Supplementary Data

Supplementary Methods

Open Field Assay

The open field assay was performed described before with slight modifications [1]. Briefly, mice were placed into the open field test chamber (40 × 40 cm, Med Associates, inc.) and the total distance animals travelled for 10 minutes was recorded and analyzed by Activity Monitor 5 software (Med Associates, inc.). The central zone was defined as a 24 x 24 cm square in the middle of test chamber.

Rotarod Assay

The rotarod assay was performed as described before with slight modifications [2]. Mice were briefly trained to maintain their position on the rotarod apparatus (TSE Systems, Inc.) before the test session. The training session consisted of a 5 minutes interval with an initial speed of 10 rounds per minute (rpm) for 110 seconds. The rotarod then accelerated linearly from 10 to 20 rpm in 80 seconds and the rod kept rotating with 20 rpm for another 110 seconds. Sixty minutes after the training session, mice were placed on the rotarod at an initial speed of 4 rpm. The test session consisted of a 7 minutes interval, during which the rotarod accelerated linearly from 4 to 60 rpm. The latency and rotation speed at which an animal fell off the rod was recorded automatically by an infrared beam located below the rotating rod.

Forced Swim Assay

The forced swim assay was performed as previously described [3]. Mice were placed individually in a transparent glass cylinder containing water (24 cm high, 14.5 cm diameter, 14 cm water depth) at 23-25°C and forced to swim. Mice were videotaped for 6 minutes, and the immobility time (time spent passively floating) was recorded for the last 4 minutes, after

discarding activity in the first 2 minutes during which an animal tries to escape. ANY-MAZE software was used to record and analyze immobility (Stoelting Co.).

Tail Flick Assay

The tail flick assay was performed as described before [4]. In brief, acute nociceptive response was measured by using an electronically controlled tail-flick analgesimeter (UGO Basile Biological Research Apparatus, 7360 Tail Flick) that integrated both a thermal nociceptive stimulus and an automated response timer. A thermal stimulus (focused light from a 20W infrared bulb as the heat source) was applied to the tips of mice tails. The time from onset of stimulation to a rapid flick or withdrawal of the tail from the heat source was recorded as tail flick latency. A maximum of 22 seconds was set as a cut off time to prevent tissue damage to the animals.

Supplementary Figures

Figure S.1. Effect of prenatal methionine on body weight in male offspring

(a) Body weight of the male offspring from age 4 weeks to 11 weeks (n = 23-25). Two way ANOVA revealed no significant drug effect ($F_{1,46} = 1.074$, $P = 0.3055$). Data are presented as means±S.E.M.

(b) Body weight of the male offspring at age 14 weeks ($n = 21$). Unpaired student test ($t = 0.2026$, $P = 0.8405$) revealed no significant drug effect. Data are presented as means±S.E.M.

Figure S.2. Effect of prenatal methionine overload in male offspring assessed by the open field, the rotarod, the tail flick and the forced swim assays

(a) Distance mice travelled in the central area of the open field box for the first 5 and 10 minutes (*n* = 12). Unpaired student test (5 min: t = 0.5913, *P* = 0.5604; 10 min: t = 0.7543, *P* = 0.4587) revealed no significant drug effect: N.S., not significant. Data are presented as means±S.E.M.

(b) Latency to fall off the rotarod in the rotarod assay (*n* = 12). Unpaired student test (t = 0.4226, *P* = 0.6767) revealed no significant drug effect: N.S., not significant. Data are presented as means±S.E.M.

(c) Nociceptive response of tail flick latency in the tail flick assay (*n* = 12). Unpaired student test (t = 1.010, *P* = 0.3235) revealed no significant drug effect: N.S., not significant. Data are presented as means±S.E.M.

(d) Immobile time in the forced swimming assay (*n* = 23-25). Unpaired student test (t = 0.3805, *P* = 0.7053) revealed no significant drug effect: N.S., not significant. Data are presented as means±S.E.M.

Figure S.3. Effect of prenatal methionine on brain weight in male offspring.

(a) Whole brain weight of the male offspring at age 14 weeks (*n* = 21). Unpaired student test (t = 4.710, *P* < 0.0001): SAL vs MET, ****P* < 0.001. Data are presented as means±S.E.M.

(b) The brain to body weight ratio of the male offspring at age 14 weeks (*n* = 21). Unpaired student test (t = 2.241, *P* = 0.0306): SAL vs MET, **P* < 0.05. Data are presented as means±S.E.M.

Supplementary tables

Table S.1. Upregulated genes of MET mice in the mRNA microarray analysis

Table S 2. Downregulated genes of MET mice in the mRNA microarray analysis

Supplemental Reference

1. Lipkind, D., et al., *New replicable anxiety-related measures of wall vs center behavior of mice in the open field.* J Appl Physiol (1985), 2004. **97**(1): p. 347-59.
2. Duangdao, D.M., et al., *Behavioral phenotyping of neuropeptide S receptor knockout mice.* Behav Brain Res, 2009. **205**(1): p. 1-9.
3. Can, A., et al., *The mouse forced swim test.* J Vis Exp, 2012(59): p. e3638.
4. Zhang, Y., et al., *A novel analgesic isolated from a traditional Chinese medicine.* Curr Biol, 2014. **24**(2): p. 117-23.

