Figure 5. Kal7 is essential for formation of cocaine place preference. In a conditioned place preference test carried out on the schedule indicated, mice of both genotypes exhibited a significant preference for the cocaine-paired side, with the response of Kal7^{KO} mice substantially attenuated compared to littermate controls at both 10 and 20mg/kg cocaine (***Middle***; p=0.025 for 10mg/kg; p=0.029 for 20mg/kg). When food was used as the unconditioned stimulus, both genotypes formed an equal preference for the food paired chamber (***Right***; p=0.64) indicating that the difference between Wt and Kal7^{KO} mice was specific to drug preference. Animals injected with saline only did not form a preference for either chamber (***Left***). (*p<0.05; **p<0.01 ANOVA) [N=6-10/group].

Figure 6.NAc spine morphology after 4 days of cocaine (20mg/kg) ***A-D.*** Representative images are shown; scale bars are 10µm. The treatment paradigm used is indicated below the images. ***E.*** Dendritic spine density was quantified as described in ***Fig. 2E***. A two-way ANOVA revealed a main effect of genotype (p=0.005) as well as a genotype x treatment interaction (p=0.003). Spine density data from ***Fig.2E*** (8 day) are replotted for comparison. ***F.*** Kolmogorov-Smirnov cumulative distribution analysis shows the same effect of genotype at baseline in these animals. ***G,H.*** However, the shorter cocaine treatment produced no main effect of treatment on spine area in either genotype [N=321-442 spines/group].

Figure S1. Comparison of fixation conditions. Due to literature suggesting that fixation strength may affect ability to visualize spines, two conditions were compared. ***A.*** Representative DiI labeled neurons from the nucleus accumbens and CA1 region of the hippocampus fixed with 4% or 1.5% paraformaldehyde. ***B.*** Low power images of nucleus accumbens slices fixed under the two conditions. A similar density of labeled neurons and processes is visible in both.

Figure S2. Graphical depiction of our method for measuring planar spine area. To achieve the most accurate measurements of spine area from collapsed z-stack images (1) we first performed a nearest neighbor deconvolution which sharpened the edges of the shaft and spines (2). To allow MetaMorph to accurately identify each spine, the dendritic shaft was manually excised from the image using the ImageJ program (3). Finally, in MetaMorph, the images were thresholded such that the spines were filled and traces were automatically drawn around each spine (4).

Figure S3. Spine length analysis. Following the 8-day cocaine treatment there was a main effect of genotype ($F_{1,48}$=23.00; p<0.0001, two-way ANOVA) but no effect of treatment ($F_{1,48}$=2.01; p=0.163) or genotype by treatment interaction ($F_{1,48}$=0.09; p=0.760). Following the four day treatment there were no main effects. A comparison of the Saline data from Wt and KO mice in the two experiments shows that there were opposite insignificant differences between the 8-day and 4-day samples. These trends are the reason for the genotype effect in the eight day but not four day experiment. Upper Brackets: ### p <0.001 2-Way ANOVA. Lower Brackets: ***p<0.001 1-Way ANOVA, NS = Not Significant.

Figure S4. Analysis of the automated stereotypy counts (fine motor counts) revealed no difference between Wt and Kal7^{KO} mice either across days (RM ANOVA $F_{(1,19)}$= 0.472; p=0.4) or on any individual day (all days p>0.245). Video analysis of a subset of animals corroborated these findings. “Fine motor counts” is a measure employed by our locomotor analysis software. It is loosely defined as repeated breaks of the same beam as would be seen with stereotypy, rather than the breaking of consecutive beams as would be seen with locomotion

Figure S5. Kal7^{KO} animals do not show an increase in locomotor activity on the conditioned place preference test day. It is noteworthy that the animals received no injection on this day. Kal7^{KO} animals were not different from Wt during the 10mg/kg test and were slightly depressed in their locomotor activity during the 20mg/kg test ($F_{1,17}$ = 9.53; p=0.007).

