

The protective role of Bax inhibitor-1 against chronic mild stress through the inhibition of Monoamine oxidase-A

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Supplementary Information

Forced Swimming Test

Each animal was placed in a beaker (18 cm diameter, 24 cm depth) filled with water (temperature 25-26°C) to a height of 16 cm. Immobility (floating with only small movements to keep balance) and swimming were scored throughout the last 4 minutes of the 6-minute test period. A trained observer who was blind to the treatment paradigm conducted the scoring.

Determination of target mRNA by semi-quantitative reverse transcriptase-PCR (RT-PCR)

Total RNA from treated mouse brain tissue was prepared with TRIZOL reagent according to the manufacturer's protocol and digested with RNase-free DNase to clear residual genomic DNA. First strand cDNA was reverse-transcribed from 1 µg of total RNA using oligo-(dT) (SuperScriptTMIII First-Strand Synthesis System, Invitrogen). The sequences of the primers used for RT-PCR were as follows: mouse MAO-A: 5' TCA ATG TAG CCA CTC CAC TGT 3' (forward); 5' TTG GGG ATA AAG TGA AGC TGA 3' (reverse); mouse MAO-B: 5' GTG GTA TGT GAA GCA GTG TGG 3' (forward); 5' TCA GTG CCT GCA AAG AAA ATC 3' (reverse). PCR conditions were as follows: 1 cycle of 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 54°C for 45 sec, and 72°C for 90 sec with a final cycle of 72°C for 10 min. Ten µL of PCR products were separated on a 1% agarose gel in 1X TAE (40 mM Tris, pH 8.0, 20 mM acetic acid, 1 mM EDTA) buffer and visualized using ethidium bromide staining.

Supplementary Figure Legends

Supplementary Figure 1. Experimental design for chronic mild stress.

Supplementary Figure 2. Body weight of the four experimental groups over the 6 weeks of chronic mild stress. BI-1, Bax inhibitor.

Supplementary Figure 3. Effects of chronic mild stress in the forced swimming test.

Swimming time during the forced swimming test was analyzed in BI-1^{+/+} and BI-1^{-/-} mice after 2- and 6-weeks of chronic mild stress.

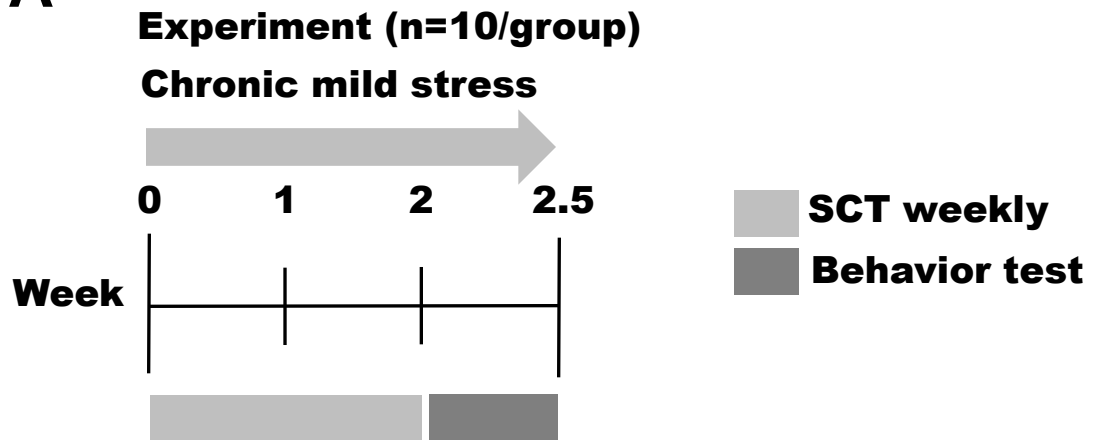
Supplementary Figure 4. Effects of chronic mild stress on MAO-A and MAO-B RNA and protein expression. (A) RT-PCR from BI-1^{+/+} and BI-1^{-/-} mouse brain RNA was performed after 2- and 6-weeks of chronic mild stress. (B) Immunoblots from BI-1^{+/+} and BI-1^{-/-} mouse brain lysates were performed with anti-MAO-A, -B, and β-actin antibody after 2- and 6-weeks of chronic mild stress. Although cropped blots were used, the gels were run under the same experimental conditions.

Supplementary Table 1. Spontaneous locomotor activities after 2- and 6-weeks of chronic mild stress.

