

Supplementary Information

Protein restriction during pregnancy alters *Cdkn1c* silencing, dopamine circuitry and offspring behaviour without changing expression of key neuronal marker genes

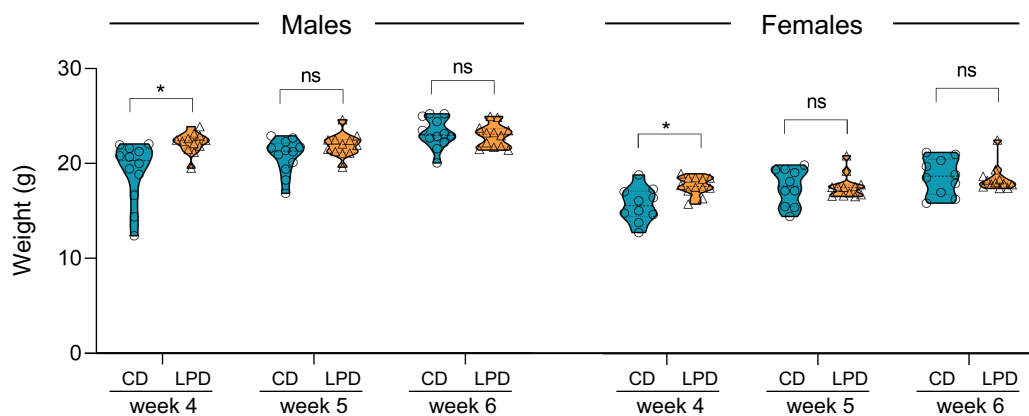
Prodani, C. *et al.*

