The 2nd MCOPS12 Patient / Family Meeting

October 21, 2024

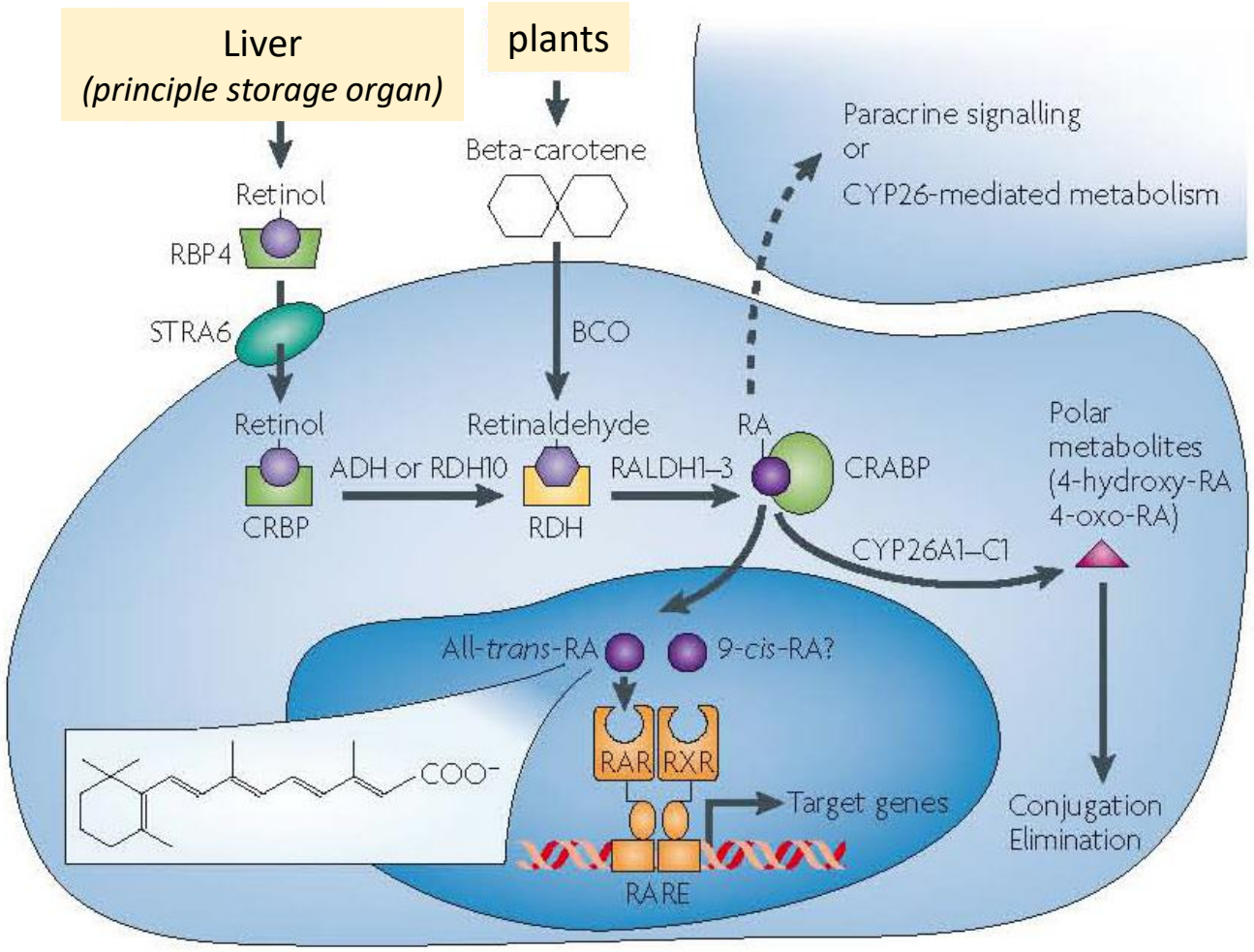


Wojciech KRĘŻEL

Institute of Genetics and Molecular and Cellular Biology

ILLKIRCH, France

Complexity of vitamin A signaling



Enzymes convert inactive precursors (retinol, carotenoids) **into active retinoids**

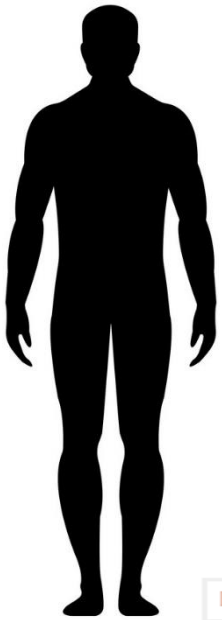
Nuclear Receptors:

- **Retinoic Acid Receptors** α, β, γ
- **Retinoid X Receptors** α, β, γ
- other dimerisation partners of RXRs**: PPARs, TRs, VDR …**

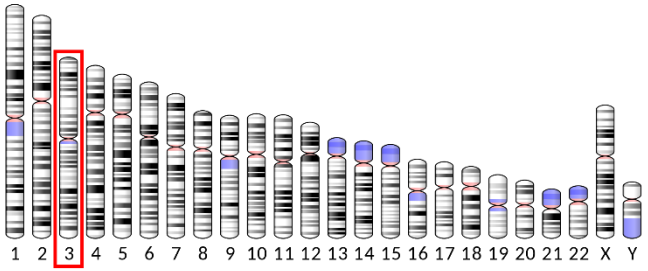
Toolbox (loss/gain of function) **: pharmacology / genetic mouse models / viral vectors / biological chemistry**

RARβ - high conservation between human and mouse (sequence)

Retinoic acid receptor beta (RARβ)



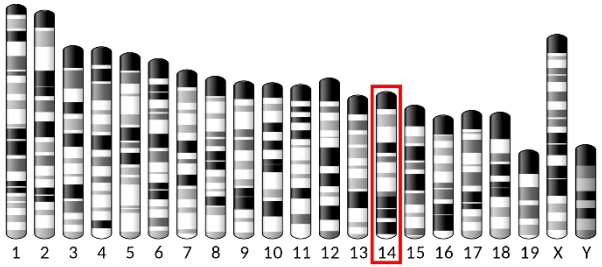
RARβ



RARβ 455 amino acids



Rarβ



RARβ 482 amino acids

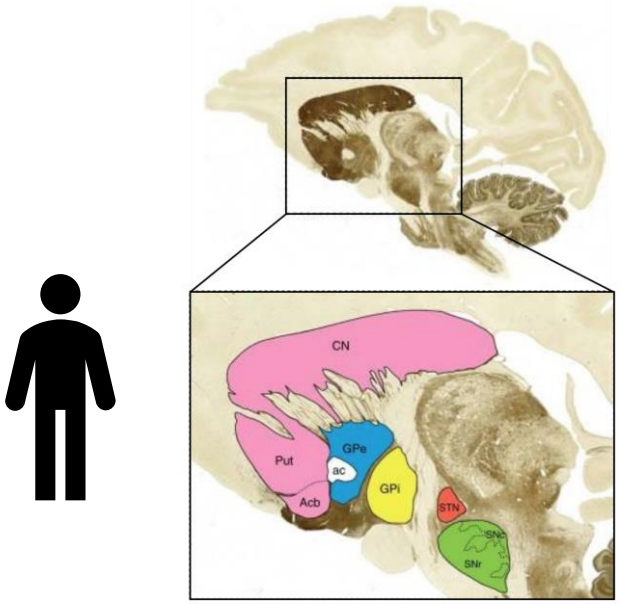
P22605 RARB_MOUSE - **Retinoic acid receptor beta** Mus musculus (Mouse)

E-value: 0.0
Score: 2333
Ident.: 92.3%
Positives : 93.2%
Query Length: 455
Match Length: 482

