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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

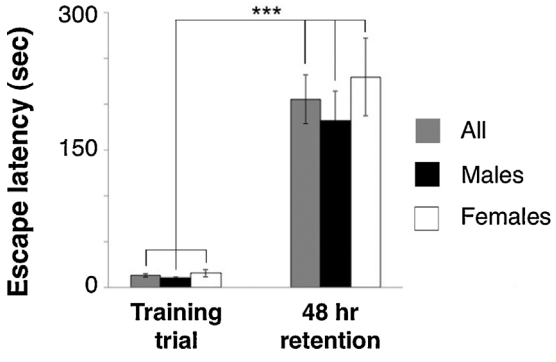

Fig. 1. All rats exhibited significantly longer escape latencies 48 h following a single inhibitory avoidance learning trial (***$p < 0.0001$), with no difference between the genders ($p > 0.05$). All $MgCl_2$ treatment doses have been collapsed within sexes. Values reported are means ± SEM.

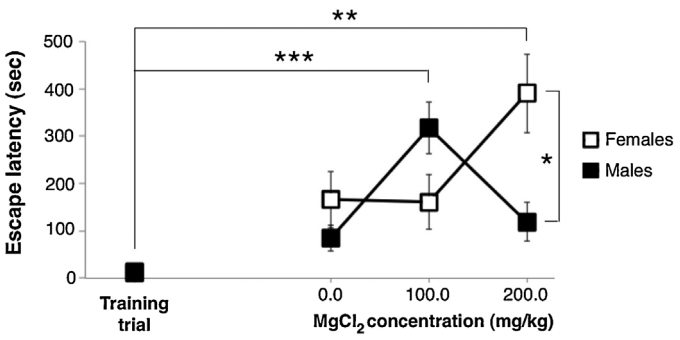

Fig. 2. Post-training administration of $MgCl_2$ enhanced memory for the inhibitory avoidance task in both male and female FBN rats. Male rats that received immediate post-training injections of $MgCl_2$ (100.0 mg/kg) had significantly longer escape latencies than control rats (***$p < 0.001$). Female rats receiving immediate post-training injections of $MgCl_2$ (200.0 mg/kg) had significantly longer escape latencies than control rats (**$p < 0.05$) or than male rats treated with the same concentration of $MgCl_2$ (*$p < 0.05$). Values reported are means ± SEM.

as the independent variable (Fig. 2). Significant interactions were further analyzed by Fisher *post hoc* analyses or un-paired t-tests. We used an un-paired *t*-test to compare differences between genders.

2.6.2. Sensorimotor testing

To compare mean latencies on the 4 sensorimotor tasks (narrow beam, wire hang, 45° incline, and blind alley), we used a one-way ANOVA with the task latency as the dependent variable and drug treatment group as the independent variable. Significant interactions were further analyzed by Fisher *post hoc* tests.

3. Results

3.1. Male and female FBN rats exhibited memory for inhibitory avoidance conditioning

As seen in Fig. 1, all rats exhibited memory for the aversive foot shock paired with entry to the dark compartment. There were no significant differences in initial escape latencies when data from all doses were compared, when data from all males were compared, or when data from all females were compared ($p > 0.05$). After collapsing across conditions and genders, every rat tested exhibited longer escape latencies when retested 48 h later ($t(47) = -7.33$, $p < 0.0001$), indicating that all rats exhibited memory for the earlier aversive paired footshock experience.

Male rats initially exposed to the IA apparatus took an average of 10.9 ± 0.72 s to escape to the dark compartment. Forty-eight hours after acquisition of the inhibitory avoidance task, male rats showed significantly longer retention escape latencies ($t(23) = -5.22$, $p < 0.0001$, 181.7 ± 32.87 s) compared to their initial escape latencies.