Supplementary Figures S1-S4

Legends for Supplementary Videos S1-S4

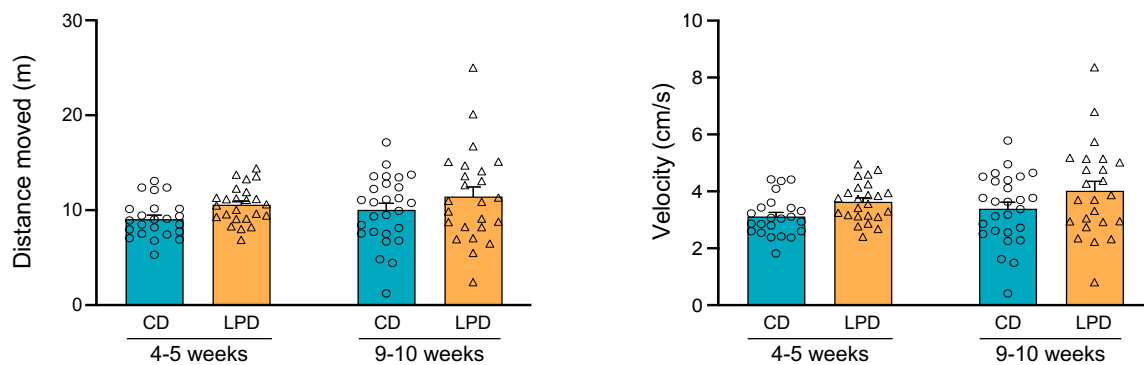
Figure S1

A



B

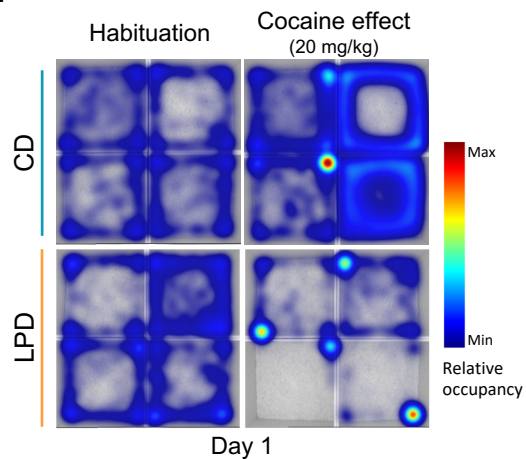
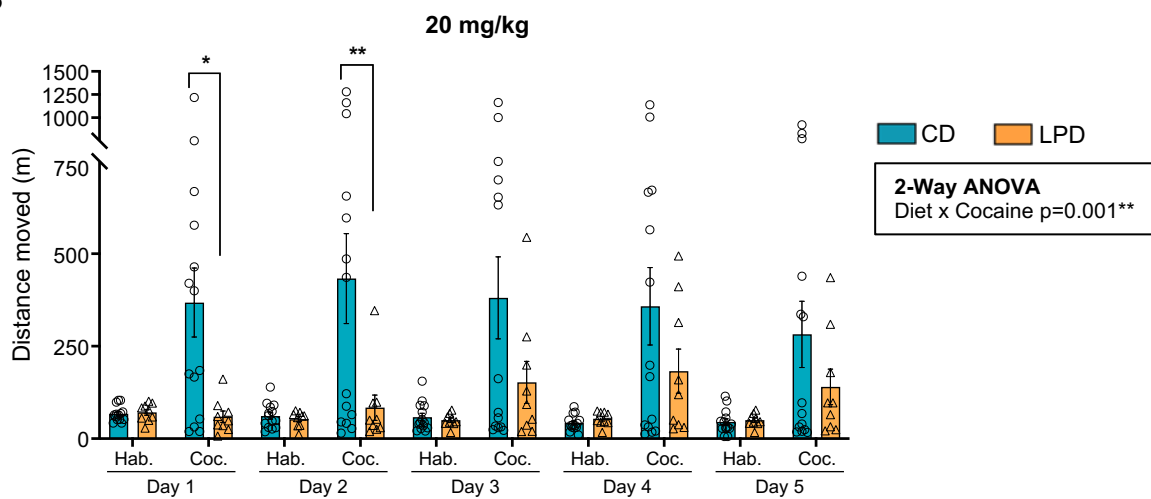
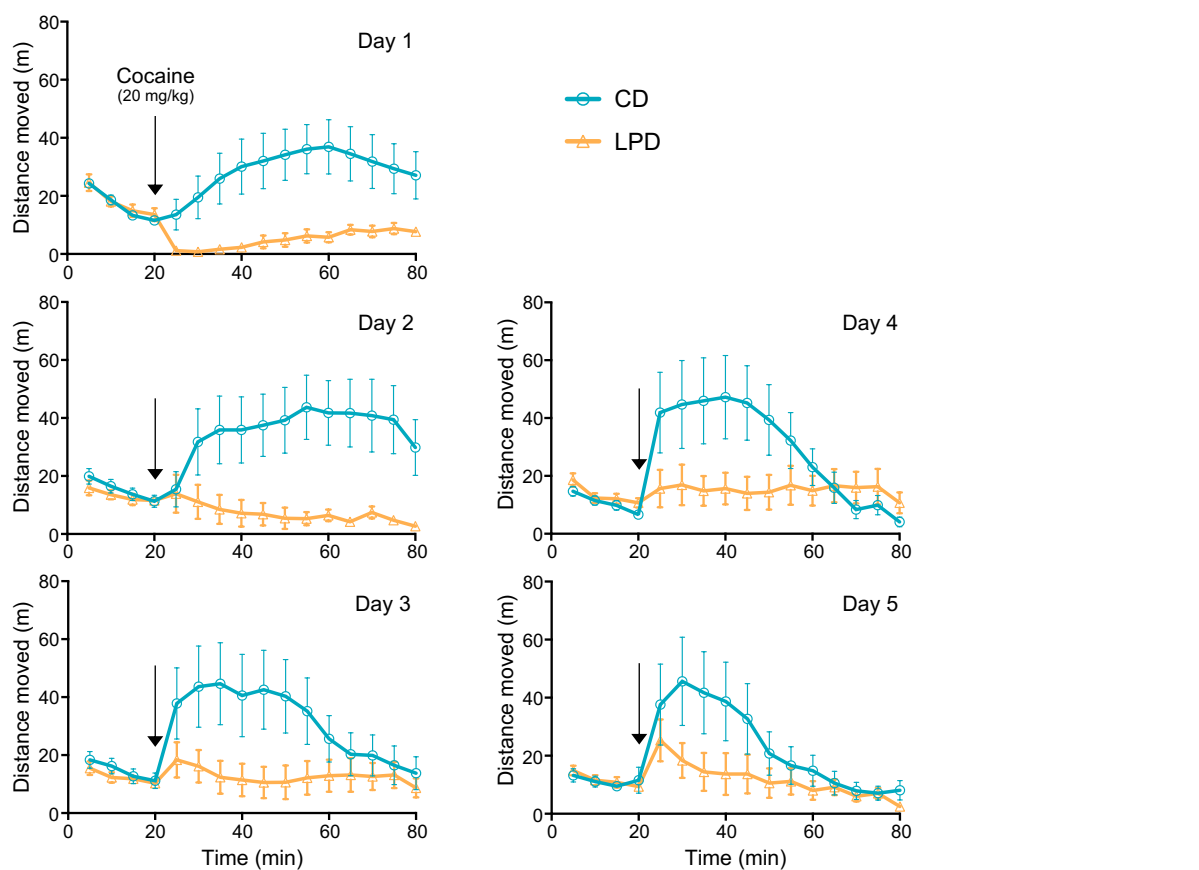
Elevated O-Maze