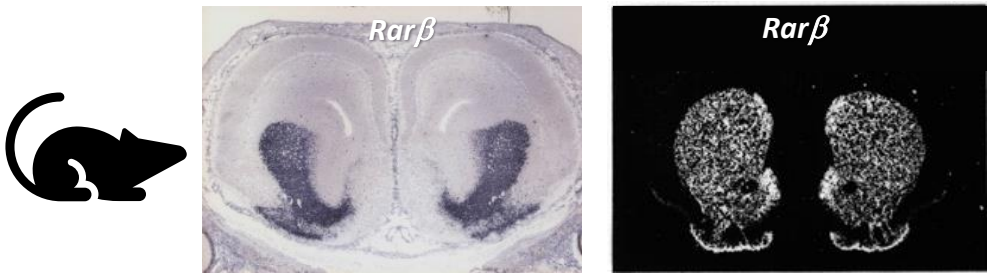
92.3% Similarity



RARβ - high conservation between human and mouse (expression)



The Human Protein Atlas,
Allen Brain Atlas and unpublished



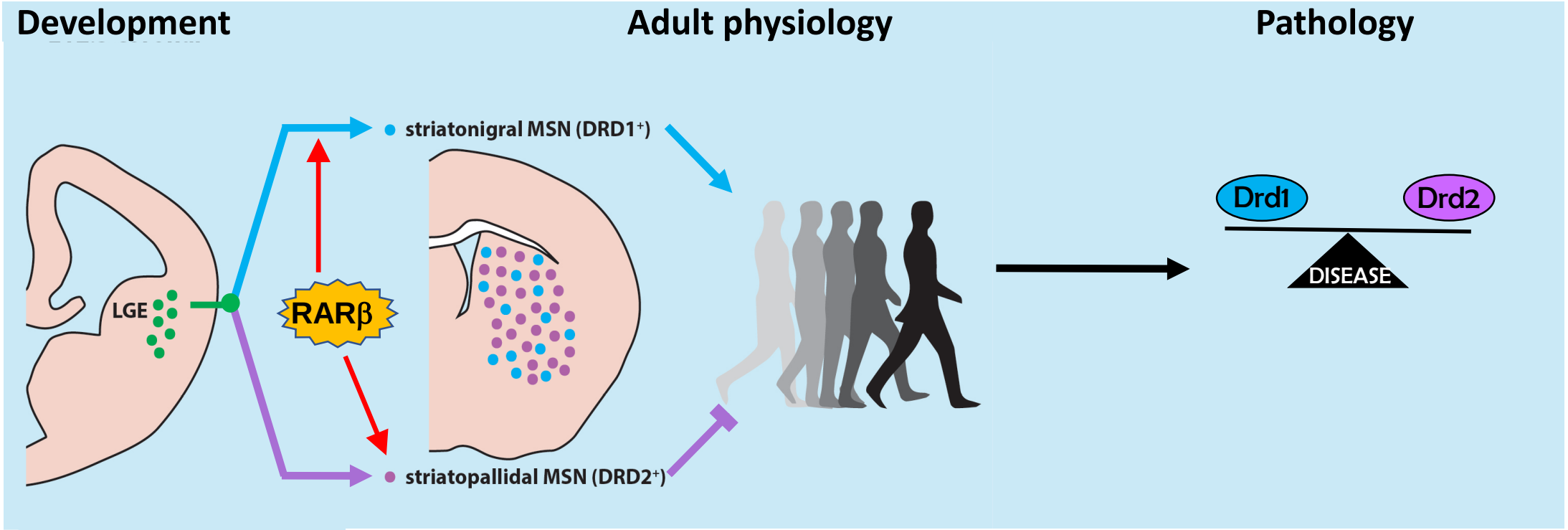
E16.5 dpc *Adult*

Krezel et al., Neurosc.1998 (mRNA)
Niewiadomska-Cimicka et al., 2017 (protein)

- **RARβ** is highly expressed in the **developing and adult striatum** in mouse and human

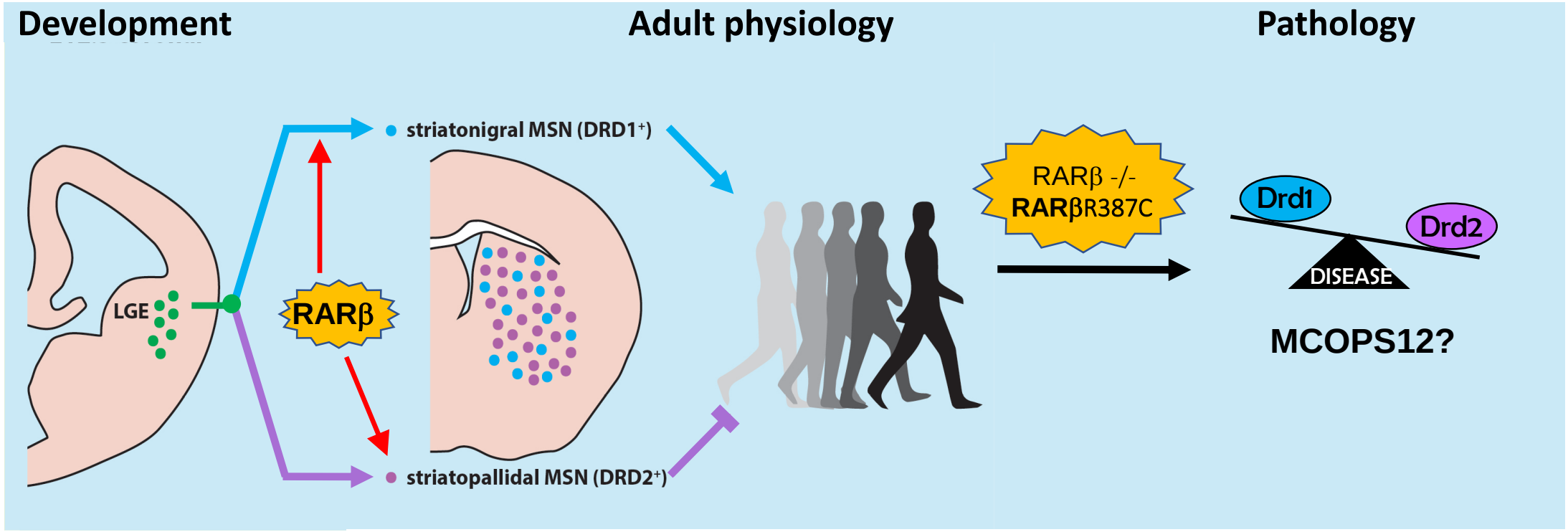
❖ **Mouse is suitable animal species to study RARb functions and model RARb-related diseases**
- ➢ **Developmental vs physiological functions ?**
- ➢ **Cellular and molecular mechanisms ?**

Retinoids, RARs/RXRs, and striatal functions



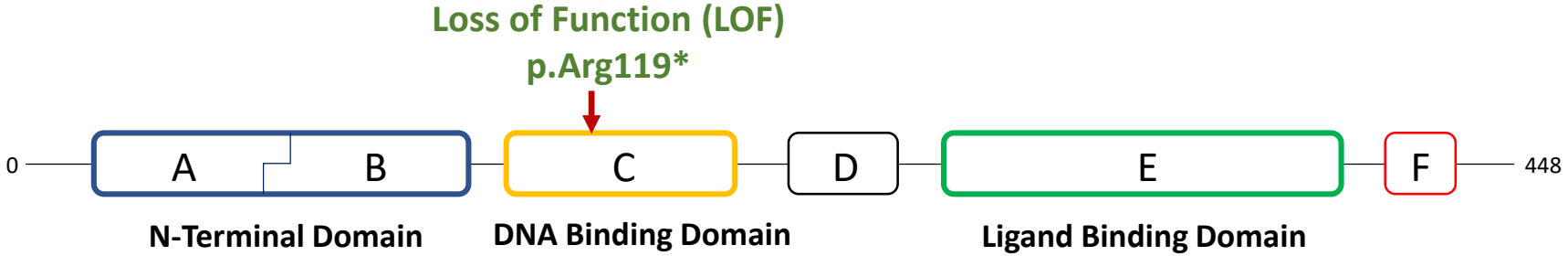
- Striatal functions : motor control, cognitive functions, habits, affect…
- Striatum controls brain activities through balanced signaling of striatonigral and striatopallidal project **M**edium **S**piny **N**eurons (MSNs)
- Dopamine controls both types of MSNs through Drd1 and Drd2 receptor

Retinoids, RARs/RXRs, and striatal functions



➢ **Do RARβ mutations lead to unbalanced dopamine signaling and striatal functions ?**

What *Rarβ* $^{-/-}$ mice tell us about MCOPS12 ?



