Erythrocyte intracellular Mg^{2+} concentration as an index of recognition and memory

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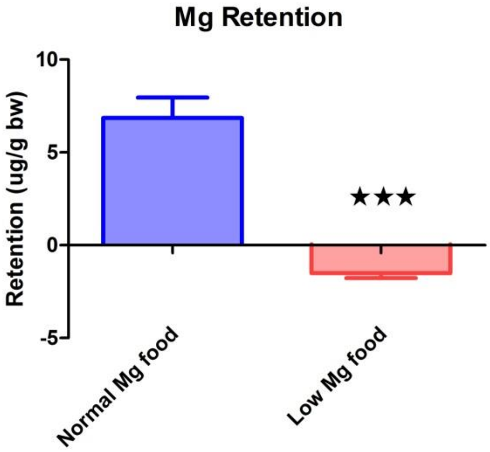
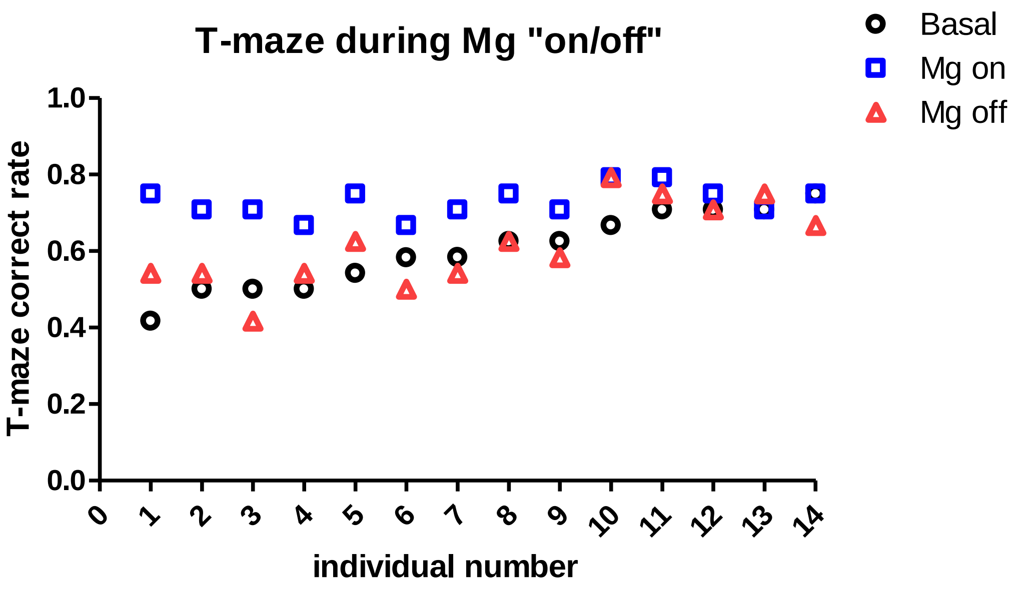
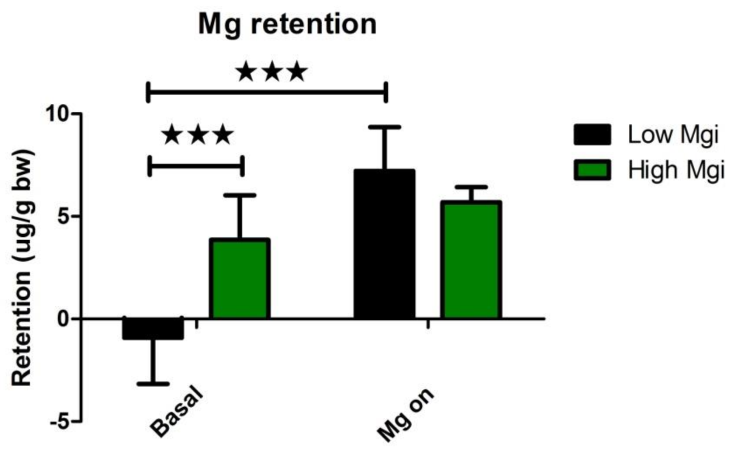
Supplementary information



