SUPPLEMENTAL INFORMATION FOR

SLC30A10 manganese transporter in the brain protects against deficits in motor function and dopaminergic neurotransmission under physiological conditions

Cherish A. Taylor, Stephanie M. Grant, Thomas Jursa, Ashvini Melkote, Rebecca Fulthorpe, Michael Aschner, Donald R. Smith, Rueben A. Gonzales, Somshuvra Mukhopadhyay

Supplemental figures S1-S5

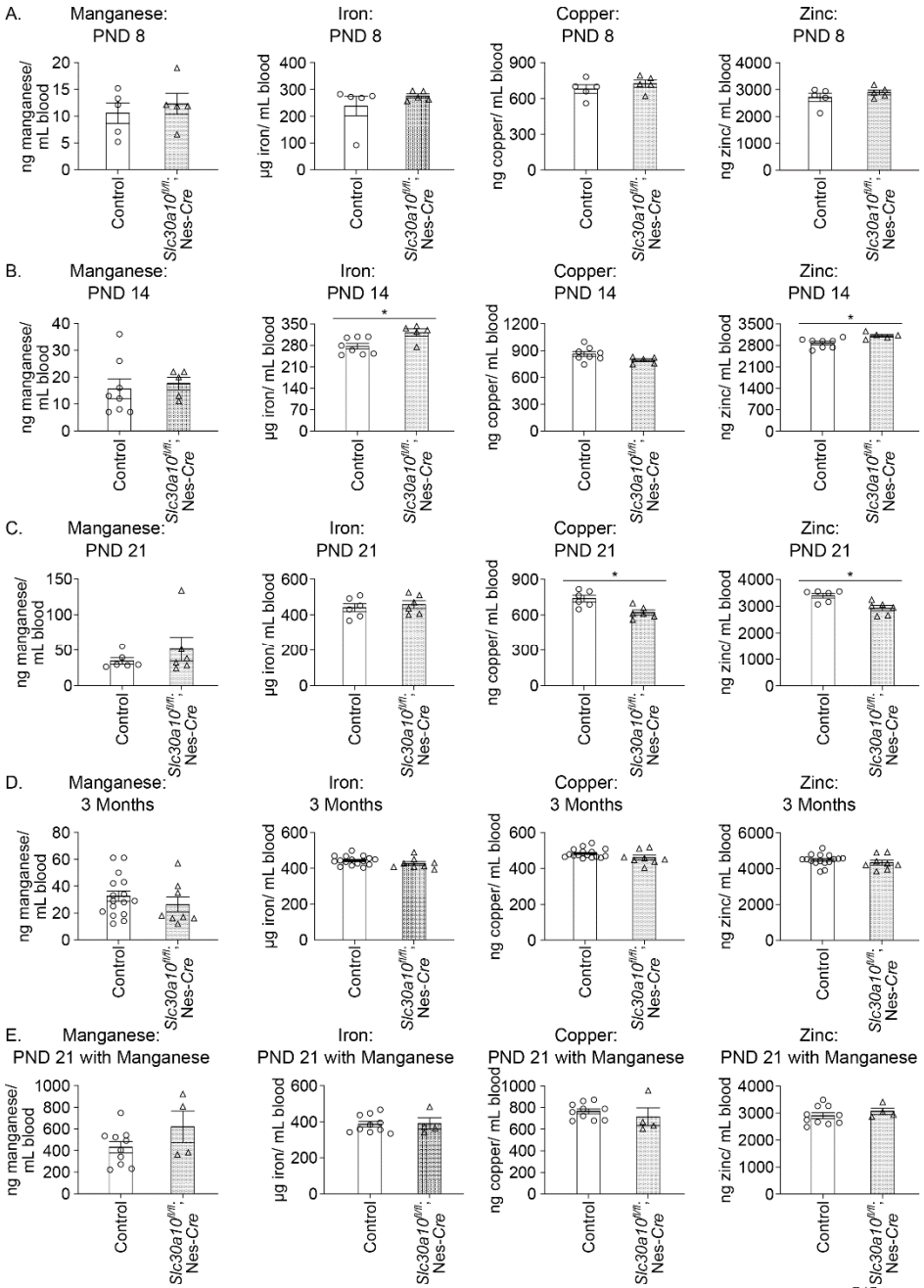

Figure S1. Blood metal levels of pan-neuronal/glial knockouts (*Slc30a10*$^{fl/fl}$; Nes-*Cre*) are comparable to littermate controls.