Caron et al., 2023

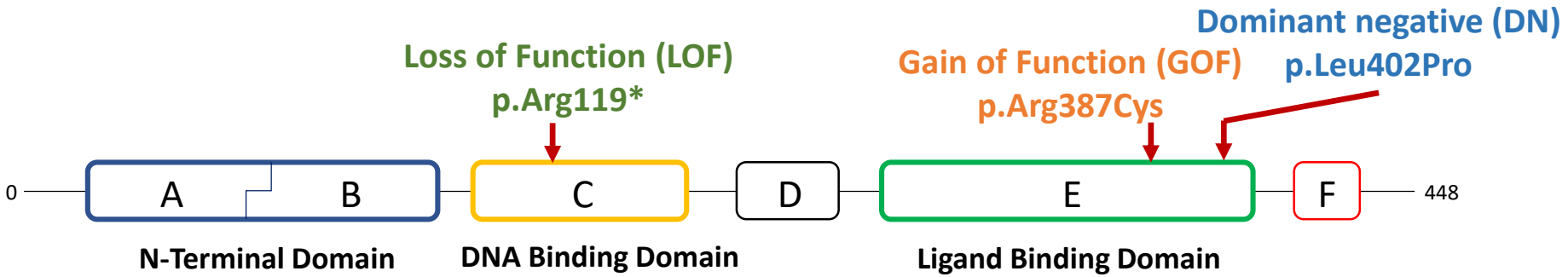
Model of LOF



Ghyselinck et al., 1997

- ***RARβ*** $^{-/-}$ **mice carrying genetic deletion of RARb - a « surrogate » model of LOF mutation found on MCOPS12 patients**
- **Need for mice models carrying MCOPS12 causing RARb variants**

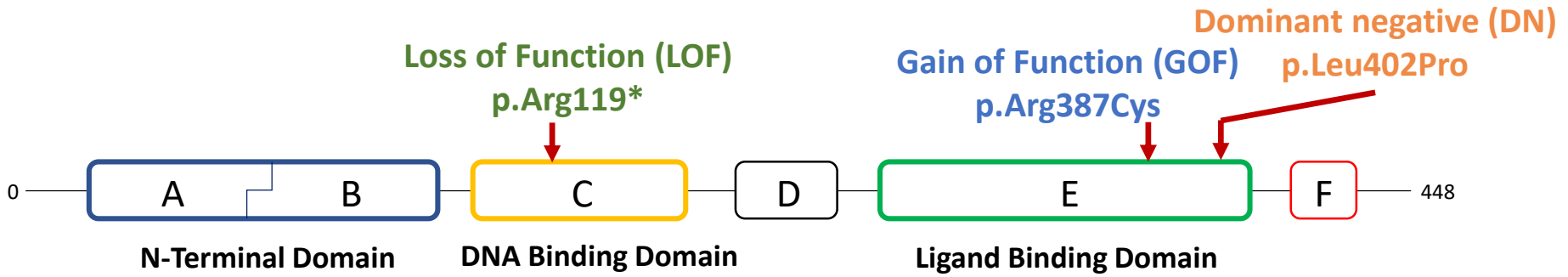
Allelic series of RARβ mutant mice models to study MCOPS12



