FST performance (i.e. animals swim more than controls on the second occasion that they are forced to swim, see Porsolt et al. [44]),

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the question arises: do antidepressants impair memory? Animal studies using tests well established in the literature as memory tests could help answer this question. Inhibitory (or passive) avoidance has been widely used for testing the effects of drugs on memory and the rationale for its use has been well documented [17]. In the step-through version, used in the present experiments, the animal must inhibit crossing to the dark compartment of a box to avoid a footshock [6].

In a previous study, acute administration of amitriptyline, a mixed serotoninergic and noradrenergic uptake inhibitor with a strong anticholinergic effect [16], produced retrograde amnesia (referred to as such because the drug was administered after training) on inhibitory avoidance, seemingly unrelated to anxiolytic effects [37]. The results of this work agree with those of Kumar and Kulkarni [23]. Other authors have also found anterograde impairment (referred to as such because the drug was administered before training) of inhibitory avoidance in rats [53,55]. As far as we know, no contradictory data have been reported, and the effects of chronic administration have not been studied. Amitriptyline was selected for the present work because it clearly impairs inhibitory avoidance in experiments as carried out in laboratory [12]. 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 [16].

The present study investigated whether or not piracetam counteracted the effect of acute and chronic amitriptyline on inhibitory avoidance. Piracetam (whose mechanism of action is still unclear but appears to be a non-specific activator of neuronal excitability [18]) has controversial results as a nootropic drug in humans [19], but there is strong evidence that it counteracts the amnesic effects of a variety of drugs in animals, e.g. [7]. Two separate experiments were performed involving, respectively, acute and chronic administration of amitriptyline. Independent groups of subjects were used for each pharmacological treatment. In the second experiment, animals were also subjected to one session in the elevated plus-maze [13] to test the effects of the same substances on spontaneous motor activity and anxiety in order to assess whether the effects of amitriptyline on avoidance performance may reflect general behavioural changes. Male and female mice were used in both experiments because sex differences are evident in the inhibitory avoidance behaviour of non-treated animals, with females showing longer latencies than males [30], the effects of antidepressants on inhibitory avoidance [30,31], and the pharmacokinetics of antidepressants in human beings [15].

## 2. Materials and methods

### 2.1. Animals

Subjects were CD1 mice that were 42 days old and obtained from CRIFFA, Lyon, France. Animals were housed in groups of four in standard plastic cages, stored in a temperature-controlled room ($21 \pm 2\,^{\circ}C$) and under a reversed light–dark cycle (07:30–19:30 lights off). Food and tap water were available ad libitum. Animals were marked for individual recognition. They were subjected to the pharmacological treatments and behavioural tests during the dark phase of the light cycle. The experimental protocol and the use of animals are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### 2.2. Drugs

Amitriptyline hydrochloride and piracetam (Sigma–Aldrich Química, Madrid, Spain) were diluted in saline solution (0.9% NaCl). The mice received physiological saline (*S*), 100 mg/kg of piracetam (*P*) or 30 mg/kg of amitriptyline (*A*) in a volume of 0.01 ml/g. The selected dose of piracetam is intermediate and frequently administered in similar studies, e.g. [40]. The selected dose of amitriptyline is rather high, nevertheless it can be found in literature, e.g. [1], and its human equivalent dose [14] is within the range of normal clinical use dosage [3].

### 2.3. 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 cm × 9.5 cm × 16.5 cm) separated by an automatic sliding door. There was a light (24 V, 10 W) in the ceiling of the starting side, which was painted white (light intensity of 290 lx at the 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 enclosed arms (30 cm × 5 cm, and 30 cm × 15 cm × 5 cm, respectively) extending from a common central square (5 cm × 5 cm) and elevated 50 cm above floor level on five pedestals. The maze floor was made of black Plexiglas while the walls of the enclosed arms were made of clear Plexiglas. The external sides of the walls were covered with black paper. The illumination in the experimental room consisted of four neon tubes fixed on the ceiling (light intensity of 110 lx at 50 cm above floor level). A video camera (SONY Handycam CCD-TR401E, Sony Corporation, Tokyo, Japan) was employed to record the elevated plus-maze task.

### 2.4. Experimental procedures

In Experiment 1 (see Fig. 1 for a schema of the procedure), mice were randomly assigned by sex to one of four groups ($N = 10$–12): namely physiological saline–physiological saline (SS), physiological saline–amitriptyline (SA), piracetam–physiological saline (PS), or piracetam–amitriptyline (PA). The training phase consisted of one trial of inhibitory avoidance that was carried out 1 h after animals received *S* or *P*. The trial began with a 90 s adaptation period to the apparatus in the light compartment. The door between the two compartments remained closed during this period. Subsequently, the door was removed and the mouse could stay in the light side for a maximum of 300 s. If it entered the dark compartment, however, an inescapable footshock of 0.7 mA was delivered for 5 s. The