Supplementary Figure S1 | LPD-exposure in pregnancy impacts juvenile offspring weight.

Corresponding to Figure 2.

- (A)** Animals exposed to maternal CD or LPD in gestation were weighed at 4, 5 and 6 weeks of age. At 4 weeks, male (left) and female (right) LPD-exposed offspring (orange) weighed significantly more than CD-exposed (blue) offspring, but these differences normalised with age. Two-way repeated measure ANOVAs: Males (Age x Diet $p < 0.0001$, Age $p < 0.0001$, Diet $p = 0.0721$, Subject $p < 0.0001$), Females (Age x Diet $p < 0.0001$, Age $p < 0.0001$, Diet $p = 0.3405$, Subject $p < 0.0001$); Sidak's multiple comparisons tests (Males $*p_{adj} = 0.0228$, Females $*p_{adj} = 0.0223$); Males $n = 12$, Females $n = 10$; violin plots show median and quartiles.
- (B)** Analysis of distance moved (left) and velocity (right) during elevated O-maze testing. Diet had a significant effect both on distance moved (Two-way ANOVA: Diet $p = 0.0343$, Age $p = 0.1782$, Interaction $p = 0.9344$) and on velocity (Two-way ANOVA: Diet $p = 0.0144$, Age $p = 0.1553$, Interaction $p = 0.7939$). However, Sidak's multiple comparisons tests did not reveal any significant pairwise differences between diet groups or age groups ($p_{adj} > 0.05$; 2 families of 2 comparisons per analysis). CD 4-5 weeks $n = 23$, LPD 4-5 weeks $n = 24$, CD 9-10 weeks $n = 27$, LPD 9-10 weeks $n = 24$; error bars = SEM.

Figure S2**A****B****C**

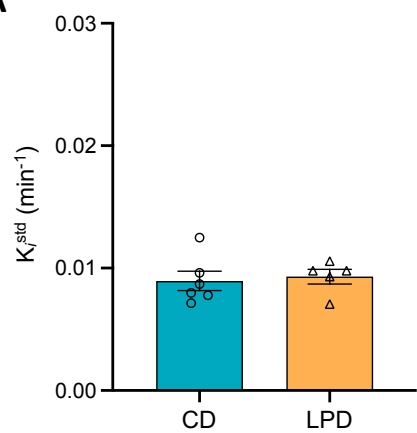
Supplementary Figure S2 | Locomotor activity in response to cocaine administration.

