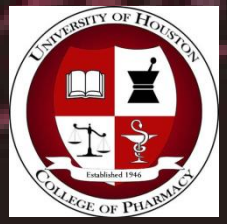
Searching for new possibilities to target neuro-developmental disorders associated with cognitive disabilities

Maria Victoria Tejada-Simon, PhD
Associate Professor
Dept. Pharmacological and Pharmaceutical Sciences, Psychology & Biology and Biochemistry

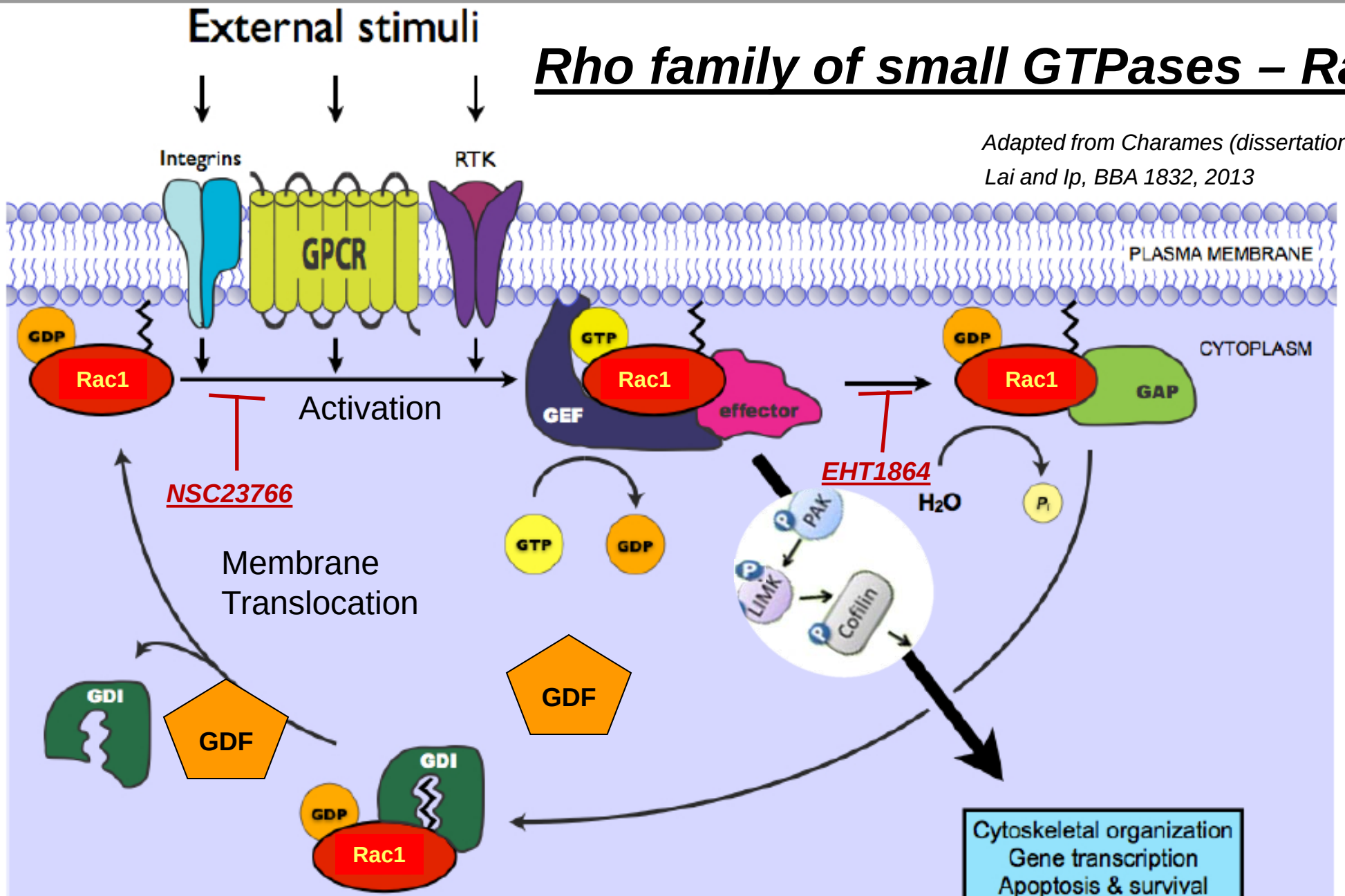


College of Pharmacy - University of Houston



Rho family of small GTPases – Rac1

Adapted from Charames (dissertation, 2010)
Lai and Ip, BBA 1832, 2013

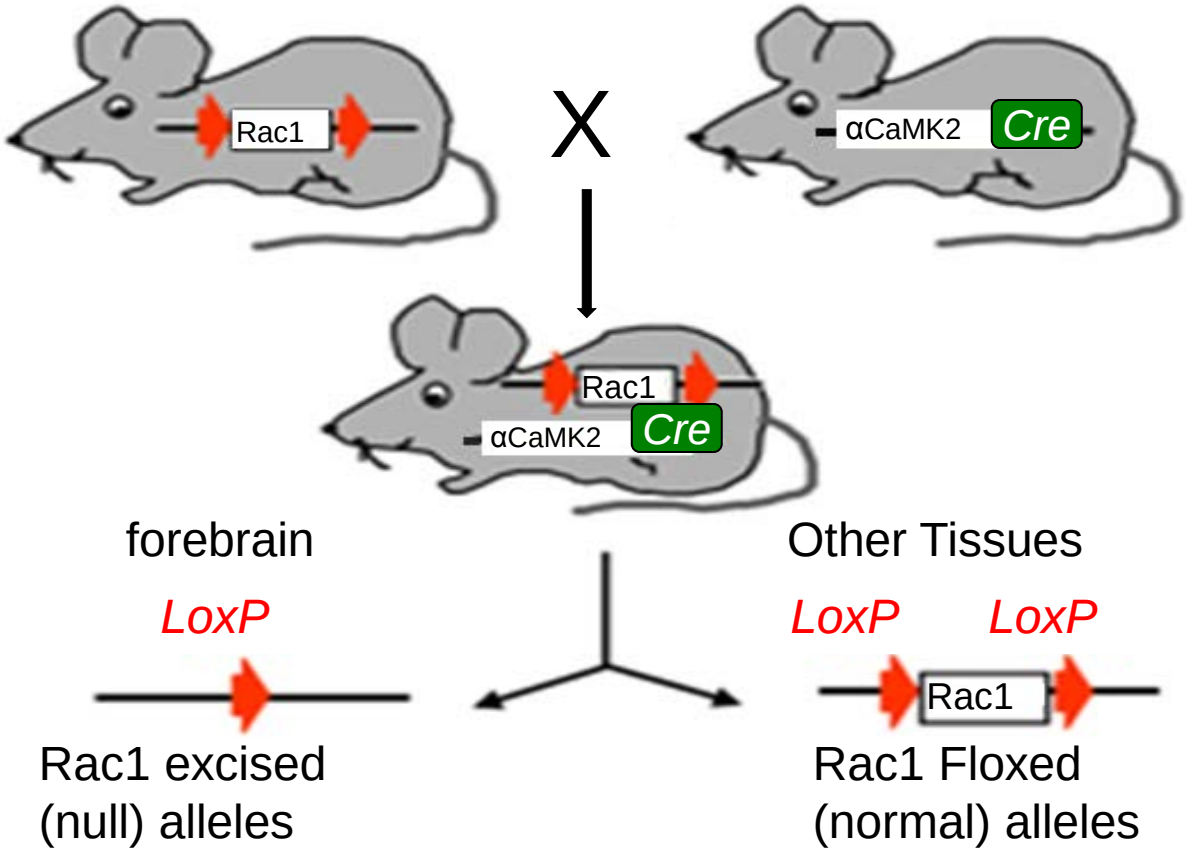


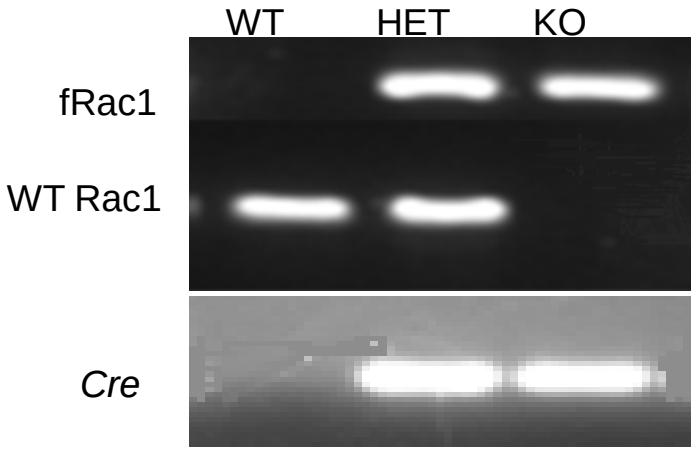
NSC23766 **- inhibitor of Rac1-Trio/Tiam1 interaction**
EHT1864 – interaction with GTP and effectors

Is Rac1 necessary for learning and memory?