Supplemental Methods

Conditioned Place Preference

A San Diego Instruments apparatus (San Diego, CA) was modified such that the two large chambers had different patterns on the walls and different textures on the floors, making them readily distinguishable. On day one, individual animals were placed in the central walkway with both doors open and allowed to explore freely for 20 minutes. The least preferred side on this pre-test day became the cocaine or food-paired side. For four days, animals were conditioned for 15 minutes after a saline injection in the morning and after a cocaine injection in the afternoon. On the final day, the animals were again allowed to freely explore all chambers. The amount of time spent on the cocaine paired side on the test day compared to the pretest day was used as the index of preference.

For food preference, the animals were food deprived for 5 days before the start of training; their weights dropped to ~85% of free-feeding. For conditioning, one side of the chamber was paired with a petri dish full of grain reward pellets (BioServ, Frenchtown, NJ) while the other was paired with an empty dish. Animals received 5 fifteen-minute sessions on each side on the same schedule as cocaine conditioning and were then given a 20 minute test similar to the cocaine animals.

Image Acquisition

Images of medium spiny neurons were taken using a Zeiss LSM 510 Meta confocal microscope. The NAc core was identified using the anterior commissure as an anatomical landmark, and medium spiny neurons within the core were identified by their morphology. Sections of dendrites (50μm) were imaged 75-125μm from the soma and after at least one dendritic branch point. Each final image consisted of a z-stack of pictures taken at 0.3 □m steps through the dendrite.

Spine Measurements

Spine length was defined as the length from the base of the spine to the farthest point, drawn using the straightest line, or pair of lines, possible. To measure spine area accurately, the methods of Svoboda and coworkers and Yuste and coworkers were adapted (1,2). First, z-stack images were processed using the nearest neighbor 2D deconvolution function in MetaMorph. Following deconvolution, the stack was collapsed to a single image using the maximum intensity function. This collapsed image was opened in ImageJ and the dendritic shaft, partially occluded spines, and extraneous background signal were carefully removed using the polygon selection tool. The image was then opened in MetaMorph and an inclusive threshold was adjusted to outline spines. Lastly, the create regions around objects tool was used to automatically trace the thresholded regions. A graphical depiction of this process is shown in **Fig. S2**.

For ANOVA analyses, the average density and length for spines on a single dendrite was considered to be an N of 1 (range = 27-86 spines per 50 □m image). For analysis of spine area using the Kolmogorov-Smirnov function, each individual spine was used as a data point (3). This type of analysis allows for equal weight to be given to each spine and is not biased by dendrites with more or fewer spines.

Biochemistry

For biochemical quantification of Kal7 in the NAc, animals were treated for 7 days with 20mg/kg cocaine. Twenty-four hours after their final injection the animals were decapitated and a punch was taken from a coronal section (2 mm thick) that included the NAc core. For Western blotting, tissue was sonicated in 1% SDS lysis buffer and analyzed on a 4-20% Tris glycine gel (Invitrogen) (4). For qPCR, tissue samples were homogenized in Trizol (Invitrogen), RNA was prepared following the manufacturer's instructions, and cDNA was prepared using random primers (**Supplementary table 1**) and either Superscript II Reverse Transcriptase (Invitrogen) or the iScript Select cDNA Kit (Bio-Rad). Quantitative polymerase chain reaction (qPCR) was performed with iQ SYBR Green supermix, using an Eppendorf Mastercycler ep realplex machine, with the standard program except that elongation time per cycle was 40 sec.

Statistics

Statistical analyses were performed using Microsoft Excel and SPSS software. Behavioral analyses were performed using one-way, two-way or repeated-measures ANOVA as appropriate. Quantification of Western blots and q-PCR was performed using unpaired t-Tests. Cumulative distribution plots for spine morphology were analyzed using the Kolmogorov–Smirnov test, all measures of spine density and morphology were also analyzed using two-way ANOVAs. The qPCR data for each sample were normalized to GAPDH, with ratios for cocaine treated animals expressed as a % of ratios for saline treated animals.