Corresponding to Figure 3.

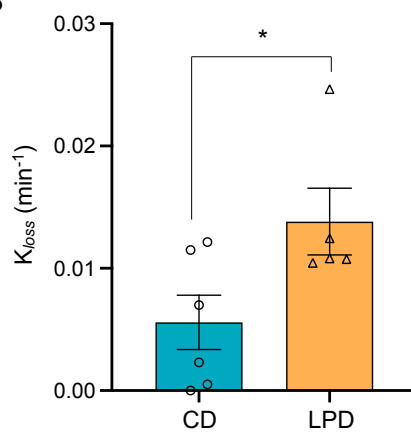
- (A)** Representative occupancy heatmaps showing locomotor activity of 4 CD- and 4 LPD-exposed adult mice before and after cocaine injection (20 mg/kg) on Day 1. For each treatment group, the movement of each mouse is summarised in an individual square.
- (B)** Quantification of distance moved during a 5-day trial involving daily administration of 20 mg/kg cocaine, for CD- (blue) or LPD- (orange) gestationally exposed adult offspring. Two-way repeated measures ANOVA revealed a significant interaction between diet and cocaine over the 5-day regime ($p=0.0010$). Sidak's multiple comparisons test confirmed no differences during habituation on any days ($p_{adj}>0.05$) but revealed significantly decreased activity in LPD mice post-cocaine on days 1 and 2 (** $p_{adj}=0.0039$, * $p_{adj}=0.0165$; 10 comparisons in total). CD $n=14$, LPD $n=9$; error bars=SEM.
- (C)** Time course comparisons (5 min intervals) of distance moved during habituation (first 20 min) and post-cocaine administration (60 min) in adult CD or LPD-exposed mice during 5 days of cocaine sensitisation, using 20 mg/kg cocaine. CD $n=14$, LPD $n=9$; error bars=SEM.

