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Supporting Information

An isotope dilution based-targeted and non-targeted carbonyl neurosteroid/steroid profiling

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Content

Table S-1. The seed file for data processing using SIEVE.

MZ	RTStart	RTStop	Description
334.274	14.7	15	3β,5α-THPROG
338.299	14.7	15	d4-3β,5α-THPROG
334.274	15	15.5	3α,5α-THPROG
338.299	15	15.5	d4-3α,5α-THPROG
347.269	14.2	14.8	5α-DHPROG
353.307	14.2	14.8	d6-5α-DHPROG
345.254	14.2	14.6	Progesterone
354.310	14.2	14.6	d9-Progesterone
332.258	14.4	14.7	Pregnenolone
336.284	14.4	14.7	d4-Pregnenolone

Table S-2. The effects of extraction buffers to the measurement of five neurosteroids using ID-TNT-carbonyl NS profiling. Each condition was tested in triplicate.

The whole brains from three adult C57BL/6J mice (male, 4-6 months) were homogenized in methanol (0.5 ml / 100 mg tissue) in the presence of internal standards. The homogenate was divided into 3 parts. For each part, the composition of extraction buffer was adjusted to either 75% methanol/25% water, 1% acetic acid in methanol, or 1.8% formic acid in methanol. Each homogenate is further homogenized before subjecting to the sample preparation procedure described above. Each condition was tested in triplicate.

(pmol/g tissue)	MeOH:water 75:25 v/v	MeOH:acetic acid 99:1 v/v	MeOH:formic acid 98.2:1.8 v/v	P value (ANOVA)
3α,5α-THPROG	6.5 ± 0.7	10.5 ± 3.5	5.0 ± 0.0	<0.01
3β,5α-THPROG	7.0 ± 0.0	8.5 ± 0.7	3.5 ± 0.7	<0.01
5α-DHPROG	33.5 ± 0.7	22.0 ± 0.0	21.0 ± 4.2	<0.01
PROG	13.0 ± 0.0	12.0 ± 1.4	11.0 ± 1.4	0.18
PREG	10. 0± 1.4	14.0 ± 1.4	24.0 ± 7.1	<0.01

Table S-3. **A cross method comparison between ID-TNT-NS profiling and GC-MS analysis.** Two samples (one from extracts of whole brains of control mice, and the other further spiked with 5pmol/g of 3α,5α-THPROG, and 3β,5α-THPROG) were analyzed using ID-TNT-NS based LC-MS, or GC-MS method. Both systems were calibrated with the calibrators carefully prepared from the same source by the same operator.

Method	Neurosteroid/steroid	Sample A (pmol/g tissue)	Sample B (Sample A + 5pmol/g tissue spike-in)	% Recovery
ID-TNT-carbonyl NS profiling	3α,5α-THPROG	8.0 ± 0.3	12.1 ± 0.3	93
	3β,5α-THPROG	18.1 ± 0.9	22.8 ± 0.4	99
GC-MS	3α,5α-THPROG	9.4 ± 1.4	12.9 ±2.2	90
	3β,5α-THPROG	20.3 ± 0.8	25.5 ± 1.6	101

Figure S-1. The effects of acute stress on cortex neurosteroids/steroids levels.

The neurosteroid/steroid profiles of cortex from adult mice (n = 4) subjected to a single exposure to raised platform for 10 min., or control animals, were analyzed using ID-TNT-carbonyl NS profiling.

A. The box-and-whisker plots of five targeted neurosteroids/steroids in the cerebellum, cortex, hippocampus, and hypothalamus from the control (clear) and stressed mice (gray). *< 0.05 (independent t test). The plots reveal that stress produced a significant increase of 3α,5α-THPROG, 3β,5α-THPROG and 5α-DHPROG in the cortex and PROG in hippocampus and hypothalamus.

B. The base peak chromatograms of d4-THPROGs, THPROGs, corticosterone/11-deoxycortisol (m/z= 377.243), putative 5α-dihydrodeoxycorticosterone (m/z=363.264), putative tetrahydrodeoxycorticosterone (DHDOC)/hydroxy-THPROG (m/z=350.269) and putative hydroxyprogesterone/deoxycorticosterone (m/z=361.249) in the cortex samples from a control (in gray) and stressed (in red) animals. The y axis is in the same scale for both plots.

C. Semi-quantification of corticosterone/11-deoxycortisol in four brain regions. The plot show that stress increased corticosterone/11-deoxycortisol level in the cortex. *, p<0.01 (t test).

D. A scatter plot of corticosterone/11-deoxycortisol *vs* putative 5α-dihydrodeoxycorticosterone levels. Each of four brain regions from each mouse are displayed. Control and stressed animals are labelled in green and red, respectively. Cere: cerebellum; Hippo: hippocampus, Hypo: hypothalamus. Rho = 0.93, p < 0.001 (Pearson correlation).