Genetic approach

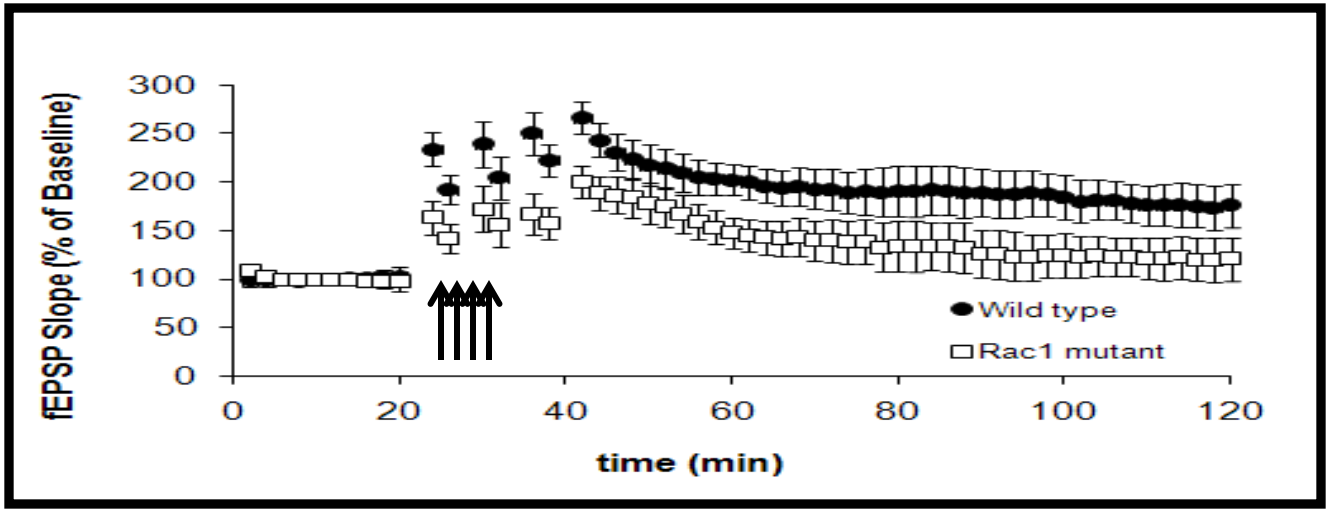
Site-specific Rac1 KO using Cre/loxP system

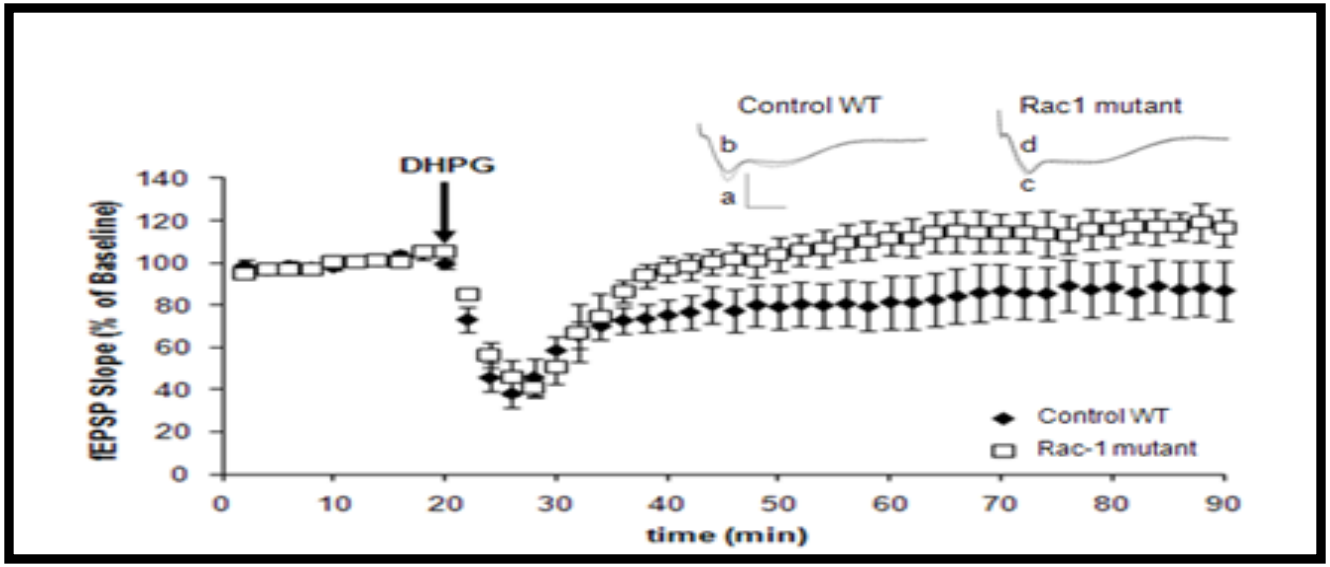




(Adapted from Chapellier et al., 2002 & Lecureuil et al., 2000)