A-E. ICP-MS analyses of blood metal levels at PND 8 (***A***), PND 14 (***B***), PND 21 (***C&E***), and 3 months (***D***) of age. For ***A***, n = 5 controls (2 males, 3 females) and 5 knockouts (3 males, 2 females). For ***B***, n = 8 controls (3 males, 4 females) and 5 knockouts (females). For ***C***, n = 6 per genotype (2 males, 4 females). For ***D***, n = 16 controls (7 males, 9 females) and 8 knockouts (5 males, 3 females). For ***E***, n = 10 controls (3 males, 7 females) and 4 knockouts (3 males, 1 female). Mice in ***A-D*** were treated with vehicle water. Mice in ***E*** were treated with Mn. Mean ± SE. *, $p < 0.05$ by *t*-test.

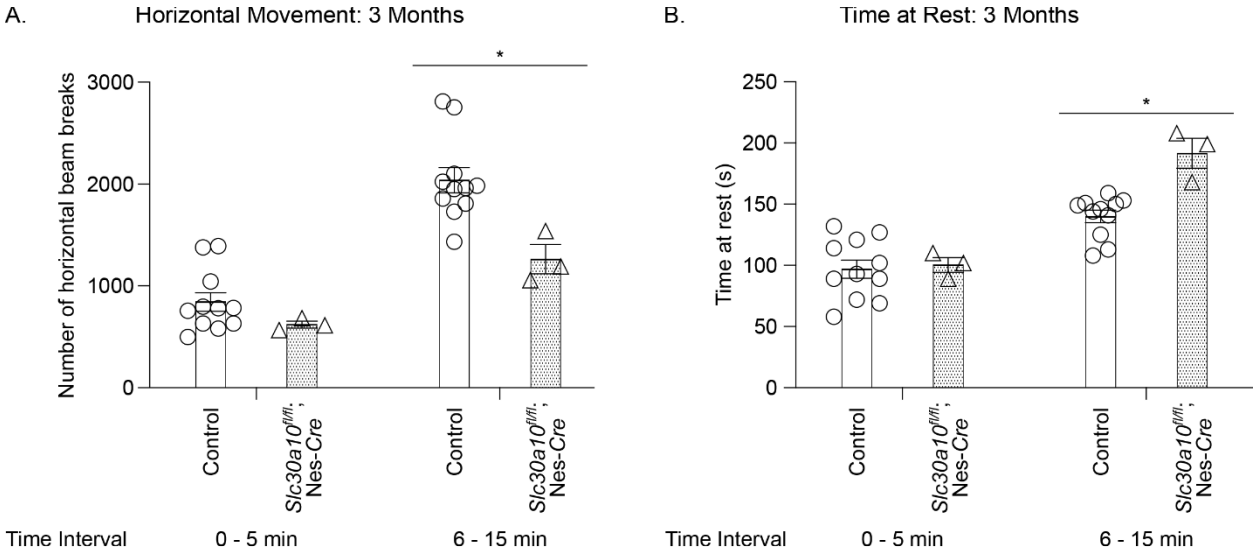

Figure S2. Pan-neuronal/glial *Slc30a10* knockouts (*Slc30a10*$^{fl/fl}$; Nes-*Cre*) exhibit reduced locomotion at 3 months of age in the absence of Mn exposure.

Open-field analyses for horizontal movement (***A***) and resting time (***B***) in 3-month-old pan-neuronal/glial *Slc30a10* knockouts or littermate controls. N = 11 controls (5 males, 6 females) and 3 knockouts (2 males, 1 female). Mean ± SE. *, $p < 0.05$ using repeated measures two-way ANOVA and Sidak's *post-hoc* analyses.