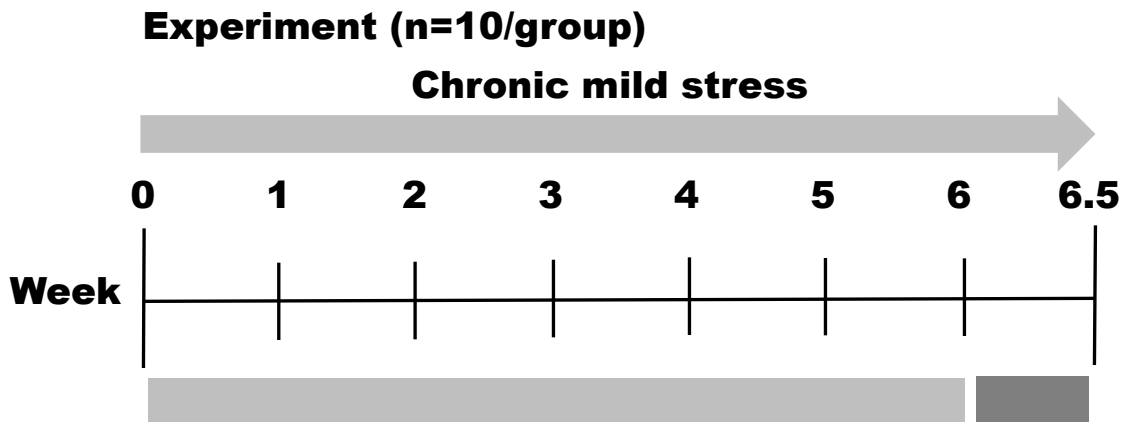
Supplementary Table 2. Analysis of serotonin, norepinephrine, and dopamine.

Supplementary Fig. 1

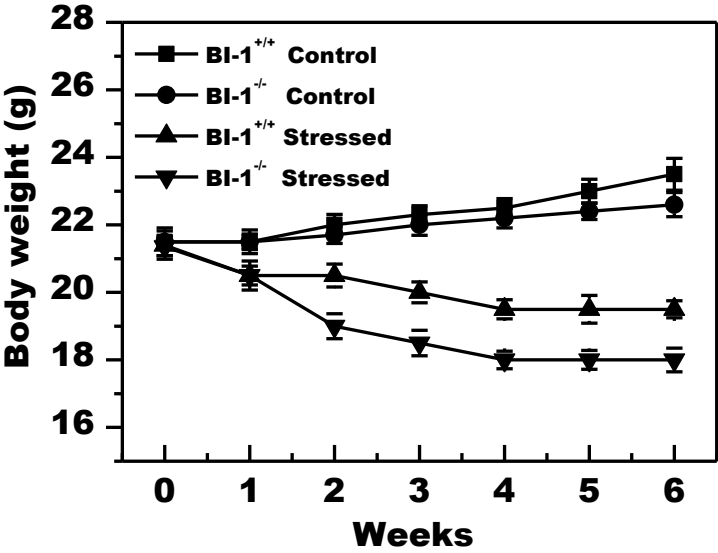
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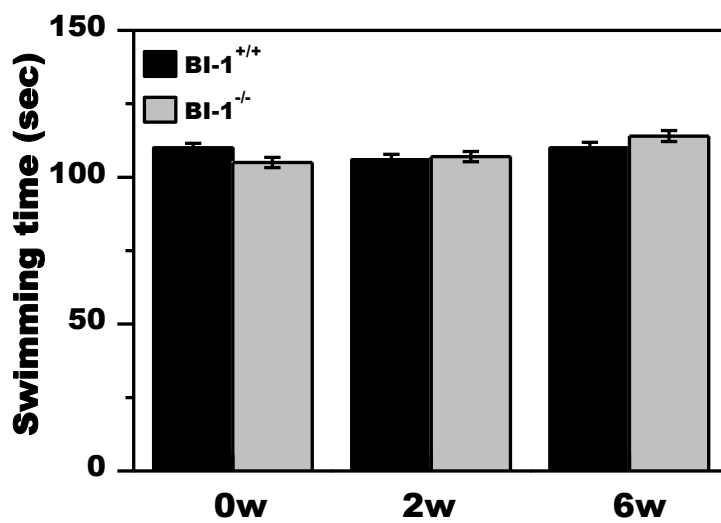
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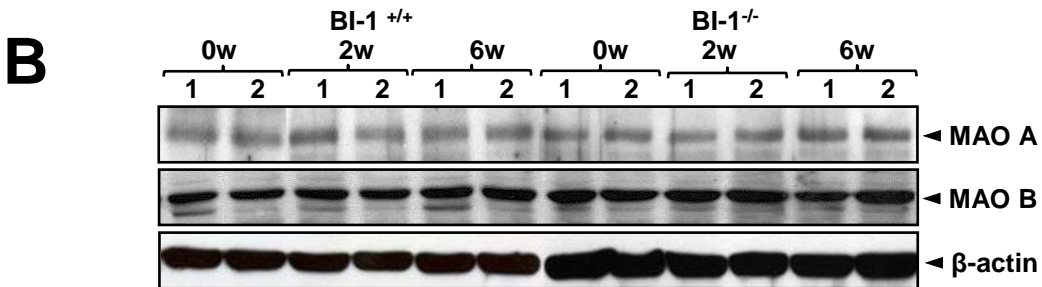
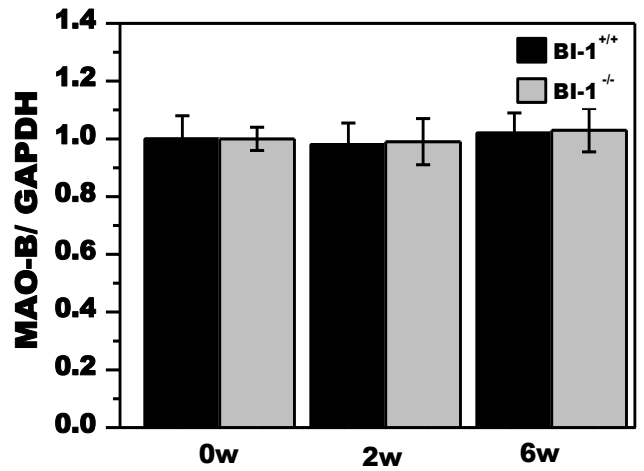
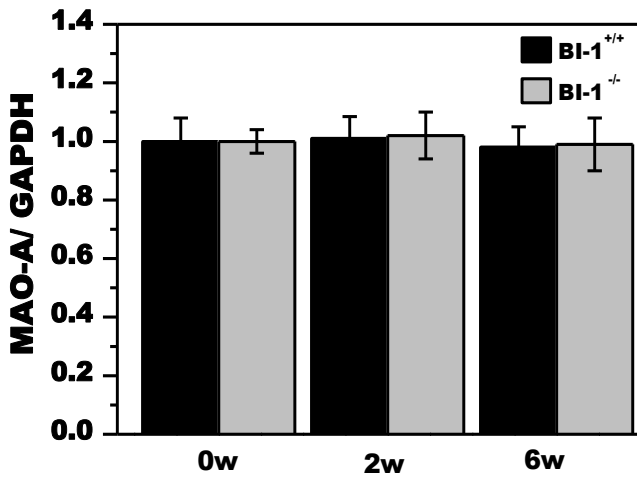
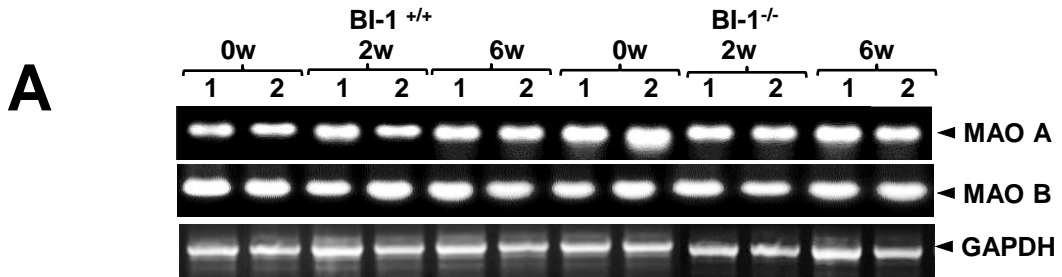
Supplementary Fig. 2