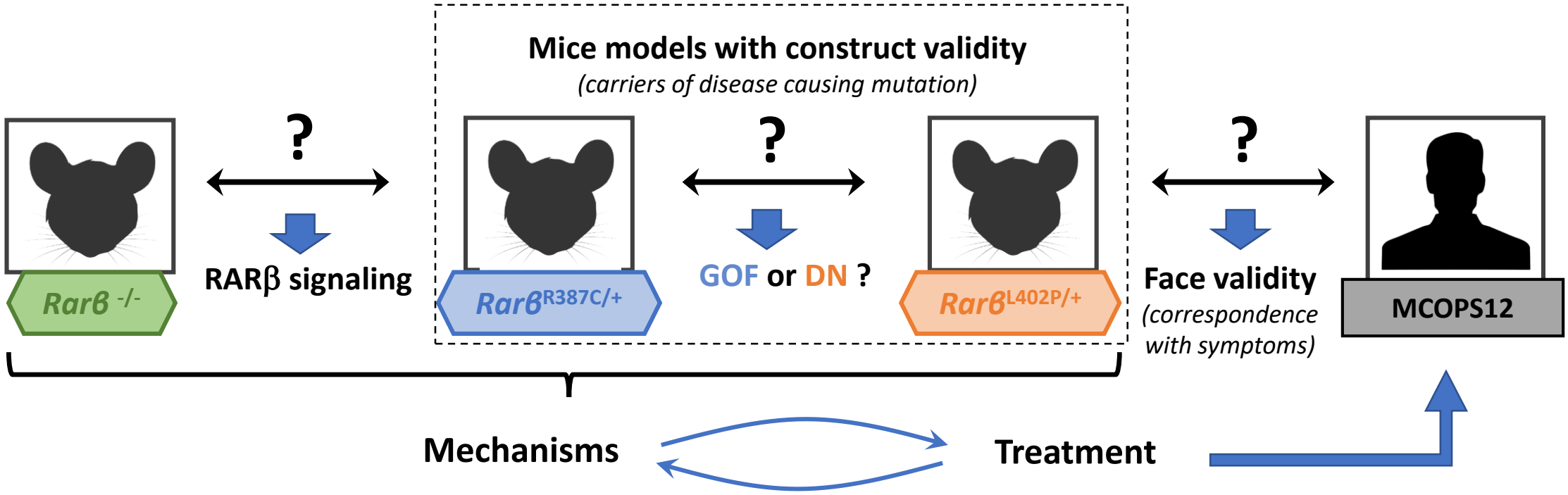
Caron et al., 2023



Allelic series of RARβ mutant mice models to study MCOPS12



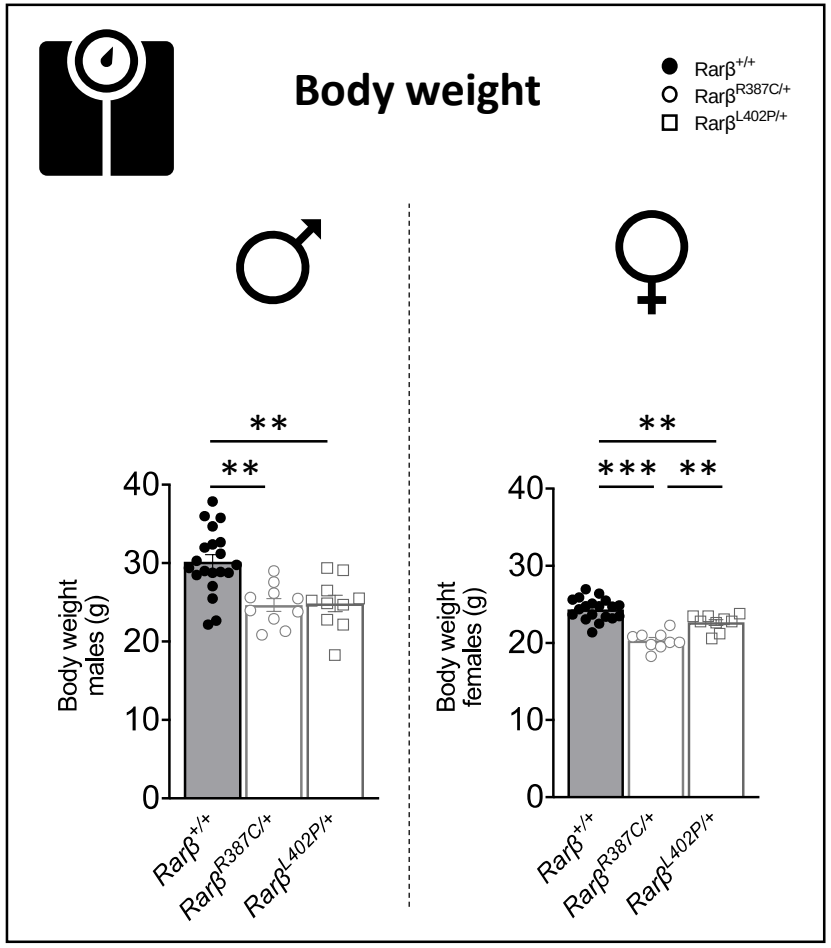
Caron et al., 2023

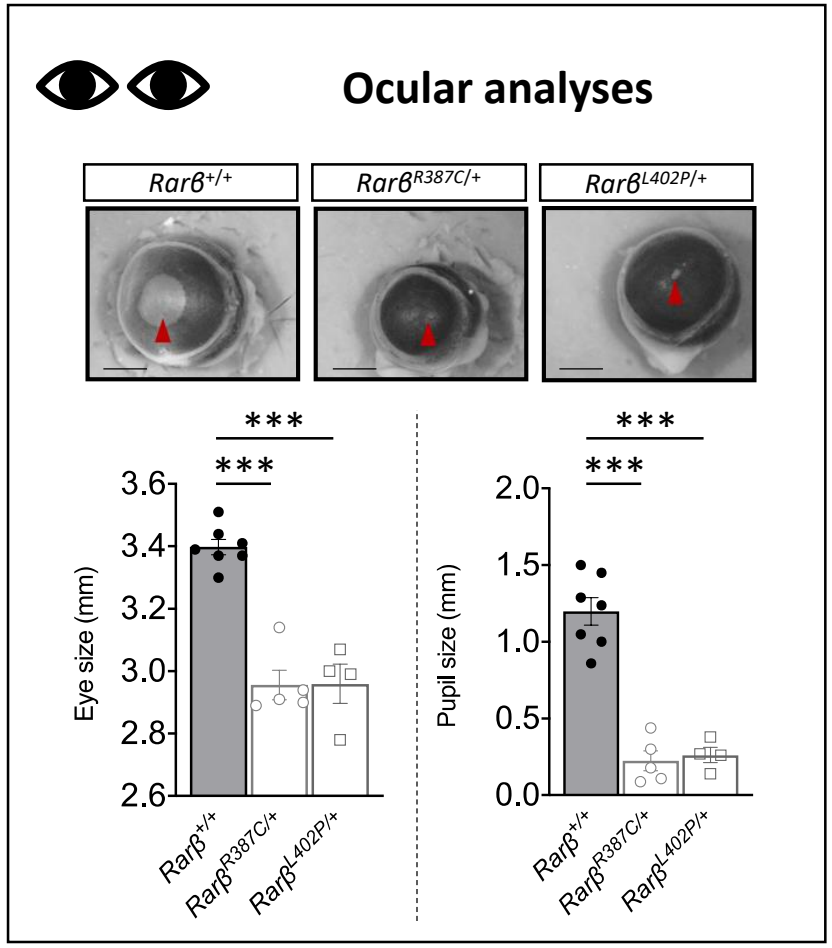


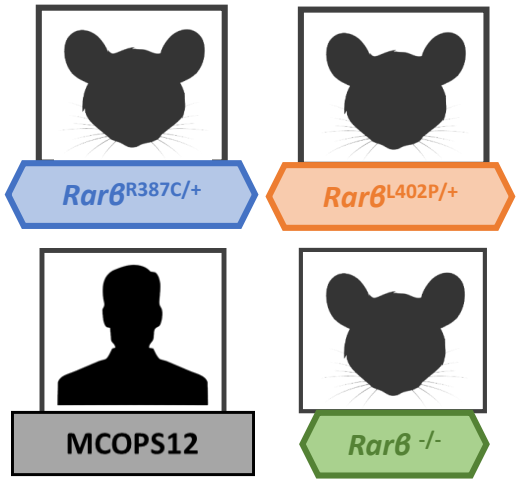
Allelic series of RARβ mutant mice reproduce key MCOPS12 symptoms



Nicolas Zinter



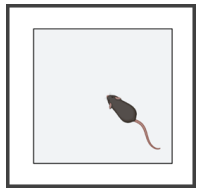


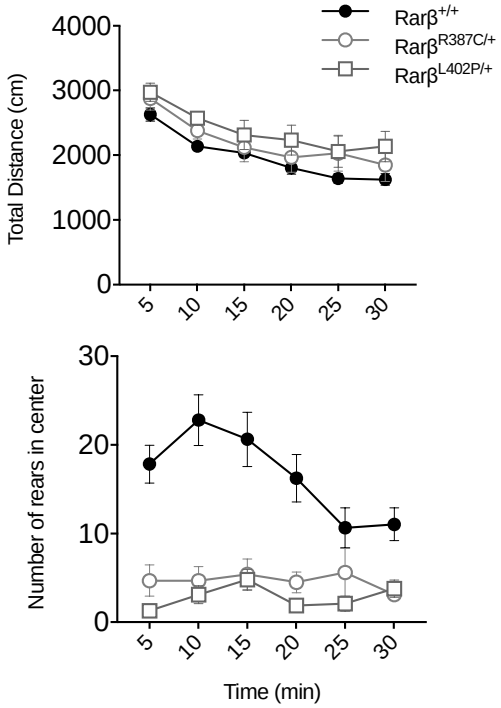


- **Males & females display lower body weight (~20%)**
- **Microphtalmia (<u>absent in *Rarβ* $^{-/-}$ mice</u>)**