Supplementary table 1. Primer pairs for Kalirin7 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were chosen to give 119-156 nt products and primer T_m of 61^oC. Maximal rates of amplification of products for every primer pair were 2.01 $\pm$ 0.08 per cycle.

gene	primer name	sequence	Tm (^oC)	nt
mGAPDH NM_008084.2	mGAPDH-for	TTGTCAGCAATGCATCCTGCACCACC	61	119
	mGAPDH-rev	CTGAGTGGCAGTGATGGCATGGAC	61	
mKalirin-7 XP_899566.1	KalSpec-for	GCCTTTCTCAGCAAACACACTGGGG	61	156
	KalSpec-rev	ATTCCCCAGTCTGAGCCAGCTGC	61	

References

1. Koh IY, Lindquist WB, Zito K, Nimchinsky EA, Svoboda K (2002): An image analysis algorithm for dendritic spines. *Neural Comput* 14:1283-1310.
2. Tashiro A, Yuste R (2008): Role of Rho GTPases in the morphogenesis and motility of dendritic spines. *Mtds Enzymol* 439:285-302.
3. Conover WJ (1999): Statistics of the Kolmogorov-Smirnov type. In: Conover WJ, editor. *Practical nonparametric statistics*. New York: John Wiley & Sons, Inc., pp 428-473.
4. Ma XM, Kiraly DD, Gaier ED, Wang Y, Kim EJ, Levine ES, *et al* (2008): Kalirin-7 is required for synaptic structure and function. *J Neurosci* 28:12368-12382.

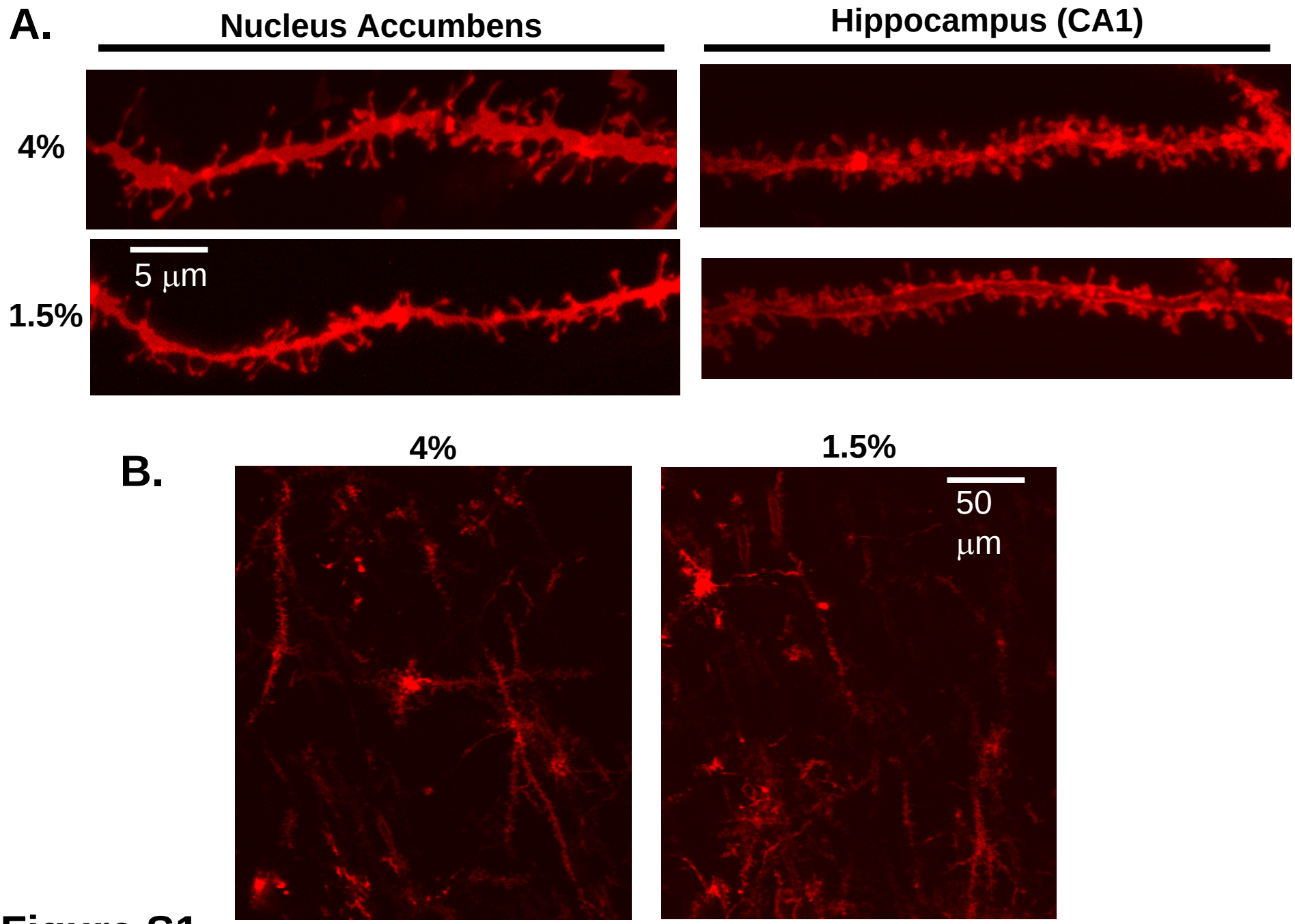
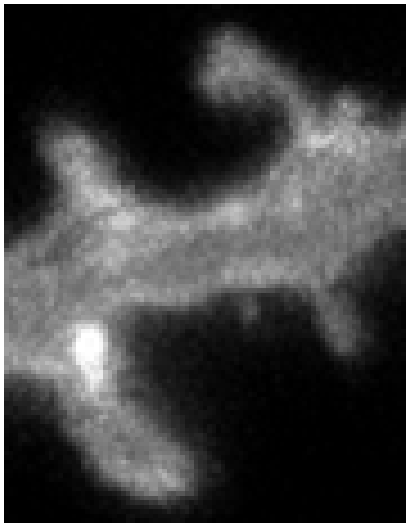