Figure S-2. The effect of finasteride on neurosteroid/steroid profiles in the cerebellum, cortex, hippocampus and hypothalamus.

Mice (n=4, each group) were treated with 50mg/kg finasteride (prepared in 20% 2-hydroxypropyl)-β-cyclodextrin in saline), 20% 2-hydroxypropyl)-β-cyclodextrin in saline, or

sham by a single *s.c.* injection. Two hours following the procedure mice were sacrificed and the hypothalamus, cerebellum, cortex and hippocampus dissected from each and neurosteroids were analyzed using the ID-TNT-carbonyl-NS profiling. The expression of five targeted neurosteroids/steroids in hypothalamus tissues from animals treated with finasteride, saline or sham are shown as box whisker plots. *, $p < 0.01$ vs Sham and Saline; **, $p < 0.05$ vs Saline (one-way ANOVA or Kruskal-Wallis test). Note that finasteride treatment drastically increased PREG levels in all brain regions.

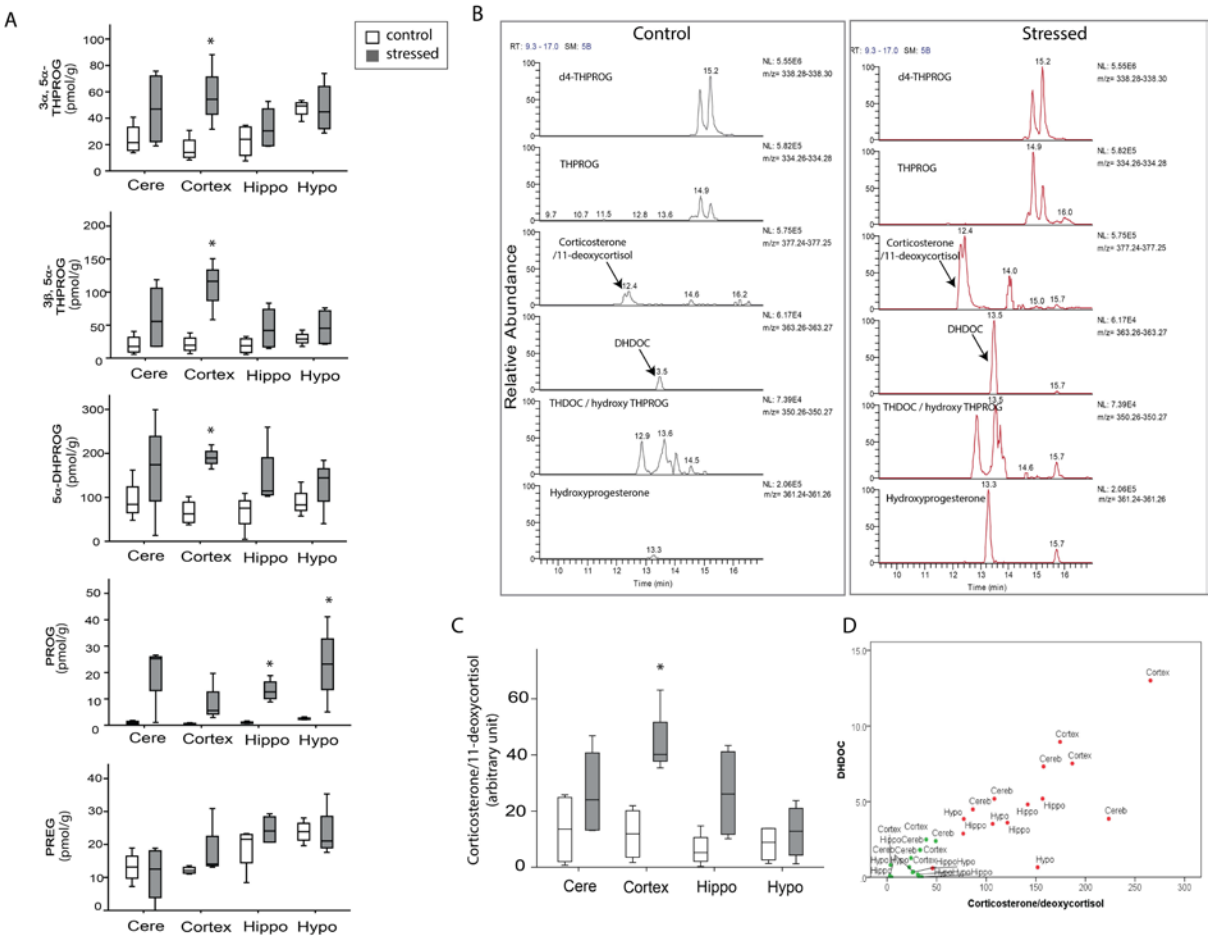
A
control
stressed
3α, 5α-THPROG (pmol/g)
3β, 5α-THPROG (pmol/g)
5α-DHPROG (pmol/g)
PROG (pmol/g)
PREG (pmol/g)
Cere
Cortex
Hippo
Hypo
B
Control
Stressed
Relative Abundance
d4-THPROG
THPROG
Corticosterone /11-deoxycortisol
DHDOC
THDOC / hydroxy THPROG
Hydroxyprogesterone
Time (min)
C
Corticosterone/11-deoxycortisol (arbitrary unit)
D
DHDOC
Corticosterone/deoxycortisol

