Kalirin 7, a Rho GEF, Plays an Essential Role in the Response to Chronic Cocaine in Mice

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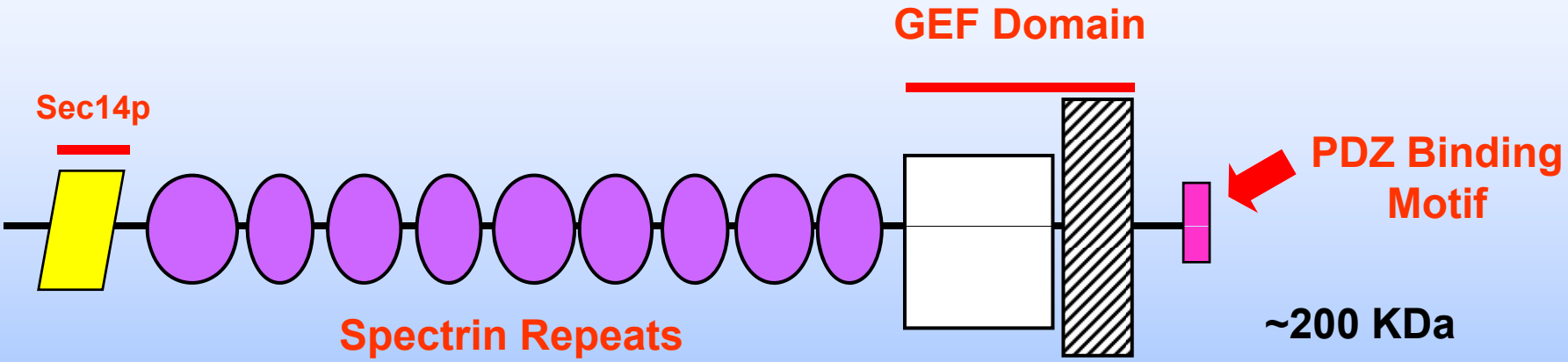
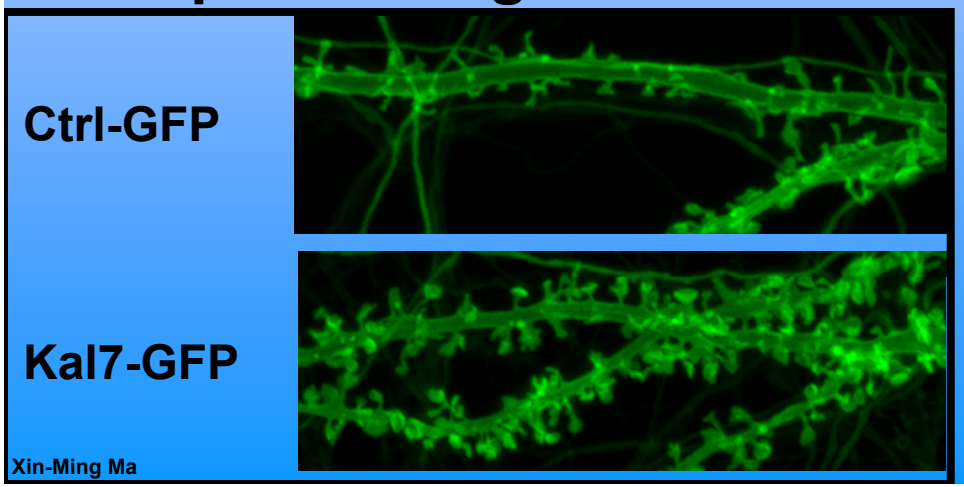
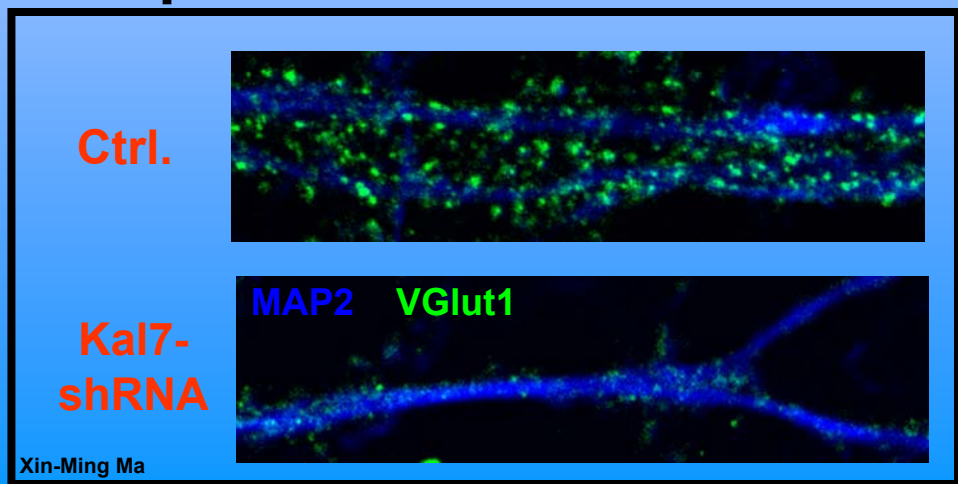
Kalirin-7

- Is the predominant CNS splice form of the alternatively spliced Kalirin gene
- Is a Rho-GEF localized to the PSD via a PDZ binding motif



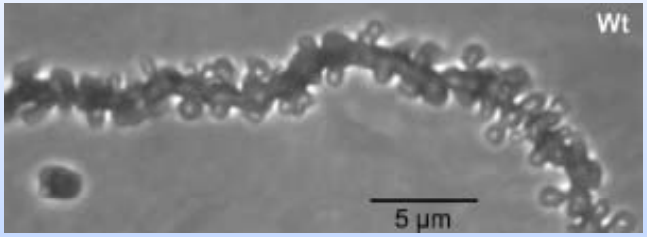
- Numerous *in vitro* experiments have shown Kalirin-7 to be an important regulator of dendritic spine formation

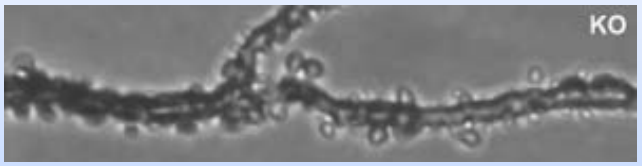


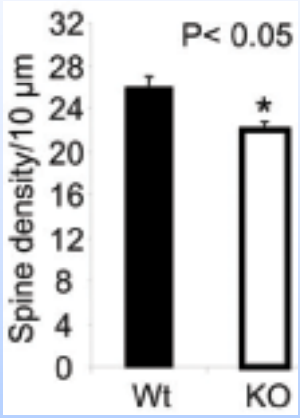


Kalirin-7 continued

Recent development of the Kalirin-7 knockout mouse has allowed us to demonstrate that Kalirin-7 is also essential for normal synapse formation *in vivo*