Figure S1

Spine area measurements in Metamorph



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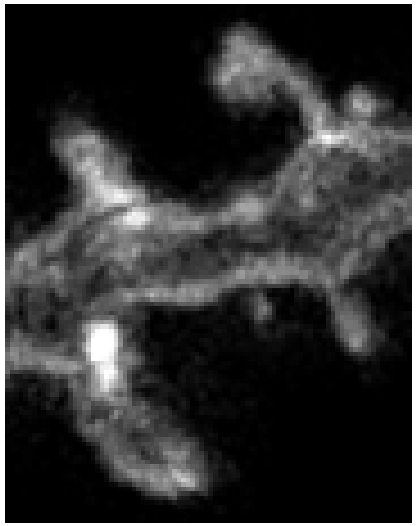
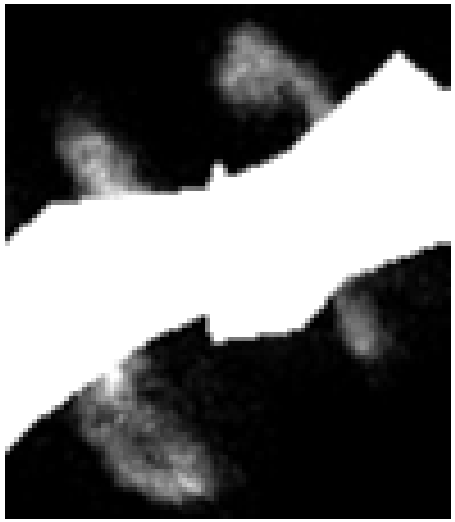
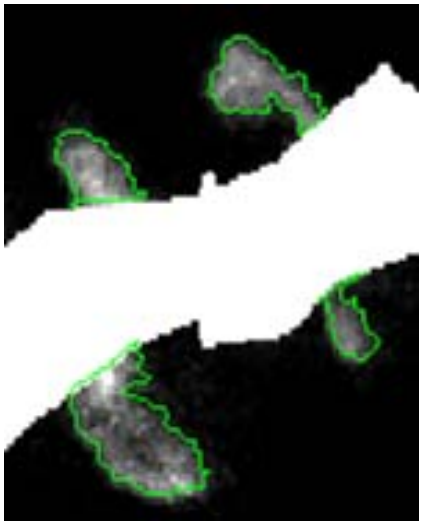