Supplementary Fig. 3



Supplementary Fig. 4



Supplementary Table. 1

Spontaneous locomotor activities after 2- and 6-week treatment of chronic mild stress

	Group	Distance traveled (cm)	Locomotor time (seconds)
2weeks	BI-1 ^{+/+} con	20802.485 ± 3188.00039	1752.23 ± 369.36238
	BI-1 ^{-/-} con	21203.425 ± 2956.20839	1928.2 ± 333.85052
	BI-1 ^{+/+} stressed	26777.88 ± 5580.03368	2159.9 ± 250.88011
	BI-1 ^{-/-} stressed	26063.55 ± 4897.95897	2381.1 ± 246.21458
6weeks	BI-1 ^{+/+} con	37613.96 ± 6188.00039	2926.8 ± 469.36238
	BI-1 ^{-/-} con	35426.59 ± 8580.03368	2880.8 ± 523.85052
	BI-1 ^{+/+} stressed	49455.35 ± 2956.20839	4030.8 ± 486.88011
	BI-1 ^{-/-} stressed	63369.68 ± 4897.95897	4846.2 ± 506.21458

Supplementary Table 2

	Group	Serotonin	Norepinephrine	Dopamine
2weeks	BI-1 ^{+/+} con	3055.48 ± 136.32	4562.37 ± 95.26	5294.31±133.92
	BI-1 ^{-/-} con	3041.19 ± 100.88	4466.64 ± 143.34	5030.15±142.91
	BI-1 ^{+/+} stressed	3235.73 ± 199.82	4442.6 ± 52.28	5374.18±103.01
	BI-1 ^{-/-} stressed	3008.47 ± 102.45	4518.48 ± 155.57	5105.76±153.55
6weeks	BI-1 ^{+/+} con	3183.52 ± 120.76	4260.54 ± 155.59	5298.43±182.58
	BI-1 ^{-/-} con	3270.71 ± 107.78	4325.60 ± 129.75	5103.36±161.44
	BI-1 ^{+/+} stressed	3282.94 ± 126.58	4614.93 ± 131.52	5347.47±144.50
	BI-1 ^{-/-} stressed	2627.56 ± 108.31	4053.03 ± 134.13	4370.94±129.74