Chronic Dietary Magnesium-L-Threonate Speeds Extinction of a Conditioned Taste Aversion

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Abstract

Elevation of brain magnesium has been shown to enhance synaptic plasticity and extinction of conditioned fear memories (Slutsky et al., 2010; Abumaria et al., 2011). This experiment examined the generalizability of this phenomenon by studying the effects of magnesium-L- threonate (MgT) on conditioned taste aversion (CTA) extinction. Adult male Sprague-Dawley rats were habituated to a 23-hour water deprivation cycle for 5 days and were given water + MgT (10mg/ml) to drink for 3 additional days. Rats then acquired a CTA following the taste of a Conditioned Stimulus [CS; 0.3% saccharin + 16mg/ml MgT (SAC+MgT)] paired with an injection of an Unconditioned Stimulus [US; 81 mg/kg (i.p.) Lithium Chloride (LiCl)]. All rats were maintained on the same water deprivation schedule and drank a water + MgT solution (558.87±10.33mg/kg/day; Mean±SEM) for up to 1 hour/day over the next 31 days. For 14 additional days, some animals continued water + MgT treatment, but others drank water only to allow MgT to be eliminated from the body (Slutsky et al., 2010). We then employed 2 different extinction paradigms: (1) CS-Only (CSO), in which SAC was presented, every-other day, or (2) Explicitly Unpaired (EU), in which both SAC and LiCl were presented, but on alternate days. EU extinction procedures have been shown to speed CTA extinction and reduce spontaneous recovery of the aversion (Mickley et al., 2009). Throughout extinction, half of the rats in each group continued to drink 639.69±9.74 mg/kg/day (Mean±SEM) of MgT (now in SAC or supplemental water+MgT solution), whereas the other half drank SAC only/water only until SAC drinking reached 100% of baseline (asymptotic extinction). MgT-treated rats exhibited a stronger CTA than did the controls as evidenced by lower SAC consumption on the first day of extinction. MgT generally enhanced the rate of extinction. This effect was seen most dramatically in the steep slopes of the extinction curves for MgT-treated rats experiencing the CSO extinction procedure. Furthermore, the MgT-treated rats did not show as dramatic a spontaneous recovery (SR) of the CTA 30 days later – indicating that the extinction procedure was more effective for these animals. Our data suggest that long-term dietary MgT enhances the acquisition and extinction of a CTA and inhibits SR of the learned aversion.

Introduction

- Magnesium (Mg^{2+}) is the fourth most abundant ion in the body and it is essential for the proper functioning of the nervous system. One major action of Mg^{2+} is modulating the voltage-dependent block of NMDA receptors - which is critical for synaptic plasticity (Mayer et al., 1984; Nowak et al., 1984).
- Mg^{2+} deficiency impairs fear conditioning in mice (Bardgett et al., 2005), but an increase in brain magnesium enhances both short-term synaptic facilitation and long-term potentiation and also improves learning and memory functions (Slutsky et al., 2010). Further, an elevation of brain magnesium enhances extinction of conditioned fears specifically, i.e., without impairing or enhancing the original memory formation (Abumaria et al., 2011).
- Our laboratory has used the conditioned taste aversion (CTA) paradigm (Garcia, Kimeldorf , & Knelling, 1955) to create a robust, aversive memory that causes the animal to refuse the CS of saccharin (Mickley et al., 2004).
- CTAs are extremely robust and extinction may be slow, creating an interesting model of other hard-to-extinguish defensive reactions to conditioned fears (Mickley et al., 2004).
- The way in which a fear is extinguished has much to do with whether or not a relapse or spontaneous recovery of the fear will occur. We have explored two different paradigms in which extinction of an established CTA occurs:
  - CS-Only (CSO): Presentation of only the CS every other day.
  - Explicitly Unpaired (EU): CS and US are given, unpaired, on alternating days.
- Exposure to the EU paradigm produces a more-rapid reacceptance of the once-aversive CS (saccharin) and makes spontaneous recovery of the CTA less potent (Mickley et al., 2009).
- Here we employed a novel magnesium compound (magnesium-L-threonate; abbreviated as: MgT; brand name Magtein™) to elevate brain magnesium via chronic oral supplementation.
- **Hypotheses:**
  - MgT will speed up extinction of a CTA when either CSO or EU extinction methods are used.
  - MgT supplementation will reduce the spontaneous recovery (SR) of a CTA; rats that experienced EU extinction procedures will show the greatest reduction.

Methods

Pilot studies evaluating the acceptability of water+MgT and Saccharin+MgT

1. Rats are equally accepting of 2 doses of water+MgT and water only. Twenty-three-hour fluid deprived naïve males Sprague-Dawley rats (N=8/group) were offered either 10mg/ml water+MgT or 16mg/ml water+MgT on two successive days. These concentrations represent the lowest and highest concentrations of MgT that we employed in our main experiment.