Female rats initially exposed to the IA apparatus took an average of 15.8 ± 4.04 s to escape to the dark compartment. Females showed significantly longer retention escape latencies ($t(23) = -5.19$, $p < 0.0001$, 229.8 ± 42.37 s) when tested 48 h after acquiring the IA task compared to their initial escape latencies.

3.2. Post-trial $MgCl_2$ enhanced memory in both male and female rats

As seen in Fig. 2, the non-specific Ca^{2+}-channel blocker $MgCl_2$ administered immediately post-trial enhanced memory 48 h later for aversive inhibitory avoidance conditioning in both male and female rats. Rats in each treatment condition exhibited memory 48 h later for the aversive foot shock paired with entry to the dark compartment, with the dose–response curve taking a general inverted-U shape (higher doses of $MgCl_2$ were not assessed, since preliminary testing indicated doses >300 mg/kg could be fatal to a percentage of test subjects). When retested 48 h after inhibitory avoidance conditioning, $MgCl_2$ dose-dependently increased escape latencies in male rats compared to controls, as determined by one-way ANOVAs ($F_{2,21} = 8.594$; $p = 0.0019$). The greatest enhancement of escape latencies for males was seen at a dose of 100.0 mg/kg $MgCl_2$ (Fisher test: $p = 0.001$; Fig. 2). Similarly, $MgCl_2$ dose-dependently increased retention escape latencies in female rats compared to their controls (one-way ANOVA: $F_{2,21} = 3.63$; $p = 0.044$). The greatest enhancement of escape latencies for females was seen at a dose of 200.0 mg/kg $MgCl_2$ (Fisher test: $p = 0.03$; Fig. 2).

A leftward shift occurred in the $MgCl_2$ dose–response curve, comparing enhancement of memory for male rats to that of female rats (Fig. 2). Optimal male memory enhancement required a lower dose of $MgCl_2$ than optimal female memory enhancement. Females administered 200.0 mg/kg $MgCl_2$ showed significantly enhanced memory for the aversive inhibitory avoidance task (exhibited longer escape latencies) compared to their male counterparts ($t(12) = 2.93$, $p = 0.01$), with no significant differences between genders at lower doses (0.0 mg/kg: $t(15) = 1.19$, $p = 0.25$; 100.0 mg/kg: $t(15) = -1.96$, $p = 0.07$).

3.3. $MgCl_2$ had no effect on sensorimotor performance by FBN rats

To verify that the $MgCl_2$-induced enhancement of memory (increases in retention latency) were not simply due to impairments in sensorimotor functions, we tested each rat on four sensorimotor tasks immediately after retention testing (Fig. 3). Administration of $MgCl_2$ had no effect on performance of any of the sensorimotor tasks in male rats, as determined by one-way ANOVAs (wire hang: $F_{2,24} = 1.414$, $p = 0.2628$; narrow beam: $F_{2,24} = 1.528$, $p = 0.2374$; 45 °C incline: $F_{2,23} = 1.564$, $p = 0.2307$; blind alley: $F_{2,24} = 0.213$, $p = 0.8099$). Similarly, $MgCl_2$ administration had no effect on any of the sensorimotor tasks in female rats, as determined by one-way ANOVAs (wire hang: $F_{2,24} = 0.507$, $p = 0.6084$; narrow beam: $F_{2,24} = 0.002$, $p = 0.9981$; 45 °C incline: $F_{2,24} = 0.245$, $p = 0.7844$; blind alley: $F_{2,24} = 0.120$, $p = 0.8871$).

4. Discussion

Both male and female FBN rats successfully remembered a single-trial paired aversive event in an inhibitory avoidance task (see Fig. 1). The non-specific Ca^{2+}-channel blocker $MgCl_2$ dose-dependently increased escape latencies when rats were retested 48 h post-training (Fig. 2), an operational measure of memory enhancement for the task. The memory enhancement produced by $MgCl_2$ was seen in both males and females, with an approximate two-fold leftward shift in the optimal dose from the dose–response curves for males *vs.* females. Females showed better memory for the inhibitory avoidance task (longer escape latencies) than males, even when treated with the vehicle. While no significant effects of the calcium-channel blocker were observed on sensory or motor functions when tested 48 h later, doses higher than 200.0 mg/kg of $MgCl_2$ were not used due to potentially deleterious or fatal side effects.