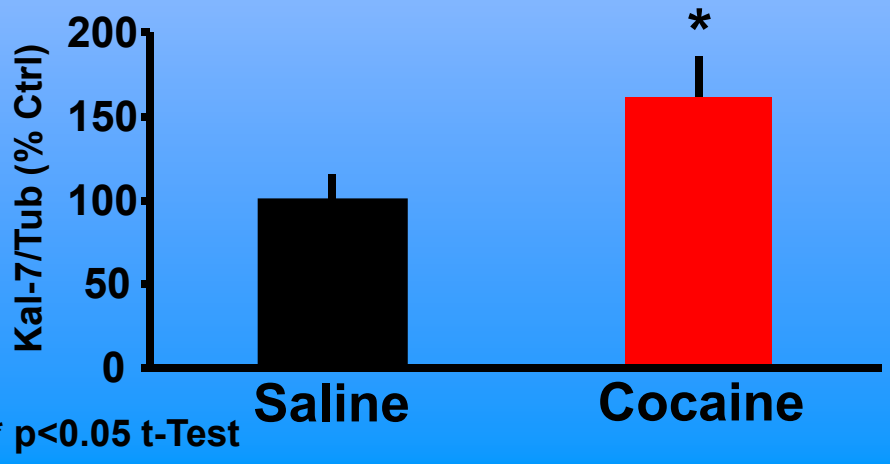




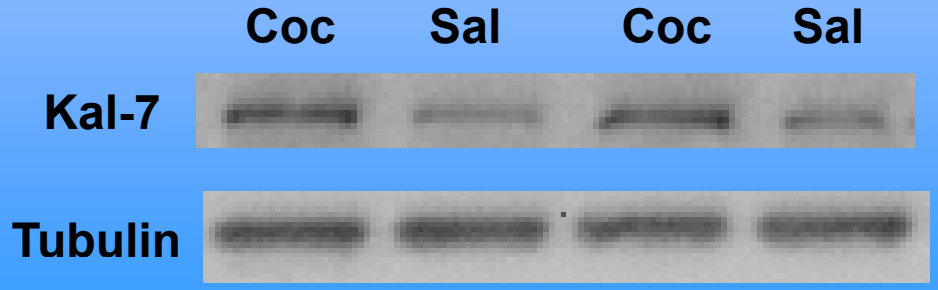
Kalirin-7 ⟶ ↑Spines

Ma and Kiraly et al., *JNeurosci* 2008

Chronic cocaine leads to upregulation of Kalirin-7 in the Nucleus Accumbens of both rats and *Wt mice* (20mg/kg x 8 days)

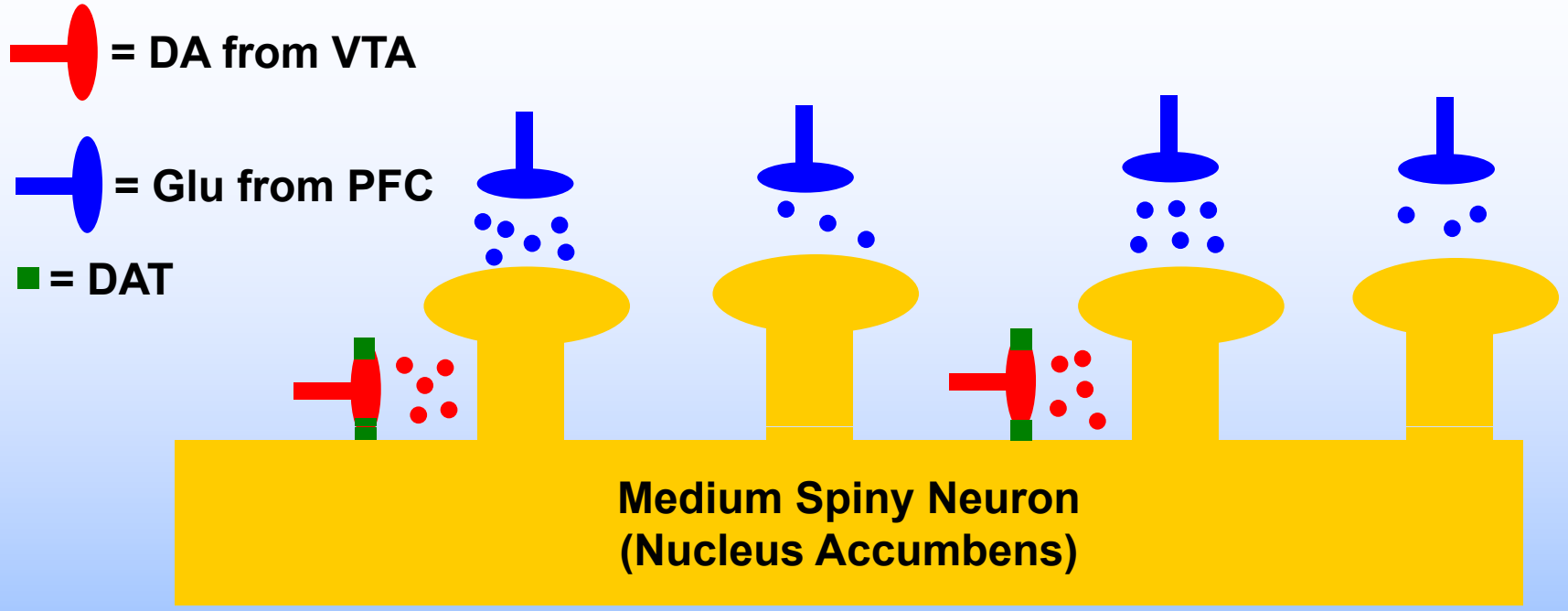


*** p<0.05 t-Test**



Cocaine ⟶ ↑Kalirin-7

Cocaine and Dendritic Spines

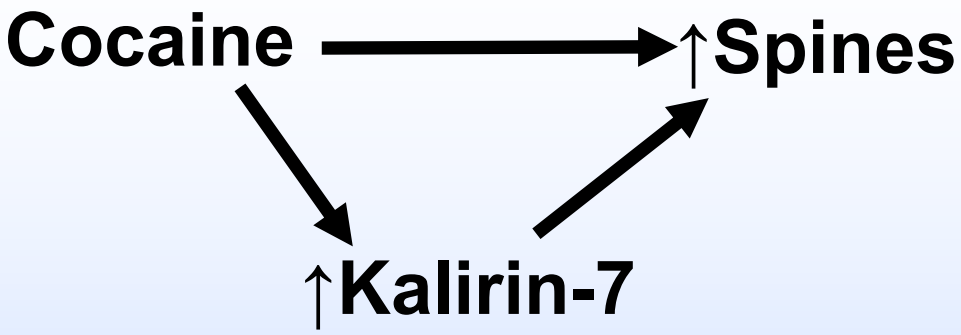


A dramatic increase in dendritic spines in the NAcc is seen after experimenter-administered[1] or self-administered cocaine[2]

This increase in spines has been reported to last as long as 3.5 months in rodents[3]

1) Robinson and Kolb, *Neuropharmacology* 2004
2) Robinson et al., *Synapse* 2001
3) Kolb et al., *PNAS* 2001