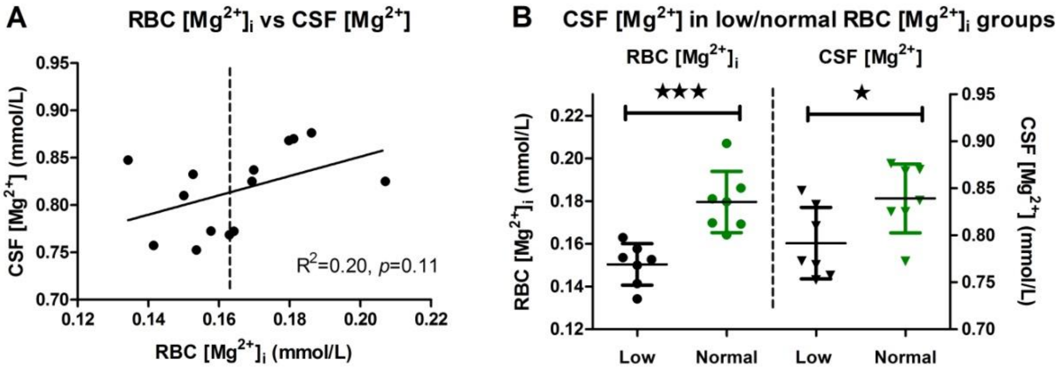
Supplementary Figure S1: Mg^{2+} intake deficiency induced a decrease in body Mg retention. The Mg retention (method see below) in low Mg^{2+} diet group dramatically dropped from positive to a negative value, indicating a Mg^{2+} loss from body.



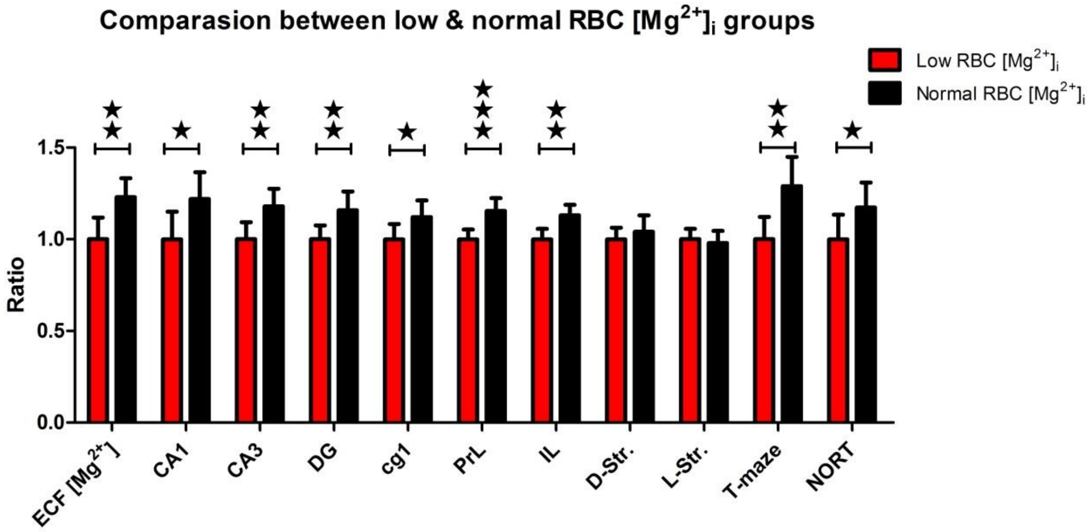
Supplementary Figure S2: Administration of MgT showed less effect on aged rats with higher basal memory status. The x-Axis represents individual rat numbers, which were arranged in ascending order based on their basal values (black).