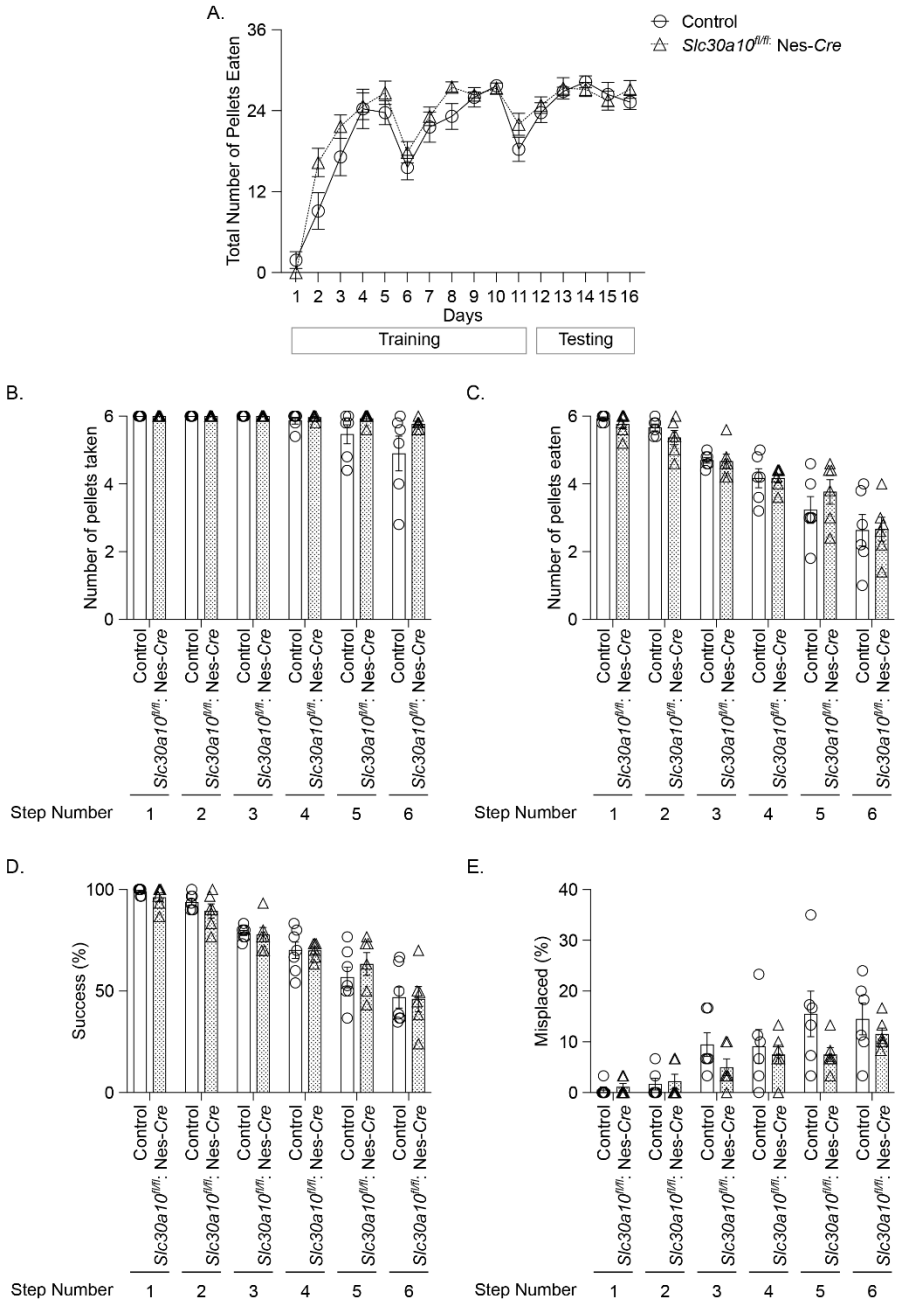

Figure S3. Skilled forelimb performance in the Montoya staircase test of pan-neuronal/glial *Slc30a10* knockouts (*Slc30a10*$^{fl/fl}$; Nes-*Cre*) is comparable to littermates.

A-E. The Montoya staircase test was used to measure skilled forelimb performance in mice that were not exposed to Mn by assaying for the total number of pellets eaten during both training (Days 1-11) and testing (Days 12-16) (***A***), the average number of pellets taken per step during testing (***B***), the average number of pellets eaten during testing (***C***), the success rate (***D***), and the percent of pellets misplaced (***E***). N = 6 mice (3 males, 3 females) per genotype per day or step. Mean ± SE. $P > 0.05$ for genotype-specific differences using a mixed-effects analysis (***A***) or repeated measures two-way ANOVA (***B-E***) and Sidak's *post-hoc* analyses.