- **10mg/ml water+MgT = 19.67±0.82ml (Mean±SEM);**
- **16mg/ml water+MgT = 20.81±0.85ml;**
- **Water Only = 19.50±3.09ml**

2. Rats are equally accepting of 2 doses of SAC+MgT (0.3% Saccharin) and SAC only. Twenty-three-hour fluid deprived naïve males Sprague-Dawley rats were offered either 0.3% SAC only (N=10) or 0.3% SAC+MgT (16mg/ml; i.e., the highest concentration we used in our main study) (N=11) over 3 successive days.

- **SAC only = 17.57±1.29ml (Mean±SEM);**
- **SAC+MgT = 17.84±1.25ml**

3. Determining levels of baseline/familiar SAC+MgT (16mg/ml) drinking as a means to evaluate the degree to which the rats in this study had extinguished their CTA.

Twenty-three-hour fluid deprived naïve males Sprague-Dawley rats (N=11) were used to determine baseline SAC+MgT consumption by averaging the volume consumed on the third day of exposure.

- **SAC+MgT = 17.84±1.25ml (Mean±SEM)**

Consequently, this volume of SAC+MgT (17.84 ml) was taken to represent 100% of baseline consumption and was used as the criterion for asymptotic extinction in this study.

Table 1. Experimental design, Group Nomenclature and Ns

Extinction Treatment	MgT Treatment: MgT	MgT Treatment: Controls
CS-Only Extinction (CSO)	CSO MgT N=/6/9/9	CSO Controls N=/6/9/8
EU-Extinction (EU)	EU MgT N=/6/8/8	EU Controls N=/6/9/9

* The Ns separated by slashes represent the number of animals in the study during the conditioning/extinction/SR phases of the experiment. One sick rat was removed from the study.

Table 2. Timeline, Phases, and Stages of the Experiment

Phases	H2O Deprivation Acclimation	Pre-Conditioning	CTA Acquisition	Extended MgT Treatment	MgT Wash-out phase	Extinction	H2O only latency	Spontaneous Recovery Test
Total # of days	5 days	3 days	1 day	31 days	14 days	Up to15 days	30 days	1 day
Magtein (MgT) Concentration	None	Water+MgT (10mg/ml)	SAC+MgT (16mg/ml) + LiCl[†]	Water+MgT: Concentration adjusted weekly to maintain mean body weight [Range = 10mg/ml – 12.1mg/ml]	Water+MgT: Concentration adjusted weekly to maintain mean body weight [Range = 12.2mg/ml – 13.4mg/ml]	SAC+MgT (16mg/ml)	None	SAC+MgT (16mg/ml)
STAGES		STAGE ONE: All animals received the same treatment			STAGE TWO: Wash out: EU Controls and CSO Controls received only water; EU MgT and CSO MgT rats received Magtein in water. Extinction: Animals in EU MgT and CSO MgT groups received Magtein in 0.3% saccharin; EU Controls and CSO Controls received only 0.3% saccharin		STAGE THREE: All animals received the same treatment	

[*] MgT = Magnesium-L-threonate; Magtein™
[†] SAC = 0.3% saccharin solution
[‡] LiCl = 81mg/kg Lithium Chloride, i.p.

Results

MgT Promotes the Consolidation or Retrieval of a Conditioned Taste Aversion (CTA)

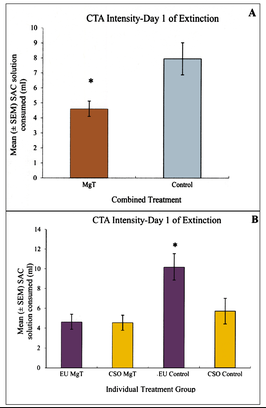

Figure 1. Saccharin drinking on the first day of extinction indicates that MgT treatments that began the day after conditioning enhance the consolidation of a CTA (Panel A). The effect is seen most prominently between the rats in the EU extinction groups (Panel B). * p < 0.05.

MgT Increases the Rate of CTA Extenction

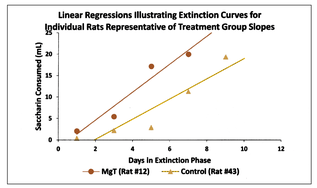

Figure 2. Slopes of the CTA extinction curves are significantly steeper in rats that received MgT treatments for an extended time immediately before and during extinction. These are regression lines fit to data collected from 2 individual rats that represent the group data. The average slopes (3.34±0.44; Mean±SEM) of the extinction curves for MgT-treated rats were significantly steeper than those of rats that were not drinking MgT in their SAC solution (Mean±SEM = 2.40±0.28) [$F(1,31) = 4.33$, $p = 0.05$]. The ratio between these 2 representative slopes (slope of MgT Rat #12/slope of control Rat #43 = 1.39) is the same as that of the mean slopes for those 2 groups of animals – indicating that these individuals represent the groups from which they were selected.