Allelic series of RARβ mutant mice reproduce key MCOPS12 symptoms - behavior

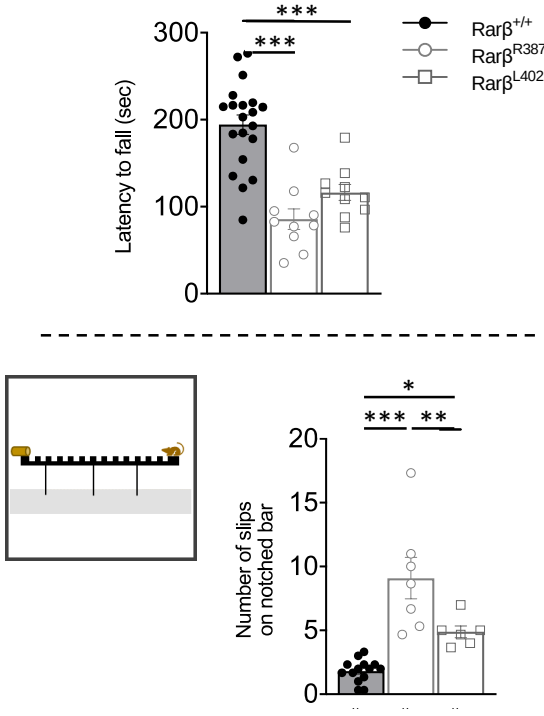
Novelty-induced locomotor activity





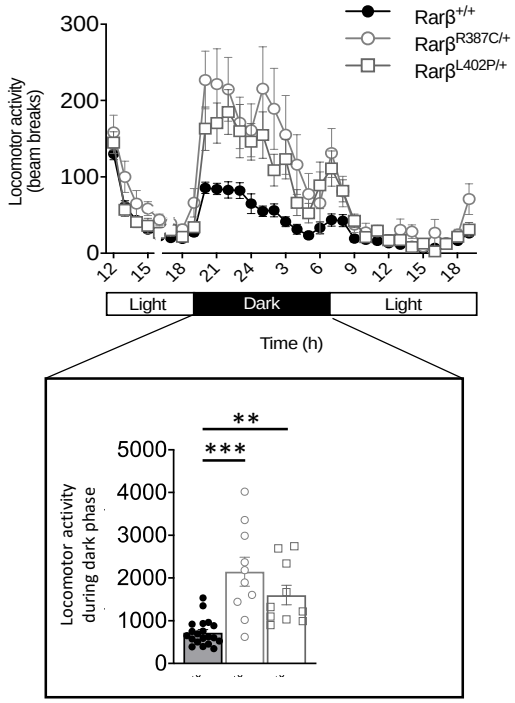
Motor coordination

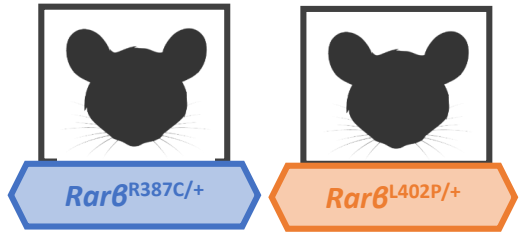




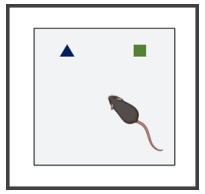
Spontaneous locomotor activity



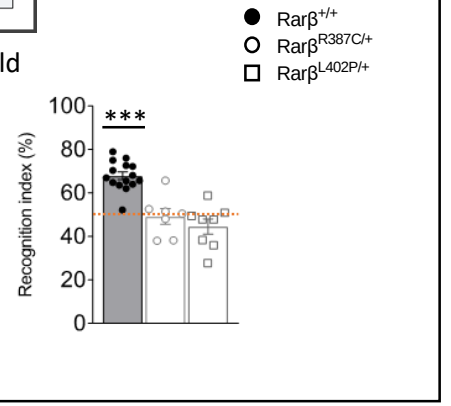




Cognition/Memory



12-weeks-old



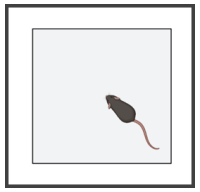
Motor coordination deficits

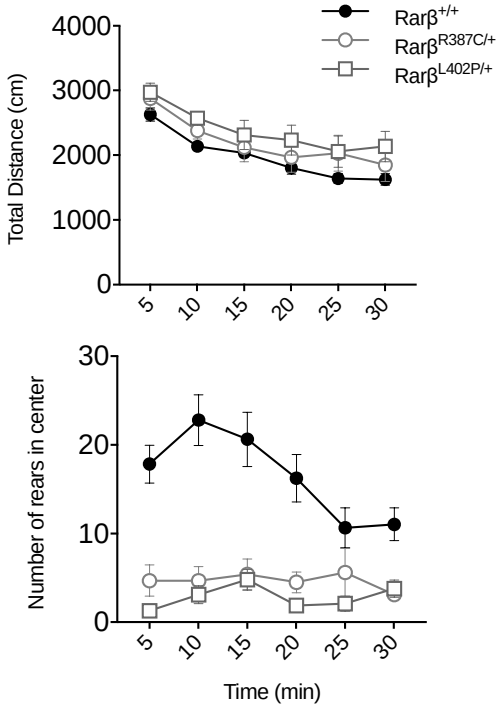
Spontaneous hyperactivity during night phase of light/dark cycle