Cocaine → ↑Spines



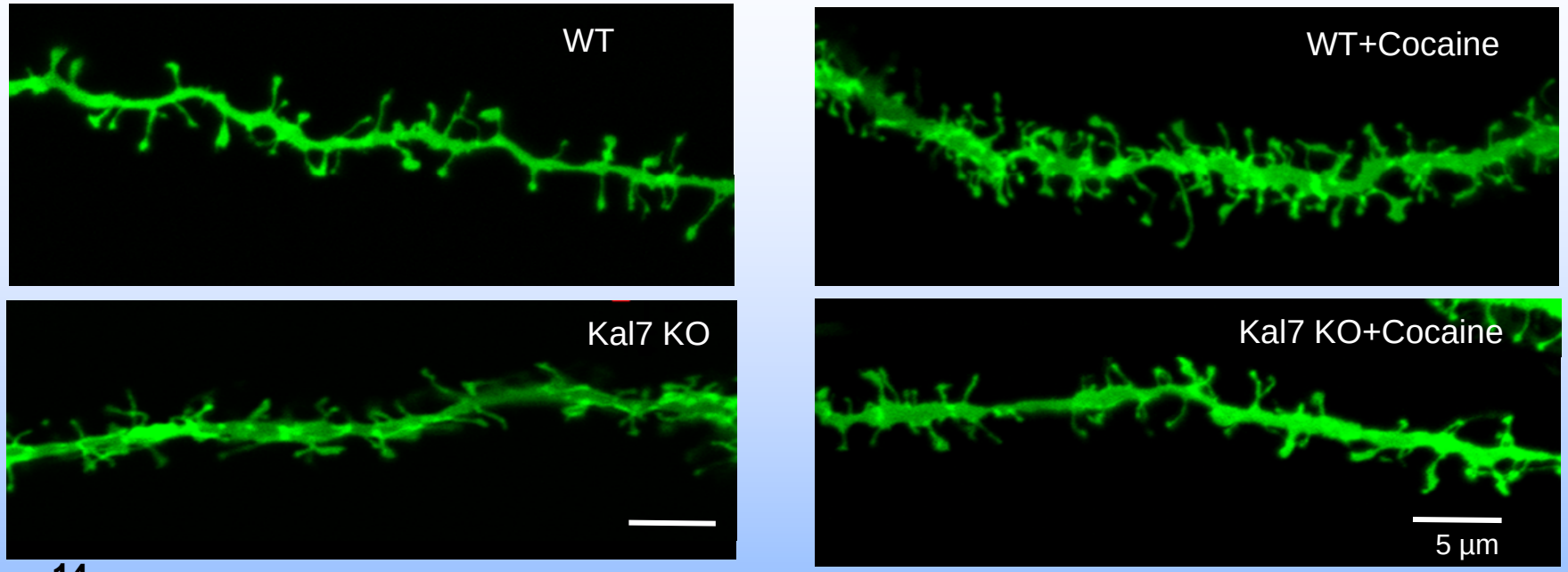
2 Main Questions:

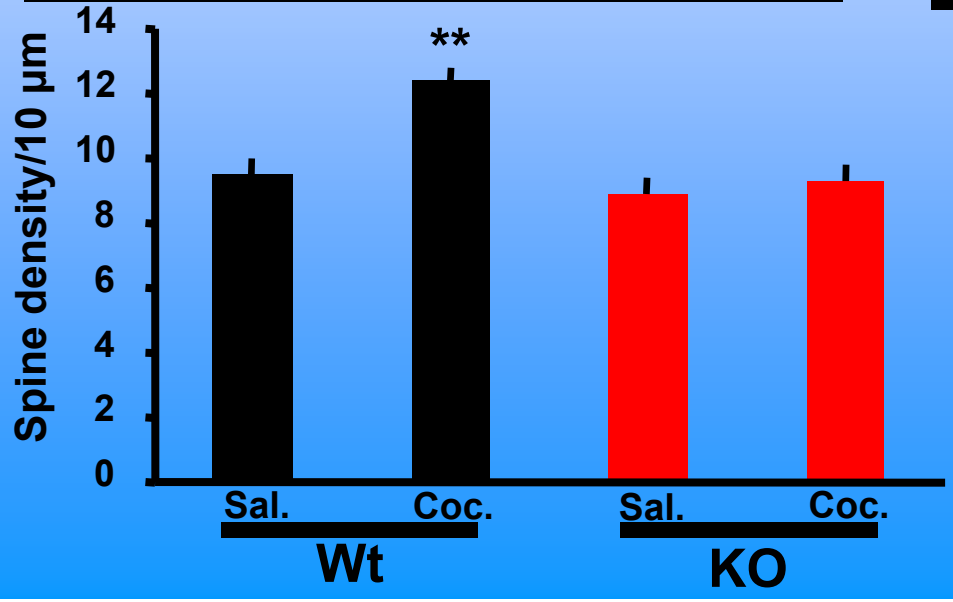
1) Are cocaine's effects on spines mediated through a Kalirin-7-dependent pathway?

2) What role do Kalirin-7 and/or these changes in spines play in cocaine-induced behaviors?

How does lack of Kalirin-7 affect cocaine-induced spine changes?

Procedure: Young adult mice given 20mg/kg I.P. once daily for 8 days, then perfusion fixed and the nucleus accumbens diolistically labeled





Cocaine

↓

↑Kalirin-7

↓

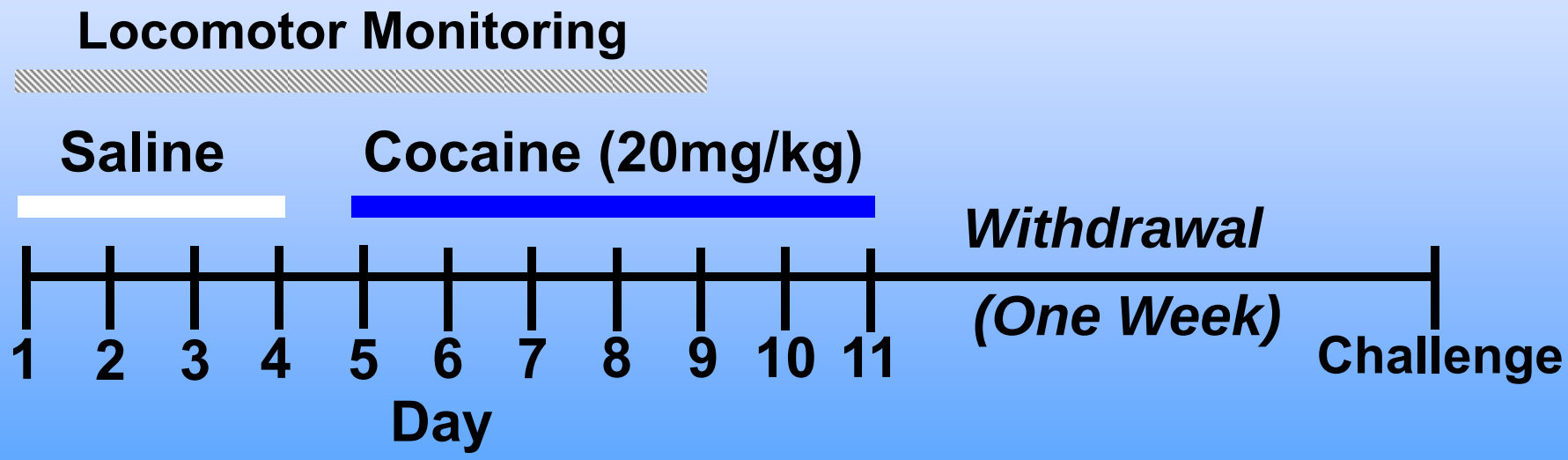
↑Spines

How do these changes affect behavioral response?