Figure S.1.

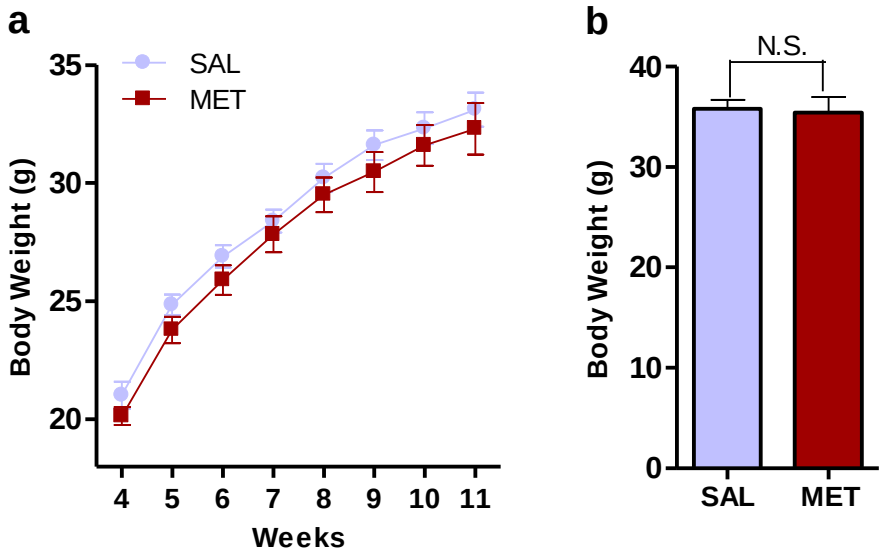


Figure S.2.

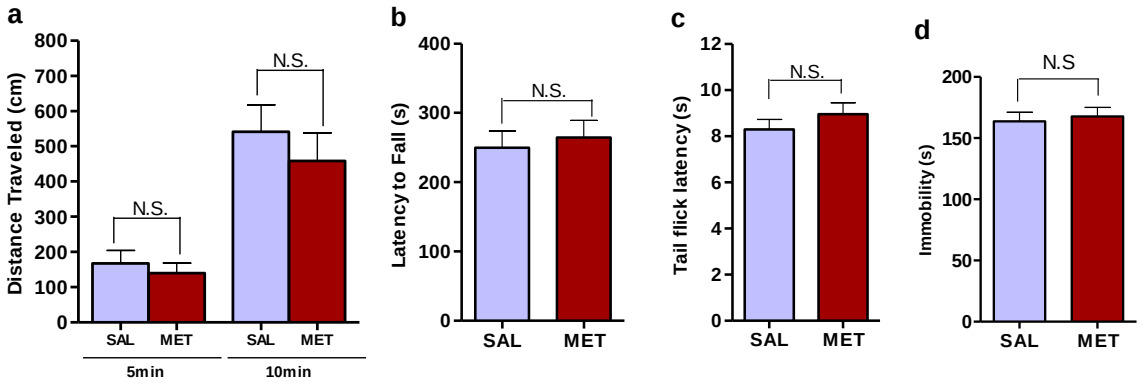
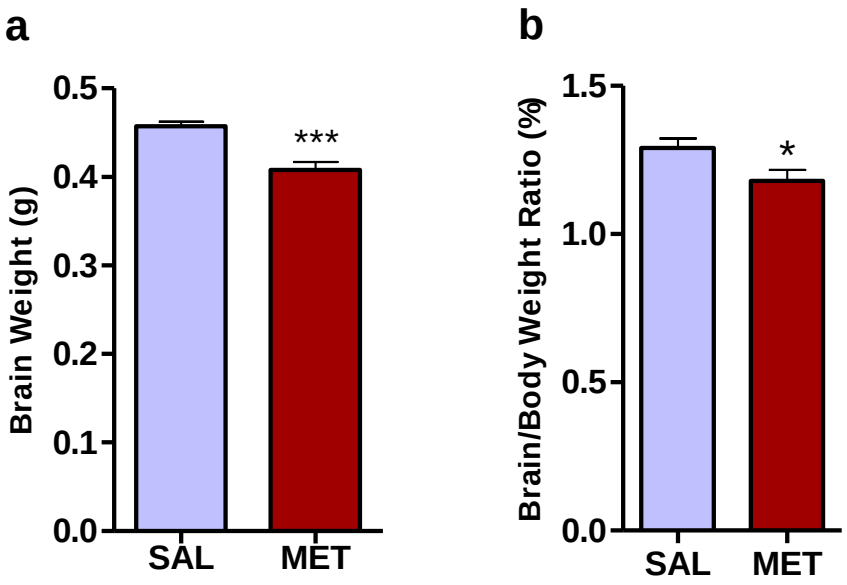


Figure S.3.


a
Brain Weight (g)
0.5
0.4
0.3
0.2
0.1
0.0
***
SAL
MET
b
Brain/Body Weight Ratio (%)
1.5
1.0
0.5
0.0
*
SAL
MET