Memory deficits

Allelic series of RARβ mutant mice reproduce key MCOPS12 symptoms - behavior

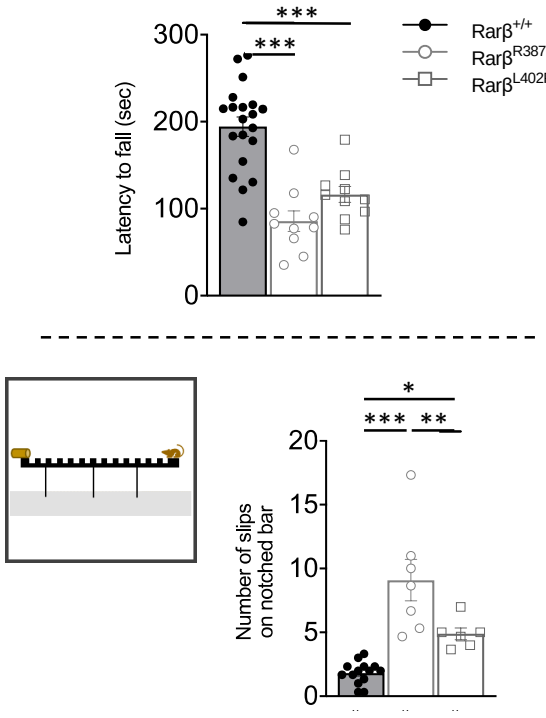
Novelty-induced locomotor activity





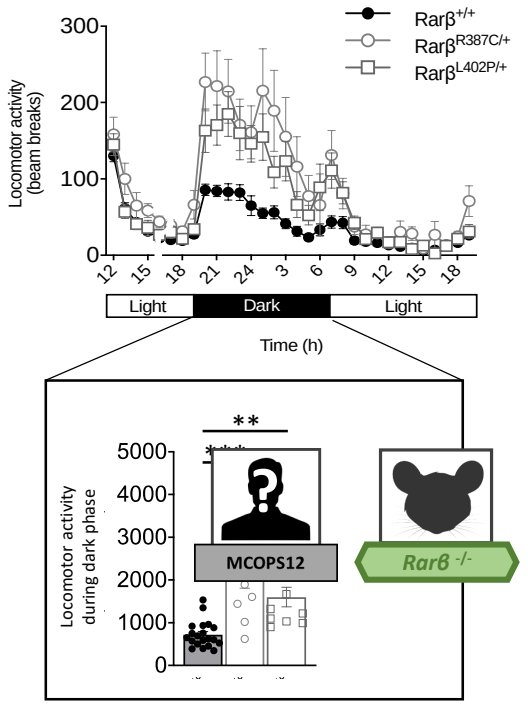
Motor coordination





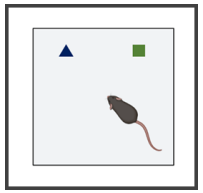
Spontaneous locomotor activity



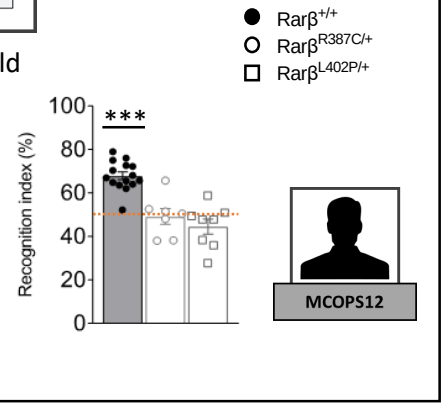




Cognition/Memory

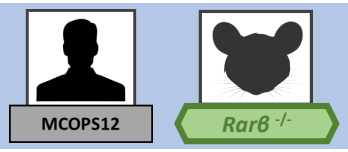


12-weeks-old



Memory deficits

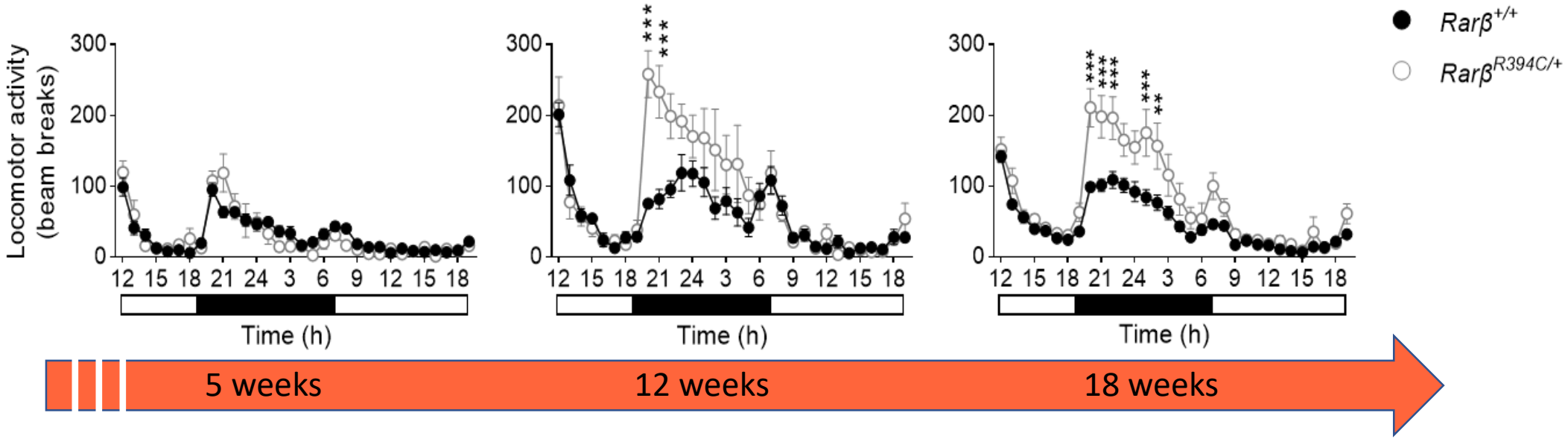
Motor coordination deficits



Spontaneous hyperactivity during night phase of light/dark cycle

Progressive behavioral deficits point to a window of opportunity for MCOPS12 treatment

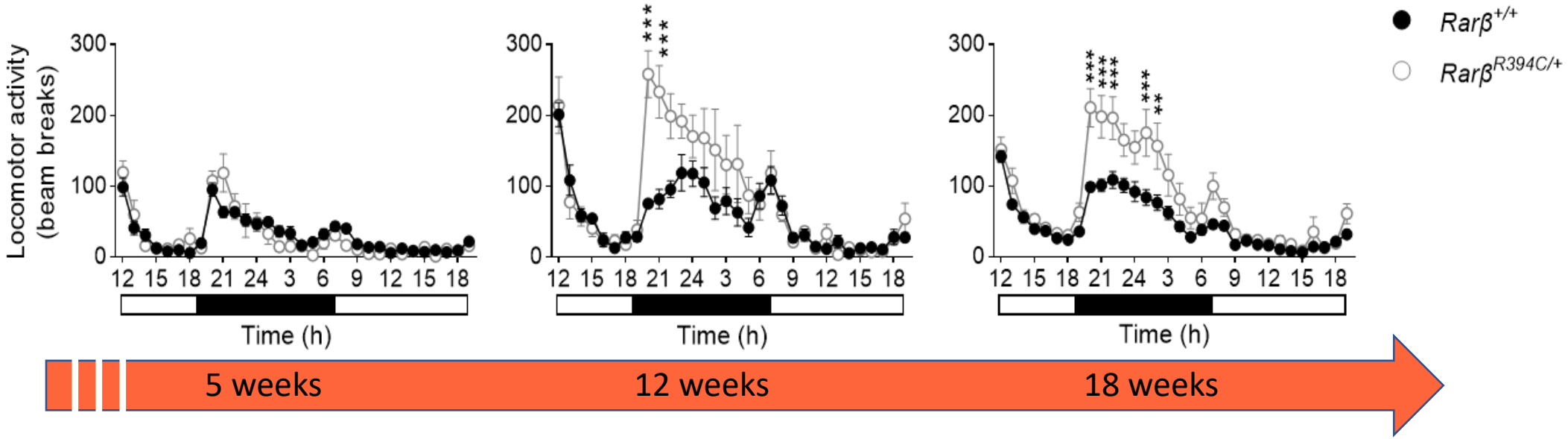
An exemple of progressive behavioral abnormalities in *RARβ*$^{R394C/+}$ mice - hyperactivity



Zinter et al. in preparation

Progressive behavioral deficits point to a window of opportunity for MCOPS12 treatment

An exemple of progressive behavioral abnormalities in *RARβ*$^{R394C/+}$ mice - hyperactivity



Progressive phenotypes in mice models *(age of onset)*:

- Sponatneous hyperactvity *(8-10 weeks)*
- Memory deficits *(8-10 weeks)*
- Growth defcits *(3-4 weeks)*

Developmental phenotypes in mice models *(age of onset)*:

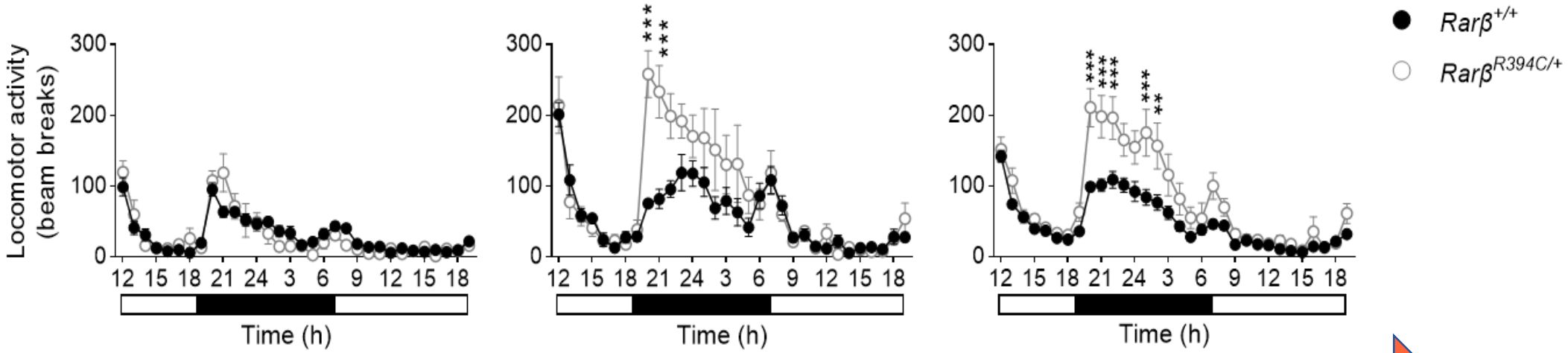
- Microphtalmia *(pre-natal)*
- Coordination deficits *(post-natal day P0 - P21?)*

❖ ***RARβ***$^{R394C/+}$ **mice displays more severe phenotype than RARβ loss of function mutation which is progressive**