Locomotor Sensitization

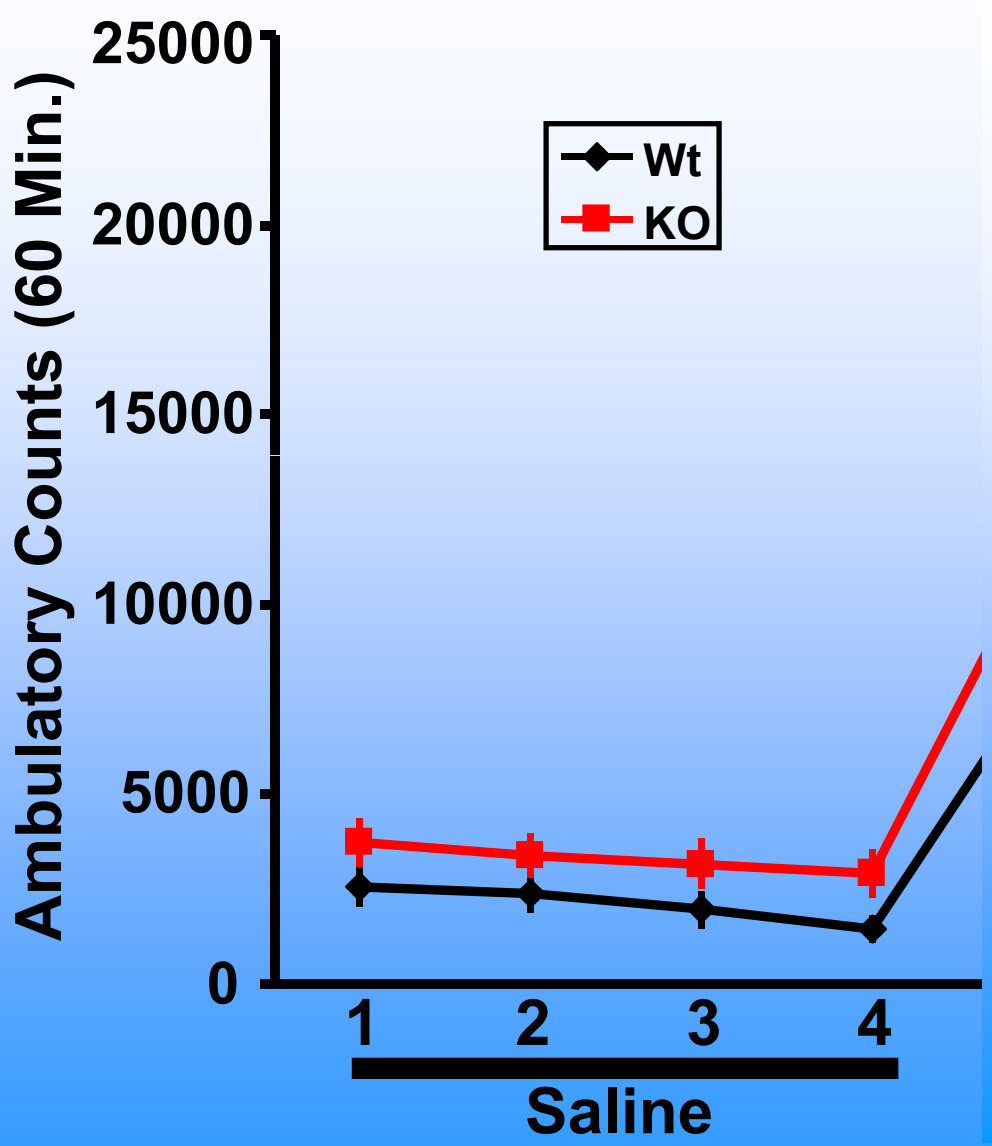
- Cocaine-induced increase in locomotion
- Common model for addiction, produces very persistent changes
- Response can be seen for up to a year in rodents[1] and longer in primates[2]

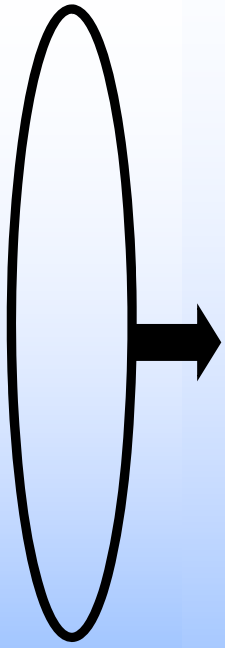
Protocol



1) Paulson et al., *Psychopharmacology* 1991
2) Castner and Goldman-Rakic, *Neuropsychopharmacology* 1999

