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Research report

# Sex- and dose-dependent effects of post-trial calcium channel blockade by magnesium chloride on memory for inhibitory avoidance conditioning

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## HIGHLIGHTS

- Memory for aversive inhibitory avoidance (IA) conditioning is affected by $Ca^{2+}$ influx.
- $MgCl_2$ dose-dependently enhanced memory for inhibitory avoidance conditioning.
- $MgCl_2$ enhanced long-term memory at a lower dose in males than females.

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## ABSTRACT

Calcium influx through voltage-dependent $Ca^{2+}$ channels is critical for many neuronal processes required for learning and memory. Persistent increases in cytosolic intracellular $Ca^{2+}$ concentrations in aging neurons are associated with learning impairments, while small transient subcellular changes in intracellular calcium concentrations play critical roles in neural plasticity in young neurons. In the present study, young male and female Fisher 344 × Brown Norway (FBN) hybrid rats were administered different doses of magnesium chloride (0.0, 100.0, or 200.0 mg/kg, i.p.) following a single inhibitory avoidance training trial. Extracellular magnesium ions can non-specifically block voltage-gated calcium channels, and/or reduce the calcium conductance gated *via* glutamate and serine's activation of neuronal NMDA receptors. In our study, magnesium chloride dose-dependently enhanced memory compared to controls (significantly increased latency to enter a dark compartment previously paired with an aversive stimulus) when tested 48 h later as compared to controls. A leftward shift in the dose response curve for memory enhancement by magnesium chloride was observed for male compared to female rats. These findings provide further insights into calcium-dependent modulation of aversive memory, and should be considered when assessing the design of effective treatment options for both male and female patients with dementia or other memory problems.



## 1. Introduction

Many neuronal processes depend on rapid intracellular $Ca^{2+}$ regulation, including synaptic plasticity, as do the macroscopic mechanisms based upon them, including learning and memory consolidation. Normally, a multitude of proteins tightly regulate cytosolic $Ca^{2+}$ levels, but intracellular $Ca^{2+}$ becomes dysregulated during normal aging and dementia [1,2], associated with impairments in learning and memory consolidation [3–5].

Non-specific voltage-gated $Ca^{2+}$-channel blockers, including $Mg^{2+}$, facilitate spatial memory [6–8]. Enhanced extracellular magnesium can decrease $Ca^{2+}$-dependent post-burst afterhyperpolarization (AHP) amplitudes (decreases which are associated with improved learning and memory) [9–12], enhance memory for behavioral tasks [6–8,13], and effectively reduce calcium influx *via* NMDA receptors [14–16].

Although $Ca^{2+}$ regulation is critically important for learning and memory, neuronal $Ca^{2+}$ is differentially regulated between males and females [17–21]. The sex hormone 17β-estradiol (E2) decreases $Ca^{2+}$-dependent AHP amplitudes recorded intracellularly [17,18,21]. When applied *in vitro*, E2 increases intrinsic excitability, and decreases AHP amplitudes [20]. While sex-differences in learning and memory have been reported across many species—from rats and mice to humans—these differences have long been a point of contention in the literature [22–27].

*Abbreviations:* AHP, post-burst afterhyperpolarization; E2, 17β-estradiol; FBN, Fisher 344 × Brown Norway hybrid; IA, inhibitory avoidance.

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Since neuronal $Ca^{2+}$ levels are differentially regulated in males and females [19,28], it is of general importance to understand whether sex-related differences occur in the effects of $Ca^{2+}$-channel blockade on learning and memory.

To assess the role of calcium influx on memory for single-trial inhibitory avoidance conditioning, rats were given a single paired training trial, and sex- and dose-dependent effects of a single post-trial injection of $MgCl_2$ were assessed on memory retention 48 h later. Immediate post-retention sensorimotor testing was used to demonstrate that any observed effects were solely upon memory for the conditioning task, and not upon other behavioral variables. The effects of $MgCl_2$ have not previously been tested on memory for this inhibitory avoidance task. The aim was to assess whether $Mg^{2+}$ treatment would improve memory for this task, and to determine if sex differences were observed between male and female rats.

## 2. Materials and methods

### 2.1. Subjects

A total of 48 locally bred young (~2–4 month old) male and female Fisher 344 × Brown Norway hybrid (FBN) rats were socially housed in a temperature-controlled environment (~22 °C) with a 12 h light/dark cycle and met all inclusion criterion defined below. Care followed established protocols approved by the Institutional Animal Care and Use Committee of the University of Texas at Dallas in accordance with NIH Animal Welfare guidelines. *Ad libitum* access to both food and water were available in the rats' home cages. The experimenters were blind as to the identity of individual rat's drug treatments until data was collected and assessed, with cohorts from each litter pseudorandomly assigned to each treatment group.