2) Nearest neighbors
2-D deconvolution



3) Dendrite removed



4) Traces around spines

Figure S2

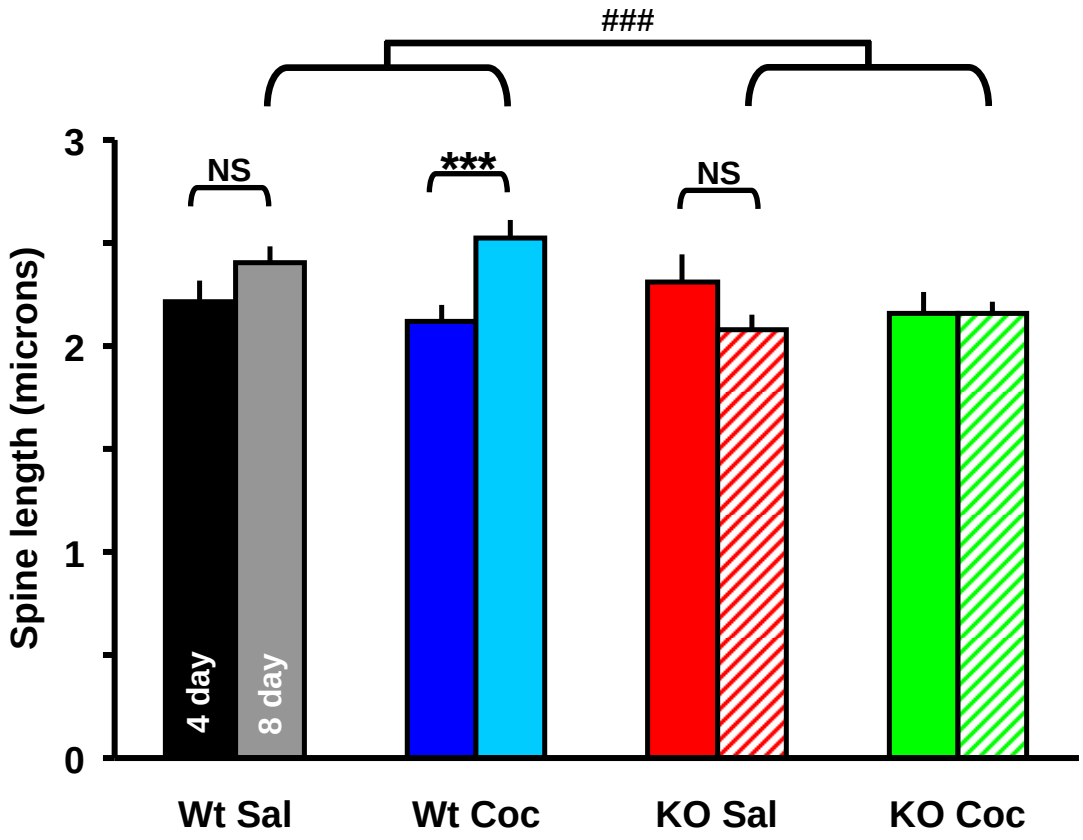

###
NS
***
NS
3
2
1
0