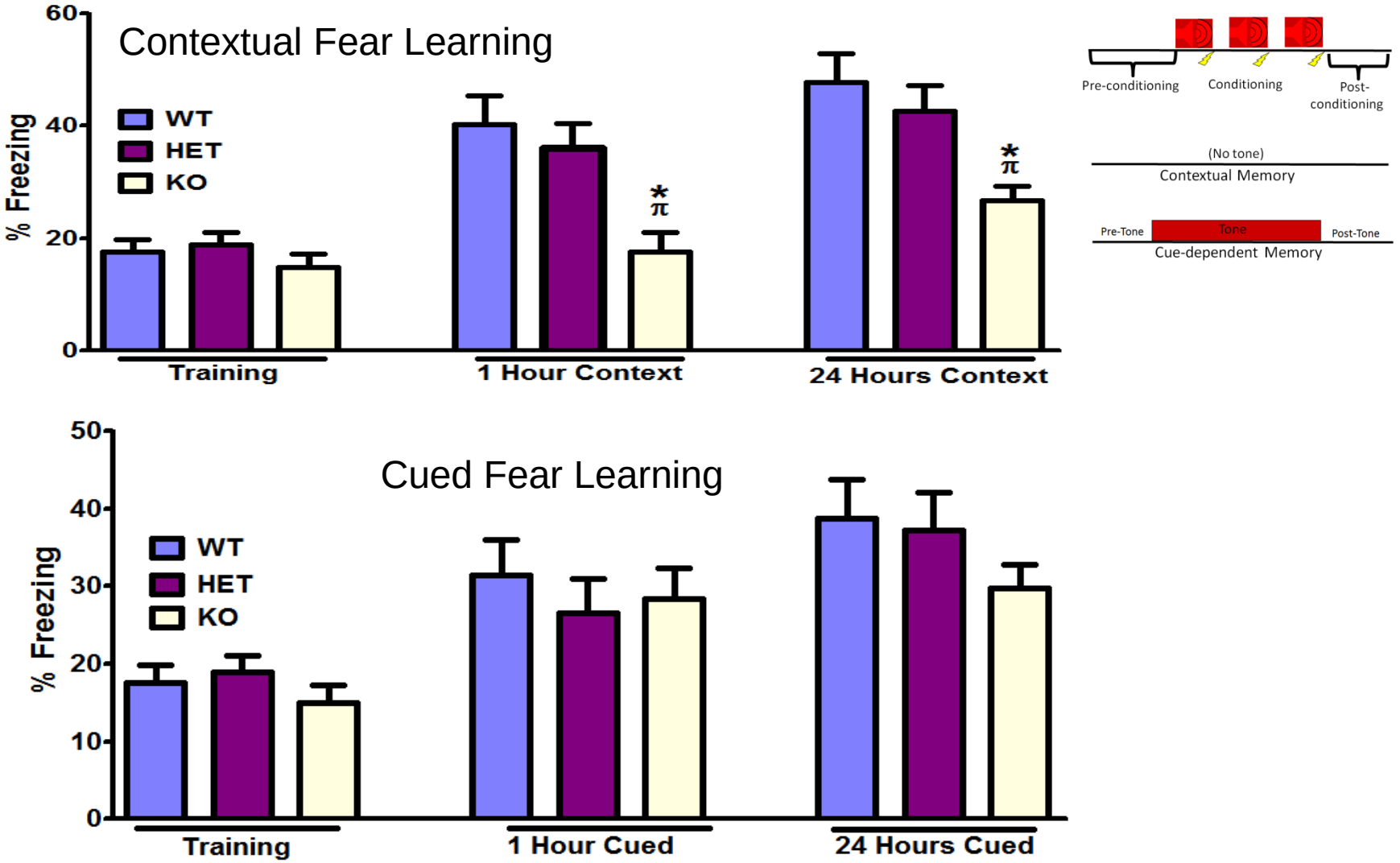
Loss of Rac1 in Long-term plasticity





Bongmba et al., 2011
Tejada-Simon and Bongmba, 2012

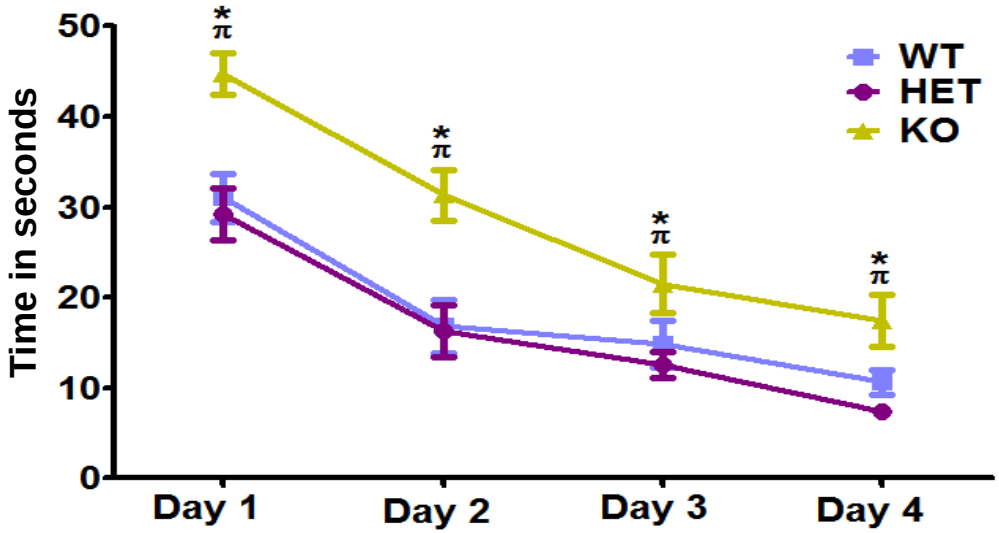
Loss of Rac1 in Associative Learning

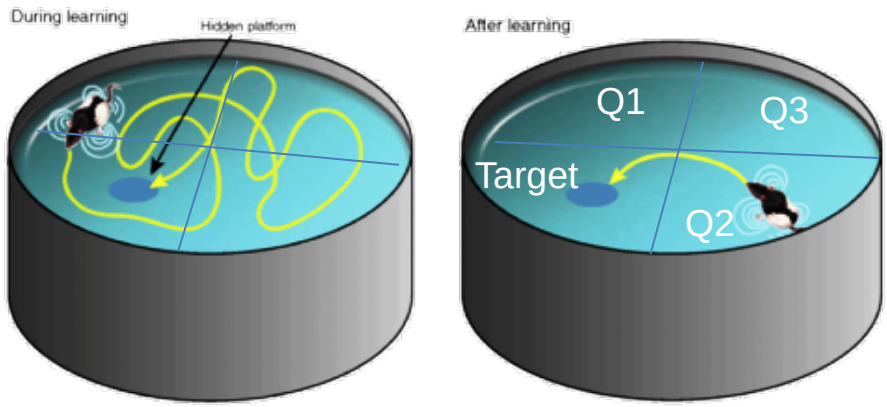


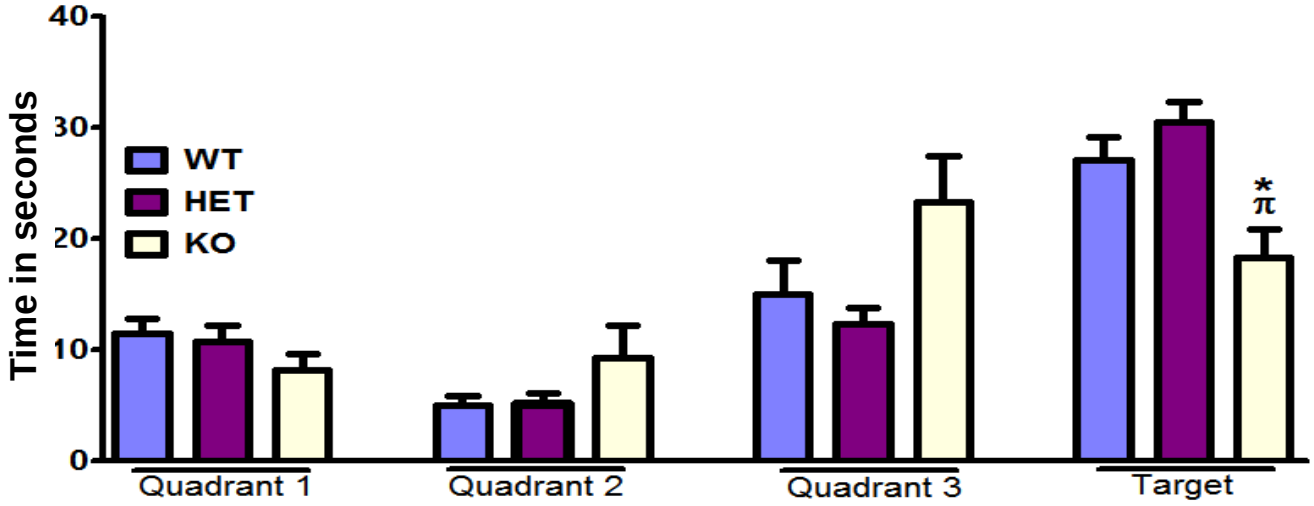
* denotes significance respect to WT; π denotes significance respect to HET ($p<0.05$, WT=17, HET=15, KO=17)

Tejada-Simon and Bongmba, 2012

Loss of Rac1 in Spatial Learning





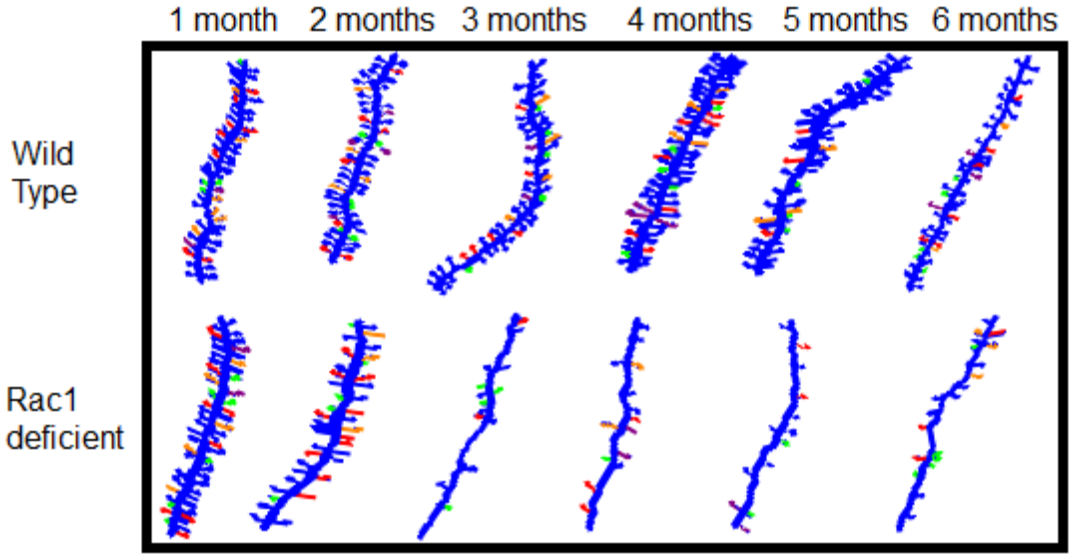


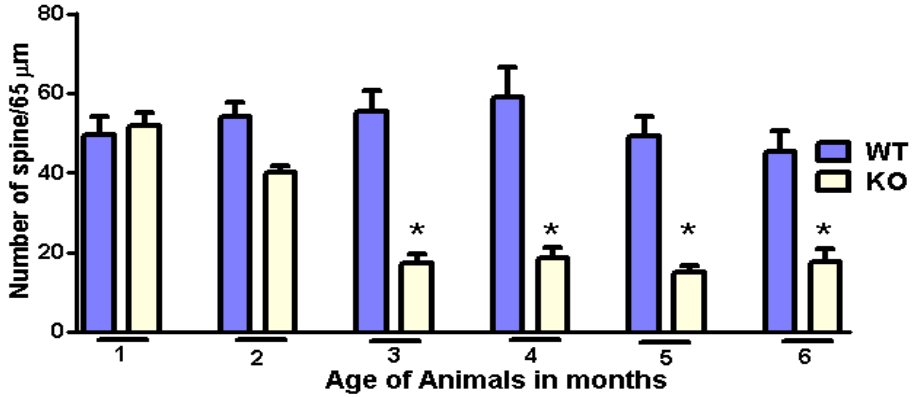
* denotes significance respect to WT; π denotes significance respect to HET ($p<0.05$, WT=17, HET=15, KO=17)