Ca^{2+} regulation is critically important for synaptic plasticity, learning, and memory. We showed that a non-specific Ca^{2+}-channel blocker can enhance consolidation of memory, as evidenced by longer escape latencies 48 h following training on single-trial inhibitory avoidance conditioning. These findings are consistent with numerous other studies demonstrating that memory is enhanced by calcium antagonists [29–32].

Our data are consistent with the hypothesis that Ca^{2+}-channel blockers facilitate memory for an aversive inhibitory avoidance task. We found that post-training injections of $MgCl_2$ (Fig. 2) dose-dependently enhanced memory when retested 48 h later. This is consistent with earlier studies showing that Ca^{2+}-channel blockers decrease AHP amplitudes [13,33–35] and NMDA receptor activation [14–16] and facilitate memory [6,36,37] in other tasks, including fear conditioning [38] in normal animals, as well as improving Morris water maze spatial learning [7,39] following traumatic brain injury. Additionally, dietary administration of $MgCl_2$ also facilitated memory following traumatic brain injury, so the memory-enhancing effect is not limited to systemic injections nor solely to enhancement of memory in the intact brain [40]. While some previous studies have found that repeated administration of $MgCl_2$ *via* either dietary supplementation [40] or daily injection [39] can produce memory impairment, in our hands, post-training administration of a single treatment with the non-specific calcium channel blocker $MgCl_2$ enhanced memory, with significant enhancement in males at doses of 100.0 mg/kg and in females at 200.0 mg/kg. While non-specific Ca^{2+}-channel blockers like Mg^{2+} [40,41] can produce deleterious side effects (including, with repeated administration, memory impairments), no impairments nor any sensory or motor effects that would affect retest performance were observed across the range of memory-enhancing doses tested in our experiments.

Ca^{2+} influx is important for learning and memory, and may be differentially regulated in male and female brains. The sex hormone, estradiol (E2), alters multiple Ca^{2+}-dependent processes in neurons. When E2 is applied to CA1 pyramidal neurons, it enhances the intrinsic excitability of neurons and decreases post-burst AHP amplitudes [17,18,20,21]. In pyramidal neurons from ovariectomized (OVX) females, AHP amplitudes were larger than those from OVX + E2 treated females [21]. Similarly, when 17β-estradiol benzoate was added to neurons from aged female rats, there was a decrease in AHP amplitude compared to aged controls [17]. These findings are consistent with our data indicating that calcium channel blockers enhance memory at different doses in males than females; however, further study is needed to determine the specific neural mechanisms that account for our findings.

Many studies have found that males and females learn tasks differently [22–25]. In a study by Maren et al. [25], male rats that received tone CS and shock US pairings acquired a conditioned response faster than female rats. When male and female rats were gonadectomized, or gonadectomized then injected with either estrogen (for the males) or testosterone (for the females), the feminized males showed a decrease in spatial learning, while the masculinized females showed enhanced spatial maze learning similar to normal males [22]. In our hands, we found that females retained the memory of the footshock after inhibitory avoidance conditioning better than males, as evidenced by longer escape latencies when retested 48 h later. It has been shown that the "male" advantage seen in many learning tasks can be greatly reduced if female rats are habituated to a task beforehand, if cues are available, if rats are group housed, or even depending on the strain of rat used [42,43]. Since our animals were group housed and well handled beforehand, these particular variables could have contributed to the female memory advantage observed in our own experiments.

