Supplementary Data

Early Postnatal Manganese Exposure Causes Arousal Dysregulation and Lasting Hypofunctioning of the Prefrontal Cortex Catecholaminergic Systems

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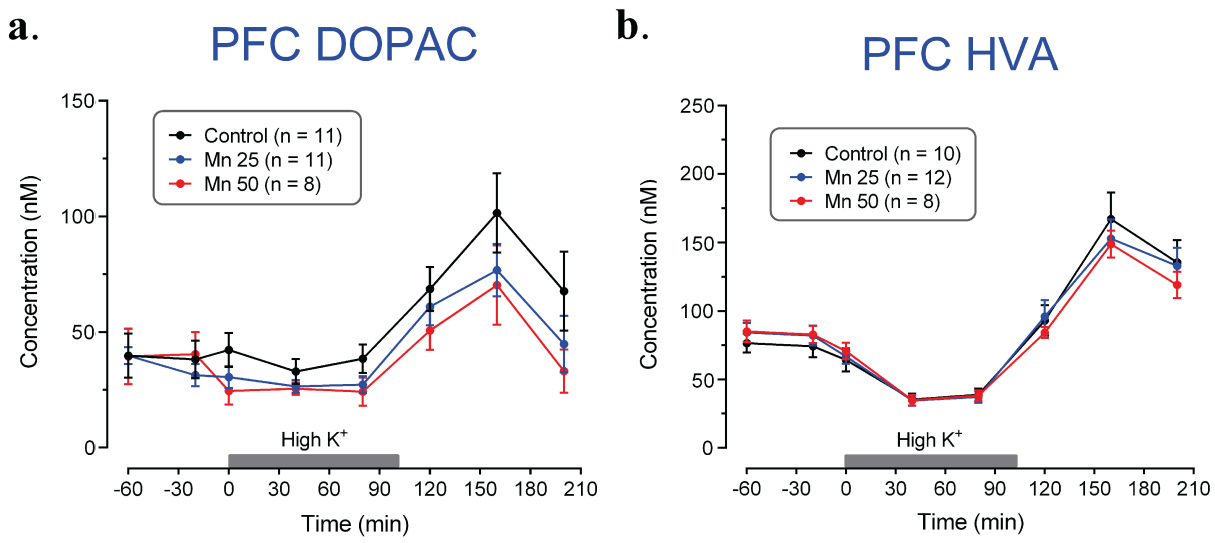
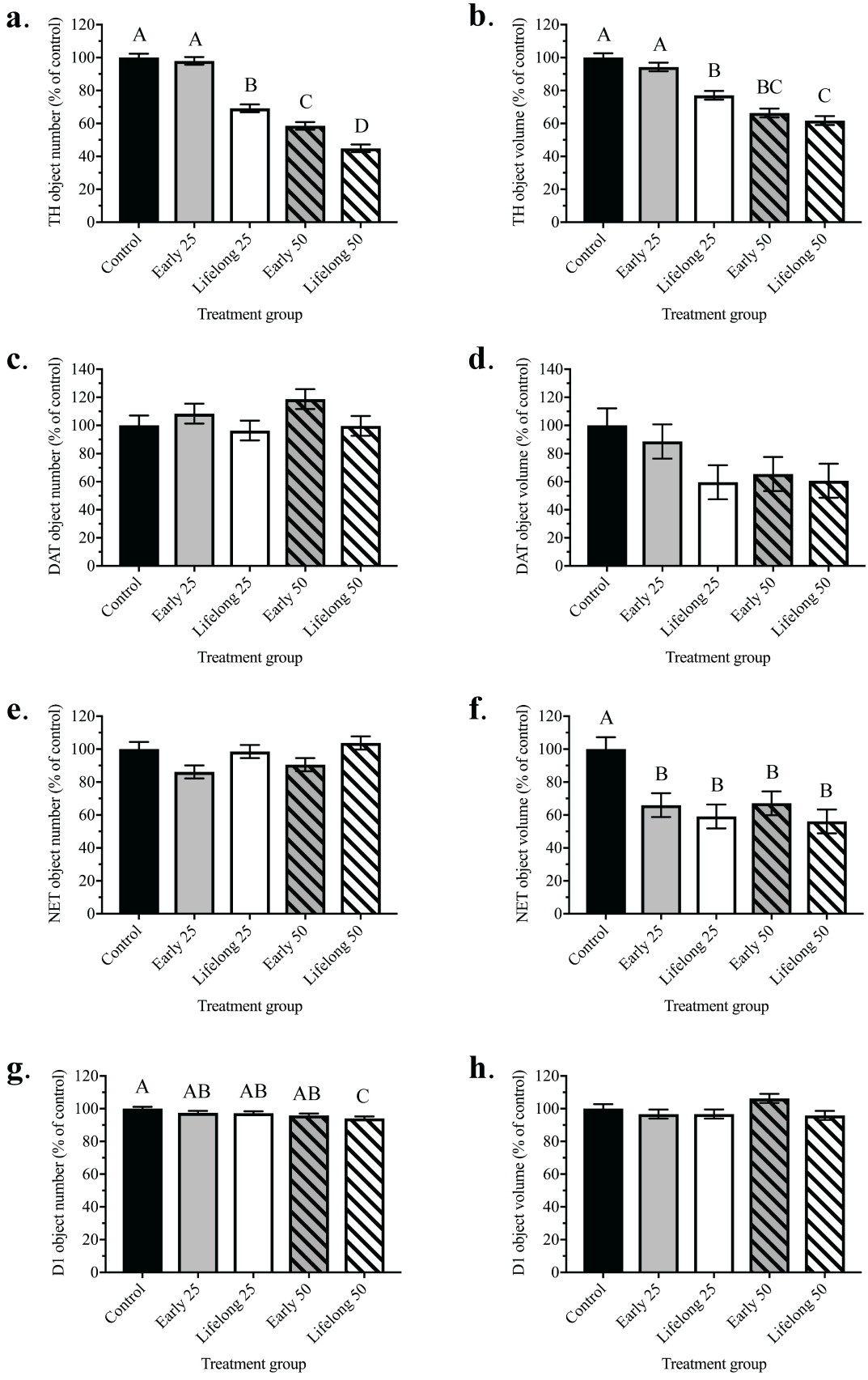
Supplementary Table 1. Basal concentrations of NE, DA, and their metabolites in the mPFC.