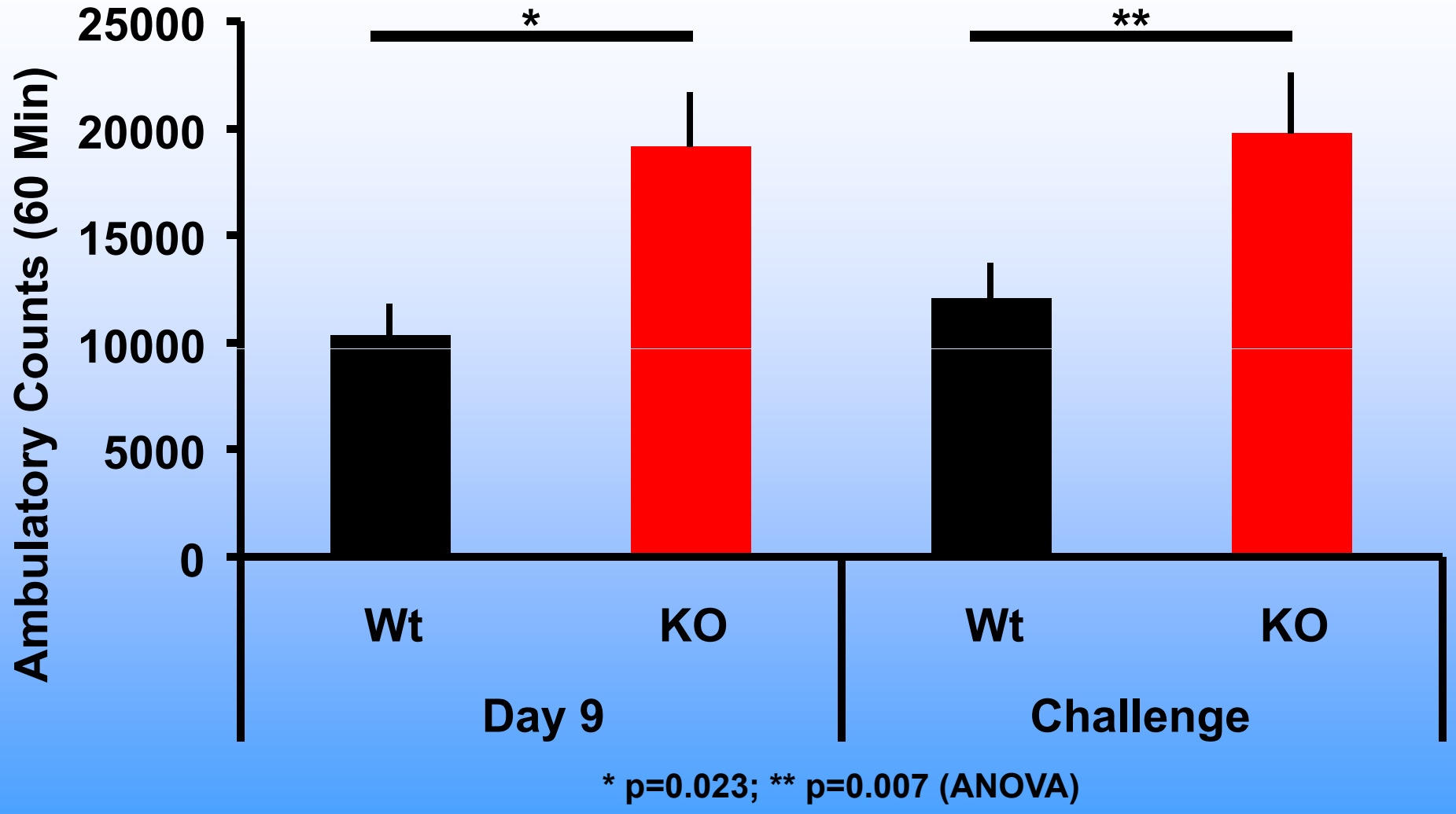
Locomotor Sensitization





**p≤0.01; *p≤0.02

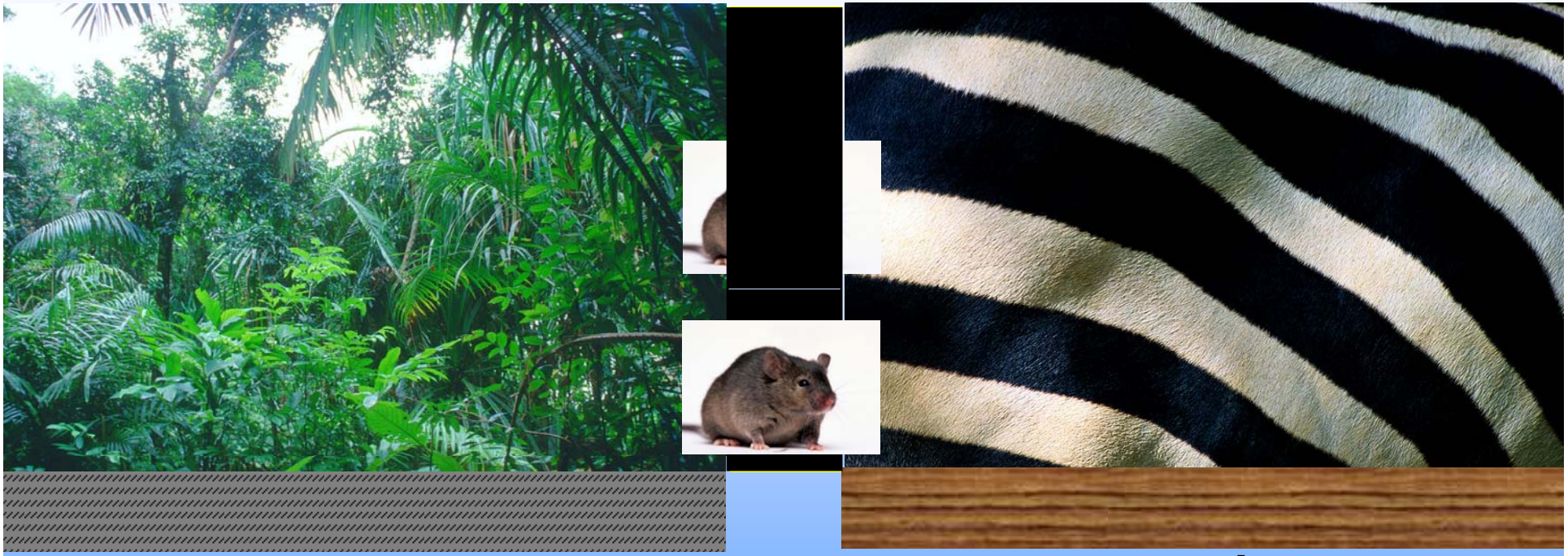
The increased sensitization persists after one week of withdrawal.



Kalirin-7 is essential for "normal" locomotor response to cocaine.
(↑Spines = Protective?)