Sex differences are seen not only in rodent studies, but also in human studies. Depending on the type of memory being compared, human females perform significantly better on verbal memory and facial recognition tasks [44]. Similar to the results from rodent memory studies, human males have been reported to have better memory for spatial information than females [44]. Piper et al. [45] used a virtual version of the water maze task to observe differences in spatial memory in human males and females. They used the "Memory Island" task, which simulates an island with 4 quadrants, similar to the Morris water maze task often used to assess rodent behavior. Males reached the objective both faster and more efficiently than their female counterparts [45]. Females appear to use more landmark information while males use more geometric information to navigate through a virtual water maze task [46].

Our results indicate that post-training administration of $MgCl_2$ facilitates memory for aversive single-trial inhibitory avoidance conditioning in both male and female FBN rats. A higher concentration of $MgCl_2$ was required to produce a maximal enhancement in female rats compared to male rats, despite the fact that female escape latencies were longer than those of males at all doses tested

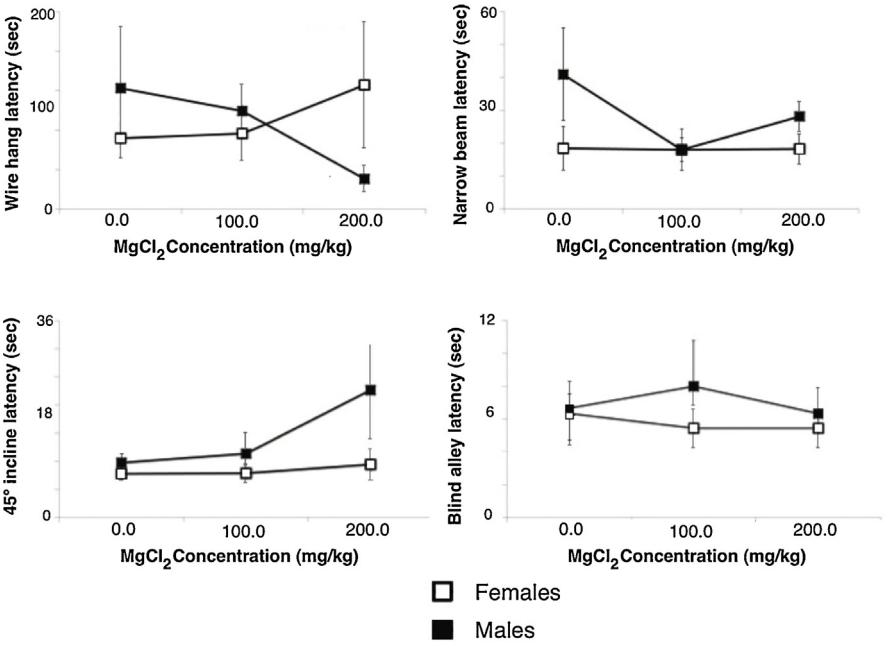

Fig. 3. Immediately following 48 h retention escape latency testing, no significant residual effects of post-trial $MgCl_2$ administration were observed ($p > 0.05$) on sensorimotor performance in either male or female FBN rats tested on the (A) wire hang, (B) narrow beam, (C) 45° incline, or (D) blind alley tasks. All values reported in (A–D) are means ± SEM.

(including controls). This finding could be of importance for clinical studies seeking to determine effective treatment doses to alleviate memory impairments impacted by cytosolic Ca^{2+}, and emphasizes the need to differentiate between male and female patients with memory problems, which can range from mild cognitive impairment up to dementia.

Our data show differences in memory retention between males and females for aversive inhibitory avoidance conditioning. The non-specific Ca^{2+} channel blocker $MgCl_2$ dose-dependently enhanced memory for the IA task, with males requiring a two-fold lower concentration than females to maximize memory enhancement (100.0 mg/kg *vs.* 200.0 mg/kg, respectively). These results provide insight into sex-dependent differences in learning and memory, and the role of calcium influx in these differences.

Conflict of interest

None of the authors has any conflict of interest to disclose.

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