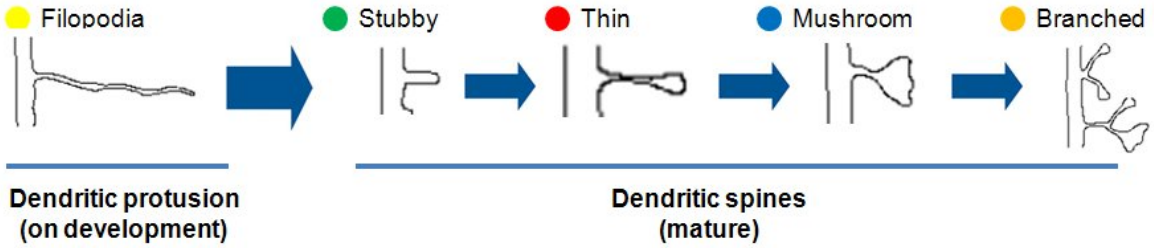
Tejada-Simon and Bongmba, 2012

Is Rac1 necessary for learning and memory?

Genetic approach







Loss of Rac1 Reduces Spine Density

Tejada-Simon and Bongmba, 2012

A possible role for Rac1 GTPase in neuro-developmental disorders associated with deficiencies in spine morphology and cognitive disabilities?

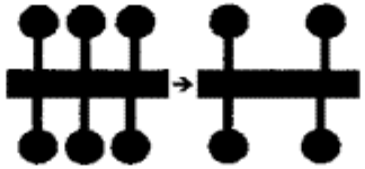
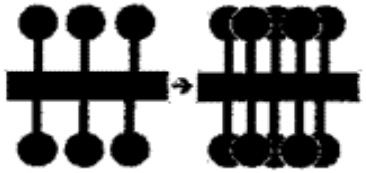
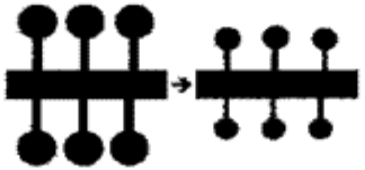
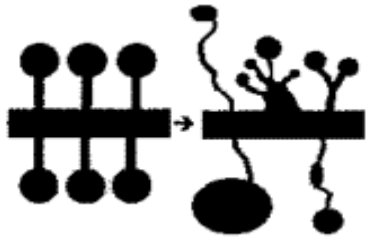
Mental retardation genes involved in Rho GTPase regulation and signaling

Gene	Locus	Protein	Function	Mutations
Nonsyndromic X-linked MR (MRX)				
OPHN1	Xq12	Oligophrenin-1	Rho family GAP. Knockdown causes decreased spine length.	T(X;12) translocation, loss of function. MRX60 family: 1-bp deletion causing frameshift; mRNA dramatically reduced.
PAK3	Xq22	PAK3	Ser/Thr kinase. Rac1/Cdc42 effector.	MRX30 family: R419X truncation, abolishes kinase activity. MRX47 family: R67C missense mutation near CRIB domain A365E missense mutation.
ARHGEF6	Xq26	αPIX, Cool-2	GEF for Rac1/Cdc42. Interacts with PAK.	T(X;21) translocation, loss of function. MRX46 family: Mutation in intron 1 causes exon skipping of exon 2 resulting in 28 amino acid deletion.
FGD1	Xp11	FGD1	GEF for Cdc42.	P312L missense mutation.
Syndromic X-linked MR (MRXS)				
OCRL1 Lowe syndrome	Xq24	OCRL1	Rac GAP. Also encodes a PIP_2 5-phosphatase.	Numerous, mostly loss of function. Several mutations in Rho GAP domain.
FMR1 Fragile X syndrome	Xq27	FMRP	RNA binding protein that interacts with CYFIP, a Rac effector, to regulate protein translation and actin dynamics.	CGG repeat expansion in 5′ UTR of *FMR1* gene.
Autosomal Syndromic MR				
MEGAP 3p–syndrome	3p25	MEGAP, WRP, srGAP3	GAP for Rac and Cdc42. Interacts with WAVE and Robo.	T(X;3) translocation to identify gene; terminal deletions of several megabases causes $3p^-$ syndrome.
LIMK1 Williams syndrome	7q11	LIMK1	Ser/Thr kinase. Downstream of Rac and RhoA, phosphorylates and inactivates cofilin.	Large heterozygous deletion encompassing ~20 genes including elastin gene.

For references, see main text. (CYFIP) Cytoplasmic FMRP interacting protein; (Cool-2) cloned out of library 2; (FGD1) Faciogenital dysplasia protein 1; (FMRP) fragile X mental retardation protein; (GAP) GTPase activating protein; (GEF) guanine nucleotide exchange factor; (LIMK1) LIM kinase 1; (MEGAP) mental disorder-associated GAP; (OCRL) oculocerebrorenal syndrome of Lowe; (PAK3) p21 activated kinase 3; (PIX) PAK interacting exchange factor; (srGAP3) Slit-Robo GAP3; (WRP) WAVE-associated Rac GAP protein.

Govek et al., 2005

Neurological disorders with Dendritic Spine Abnormalities

Spine Abnormality		Example of Disorder
Decrease in Spine Density		Alzheimer Disease Alcohol abuse
Increase in Spine Density		**Fragile X syndrome** Sudden Infant death Syndrome
Reduction in Spine Size		Schizophrenia Down Syndrome
Distortion of Spine Shape		Epilepsy Alcohol abuse

Adapted from Fiala et al 2002

Fragile X Syndrome

- Most common form of genetically transferred intellectual disability
- Common cause of autism
- Affects 1:4,000 to 1:8,000 males; less common in females and symptoms less severe





Peripheral phenotypes
- *elongated face*
- *wide forehead*
- *enlarged ears*
- *stereotypic behavior*
- *irritable bowel syndrome*
- *esophageal reflux*

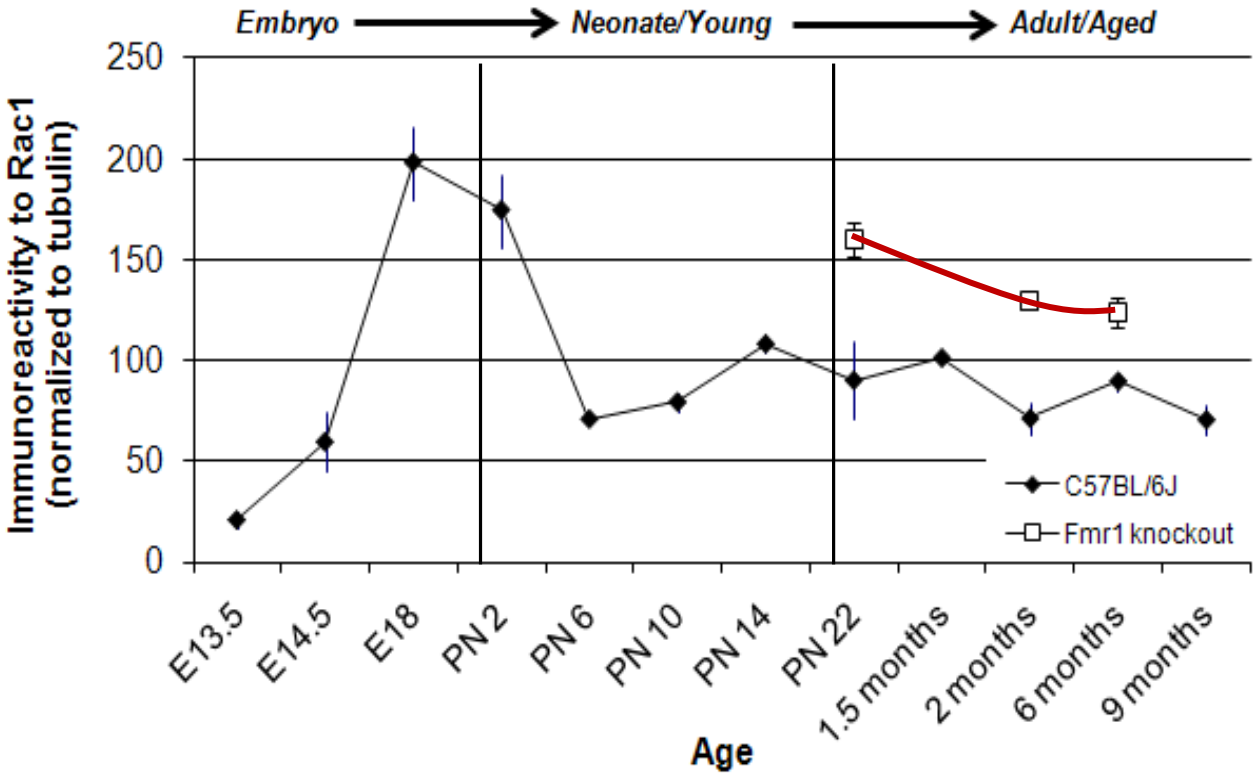
- Caused by absence of fragile X mental retardation protein (FMRP)
- CGG repeat disease → methylation → results in silencing of *fmr1* gene (X chromosome)

