Male-Specific Cyclic Adenosine Monophosphate Signaling in the Hippocampus Controls Spatial Memory Deficits in a Mouse Model of Autism and Intellectual Disability

SUPPLEMENTAL INFORMATION

Supplemental Table S1. Antibodies used in this study

Antigen	Product #	Concentration	Vendor
Cc2d1a	ab68302	1:1,000	AbCam
Cc2d1b	20774-1-AP	1:600	Proteintech
total PDE4D	PDE4D5-451AP	1:250	FabGennix Intl.
pPDE4D Ser-190	PPD4-440AP	1:100	FabGennix Intl.
total PKA	5842	1:1,000	Cell Sign Tech
pPKA Thr-197	5661	1:500	Cell Sign Tech
total CREB	9197	1:400	Cell Sign Tech
pCREB Ser-133	p1010-133	1:500	PhosphoSolutions
beta-actin	ab6276	1:10,000	AbCam

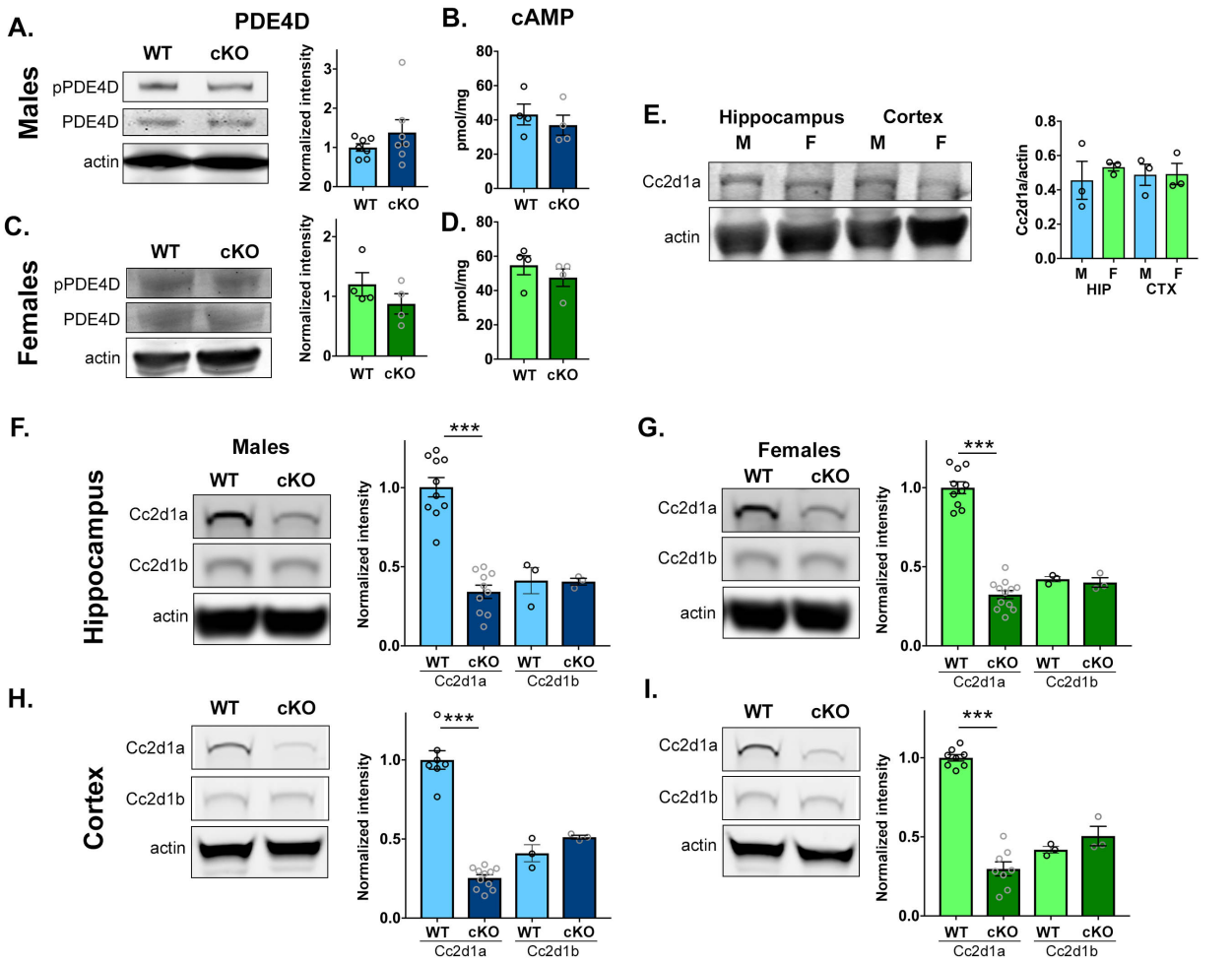
Supplemental Table S2. Analysis of basic motor and somatosensory function in *Cc2d1a* cKO mice

Genotype	Sex	Weight (g)	Righting reflex (s)	Wire hang (s)	Stride and gait	Tail pinch[a]	Visual reflex[b]
WT	F	27.64± 0.57	<1 s	59.36± 0.32	Normal	13/14	14/14
cKO	F	26.58 ± 0.88	<1 s	59.67 ± 0.24	Normal	10/10	10/10
WT	M	32.62 ± 0.72	<1 s	59.15 ± 0.26	Normal	16/20	20/20
cKO	M	32.08 ± 1.04	<1 s	59.12 ± 0.31	Normal	14/17	17/17

[a]Fraction of animals that responded to tail pinch. [b]Fraction of animals that responded with visual stimulus.

Supplementary Table S3. Summary data for hidden platform (HP) performance in *Cc2d1a* cKO males and females (Male data from Oaks et al, (1) * p<0.05)

Genotype	HP1	HP2	HP3	HP4	HP5	N=
WT F	23.4±2.5	17.0±1.8	13.0±1.8	13.7±2.0	10.9±1.4	20
cKO F	24.7±2.7	19.6±2.4	17.5±2.6	14.3±2.4	9.2±1.4	17
WT M	20.2±2.3	11.9±1.6	9.1±1.4	10.1±1.3	7.3±0.8	14
cKO M	20.6±2.9	20.7±3.2 *	16.0±3.5 *	11.7±1.9	9.7±2.7	15



Supplemental Figure S1. Control studies to test for spatial- and sex-specificity of signaling deficits caused by loss of Cc2d1a A-D. No changes are found in PDE4D phosphorylation measured by Western blot in males and females (**A.** and **C.**) and cAMP levels measured by ELISA (**B.** and **D.**) in the mouse cortex. **E.** CC2D1A expression levels are the same in male and female WT brains in the hippocampus and cortex. **F-I.** Reduction in CC2D1A expression is comparable in males and females in the cortex and hippocampus showing that sex-specific signaling differences are not due to residual CC2D1A activity in the female hippocampus. CC2D1B is expressed at lower levels than CC2D1A and is not affected. Note that CC2D1A expression is not completely ablated because the CaMKIIa promoter is primarily active in excitatory neurons causing conditional CC2D1A removal only in that population, and probably leaving CC2D1A in interneurons intact.

Supplemental Reference

1. Oaks AW, Zamarbide M, Tambunan DE, Santini E, Di Costanzo S, Pond HL, *et al.* (2017): Cc2d1a Loss of Function Disrupts Functional and Morphological Development in Forebrain Neurons Leading to Cognitive and Social Deficits. *Cereb Cortex*. 27: 1670–1685.