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Chronic dietary magnesium-L-threonate speeds extinction and reduces spontaneous recovery of a conditioned taste aversion

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ABSTRACT

Elevation of brain magnesium enhances synaptic plasticity and extinction of conditioned fear memories. This experiment examined the generalizability of this phenomenon by studying the effects of a novel magnesium compound, magnesium-L-threonate (MgT), on conditioned taste aversion (CTA) extinction and spontaneous recovery (SR). Adult male Sprague–Dawley rats were maintained on a 23-hour water deprivation cycle and acquired a CTA following the taste of a CS [0.3% saccharin + 16 mg/ml MgT (SAC + MgT)] paired with a US [81 mg/kg (i.p.) lithium chloride (LiCl)]. Following CTA acquisition, rats drank a water + MgT solution 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. 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. Throughout extinction, half of the rats in each group continued to drink MgT (now in SAC or supplemental water + MgT solution), whereas the other half drank SAC only/water only until SAC drinking reached ≥90% of baseline (asymptotic extinction). Rats receiving MgT just before/during extinction drank less SAC on the first day of extinction suggesting that they had retained a stronger CTA. MgT enhanced the rate of extinction. Furthermore, the MgT-treated rats showed a relatively modest 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 may enhance the consolidation/retention of a CTA, speed extinction, and inhibit SR of this learned aversion.

1. Introduction

Magnesium (Mg^{2+}) is known to play a major role in cellular metabolism (Lin et al., 2002) and is critical for nervous system functioning (Paymaster, 1976; Furukawa et al., 2009). Aberrations in Mg^{2+} homeostasis leads to biochemical dysregulation and may contribute to psychological and neurological disorders such as depression (Whittle et al., 2011; Murck, 2002; Rasmussen et al., 1989; Singewald et al., 2011), Parkinson's Disease (Shindo et al., 2011) and glaucoma (Crish et al., 2012). Magnesium deficiency impairs fear conditioning in mice (Bardgett et al., 2005). Moreover, treatment with magnesium sulfate ($MgSO_4$) may produce therapeutic benefits as they enhance the metabolic response to energetic stresses induced by hypoxia, ischemia and traumatic brain injury (Wang et al., 2012; Vink et al, 2003; Goni-de-Cerio et al., 2012).

Mg^{2+} also modulates the voltage-dependent block of N-methyl-D-aspartate (NMDA) receptors, controlling their opening during coincidence detection — a function that is critical for synaptic plasticity (Mayer et al., 1984; Nowak et al., 1984). *In vitro* studies have shown that increasing Mg^{2+} concentrations in extracellular fluids can enhance synaptic plasticity of cultured hippocampal neurons (Slutsky et al., 2004). Subsequent *in vivo* experiments revealed that increases in brain Mg^{2+} enhanced short-term synaptic facilitation and long-term potentiation as well as spatial memory (Slutsky et al., 2010). The underlying mechanisms of these physiological and cognitive changes are still being investigated but current evidence suggests that chronic increases in extracellular Mg^{2+} cause a compensatory upregulation of NR2B NMDA receptors to counterbalance the sustained blockade of NMDA receptor channels (Slutsky et al., 2010). Similar mechanisms of homeostatic plasticity have been reported in other neural systems (for review, see Turrigiano, 2008).

These benefits in cognition and control of emotions follow chronic enhancements of brain Mg^{2+} (Abumaria et al., 2009, 2011). However, there are several practical challenges of simply providing increased levels of elemental Mg^{2+} in diet. High levels of Mg^{2+} intake can interfere with a variety of physiological functions and induce diarrhea and lethargy (Chester-Jones et al., 1990). Moreover, central nervous system regulation of brain cerebrospinal fluid (CSF) Mg^{2+} concentrations limits blood-brain barrier penetration of peripherally administered $MgSO_4$ (for

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review, see McKee et al., 2005). However, a newly developed compound, Magnesium-L-threonate (MgT; brand name Magtein™) has been shown to significantly enhance bioavailability and produce 7–15% increases in rat CSF Mg^{2+} while other magnesium compounds tested failed to significantly elevate Mg^{2+} in CSF when compared to controls (Slutsky et al., 2010).

Thus far, two behavioral investigations have studied the effects of MgT on learning and memory. Slutsky et al. (2010) reported that MgT treatment benefits performance on working, spatial and recognition memory tasks. MgT has also been evaluated for its ability to enhance extinction of conditioned fear responses in rodents. In particular, Abumaria et al. (2011) found that increased levels of Mg^{2+} in the brain enhanced the retention of fear extinction without impairing the initial fear memory. Abumaria et al. further suggested that the retention of this extinction memory is stronger in animals with increased levels of brain Mg^{2+} due to a corresponding increase in synaptic plasticity in the hippocampus and infralimbic prefrontal cortex that accompanies activation of NMDA receptor signaling and brain-derived neurotrophic factor expression in the prefrontal cortex (Abumaria et al., 2011). If these findings are verified, effective magnesium supplements may be used to enhance the efficacy of therapy for anxiety disorders such as post-traumatic stress disorder (PTSD) or phobias, as relapse to the original fear is a common problem after therapy (Rauhut et al., 2001). To gain a full appreciation for the potential of MgT and its ability to affect learning and memory, more pre-clinical research needs to be performed to determine its effects on different types of defensive reactions to learned fears.

