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

Sharp, Sheila; Mitchell, Scott; Vallée, Monique; Kuzmanova, Elena; Cooper, Michelle; Belelli, Delia

*Published in:*
Analytical Chemistry

*DOI:*
10.1021/acs.analchem.8b00055

*Publication date:*
2018

*Document Version*
Peer reviewed version

# Supporting Information

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

Sheila Sharp[1], Scott Mitchell[1], Monique Vallée[2], Elena Kuzmanova[1], Michelle Cooper[1], Delia Belelli[1], Jeremy J. Lambert[1], and Jeffrey T.-J. Huang[1*]

[1]School of Medicine, University of Dundee, Dundee, DD1 9SY, UK

[2]Univ. Bordeaux, Neurocentre Magendie, Physiopathologie de la plasticité neuronale, INSERM U1215, F-33000, Bordeaux, France

*Short title: ID-TNT-carbonyl NS profiling*

***Corresponding author:***

*Corresponding author:

Jeffrey T.-J. Huang

Jacqui Wood Cancer Centre

School of Medicine

University of Dundee

Dundee, DD1 9SY

Tel: +44 (0)1382 386901

Email: j.t.j.huang@dundee.ac.uk

**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.

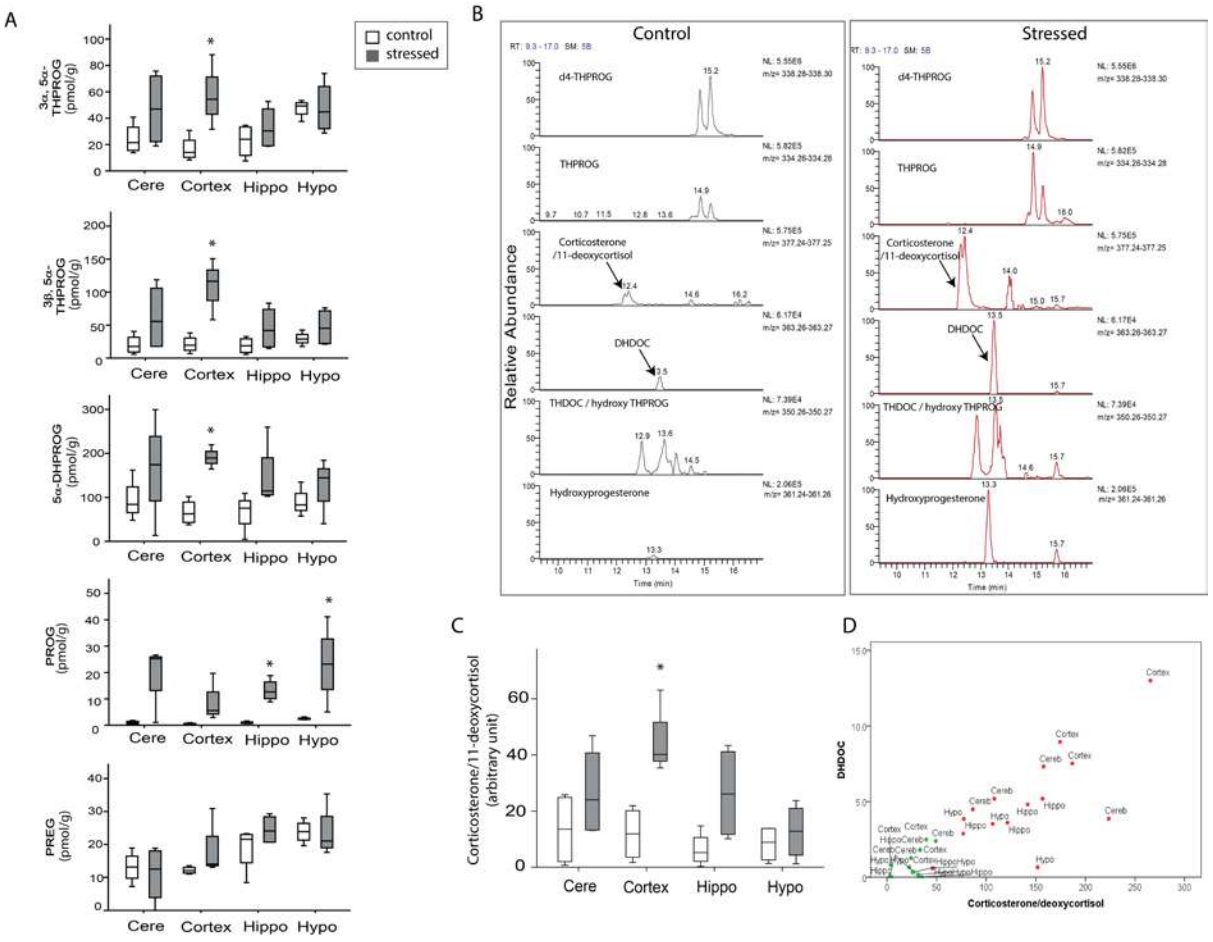
A
control
stressed
3α, 5α-THPROG (pmol/g)
3β, 5α-THPROG (pmol/g)
5α-DHPROG (pmol/g)
PROG (pmol/g)
PREG (pmol/g)
Cere
Cortex
Hippo
Hypo
B
Control
Stressed
Relative Abundance
d4-THPROG
THPROG
Corticosterone /11-deoxycortisol
DHDOC
THDOC / hydroxy THPROG
Hydroxyprogesterone
Time (min)
C
Corticosterone/11-deoxycortisol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