Figure S3

A



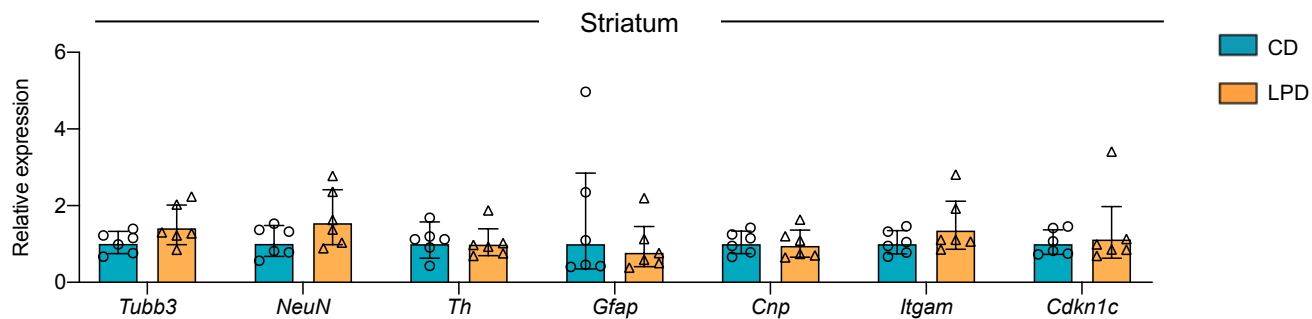
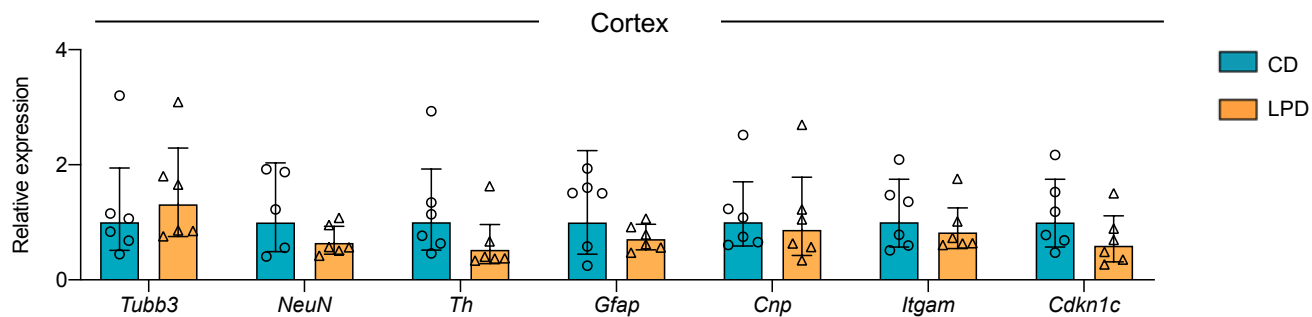
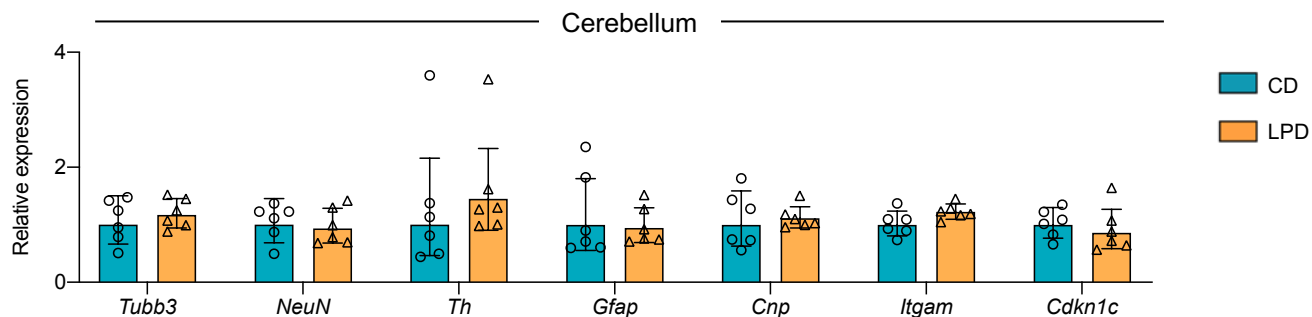
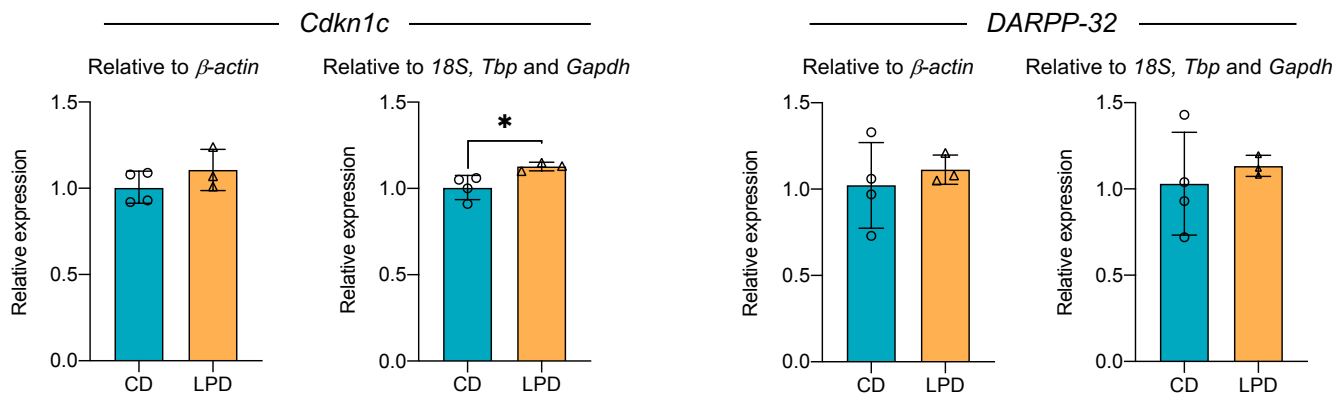
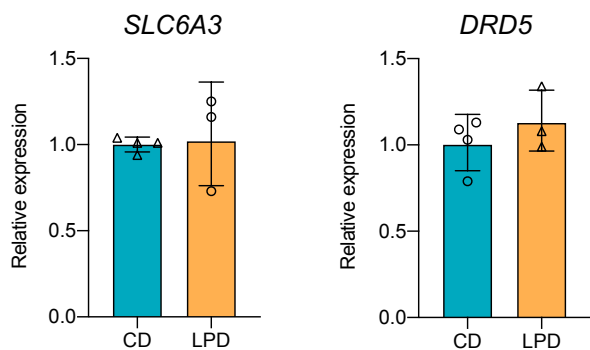
B