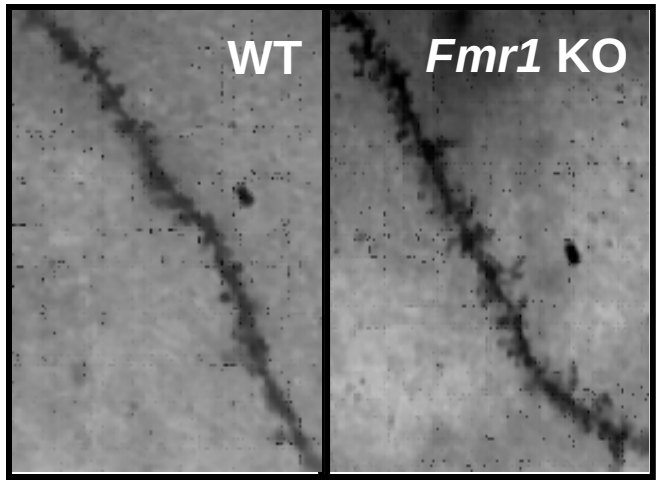
European Journal of Human Genetics (2008) ***16,*** *666–672*
Ashley et al., 1993; *Darnell et al., 2011.*

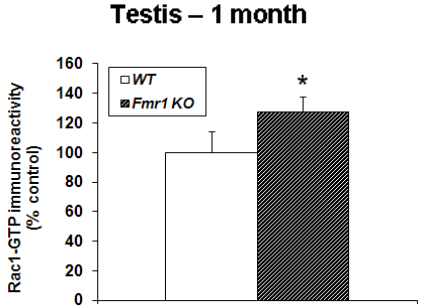
Summary of characteristic phenotypes in *FXS*

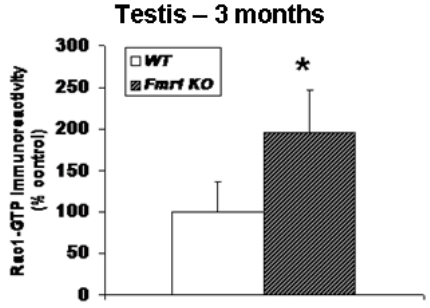
Phenotype	Fragile X syndrome
Other deficits	Language deficits, hyperactivity, attention deficits, autism Abnormal sleep, seizures, anxiety disorders, sensory-processing problems, epilepsy.
Body growth	Accelerated growth, slower after adolescence
Testicles	Post-pubertal macroorchidism
Basal protein translation	*elevated*
Dendritic spine morphology	*Increased density of thin, long “immature” dendritic spines*
Long-term potentiation	*Mild phenotype (altered cortical LTP)*
Long-term depression	*Exaggerated*