Hedonic/Motivational Value of Cocaine

Conditioned Place Preference



Saline

Day One

Day Three

Day Five

Day Seven

Cocaine

Day Two

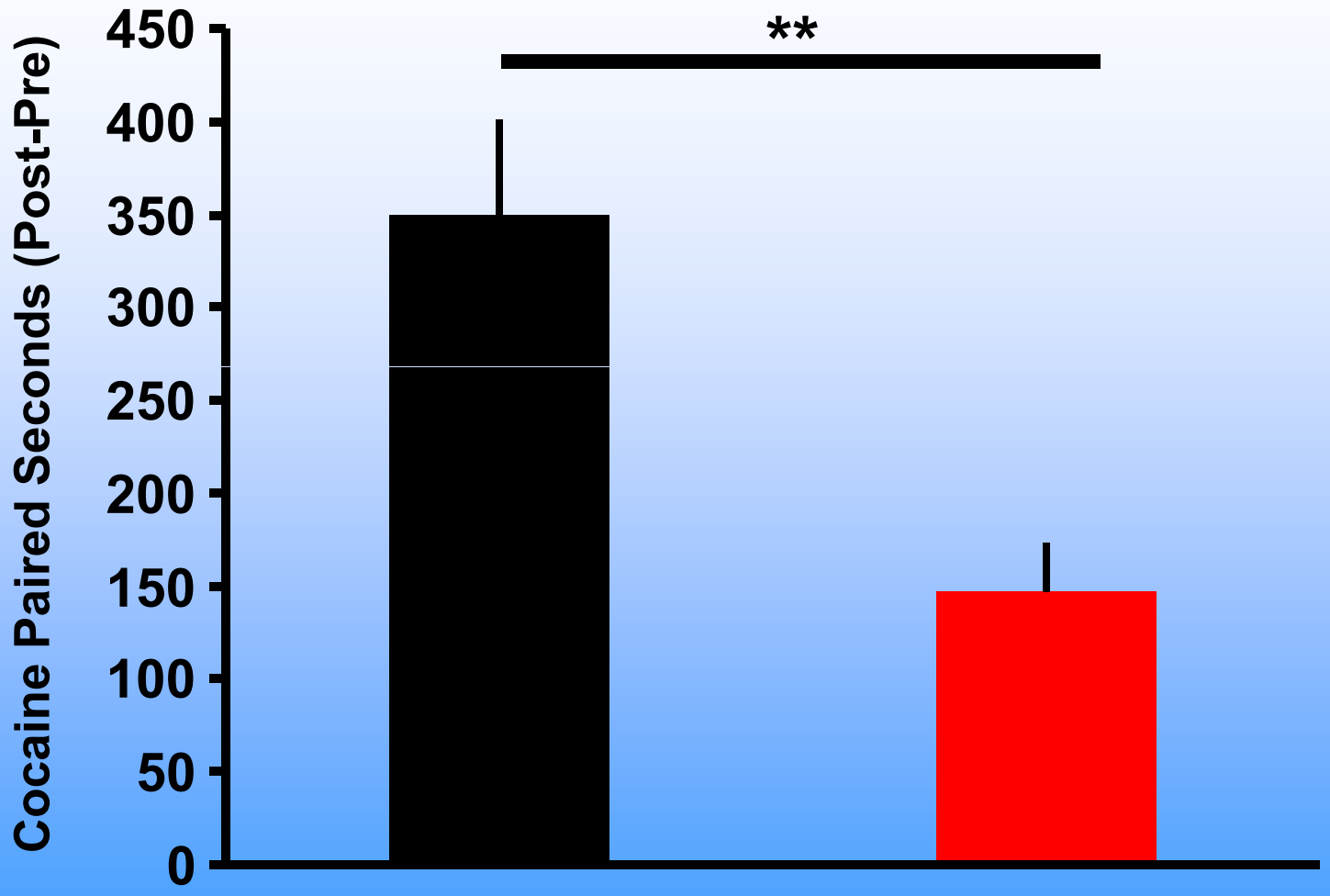
Day Four

Day Six

Day Eight

CPP Results

(20 Minute Test)

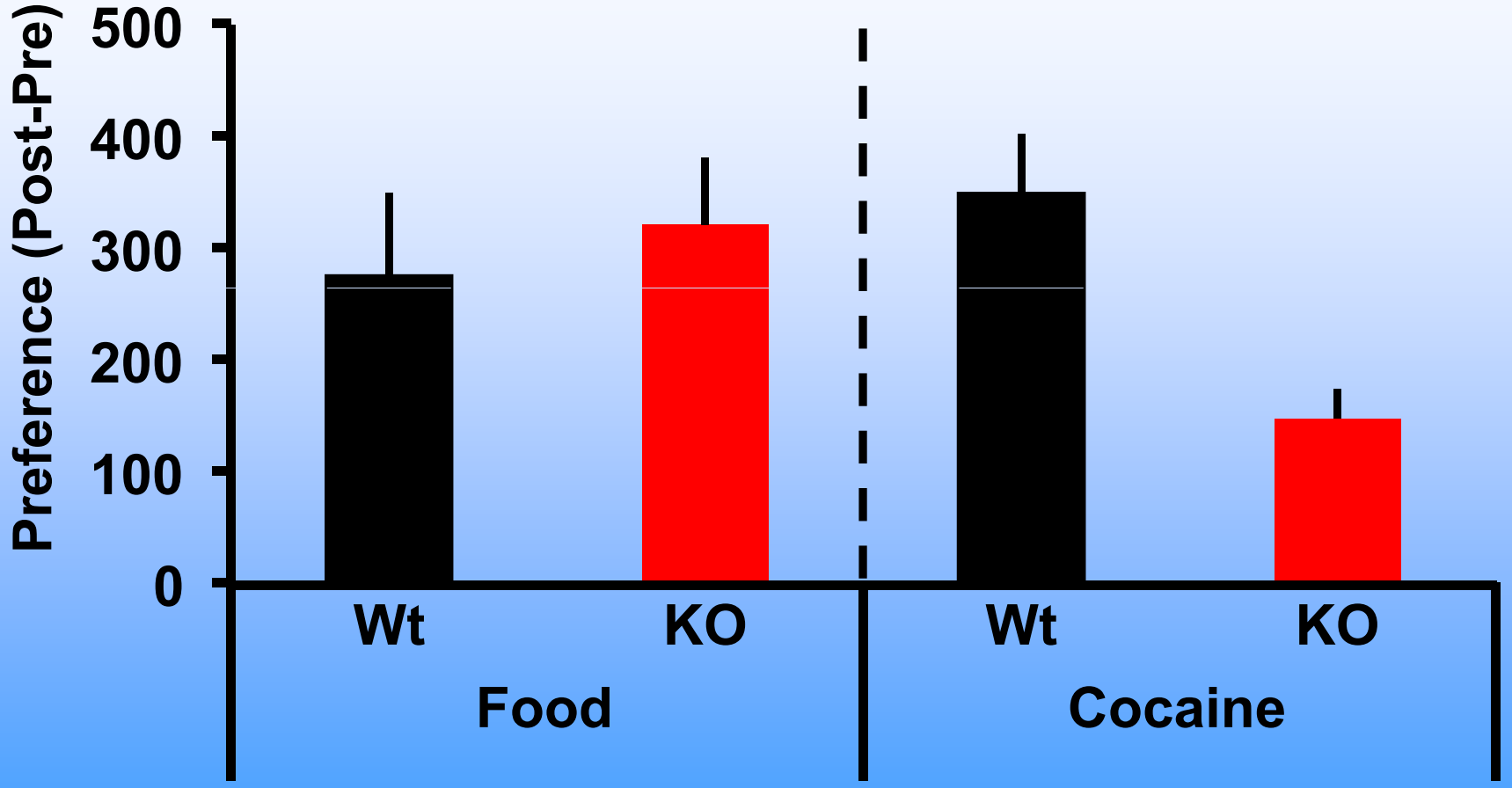


** p < 0.01 (ANOVA)

Kalirin-7 is also essential for normal motivational value of cocaine

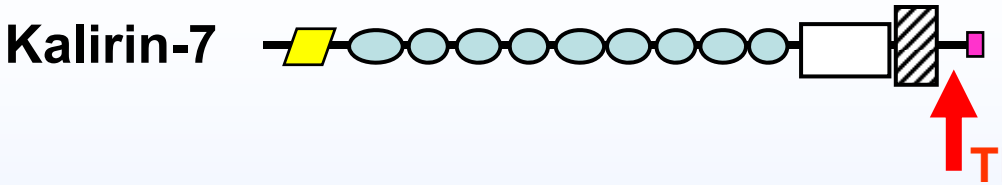
Food Place Preference

Protocol: Animals food deprived to 80% of free-feeding weight and subjected to same conditioning paradigm with grain pellets in place of cocaine.

