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Supplementary Materials for

Age- and sex-dependent effects of metformin on neural precursor cells and cognitive recovery in a model of neonatal stroke

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This PDF file includes:

Supplementary Materials

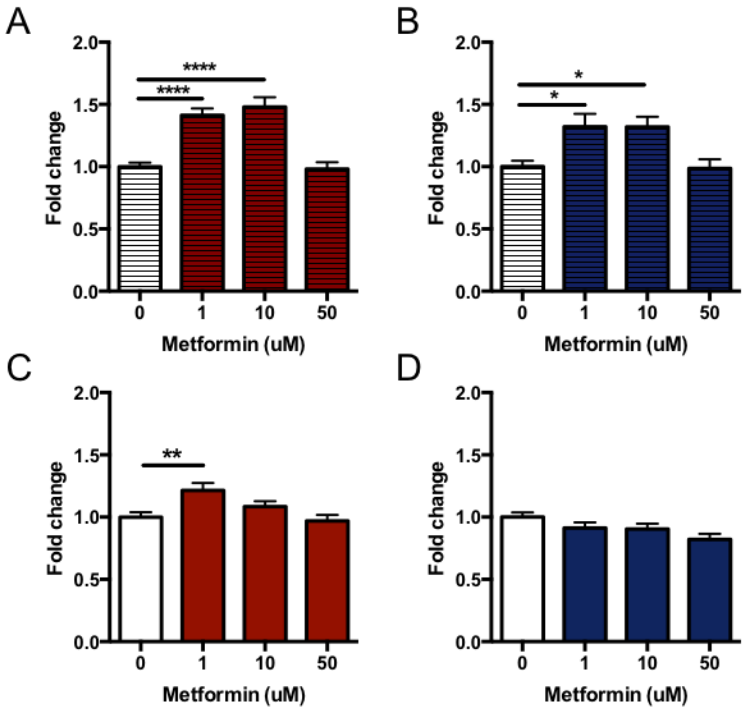

Fig. S1. Metformin at 1 µM increases the number of neurospheres in an age- and sex-dependent manner. Metformin dose response effect on the number of neurospheres from the SVZ of neonatal females (A; n=8-16, $p<0.0001$, $F_{(3,52)}=4.26$, one-way ANOVA) and males (B; n=8-15, $p<0.05$, , $F_{(3,49)}=3.21$, one-way ANOVA). Metformin dose response effect on the number of neurospheres from the SVZ of adult females (C; n=15, $p<0.01$, $F_{(3,55)}=1.18$, one-way ANOVA) and males (D; n=11, , $F_{(3,40)}=0.310$, one-way ANOVA).

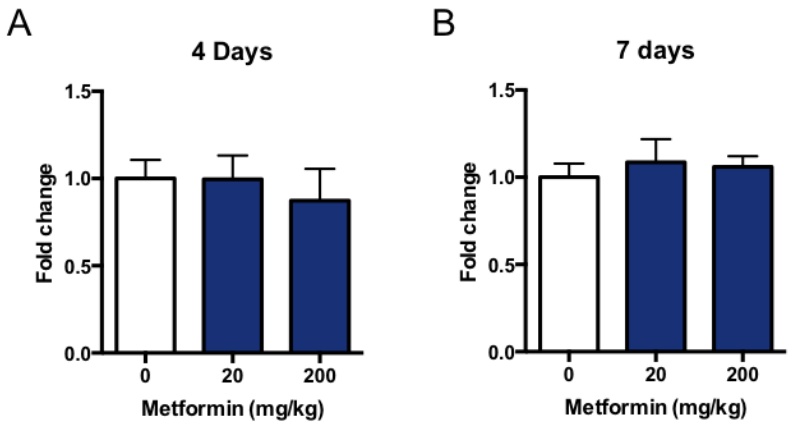

Fig. S2. Metformin has no effect on the number of neurospheres from the adult male SVZ regardless of metformin dose or duration of treatment. (**A**) Fold change in number of neurospheres from the adult male SVZ following 4 days of metformin treatment (20 and 200 mg/kg) (n=4-10, $F_{(2,25)}$=2.43, one-way ANOVA). (**B**) Fold change in the number of neurospheres from the adult male SVZ following 7 days of metformin treatment (20 and 200 mg/kg; n=4-8, $F_{(2,14)}$=1.41, one-way ANOVA).