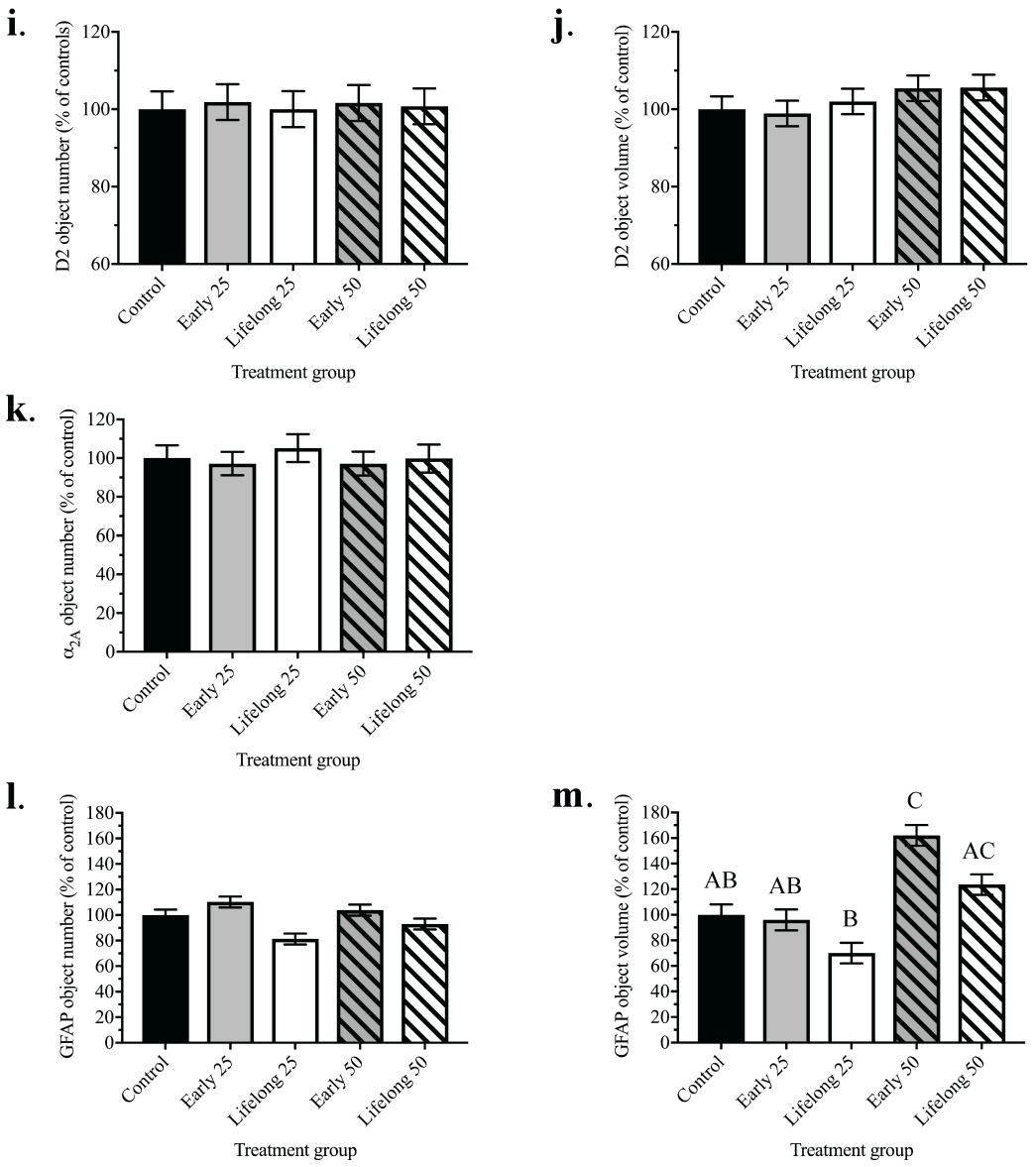
Neurotransmitter/ Metabolite	Control	25 mg Mn/kg/day	50 mg Mn/kg/day
NE	3.40 ± 0.37 (10)	2.60 ± 0.31 (12)	2.15 ± 0.41 (8)
DA	0.86 ± 0.20 (11)	0.65 ± 0.14 (12)	0.46 ± 0.06 (8)
DOPAC	39.81 ± 8.47 (11)	35.52 ± 3.96 (11)	39.93 ± 10.66 (8)
HVA	75.36 ± 7.48 (10)	83.13 ± 7.06 (12)	83.87 ± 7.10 (8)