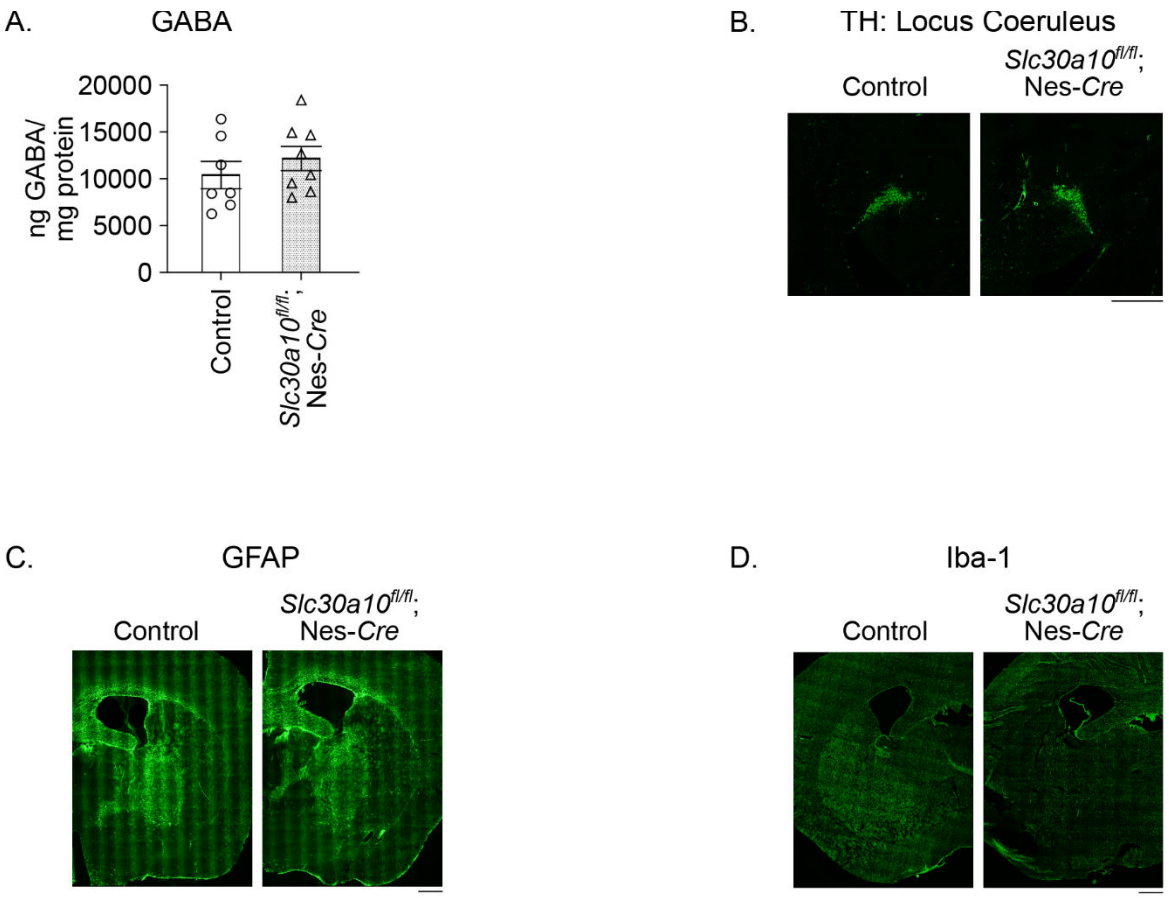

Figure S4. Changes in tissue GABA levels and evidence of noradrenergic neurodegeneration in the locus coeruleus or glial activation in the basal ganglia are not evident in pan-neuronal/glial *Slc30a10* knockouts (*Slc30a10*$^{fl/fl}$; Nes-*Cre*) under physiological conditions.

A. HPLC with electrochemical detection to measure GABA in the dorsal striatum. N = 7 controls (4 males, 3 females) and 8 knockouts (5 males, 3 females). Mean ± SE. P > 0.05 by *t*-test.
B-D. Immunofluorescence to detect tyrosine hydroxylase (TH)-positive noradrenergic neurons in the locus coeruleus; or GFAP-positive astrocytes or Iba-1-positive microglia in the basal ganglia. Bar, 500 μm.

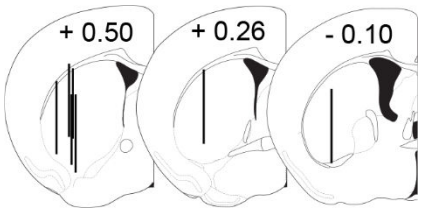

Figure S5. Histological analysis of probe locations for the *in vivo* microdialysis experiment. The number on each panel represents the anterior-posterior position relative to Bregma.