Figure S-1

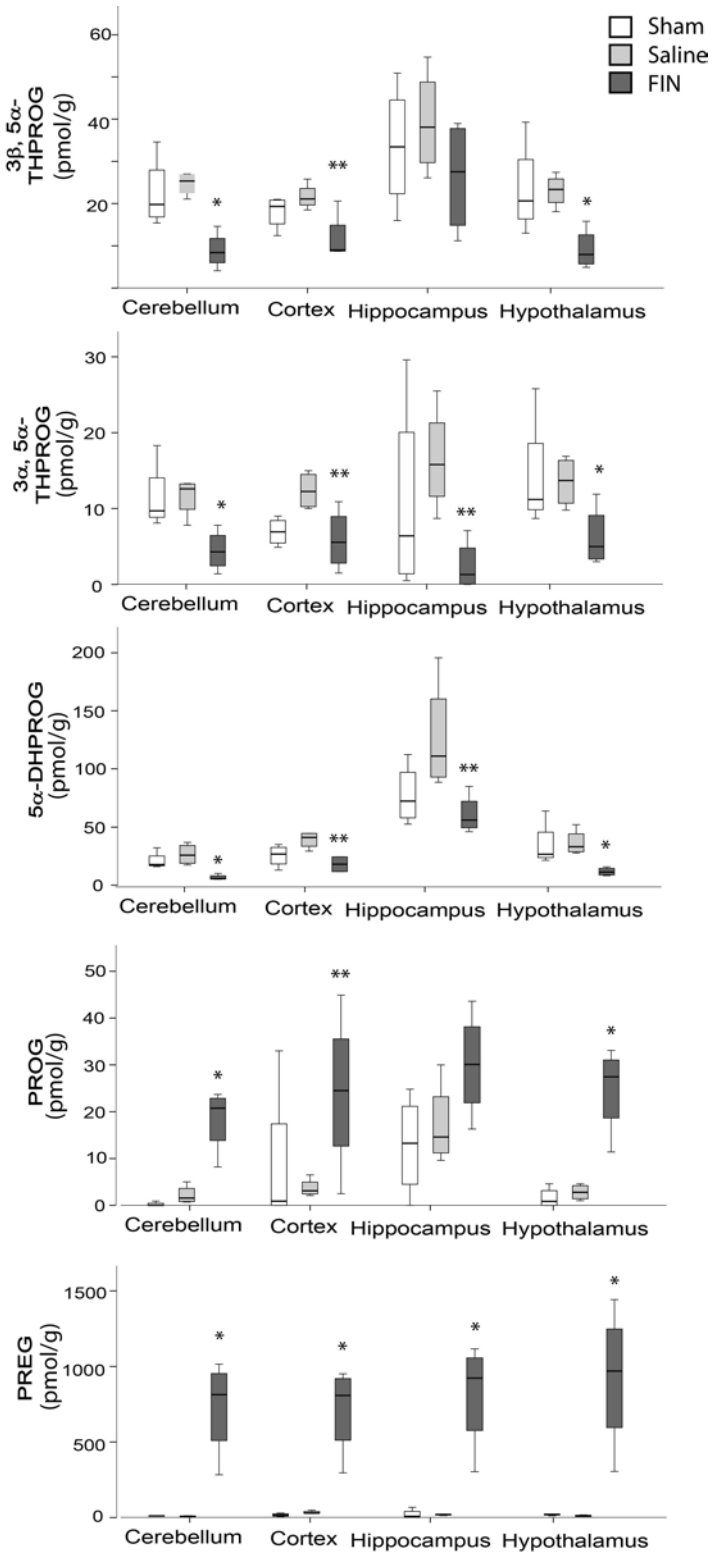
Sham
Saline
FIN
3β, 5α-THPROG (pmol/g)
3α, 5α-THPROG (pmol/g)
5α-DHPROG (pmol/g)
PROG (pmol/g)
PREG (pmol/g)
Cerebellum
Cortex
Hippocampus
Hypothalamus

Figure S-2