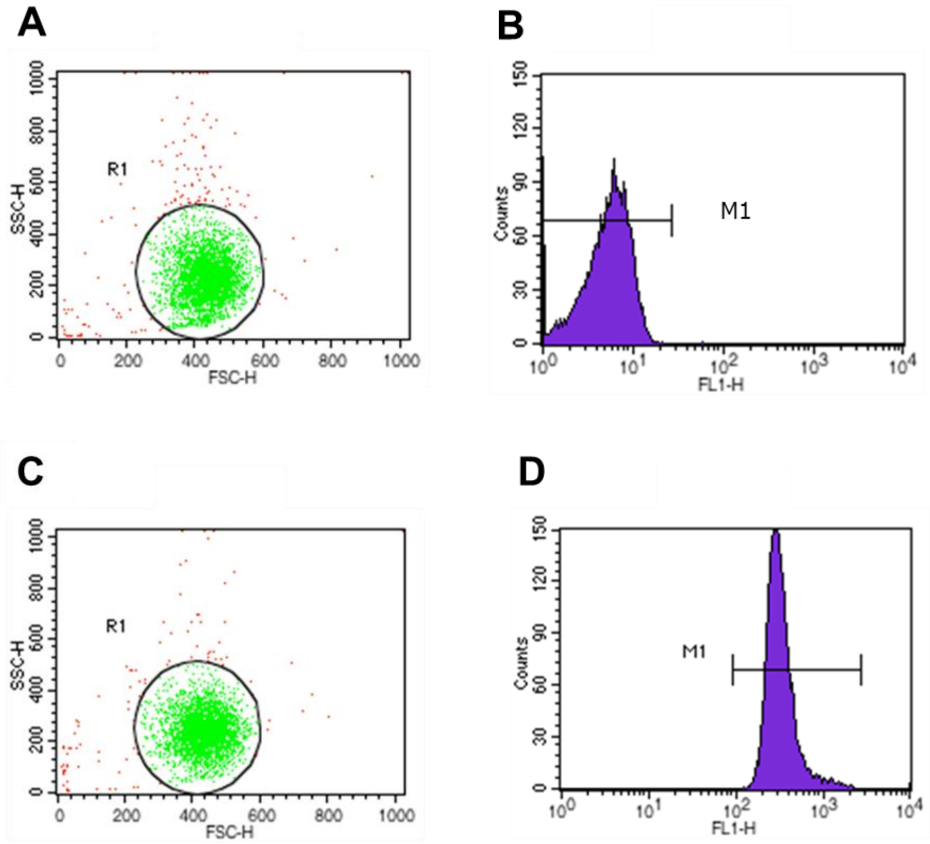
Supplementary Figure S3: Effect of MgT treatment on body Mg retention. Rats were divided into low and normal groups according to their basal RBC $[Mg^{2+}]_i$ values**.** During MgT treatment, RBC $[Mg^{2+}]_i$ was regulated by body Mg retention efficiency. Only Mg retention in the low RBC $[Mg^{2+}]_i$ group showed strong response to MgT treatment. Data are presented as mean ± SD. Two-way ANOVA was followed by Bonferroni's *post hoc* test, ***p<0.001.



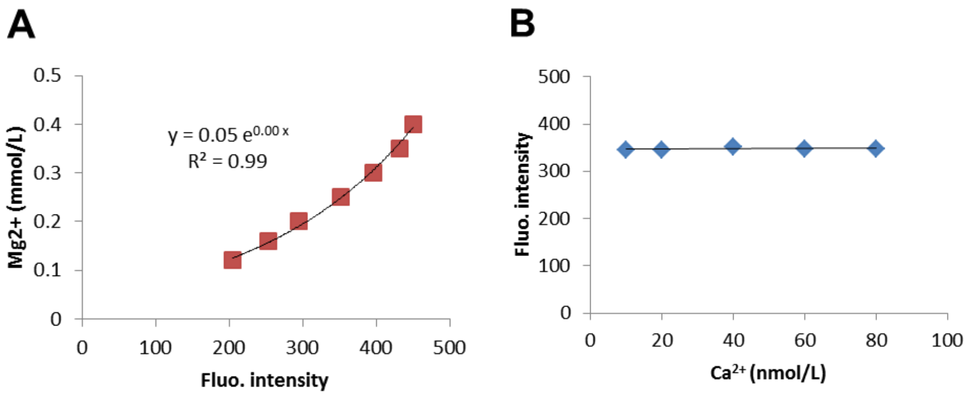
Supplementary Figure S4: Correlation between RBC $[Mg^{2+}]_i$ and CSF $[Mg^{2+}]$. (A) Linear correlation analysis between RBC $[Mg^{2+}]_i$ and CSF $[Mg^{2+}]$ in aged rats (n=14) (dotted line, rats were divided into normal and low groups according to their RBC $[Mg^{2+}]_i$ values). (B) Comparison of CSF $[Mg^{2+}]$ based on individual RBC $[Mg^{2+}]_i$ levels. Unpaired t test, *$p<0.05$, ***$p<0.001$.