### 2.2. Inhibitory avoidance (IA) conditioning

The apparatus used was a trough-shaped Plexiglas chamber (91 cm long, 15 cm deep, 20 cm wide at the top, and 6.4 cm wide at the bottom) with a sliding guillotine door to separate the light compartment (30 cm long) from the dark compartment (60 cm long, with two angled and divided metal shock plates as a floor). A lamp (20 W) was placed over the light compartment to brightly illuminate the light but not the dark compartment. All rats were handled for 5 days, 5 min/day, prior to undergoing a single paired training session. Each rat was placed in the light compartment, facing away from the dark compartment. When the rat turned around and escaped fully into the dark compartment, the door was closed and initial escape latency was recorded. When the rat reached the end of the dark compartment and turned around, a moderate footshock (0.18 mA, 1 s) was applied. Any rat that failed to vocalize or jump in response to the footshock was eliminated from further study. After another 15 s the rat was removed from the apparatus and given an immediate post-training injection of either vehicle or one of the $MgCl_2$ doses (see Section 2.3).

### 2.3. Post-trial drug treatment

Male and female FBN rats were divided into 3 treatment groups (see Table 1) and each was given an i.p. injection of either vehicle (0.9% saline) or magnesium chloride ($MgCl_2{\cdot}6H_2O$, 100.0 or 200.0 mg/kg, i.p., dissolved in 0.9% saline; Fisher Scientific).

### 2.4. Memory retention testing

Forty-eight hour later each rat was returned to the light compartment of the apparatus, and the time it took to fully enter the dark compartment (retention escape latency) was measured.

**Table 1**
Number of male and female rats tested in the different $MgCl_2$ dose treatment groups.

| $MgCl_2$ treatment | | | |
|---|---|---|---|
| Dose (mg/kg) | Number of rats | | |
| | Males | Females | Total |
| 0.0 | 8 | 9 | 17 |
| 100.0 | 9 | 8 | 17 |
| 200.0 | 7 | 7 | 14 |
| Total | 24 | 24 | 48 |

Longer escape latencies indicated better memory for the previous aversive event.

### 2.5. Sensorimotor testing

To determine if administration of magnesium chloride affected rats' sensory or motor capacity to perform an escape from the light compartment during retesting, sensorimotor function was assessed immediately following memory retention testing. Each task below was assessed once per rat, immediately after memory retention testing was concluded (*i.e.* within the same relative time interval as memory was tested).

A series of four sensorimotor tests were used. For the wire-hang test, the rat was suspended grasping by its forelimbs from a wire (200 mm thick) 40 cm above a foam cushion, and the latency to drop was recorded. On the narrow beam test, the rat was placed midway down a 1.2 m long × 2.5 cm beam that had 25 $cm^2$ wooden escape platforms on either end and was 80 cm above a foam cushion. The latency to reach either end of the beam was recorded. On the 45° incline test, the rat was placed facing downward on a 45° incline apparatus (33.5 cm long × 23.5 cm wide × 42 cm high) and the latency to turn facing upward was recorded. Subjects that continuously climbed down the 45 °C incline, rather than turning around, were excluded. Finally, in a blind alley test, the rat was placed face first into an enclosed alley (29 cm long × 9.5 cm wide × 24 cm high) with walls on three sides of the apparatus. The time it took the rat to turn around and face the opening was recorded.

### 2.6. Statistical analyses

#### 2.6.1. Inhibitory avoidance

To compare initial escape latencies to 48 h retention escape latencies, we used a paired *t*-test (Fig. 1). To analyze the effect of $MgCl_2$ on retention escape latencies, we used a one-way ANOVA with escape latency as the dependent variable and treatment group

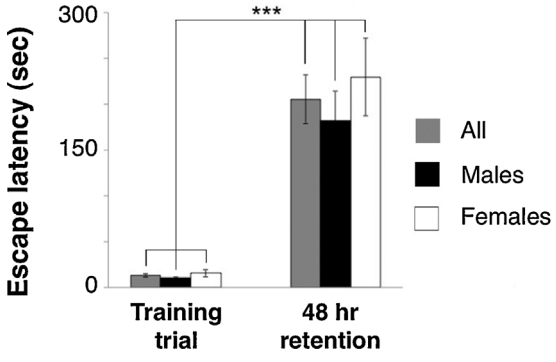


**Fig. 1.** All rats exhibited significantly longer escape latencies 48 h following a single inhibitory avoidance learning trial (***$p < 0.0001$), with no difference between the genders ($p > 0.05$). All $MgCl_2$ treatment doses have been collapsed within sexes. Values reported are means ± SEM.