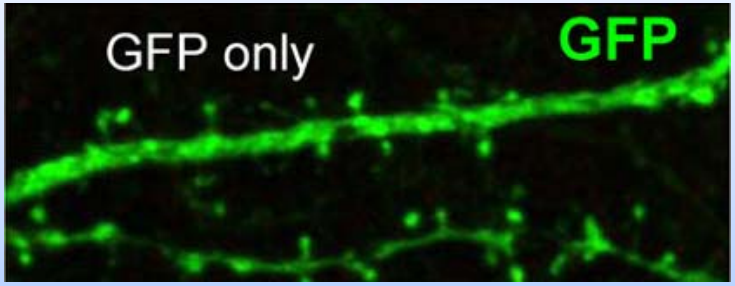


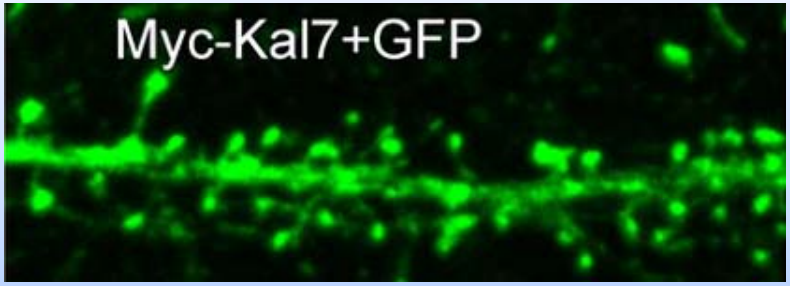
The decrease in preference is specific to cocaine and is *not* due to a global learning deficit

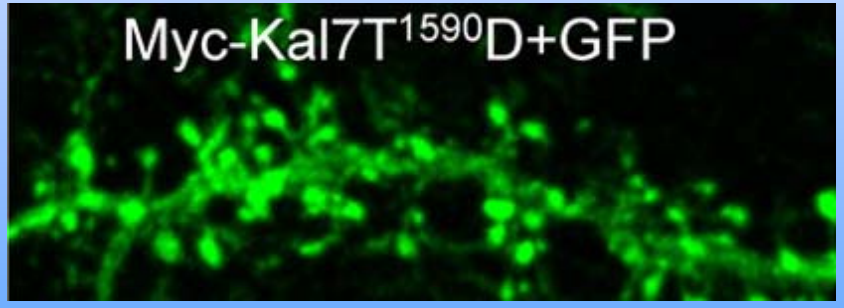
How might phosphoproteomics help?

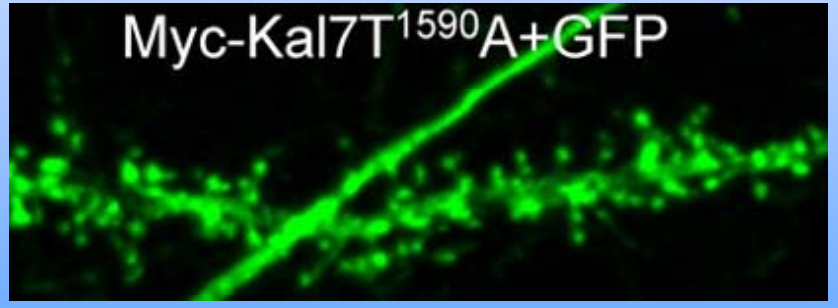


Phosphorylation at this site alters the effect that Kal7 has on the morphological properties of cultured neurons