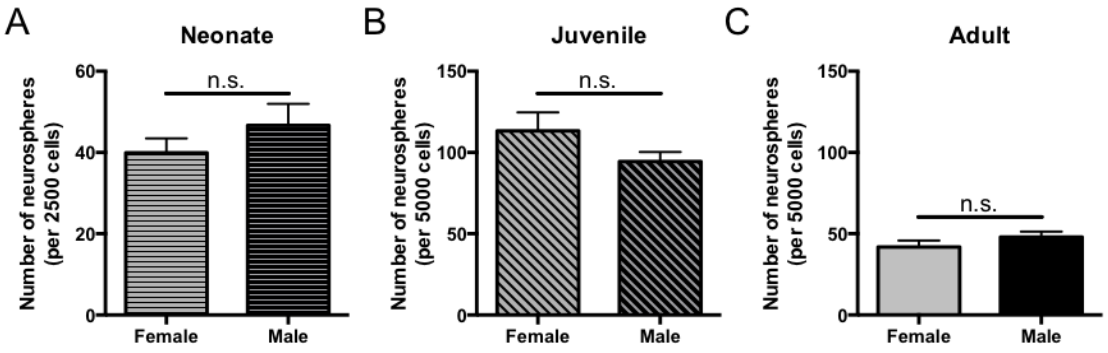

Fig. S3. The absolute number of neurospheres is the same in male and female C57 mice at each age examined. Absolute number of female (gray bars) and male (black bars) neurospheres from the SVZ of neonatal (A; n=10-11), juvenile (B; n=7-10), and adult mice (C; n=11-15, Student's t-test).

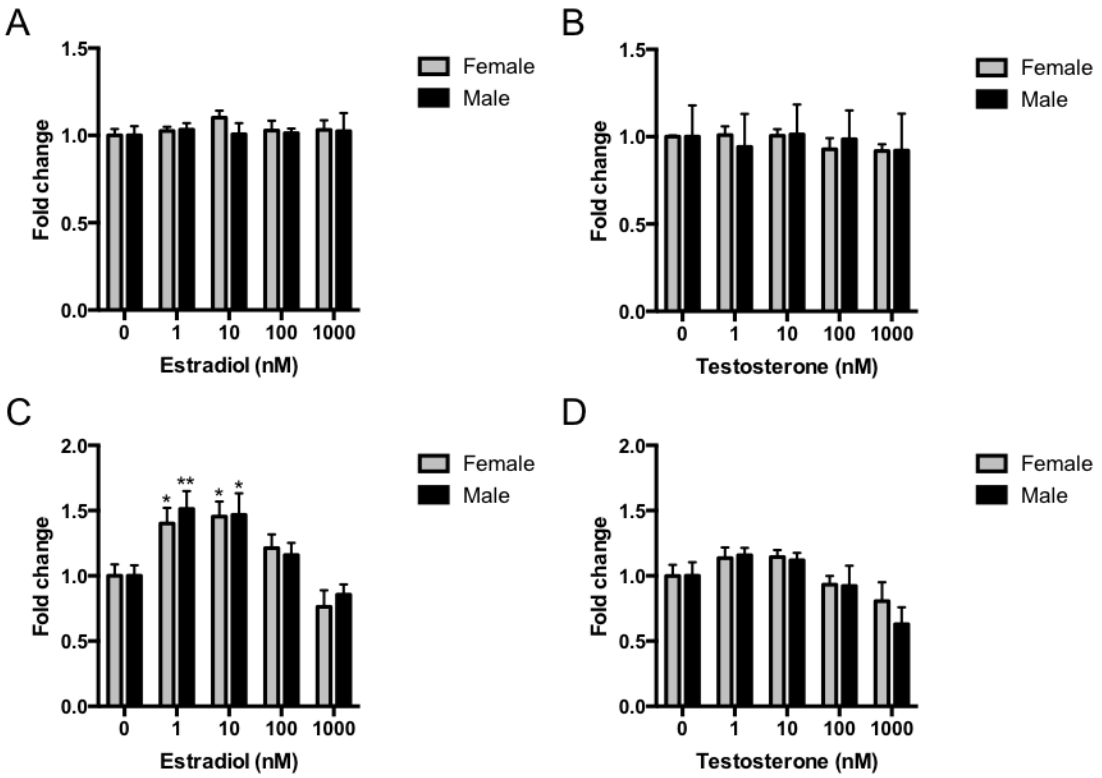

Fig. S4. Lack of sex-dependent effects of estradiol and testosterone on primary and secondary neurospheres from the SVZ of adult female and male mice. (**A**,**B**) Fold change in the number of primary neurospheres from the female and male SVZ following in vitro exposure to estradiol with no effect of dose or sex (A; n=4-5, $F_{(4,35)}$=0.283, $F_{(1,35)}$=0.446, two-way ANOVA) or testosterone with no effect of dose or sex (B; n=4-5, $F_{(4,35)}$=0.069, $F_{(1,35)}$=0.183 two-way ANOVA). (**C**,**D**) Fold change in the number of secondary neurospheres from the female and male SVZ following in vitro estradiol with an effect of dose (C; n=6-10, *p<0.05, **p<0.01, $F_{(4,84)}$=11.15, two-way ANOVA) or testosterone with an effect of dose (D; n=6-10, $F_{(4,77)}$=6.08, $F_{(1,77)}$=0.412, two-way ANOVA) exposure.