Zinter et al. in preparation

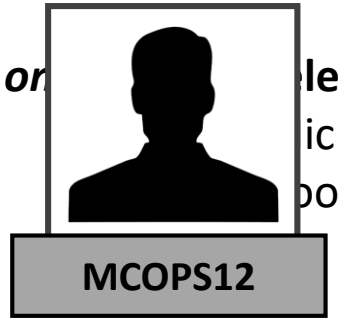
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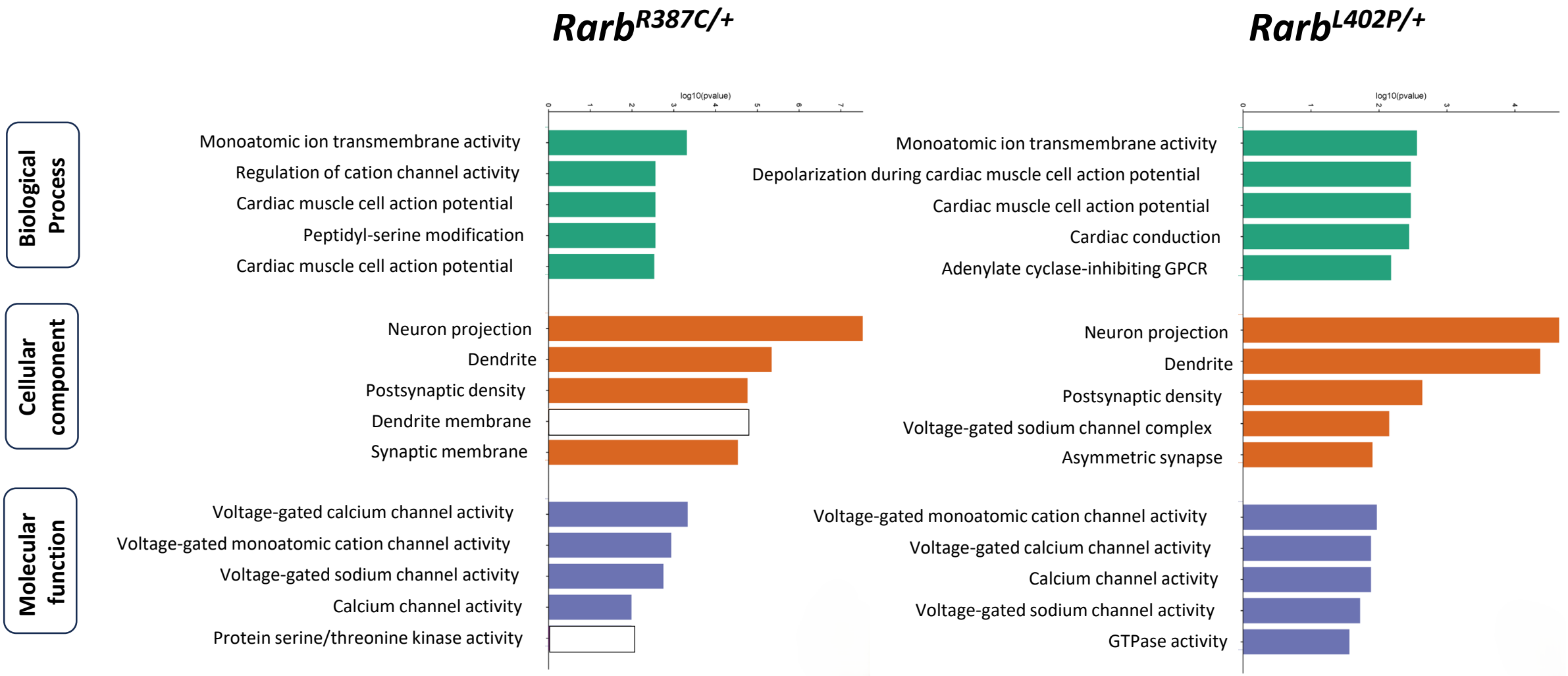
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Zinter et al. in preparation

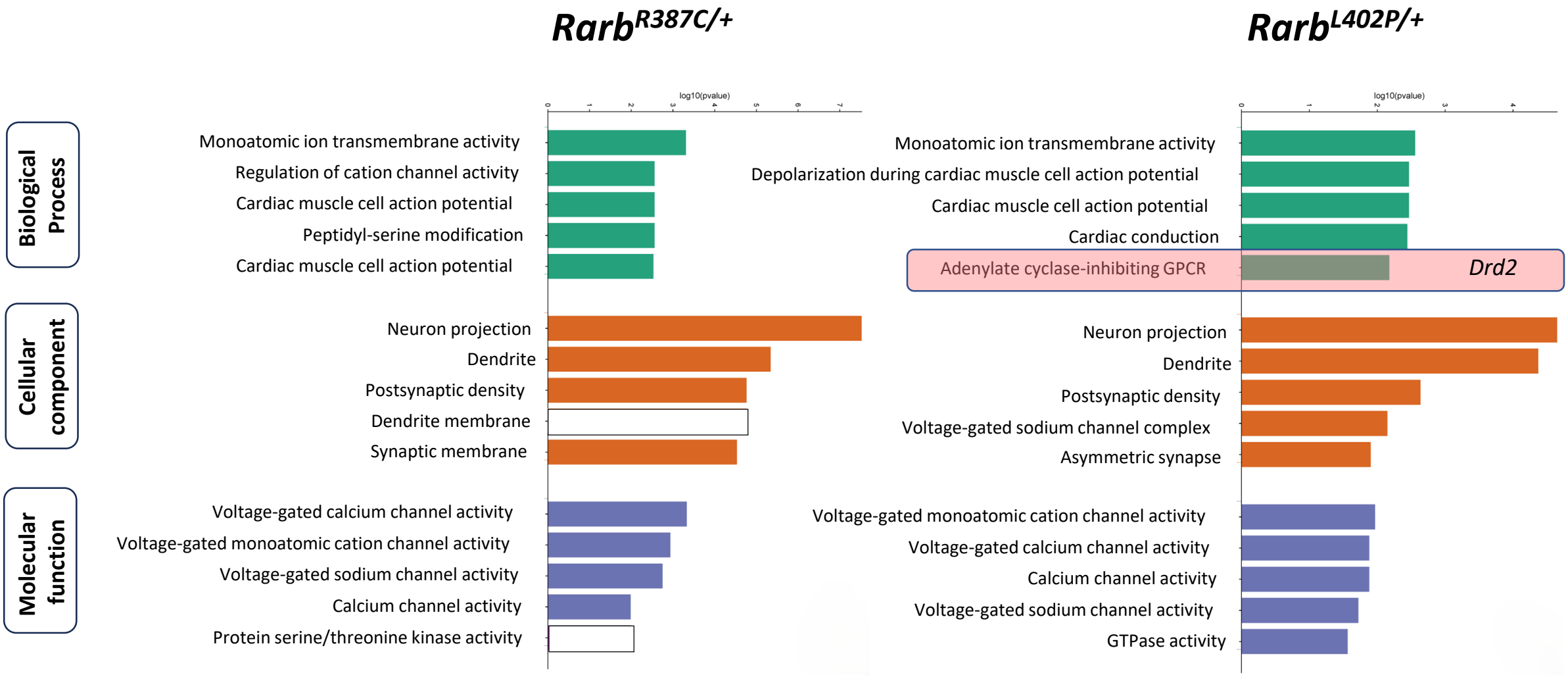
Genes differentially expressed across the various genotypes *(striatum)*