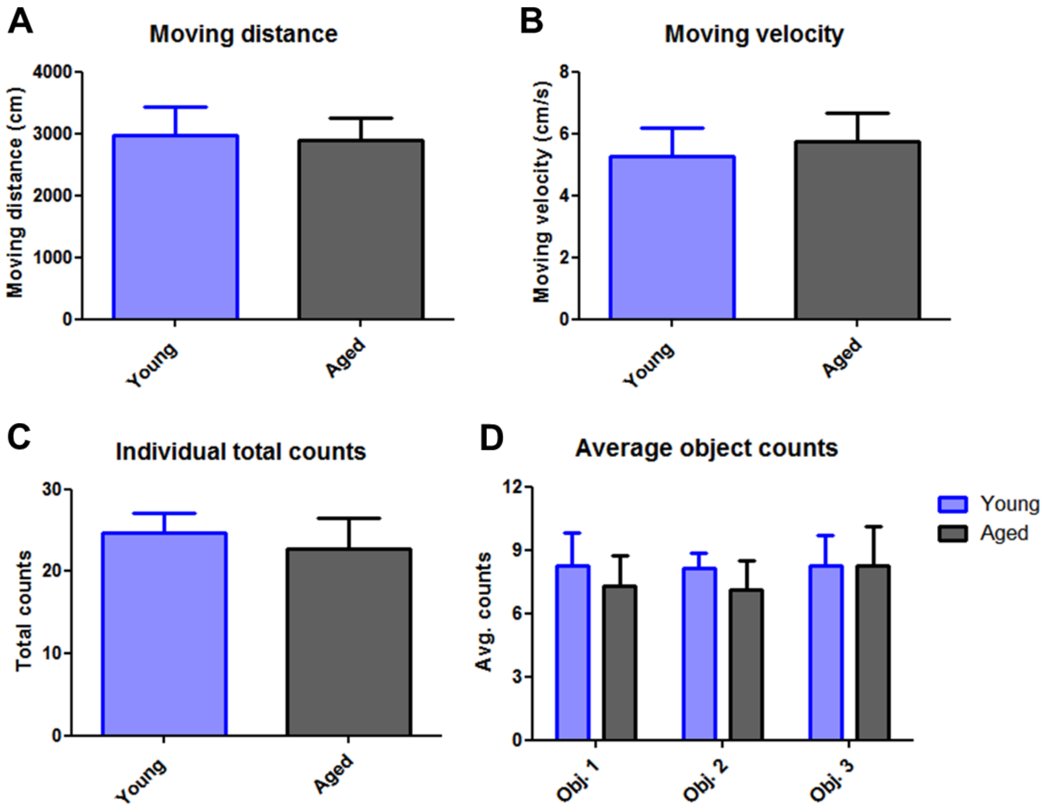
Supplementary Figure S5: Differentiation of [Mg^{2+}], synapse density, and memory levels based on individual RBC [Mg^{2+}]$_i$ values. Significant differences between low RBC [Mg^{2+}]$_i$ group and normal RBC [Mg^{2+}]$_i$ group were shown. Data are presented as mean ± SD. Unpaired t test, **p*<0.05, ***p*<0.01, ****p*<0.001.



Supplementary Figure S6: The flow cytometry images of Mag staining in erythrocyte. (A) The forward scatter (FSC)/side scatter (SSC) image of an erythrocyte sample incubated without Mag dye (control). (B) The FL1/counts image of the control. (C) and (D), the FSC/SSC and FL1/counts images of the same erythrocyte sample incubated with Mag dye loading.



Supplementary Figure S7: The calibration of MaG fluorescence intensity. (A) A fitted curve was built using fluorescence intensity as x-axis and standard [Mg^{2+}] gradient as y-axis. The real RBC $[Mg^{2+}]_i$ in each sample was calculated by inputting the fluorescence intensity value to the fitting formula. (B) The influence of $[Ca^{2+}]_i$ changes to MaG fluorescence was evaluated by incubating the erythrocytes with [Ca^{2+}] gradient (similar as $[Mg^{2+}]_i$ calibration, with fixed $[Mg^{2+}]_i$ = 0.25mM) and comparing the related fluorescence. There was not Ca^{2+} influence in erythrocyte at physiological level.



Supplementary Figure S8: Rats did not show age related deficits in motor abilities, explorative abilities and object preference. The total moving distance (A) and velocity (B) were measured in the habituation phase. There were no differences between young and aged rats (the same rats as Fig. 2~6; n=7/group). (C) In the sample phase, the total counts of all the 3 objects for each rat were calculated. There were no differences between young and aged rats, suggesting similar explorative abilities between the 2 groups. (D) Also in the sample phase, the number of counts for each object was compared. There were not significant differences among the average counts of the 3 objects, both in young and aged groups. It suggested that there was not an object preference which may interfere the experiment (rats explore one object much more/less than the other objects in the sample phase).