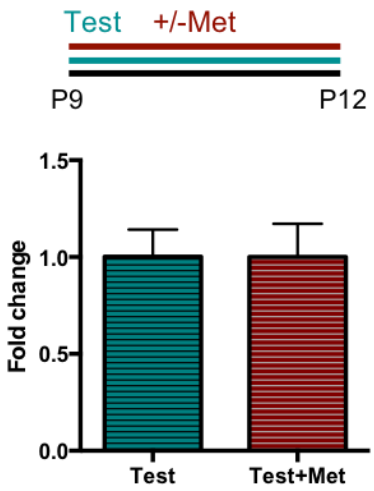

Fig. S5. Testosterone and metformin eliminate the effect of metformin on NPCs in the female SVZ. Experimental paradigm and fold change in the number of neurospheres from female neonatal mice that received testosterone or vehicle and metformin (20mg/kg) or vehicle for 4 days in vivo (n=8-9, Student's t-test).

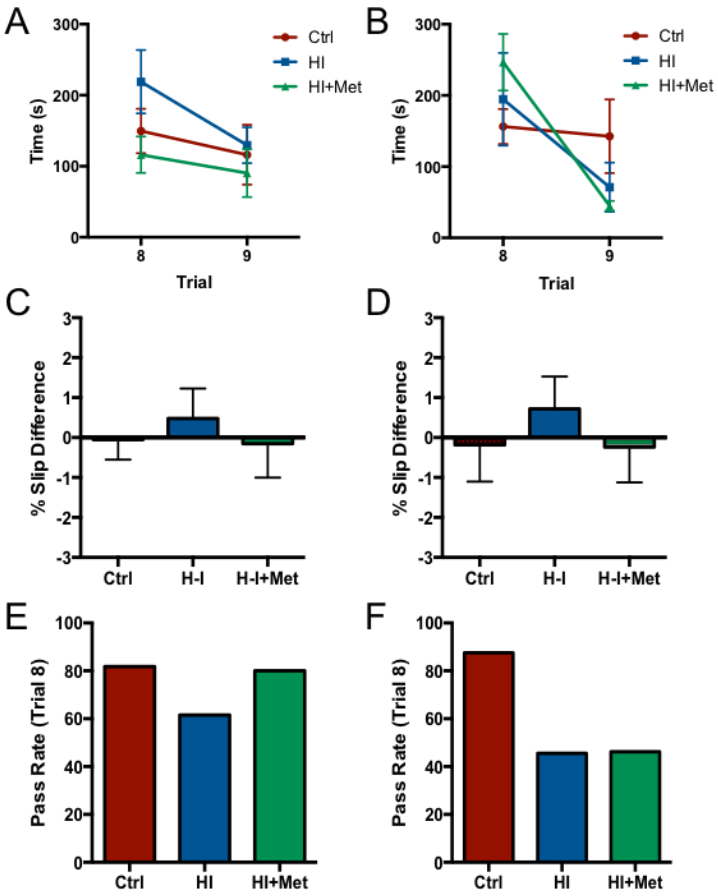

Fig. S6. Motor impairments do not account for the deficits in task acquisition in females and males. (**A**,**B**) Time to complete trials 8 and 9 in the puzzle box task in females (A; n=8-12, $F_{(1,26)}$=3.48, $F_{(2,26)}$=1.94, repeated measures two-way ANOVA) and males (B; n=5-7, $F_{(1,15)}$=12.57, repeated measures two-way ANOVA). Hence, mice that were able to acquire the task in trial 8 performed it in the same amount of time as control mice on trial 9, irrespective of whether they received an injury. (**C**,**D**) The % slip difference in the foot fault task at P42 in females (C; n=10, $F_{(2,27)}$=2.16, one-way ANOVA) and males (D; n=8-10, $F_{(2,25)}$=2.25, one-way ANOVA) showing mice do not have motor deficits in this task at later times. (**E**,**F**) Trial 8 pass rate in females (E; n=11-15) and males (F; n=8-13).