Figure S-1

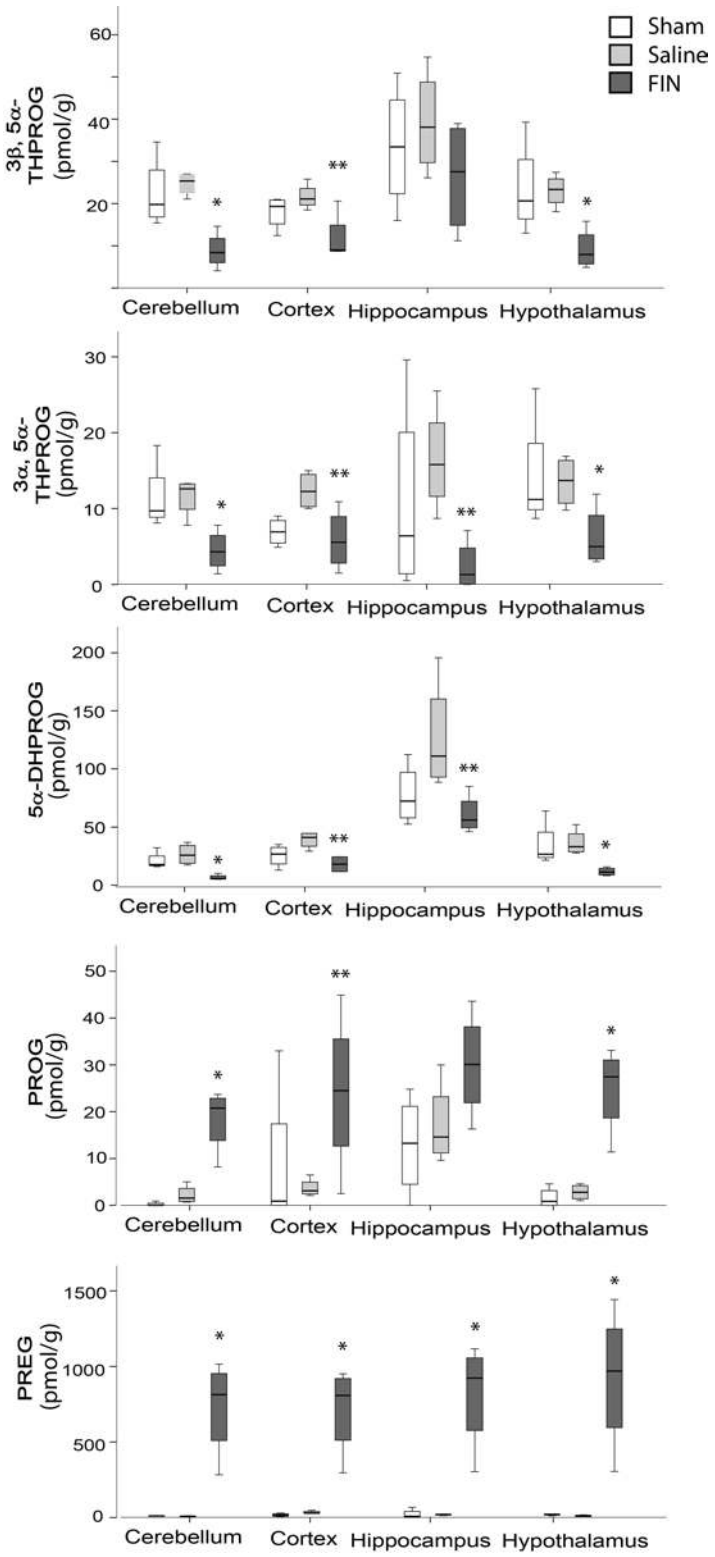
Sham
Saline
FIN
3β, 5α-THPROG (pmol/g)
3α, 5α-THPROG (pmol/g)
5α-DHPROG (pmol/g)
PROG (pmol/g)
PREG (pmol/g)
Cerebellum
Cortex
Hippocampus
Hypothalamus

Figure S-2