MgT and EU Extinction Procedures Reduced Spontaneious Recovery of a CTA

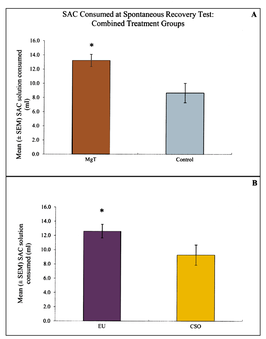

Figure 3. Mean total SAC+MgT (16 mg/ml) consumed on the day of the spontaneous recovery (SR) test. Panel A: Rats continuing to receive MgT during the "wash out" and extinction phases of the study (see Table 2) showed a significantly reduced SR of the CTA (i.e., they drank more SAC) than did controls. Likewise, rats going through the EU extinction procedure drank more SAC than CSO animals at the SR test – indicating that this extinction procedure reduced SR of the CTA (see Panel B). * = significantly more SAC consumed that the other group ($p < 0.05$).

MgT and EU-EXT Procedures Combined to Produce the Most-Potent Reduction in CTA Spontaneious Recovery

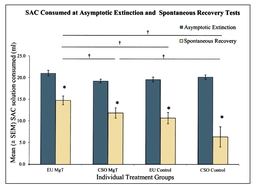

Figure 4. Mean total SAC solution consumed on the day of asymptotic extinction and SR tests. Animals in all groups reached asymptotic extinction but they exhibited varying amounts of SR. Rats that received MgT during the "wash out" and extinction phases of the study (see Table 2) and underwent EU extinction showed the mildest SR; while those in the control group and undergoing CSO extinction experienced the most severe SR. * = significantly reduced SAC consumption during SR as compared to the same group's SAC drinking on the day the achieved asymptotic extinction. † = significant differences in the SAC consumption of the groups indicated at SR test; $p < 0.05$.

Summary & Conclusions

- Our data indicate that chronic MgT treatment:
  - Facilitated consolidation of a CTA
  - Increased the rate of CTA extinction
  - Reduced spontaneous recovery of a CTA
  - Interacted with EU extinction procedures to reduce spontaneous recovery of a CTA
- MgT shows promise as a cognitive enhancing treatment that may have efficacy in reducing learned aversive responses and the fears that mediate them (Slutsky et al., 2010; Abumaria et al., 2011).
- MgT may be especially efficacious when combined with behavioral methodologies (e.g., EU extinction procedures) aimed at reducing SR following fear extinction.
- Our data are consistent with a growing animal literature suggesting that chronic MgT treatment may have clinical relevance since it has been shown to reduce learned helplessness (a model of depression) (Abumaria et al., 2009), cortical damage following trauma (Hoane et al., 2008), and cognitive deficits in a mouse model of Alzheimer's Disease (Liu et al., 2009).

References

Abumaria, N., Yin, B., Zhang, L., Zhao, L., Liu, G. Enhancement of cognitive control of emotions by elevated brain magnesium leads to anti-depressants like effect. (2009). Program. No. 549.16.2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, Online.

Abumaria, N., Yin, B., Zhang, L., Li, X.-Y., Chen, T., Descalzi, G., Zhao, L., Ahn, M., Luo, L., Ran, C., Zhuo, M., & Liu, G. (2011) Effects of elevation of brain magnesium on fear conditioning, fear extinction, and synaptic plasticity in the infralimbic prefrontal cortex and lateral amygdala. Journal of Neuroscience, 31(42), 14871–14881.

Bardgett, M.E., Schultheis, P.J., McGill, D.L., Richmond, R.E. & Wagge, J.R. (2005) Magnesium deficiency impairs fear conditioning in mice. BrainResearch, 1038, 100-106.

Garcia J., Kimeldorf, D.J., & Knelling, R.A. (1955). Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science, 122, 157-158.

Hoane, M.R., Gilbert, D.R., Barbre, A.B. & Harrison, S.A. (2008). Magnesium dietary manipulation and recovery of function following controlled cortical damage in the rat. Magnesium Research, 21, 29-37.

Liu, G., Li, W., Yu, J., Huang, X., Abumaria, N., Zhu, Y. & Chui, D. (2009). High brain magnesium reverses cognitive deficits and synaptic loss in Alzheimer's mouse model. Program. No. 116.5. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, Online.

Mayer, M.L., Westbrook, G.L., & Guthrie, P.B. (1984). Voltage dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature, 309, 261–263.

Mickley, G.A., Kenmuir, C.L., McMullen, C. A., Yocom, A. M., Valentine, E. L., Dengler- Crish, C.M., Weber, B., Wellman, J. A. & Remmers-Roeber, D. R. (2004). Dynamic processing of taste aversion extinction in the brain. Brain Research, 1061(1), 79-89.

Mickley, G.A., Disorbo, A., Wilson, G.N., Huffman, J., Bacik, S., Hoxha, Z., Biada, J.M., & Kim, Y.-H. (2009). Explicit disassociation of a conditioned stimulus and unconditioned stimulus during extinction training reduces both time to asymptotic extinction and spontaneous recovery of a conditioned taste aversion. Learning & Motivation, 40, 209- 220.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., & Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurons. Nature, 307, 462–465.

Slutsky, I., Abumaria, N., Wu, L., Huang, C., Zhang ,L., Li, B., Zhao, X., Govindarajan ,A., Zhao, M.G., Zhuo, M., Tonegawa, S., & Liu, G. (2010) Enhancement of learning and memory by elevating brain magnesium. Neuron, 65, 165–177.

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