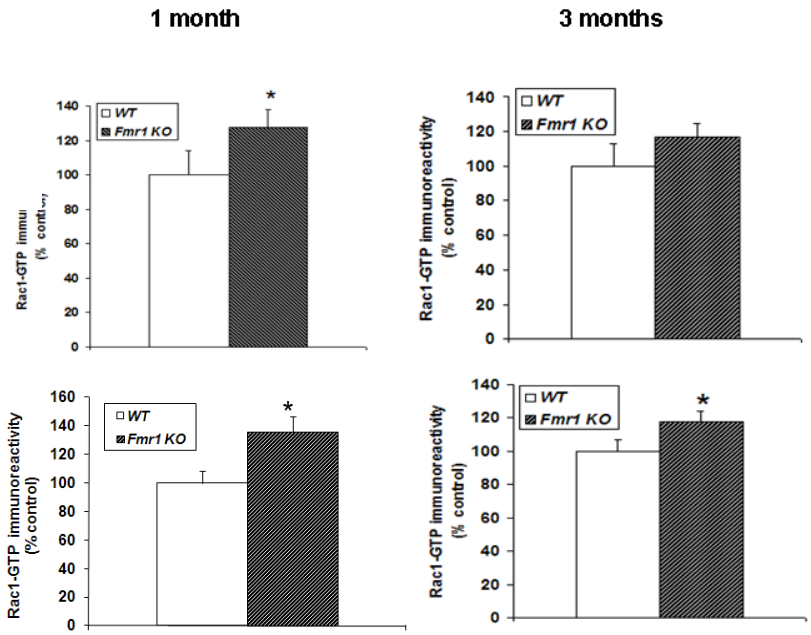
Rac1 GTPase levels are elevated in FXS





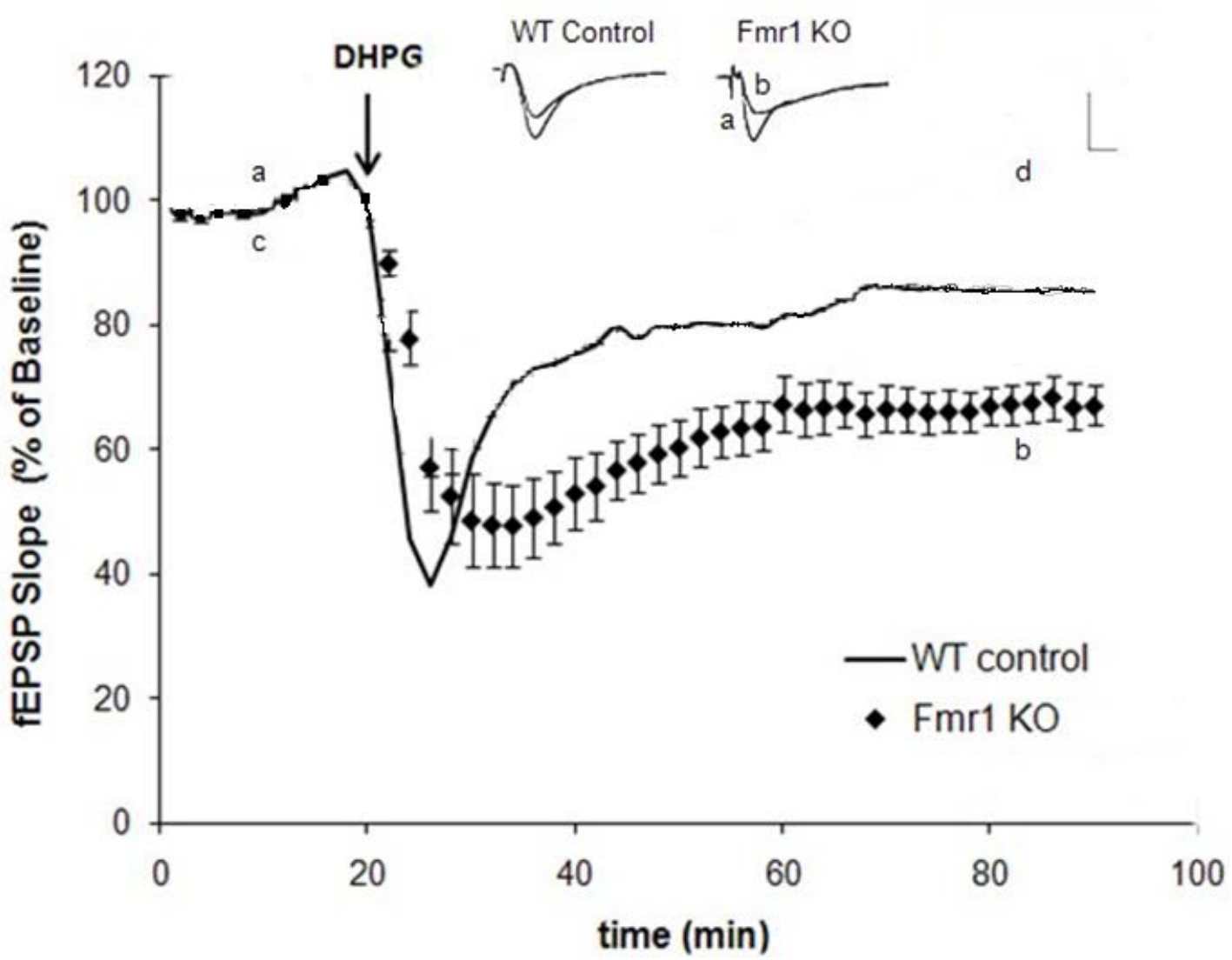






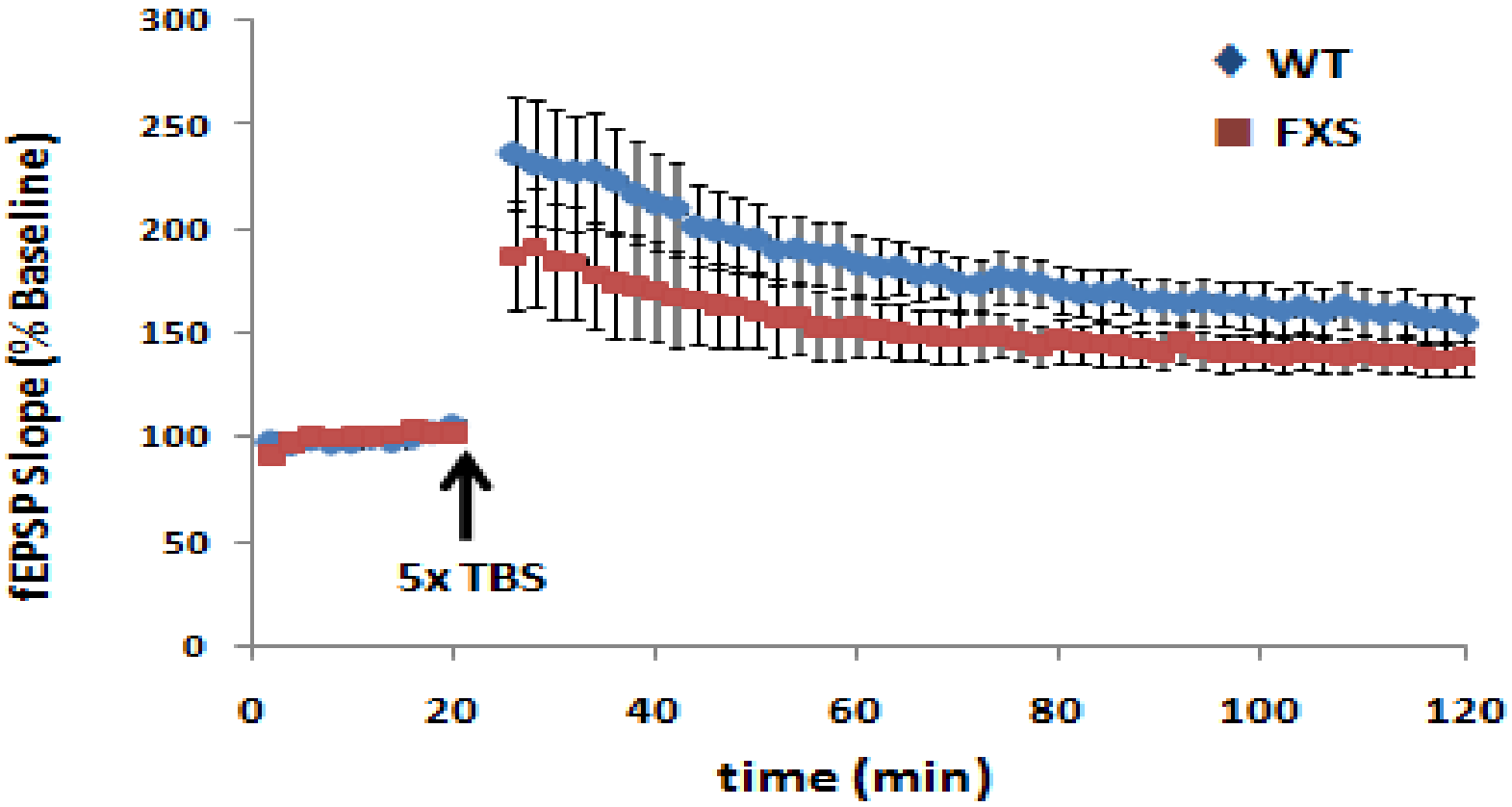
Bongmba et al., 2011

Plasticity Studies in *fmr1* KO mice by our laboratory: Exaggerated long-term depression (LTD)



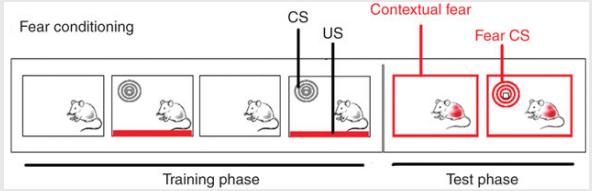
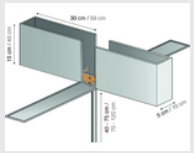
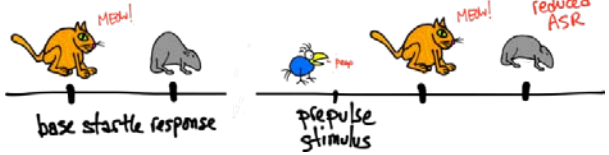
Bongmba et al., 2011

Plasticity Studies in *fmr1* KO mice by our laboratory: Deficient long-term potentiation (LTP)



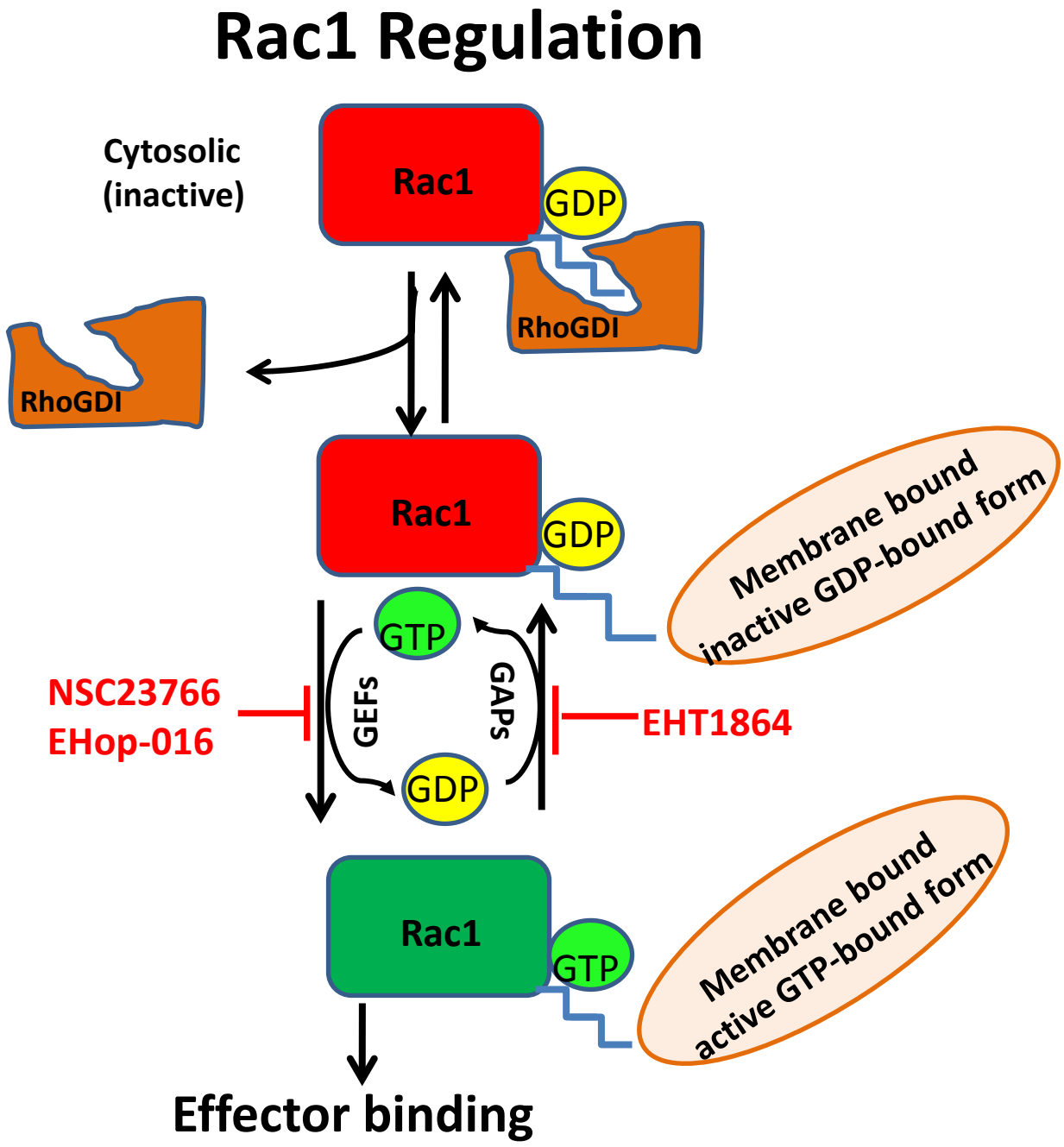
Martinez and Tejada-Simon, 2014 (unp.)