Supplementary Figure S3 | Dopamine turnover is altered in the brains of LPD-exposed offspring.

Corresponding to Figure 4.

- (A)** Comparison of CD and LPD K_i^{std} values, a measure of dopamine synthesis capacity that omits the correction for loss of radioactive metabolites. Each data point represents an animal (CD n=6, LPD n=5, with striatal values averaged per animal); error bars=SEM; two-tailed unpaired t-test, p=0.7396.
- (B)** Comparison of CD and LPD K_{loss} values, an index of dopamine turnover. Each data point represents an animal (CD n=6, LPD n=5, with striatal values averaged per animal); error bars=SEM; two-tailed unpaired t-test (*p=0.0419).

Figure S4**A****B****C****D****E**

Supplementary Figure S4 | Gene expression in adult brain of *in utero* LPD- or CD-exposed mice.

Corresponding to Figure 5.

- (A-C)** Quantitative RT-PCR comparison of *Tubb3*, *NeuN*, *Th*, *Gfap*, *Cnp*, *Itgam* and *Cdkn1c* transcript abundance in dissected adult striatum (**A**), cortex (**B**) and cerebellum (**C**) of mice that were exposed to LPD (orange) or CD (blue) *in utero*. Expression was normalised to β -actin and is plotted as fold change relative to CD. Bars show geometric mean; error bars=geometric SD; n=6 for each diet group. No significant differences were detected between CD and LPD-exposed samples, as determined by unpaired t-tests ($p>0.05$).
- (D)** Alternative quantitative RT-PCR comparisons of *Cdkn1c* or *DARPP-32* expression levels in midbrain samples using different controls for normalisation: β -actin (left) or *18S*, *Tbp*, *Gapdh* (right). Both are plotted as fold change relative to CD and bars show geometric mean; error bars=geometric SD; CD n=4, LPD n=3; two-tailed unpaired t-tests (* $p=0.0349$).
- (E)** Quantitative RT-PCR analysis of *SLC6A3* and *DRD5* using animals not previously subjected to behavioural tests. Expression was normalised to β -actin and is plotted as fold change relative to CD. Bars show geometric mean; error bars=geometric SD; CD n=4, LPD n=3; no significant differences were detected with two-tailed unpaired t-tests ($p>0.05$).

Supplementary Videos S1 and S2

Representative videos of CD-exposed (Supplementary Video S1) and LPD-exposed (Supplementary Video S2) adult mice during habituation on Day 2 of the 20 mg/kg cocaine treatment regime.

Supplementary Videos S3 and S4

Representative videos of CD-exposed (Supplementary Video S3) and LPD-exposed (Supplementary Video S4) adult mice following administration of 20 mg/kg cocaine on Day 2 of the treatment regime.