Mg retention procedure:

To assess Mg retention, animals were individually housed in metabolic cages. Animals received either normal food or Mg deficient food, with either deionized water or water containing MgT. After 3 days of habituation, urine and fecal pellets from each rat were collected on a daily basis over a continuous five-day-period. Food in each cage was weighed every day to calculate the daily Mg intake. Rats were weighed. The urine and fecal pellets were analyzed for Mg content using ICP-AES. The percentage of retention was estimated using the following equation:

$$\text{Mg Retention} = \frac{\text{Mg}_{\text{intake}} - \text{Mg}_{\text{feces}} - \text{Mg}_{\text{urine}}}{\text{Body weight}} \qquad \text{Supplementary Equation S (1)}$$

Supplementary Table S1: Mg detection in tissues

Research field	Species	Assay tech.*	Liver	Kidney	Heart	Muscle	Muscle ionized	Brain	Brain ionized	Skeleton	Intestine	Reference
Alzheimer's Disease	Human	ICP-AES						√				1
Congestive heart failure	Human	AAS			×	×						2
Genetic low Mg, Mg deficient food	Mouse	AAS		√						√		3
Hypertension, Aging	Human	NMR					√		√			4
Induced hypertension	Rat	AAS	×	√	√			√				5
Mg deficient food	Rat	AAS	×	×	×	×		×		√		6
Mg deficient food	Rat	MAPP	×	×	×	×		×		√	√	7
Migraine	Human	NMR							√			8
Preeclampsia	Human	NMR					√		√			9
Traumatic brain injury	Rat	NMR							√			10

√ and ×show the Mg indexes that have been tested in each research. √ means the Mg index effectively responding to the modification of experimental conditions. ×means no response.

*, please see abbreviations as below:

AAS, atomic absorption spectroscopy; ICP-AES, inductively coupled plasma atomic emission spectrometry; MAPP, Magnesium ammonium phosphate precipitate; NMR, nuclear magnetic resonance.

Supplementary Table S2: Mg detection in blood cells

Research field	Species	Assay tech.*	CSF	Urine	Serum	Plasma	Plasma ionized	RBC ionized	RBC membrane	RBC total	Lymphocyte total	Lymphocyte ionized	Platelet total	24h retention	Reference
Aging, hypertension, diabetes	Human	NMR						√							11
Angina	Human	CC, AAS		√	×					√	√			√	12
Anxiety/stress	Human	AES		√											13
Congestive heart failure	Human	AAS			×						×				2
Diabetes	Human	NMR						√							14
Genetic low Mg, Mg deficient food	Mouse	AAS		√		√				√					3
Hemodialysis	Human	AAS, ISE, FP				√	√			√	√	√			15
Hypertension	Human	AAS				×			√	×					16
Hypertension	Human	FP										×			17
Hypertension	Human	NMR				×		√							18
Hypertension	Human	NMR						√							19
Hypertension	Human	NMR						√							20
Hypertension	Human	FP										×			21
Hypertension, insulin inducement	Human	AAS, NMR						√		√					22
Hypertension, obesity	Human	DCPS				×				√			√		23

Research field	Species	Assay tech.*	CSF	Urine	Serum	Plasma	Plasma ionized	RBC ionized	RBC membrane	RBC total	Lymphocyte total	Lymphocyte ionized	Platelet total	24h retention	Reference
Hypomagnese-mia	Human	AAS			√						×				24
Induced hypertension	Rat	AAS								√					5
Insulin inducement	Human	NMR						√							25
Maternal and cord blood	Human	NMR, ISE					√	√							26
Mg deficient food	Rat	AAS				√									6
Mg deficient food	Rat	MAPP			√										7
Mg deficient food	Rat	CC				√									27
Mg deficient food	Calve	AAS	√			√				√					28
Mg deficient food	Rat	Isotope, ICP-MS				√	√			√	×		×		29
Migraine	Human	AAS, FP				×				√	×	√			30
Oral Mg treat	Rat	CC	√												31
Sleep deprivation	Rat	AAS, ISE				×	√								32

√ and × show the Mg indexes that have been tested in each research. √ means the Mg index effectively responding to the modification of experimental conditions. × means no response.

*, please see abbreviations as below:

AAS, atomic absorption spectroscopy; AES, atomic emission spectrometry; CC, calmagite chromometry; DCPS, direct current plasma spectrometer; FP, fluorescence probe; ICP-MS, inductively coupled plasma mass spectrometry; ISE, ion-selective electrode; MAPP, Magnesium ammonium phosphate precipitate; NMR, nuclear magnetic resonance.

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