Behavioral Studies in *fmr1* KO mice by our laboratory

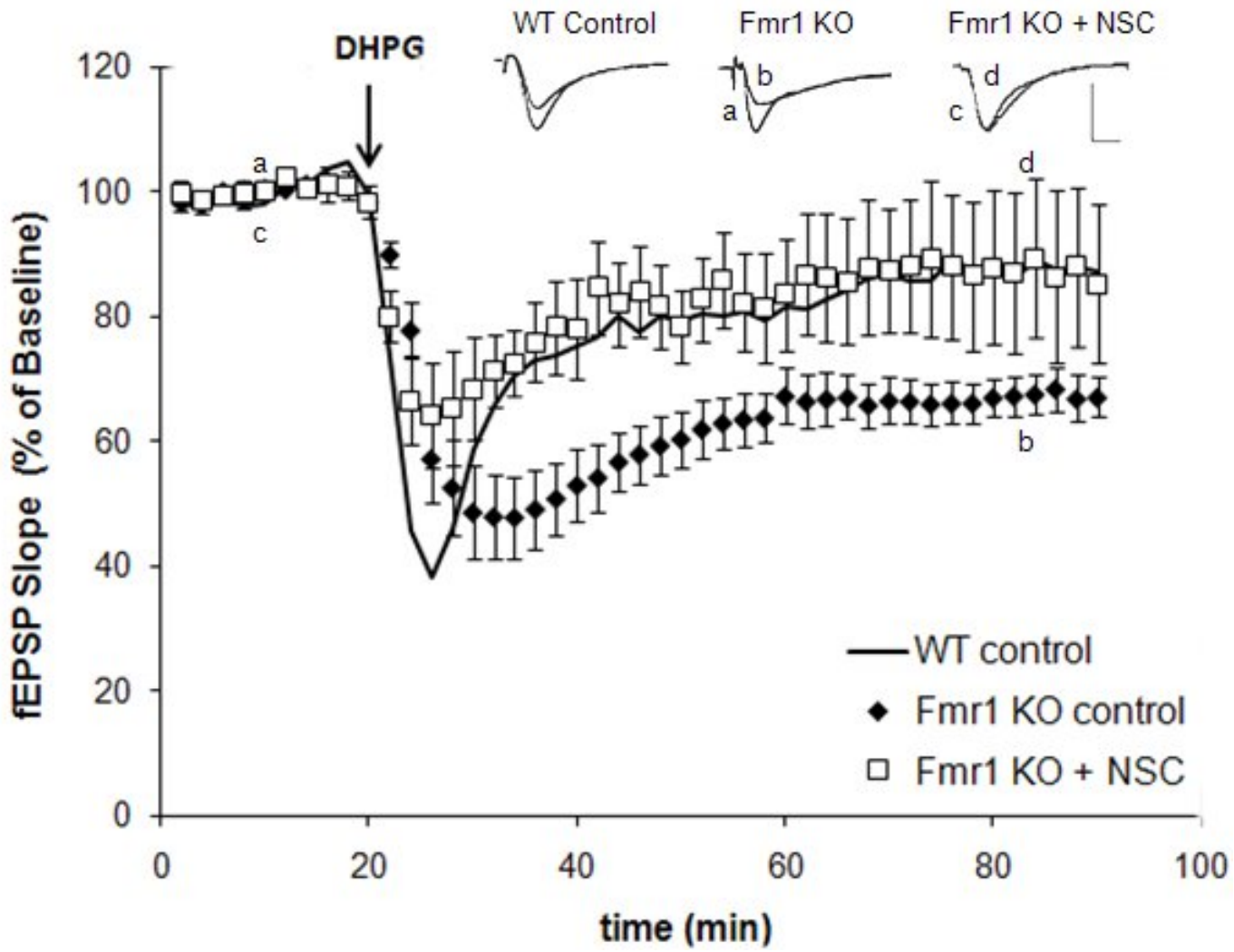
Behavioral test	Our Results	Others References
Associative fear conditioning and 24 hr testing after training 	**Impaired learning behavior and significantly deficient memory/recall compared to WT (to context and to cue)**	Olmos-Serrano et al., 2011; Zhao, 2005; Paradee et al., 1999
Audiogenic Seizures (AGS) ***Scored for wild running, seizures and death*** 	**Significantly more sensitive to AGS compared to controls (10% WT vs. 70% KO).**	Chen and Toth, 2001; Osterweil et al., 2013
Elevated Plus Maze 	**Significantly higher tendency to explore open arms suggesting anxiolytic phenotype. No hyperactivity (distance covered during the test).**	Jung et al., 2012; Yuskaitis et al., 2009
Prepulse inhibition (PPI)/Startle response) 	**Startle response significantly higher than control. PPI significantly different (from 78-90 dB) than control.**	Olmos-Serrano et al., 2011; Chen and Toth, 2001; Nielsen et al., 2002

Martinez and Tejada-Simon, 2014 (unp.)
Nguy and Tejada-Simon, 2014 (press)

Can phenotypes in fmr1 KO mice be corrected by manipulating levels of Rac1?



Can phenotypes in *fmr1* KO mice be corrected by manipulating levels of Rac1?

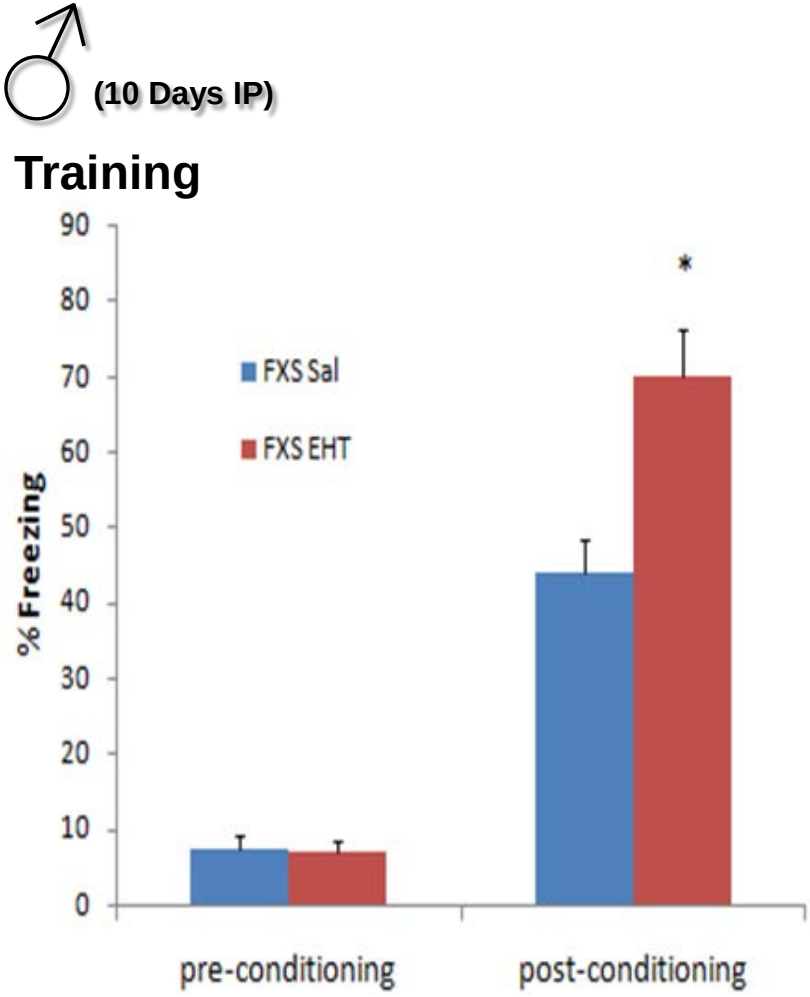


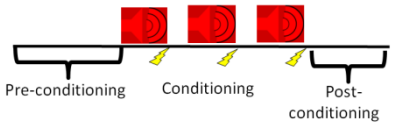
Bongmba et al., 2011

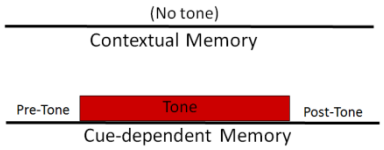
Can phenotypes in *fmr1* KO mice be corrected by manipulating levels of Rac1?