Values are mean baseline nM concentrations (prior to introduction of a high K^+ stimulus) ± SEM with group sizes in parentheses. The NE reuptake blocker desipramine (100 μM) was present in the perfusion fluid throughout. There was no significant effect of Mn exposure based on one-way ANOVA ($p > 0.05$).



Supplementary Figure S1. Mn exposure did not alter the K^+-stimulated release of DOPAC or HVA in the mPFC of young adolescent animals. Concentrations of **(a)** DOPAC and **(b)** HVA in nM as a function of time after administration of a high K^+ stimulus. There was no effect of Mn exposure on either DOPAC or HVA concentrations at 40 min after initiation of high K^+ perfusion, based on one-way ANOVA ($p > 0.05$). Data are means ± SEM (n = 8-12 animals/group).


a.
TH object number (% of control)
A
A
B
C
D
b.
TH object volume (% of control)
A
A
B
BC
C
c.
DAT object number (% of control)
d.
DAT object volume (% of control)
e.
NET object number (% of control)
f.
NET object volume (% of control)
A
B
B
B
B
g.
D1 object number (% of control)
A
AB
AB
AB
C
h.
D1 object volume (% of control)
0
20
40
60
80
100
120
140
Control
Early 25
Lifelong 25
Early 50
Lifelong 50
Treatment group



Supplementary Figure S2a – S2m. Imaris-rendered object number and object volume for proteins quantified by immunohistochemistry. Bar charts show total object number/image (left panels) and total object volume/image (right panels) of Imaris-quantified objects. Data (least squares means ± SEM) reflect 12-18 images/animal and n = 6 animals/treatment group, shown as percent of control values generated from the statistical model that included all five treatment groups. (**a, b**) tyrosine hydroxylase (TH), (**c, d**) dopamine transporter (DAT), (**e, f**) norepinephrine transporter (NET), (**g, h**) dopamine D1 receptor (D1), (**i, j**) dopamine D2

receptor (D2), (**k**) α_{2A} adrenergic receptor (α_{2A}), (**l, m**) glial fibrillary acidic protein (GFAP). Bars with different superscripts are statistically different ($p < 0.05$) based on Tukey's multiple comparisons test.