

Electronic Supplementary Material

**Sex-specific disruption of murine midbrain astrocytic and dopaminergic developmental trajectories following antenatal GC treatment**

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**Supplemental Table 1:** Results of global 3-way ANOVA for various parameters in the SNc

|                    | Parameter measured      | Source of variation     | F                 | p      |
|--------------------|-------------------------|-------------------------|-------------------|--------|
| SNc                | Nucleus volume          | Sex                     | $F_{1,80}=14.39$  | <0.001 |
|                    |                         | Level                   | $F_{3,80}=388.28$ | <0.001 |
|                    |                         | Treatment               | $F_{2,80}=0.38$   | 0.69   |
|                    |                         | Sex x Level             | $F_{3,80}=14.79$  | <0.001 |
|                    |                         | Sex x Treatment         | $F_{2,80}=9.79$   | 0.002  |
|                    |                         | Treatment x Level       | $F_{6,80}=17.11$  | <0.001 |
|                    |                         | Sex x Level x Treatment | $F_{6,80}=5.85$   | <0.001 |
|                    | GS-IR cell number       | Sex                     | $F_{1,80}=2.71$   | 0.106  |
|                    |                         | Level                   | $F_{3,80}=103.51$ | <0.001 |
|                    |                         | Treatment               | $F_{2,80}=43.78$  | <0.001 |
|                    |                         | Sex x Level             | $F_{3,80}=6.14$   | 0.001  |
|                    |                         | Sex x Treatment         | $F_{2,80}=7.25$   | 0.002  |
|                    |                         | Treatment x Level       | $F_{6,80}=8.42$   | <0.001 |
|                    |                         | Sex x Level x Treatment | $F_{6,80}=3.72$   | 0.004  |
|                    | TH-IR cell number       | Sex                     | $F_{1,80}=7.19$   | 0.009  |
|                    |                         | Level                   | $F_{3,80}=304.00$ | <0.001 |
|                    |                         | Treatment               | $F_{2,80}=1.39$   | 0.257  |
|                    |                         | Sex x Level             | $F_{3,80}=13.42$  | <0.001 |
|                    |                         | Sex x Treatment         | $F_{2,80}=13.54$  | <0.001 |
|                    |                         | Treatment x Level       | $F_{6,80}=14.32$  | <0.001 |
|                    |                         | Sex x Level x Treatment | $F_{6,80}=4.83$   | <0.001 |
|                    | TH-IR cell density      | Sex                     | $F_{1,80}=0.24$   | 0.628  |
|                    |                         | Level                   | $F_{3,80}=0.92$   | 0.439  |
|                    |                         | Treatment               | $F_{2,80}=0.71$   | 0.494  |
|                    |                         | Sex x Level             | $F_{3,80}=0.06$   | 0.980  |
|                    |                         | Sex x Treatment         | $F_{2,80}=1.03$   | 0.362  |
|                    |                         | Treatment x Level       | $F_{6,80}=0.92$   | 0.483  |
|                    |                         | Sex x Level x Treatment | $F_{6,80}=0.21$   | 0.972  |
| GS-IR cell density | Sex                     | $F_{1,80}=1.27$         | 0.264             |        |
|                    | Level                   | $F_{3,80}=1.77$         | 0.165             |        |
|                    | Treatment               | $F_{2,80}=30.81$        | <0.001            |        |
|                    | Sex x Level             | $F_{3,80}=0.53$         | 0.665             |        |
|                    | Sex x Treatment         | $F_{2,80}=0.50$         | 0.611             |        |
|                    | Treatment x Level       | $F_{6,80}=0.93$         | 0.482             |        |
|                    | Sex x Level x Treatment | $F_{6,80}=0.74$         | 0.622             |        |

Global three-way ANOVAs were performed for the substantia nigra pars compacta (SNc) to investigate the effects of antenatal glucocorticoid treatment compared with controls on the counts of cells immunoreactive for tyrosine hydroxylase (TH-IR) and glutamine synthetase (GS-IR), nucleus volumes, TH-IR and GS-IR cell distribution, and the TH-IR and GS-IR cell density.