♂ (10 Days IP)

Training





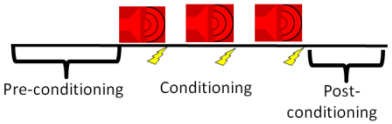


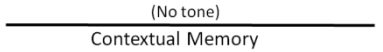
Martinez and Tejada-Simon, 2014 (unp.)

Can phenotypes in *fmr1* KO mice be corrected by manipulating levels of Rac1?

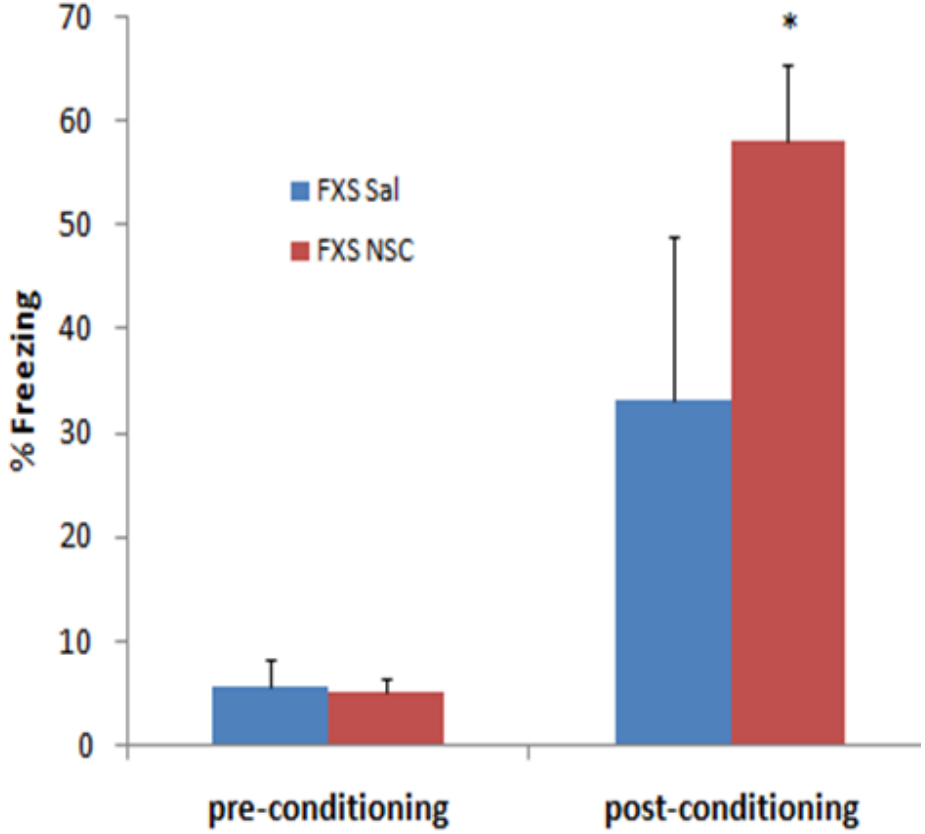


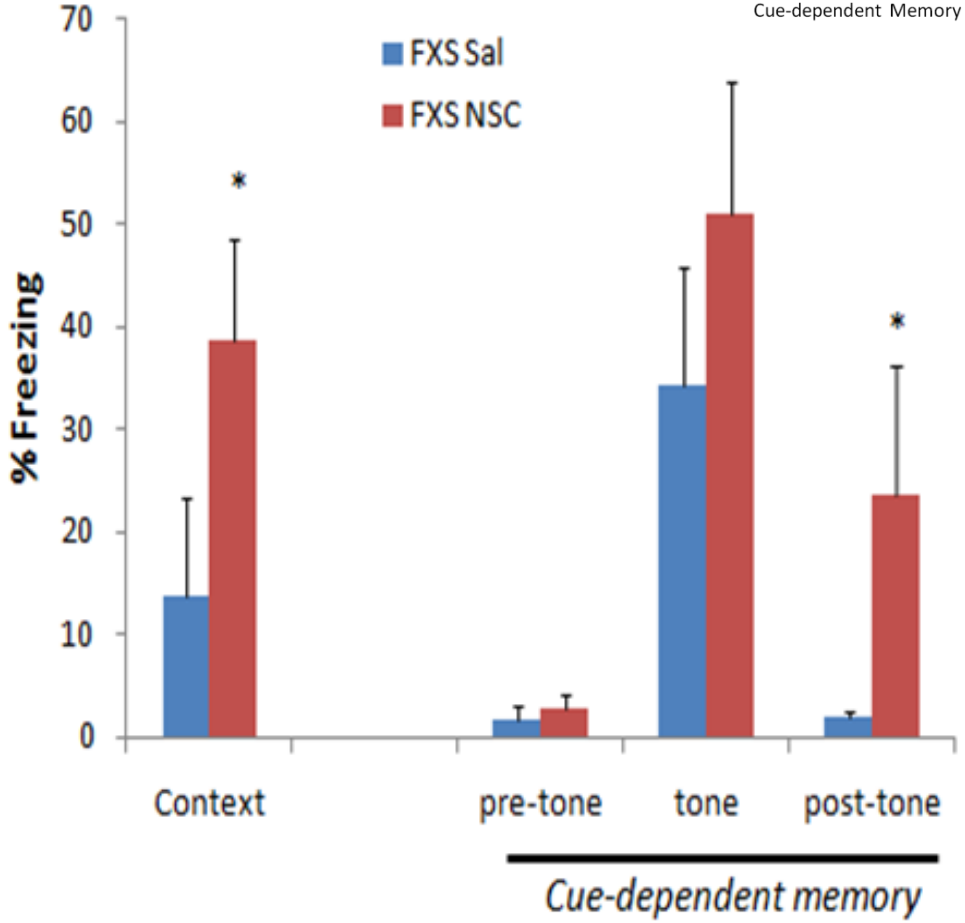
(5 Days IP)











Martinez and Tejada-Simon, 2014 (unp.)

Can phenotypes in *fmr1* KO mice be corrected by manipulating levels of Rac1?

♂ (5 Days IP)



Audiogenic Seizures

	Wild Running		Seizure		Death	
6 weeks old	#	%	#	%	#	%
WT	0/4	(0%)	0/4	(0%)	0/4	(0%)
FXS	1/ 4	(25%)	1/ 4	(25%)	1/ 4	(25%)
12 weeks old						
WT	1/9	(11%)	1/9	(11%)	0/9	(0%)
FXS	7/ 10	(70%)	7/ 10	(70%)	7/ 10	(70%)
FXS-Saline	3/ 4	(75%)	3/ 4	(75%)	3/4	(75%)
FXS-NSC	0/4	(0%)	0/4	(0%)	0/4	(0%)

Martinez and Tejada-Simon, 2014 (unp.)

Summary

Phenotype	*Can fmr1* KO phenotypes be rescued by inhibition of Rac1?
Exaggerated LTD	Rescued with NSC treatment on hippocampal slices derived from male *fmr1* KO mice
Associative learning (FC)	Better learning skills (males treated with EHT and also NSC) No LTM improvement (males treated with EHT) LTM to context and cue improved (males treated with NSC)
Audiogenic epileptic seizures	Protection from AGS/death (males treated with NSC)

Martinez and Tejada-Simon, 2014 (unp.)
Nguy and Tejada-Simon, 2014 (press)

Acknowledgements

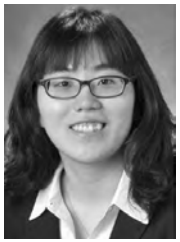
Lab members



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Daeun May Park



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Stacy Nguy, BS



Odelia Bongmba, PhD



Annie Pally, BS

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