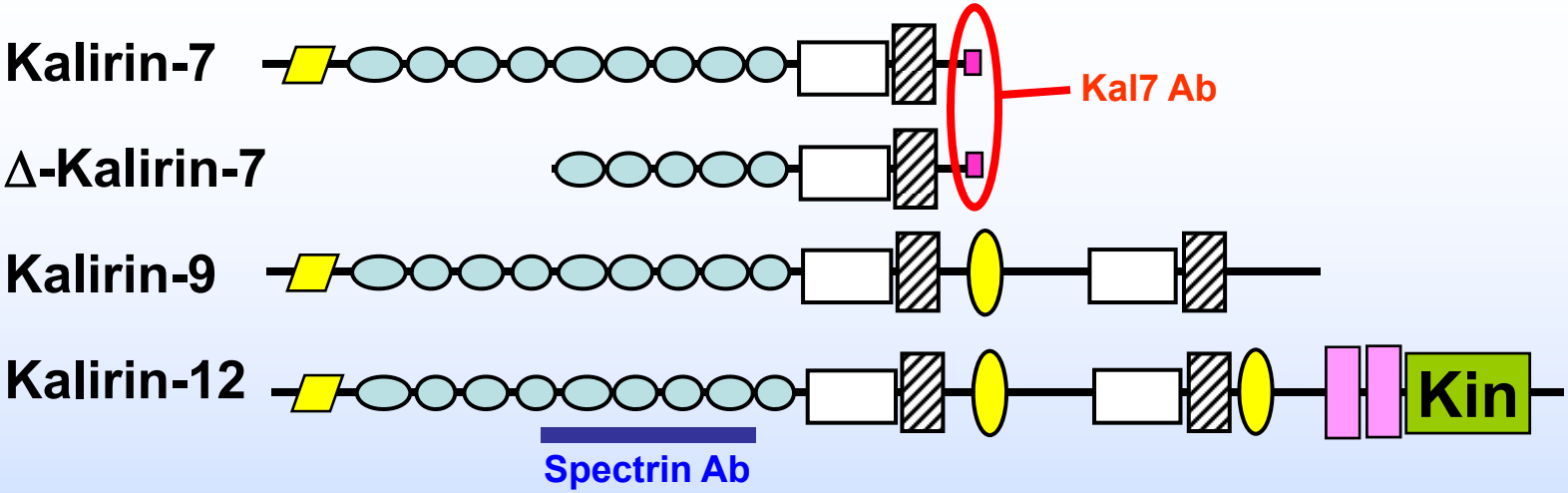
Phosphomimetic
Large spines, full heads

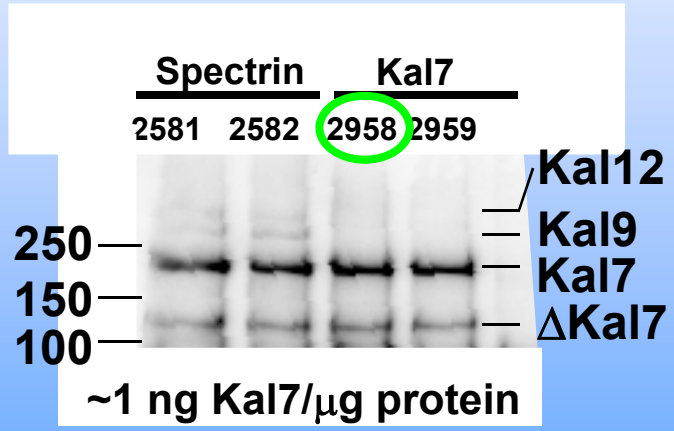
Xin et al., *J Cell Sci* 2008

Non-Phosphorylatable
Shorter spines, small heads

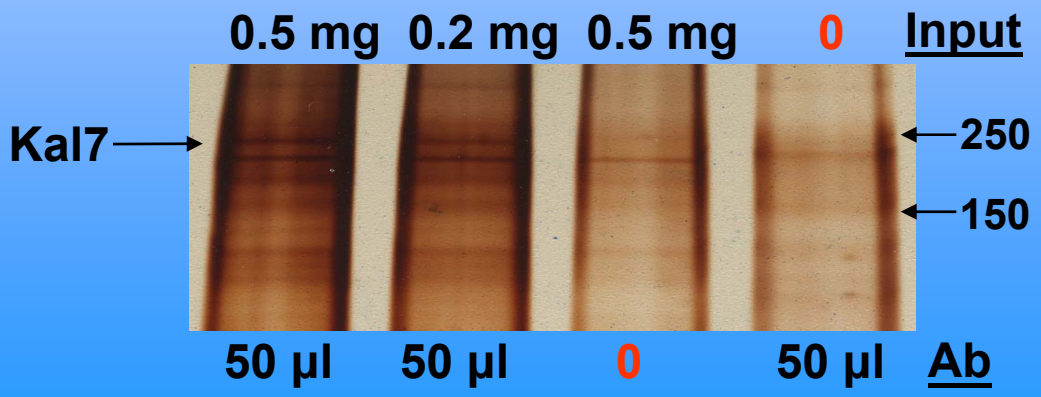
Given the important role of phosphorylation of other cocaine-regulated proteins (Creb, DARPP-32 etc.) it seemed likely that phosphorylation of Kalirin-7 may be altered by cocaine treatment.

Kalirin-7 for Proteomics

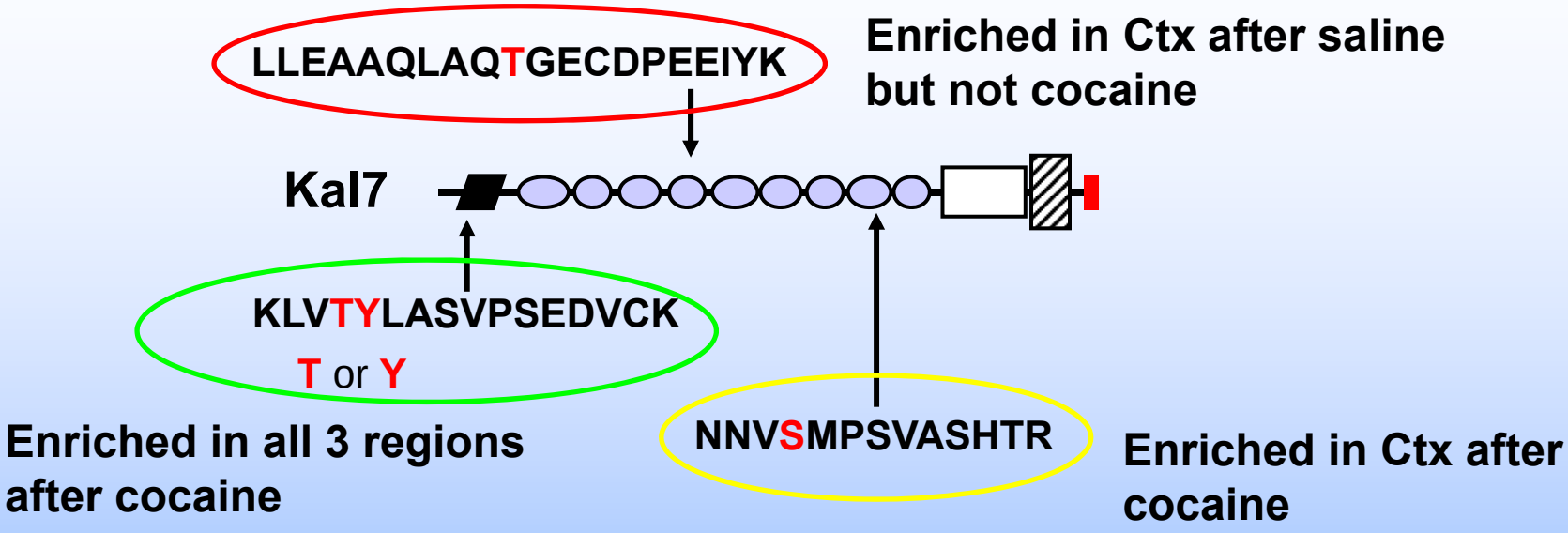




Protocol: Wt mice treated with acute 20mg/kg cocaine or saline. Sacrificed 30 min after injection. IPs done from NAc, Stri and Ctx



Saline vs Cocaine: NAcc, Striatum, Cortex



Future Directions

1) Identification of target kinases

2) Examination of chronic cocaine ± withdrawal

3) Development of phospho-specific antibodies

4) Comparison of Wt and Kal7^{KO} signaling

Thanks!

Xin-Ming Ma

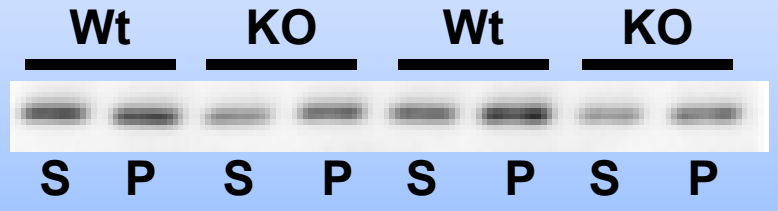
Betty Eipper
Dick Mains

Jodi Eipper-Mains

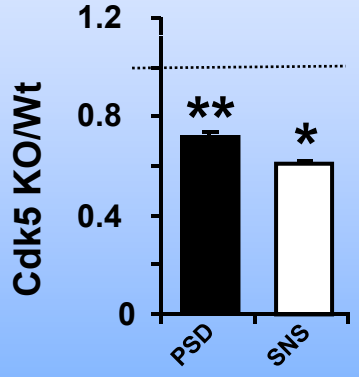
Ping Wang
Darlene D'Amato

Jane Taylor
Peter Olausson

More recently, we have shown that levels of Cdk5 are decreased in purified PSDs of Kal7^{KO} mice.



S= Synaptosome P = PSD Pellet



Ma and Kiraly et al., *JNeurosci* 2008

IPT Protocol

Male Wt C57/Bl6 Mice

Acute 20mg/kg cocaine or saline

Sacrifice 30 minutes later

Add NP40 + Kal7 antibody to

0.5-1.0mg of total protein

Precipitate with Protein A

Run SDS-PAGE

Silver Stain