**Supplemental Table 2:** Results of global 3-way ANOVA for various parameters in the VTA

|                         | Parameter measured | Source of variation     | F                | p      |
|-------------------------|--------------------|-------------------------|------------------|--------|
| VTA                     | Nucleus volume     | Sex                     | $F_{1,60}=2.96$  | 0.092  |
|                         |                    | Level                   | $F_{2,60}=97.27$ | <0.001 |
|                         |                    | Treatment               | $F_{2,60}=31.25$ | <0.001 |
|                         |                    | Sex x Level             | $F_{2,60}=27.97$ | <0.001 |
|                         |                    | Sex x Treatment         | $F_{2,60}=13.39$ | <0.001 |
|                         |                    | Treatment x Level       | $F_{4,60}=9.63$  | <0.001 |
|                         |                    | Sex x Level x Treatment | $F_{4,60}=20.20$ | <0.001 |
|                         | GS-IR cell number  | Sex                     | $F_{1,60}=0.12$  | 0.726  |
|                         |                    | Level                   | $F_{2,60}=11.84$ | <0.001 |
|                         |                    | Treatment               | $F_{2,60}=51.18$ | <0.001 |
|                         |                    | Sex x Level             | $F_{2,60}=10.27$ | <0.001 |
|                         |                    | Sex x Treatment         | $F_{2,60}=5.80$  | 0.006  |
|                         |                    | Treatment x Level       | $F_{4,60}=6.07$  | 0.001  |
|                         | TH-IR cell number  | Sex                     | $F_{1,60}=2.33$  | 0.134  |
|                         |                    | Level                   | $F_{2,60}=41.62$ | <0.001 |
|                         |                    | Treatment               | $F_{2,60}=13.85$ | <0.001 |
|                         |                    | Sex x Level             | $F_{2,60}=23.93$ | <0.001 |
|                         |                    | Sex x Treatment         | $F_{2,60}=11.60$ | <0.001 |
|                         |                    | Treatment x Level       | $F_{4,60}=11.53$ | <0.001 |
|                         |                    | Sex x Level x Treatment | $F_{4,60}=9.10$  | <0.001 |
|                         | TH-IR cell density | Sex                     | $F_{1,60}=0.005$ | 0.945  |
|                         |                    | Level                   | $F_{2,60}=0.151$ | 0.860  |
|                         |                    | Treatment               | $F_{2,60}=8.48$  | 0.001  |
|                         |                    | Sex x Level             | $F_{2,60}=3.06$  | 0.057  |
|                         |                    | Sex x Treatment         | $F_{2,60}=6.78$  | 0.003  |
|                         |                    | Treatment x Level       | $F_{4,60}=3.08$  | 0.025  |
|                         |                    | Sex x Level x Treatment | $F_{4,60}=4.86$  | 0.002  |
|                         | GS-IR cell density | Sex                     | $F_{1,60}=6.46$  | 0.015  |
| Level                   |                    | $F_{2,60}=0.94$         | 0.401            |        |
| Treatment               |                    | $F_{2,60}=72.70$        | <0.001           |        |
| Sex x Level             |                    | $F_{2,60}=6.92$         | 0.003            |        |
| Sex x Treatment         |                    | $F_{2,60}=0.22$         | 0.803            |        |
| Treatment x Level       |                    | $F_{4,60}=0.48$         | 0.753            |        |
| Sex x Level x Treatment |                    | $F_{4,60}=1.42$         | 0.246            |        |

Global three-way ANOVAs were performed for the ventral tegmental area (VTA) to investigate the effects of antenatal glucocorticoid treatment compared with controls on the counts of cells immunoreactive for tyrosine hydroxylase (TH-IR) and glutamine synthetase (GS-H), nucleus volumes, TH-IR and GS-IR cell distribution, and the TH-IR and GS-IR cell density.