Spine length (microns)
4 day
8 day
Wt Sal
Wt Coc
KO Sal
KO Coc

Figure S3

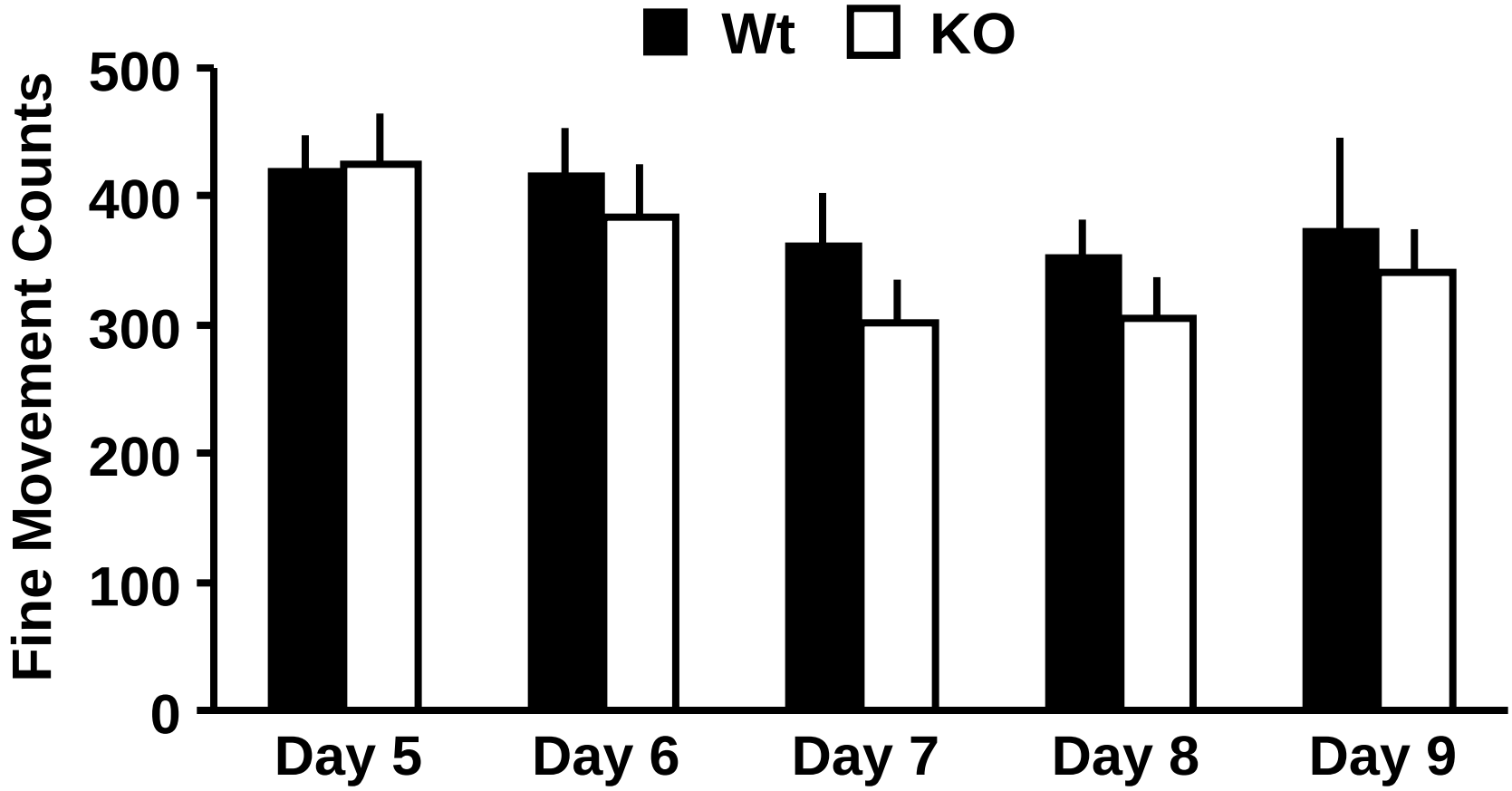

Wt
KO
Fine Movement Counts
500
400
300
200
100
0
Day 5