The purpose of the current study was to examine the ability of MgT to affect the extinction and spontaneous recovery of a conditioned taste aversion (CTA). The pairing of a novel taste (conditioned stimulus; CS) with malaise or noxious sensation (unconditioned stimulus; US) results in the formation of a CTA (Garcia et al., 1955, 1961, 1968). Although somewhat resistant to extinction, a CTA may be reduced by the repeated, nonreinforced presentation of the CS (Nolan et al., 1997; Mickley et al., 2004). Further, spontaneous recovery (SR) of the CTA (i.e., a re-occurring suppression of CS consumption) appears when the CS is presented following a sufficiently long delay after extinction (Kraemer and Spear, 1992; Rosas and Bouton, 1996; Berman et al., 2003; Mickley et al., 2007).

Here we created an aversive memory that caused our animals to refuse the conditioned stimulus of saccharin (Houpt et al., 1996; Mickley et al., 2004). This CTA was extinguished by either repeated exposure to the CS alone (CS-Only; CSO-EXT) or through the use of an Explicitly Unpaired extinction procedure (EU-EXT) which has been shown to speed up extinction and attenuate SR of the CTA (Mickley et al., 2009; Mickley et al., 2011). We hypothesized that MgT-treated rats would exhibit a faster rate of extinction than controls and show a reduced SR of the CTA.

2. Materials and methods

2.1. Subjects

This study employed 36 adult male Sprague–Dawley rats (mean weight at the start of the study ± SEM = 282.3 ± 2.7 g) obtained from Charles River Laboratories (Wilmington, MA). All subjects were handled and maintained in accordance with the Animal Welfare Act and *The Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The study was approved by the Baldwin Wallace University Institutional Animal Care and Use Committee. Animals were individually housed in plastic tub cages (20 cm × 22 cm × 20 cm deep) with wire cage tops. Each cage bottom contained corncob bedding (The Andersons, Inc., Maumee, OH). Drinking water and other fluids were administered in 50 ml sipper (ball spout) bottles. Rats had access to food *ad libitum* (Lab Diet, No. 5001, containing 0.21% Magnesium, PMI Nutrition International, Richmond, IN) (see: http://labdiet.com/pdf/5001.pdf) and were housed in a temperature-controlled room between 23 and 26 °C with a 12-hour light/dark cycle (lights on at 0600 h; off at 1800 h).

2.2. Materials

Magnesium-L-threonate powder (brand name Magtein™; MgT) was obtained from AIDP (City of Industry, CA; http://www.magtein.com/) and mixed in the rats' drinking water. Reverse osmosis (RO) water was used to mix all MgT solutions to avoid administering additional Mg^{2+} in local tap water. MgT concentrations varied from 10 to 16 mg/ml depending on the phase of the study (see below and Table 2). Fluids were consumed at will by our animals during a 1-hour period each day and the concentration of MgT liquids was adjusted based on body weights of the rats and average volume consumed in order to get as close as possible to the target dosage of 604 mg/kg/day employed by other investigators (Slutsky et al., 2010; Abumaria et al., 2011). Similar doses and time courses of MgT exposure have been shown to be effective in elevating brain magnesium and enhancing hippocampal-dependent learning and memory in rats (Slutsky et al., 2010) as well as extinction of conditioned fears (Abumaria et al., 2011). Saccharin (SAC) and lithium chloride (LiCl) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

2.3. Pilot studies evaluating the acceptability of water + MgT, Saccharin + MgT and the ability of rats to distinguish between the tastes of SAC-only and SAC + MgT

In the main studies reported here we exposed rats to various water + MgT, SAC + MgT, and SAC-only solutions and we made adjustments in the MgT concentrations as the rats' weights changed during certain phases of our experiments. MgT is colorless, tasteless, and odorless to humans (http://www.magtein.com/thequality.html). However, it was important to determine if rats would attend to the tastes of these various solutions and adjust their consumption of them. Therefore, we performed 2 pilot studies to determine if rats were equally accepting of a range of concentrations of (1) water + MgT, and (2) SAC + MgT. Moreover, in a 3rd pilot study we attempted to evaluate the extent to which our animals could distinguish between the tastes of SAC-only vs. SAC + MgT by creating a CTA to SAC only and then assessing their consumption of both solutions. Finally, we wanted to assess the baseline consumption of SAC + MgT solutions with the goal of determining if this baseline was similar/different from the SAC-only baseline consumption we established in our previous studies (see for example, Mickley et al., 2009).

In the first pilot study, 23-hour fluid-deprived naïve male Sprague–Dawley rats (N = 8/group) were given either water + MgT (10 mg/ml) or water + MgT (16 mg/ml) on two successive days. These concentrations represent the lowest and highest concentrations of MgT that we employed in our main experiment. The animals drank approximately equal volumes of each [water + MgT (10 mg/ml) = 19.67 ± 0.82 ml (Mean ± SEM); water + MgT (16 mg/ml) = 20.81 ± 0.85 ml (Mean ± SEM)] — indicating no preference for the taste of either solution. Likewise, the average water-only drinking over 2 days [19.50 ± 3.09 ml (Mean ± SEM)] was not significantly different from the water + MgT averages (see above).

This first pilot study confirmed that rats will drink essentially equal amounts of two water + MgT solutions (10 mg/ml and 16 mg/ml) and water alone. Thus MgT, in the concentrations employed in this study, appeared to be neither aversive nor more desirable to our animals than was water alone. This gave us some confidence that if MgT was combined with another more-distinctive tastant (i.e., SAC) it might not alter the salience of that stimulus (see second pilot study described below). Further, since we needed to adjust the concentration of our MgT solutions as the study progressed (in order to continue to deliver the desired dose to our growing animals throughout the main experiment described below), this reassured us that the rats would not find