Day 6
Day 7
Day 8
Day 9

Figure S4

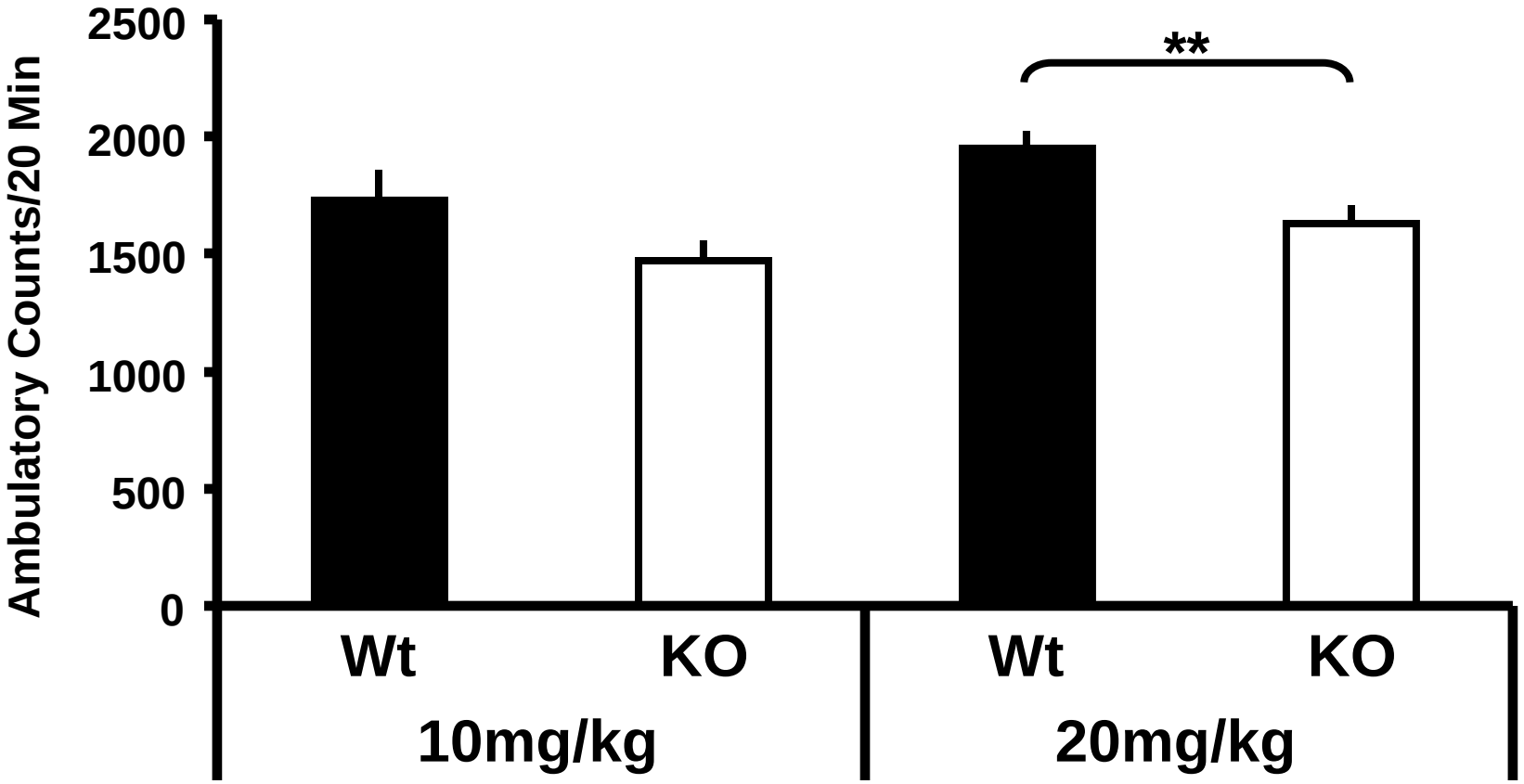

Ambulatory Counts/20 Min
2500
2000
1500
1000
500
0
**
Wt
KO
Wt
KO
10mg/kg
20mg/kg

Figure S5