	Number of Deregulated Genes		$Rarb^{R387C/+}$	$Rarb^{L402P/+}$	$Rarb^{-/-}$	$Rarb^{+/-}$
Upregulated genes	$Rarb^{R387C/+}$	*979*	NA	461 (47%)	122 (12%)	7 (0.7%)
	$Rarb^{L402P/+}$	*1186*	461 (39%)	NA	198 (17%)	12 (1.0%)
	$Rarb^{-/-}$	*411*	122 (30%)	198 (48%)	NA	39 (10%)
	$Rarb^{+/-}$	58	7 (12%)	12 (21%)	39 (67%)	NA
Downregulated genes	$Rarb^{R387C/+}$	*1386*	NA	777 (56%)	213 (15%)	15 (1.1%)
	$Rarb^{L402P/+}$	*1334*	777 (58%)	NA	253 (19%)	21 (1.6%)
	$Rarb^{-/-}$	*390*	213 (55%)	253 (65%)	NA	31 (8%)
	$Rarb^{+/-}$	*41*	15 (37%)	21 (51%)	31 (76%)	NA

Gene Ontology (GO) analysis of downregulated direct targets of Rarb

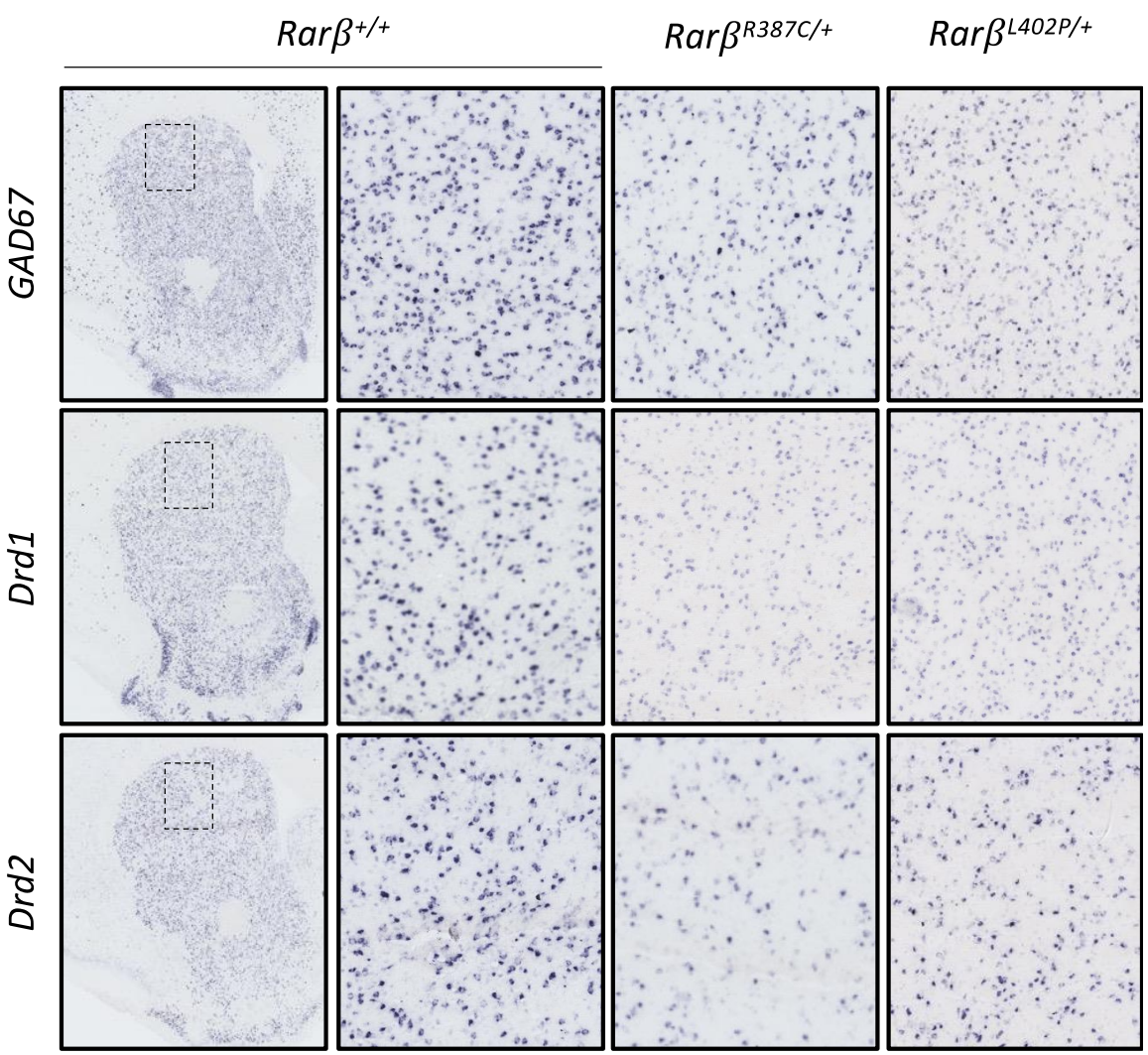


Gene Ontology (GO) analysis of downregulated direct targets of Rarb

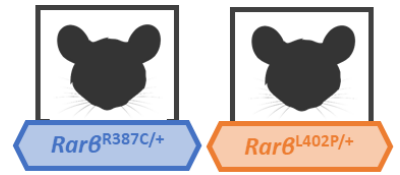


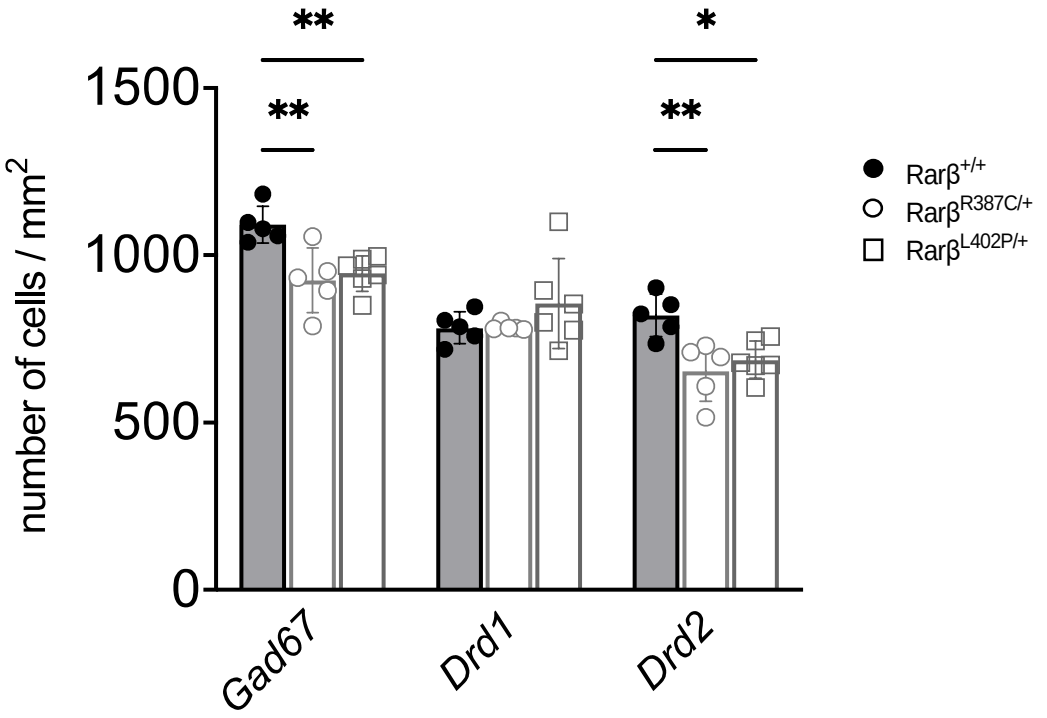
Expression of Drd2+ in spMSNs *vs* presence of Drd2+ spMSN?

Reduced number of Drd2+ spMSN in the striatum of mouse models of MCOPS12

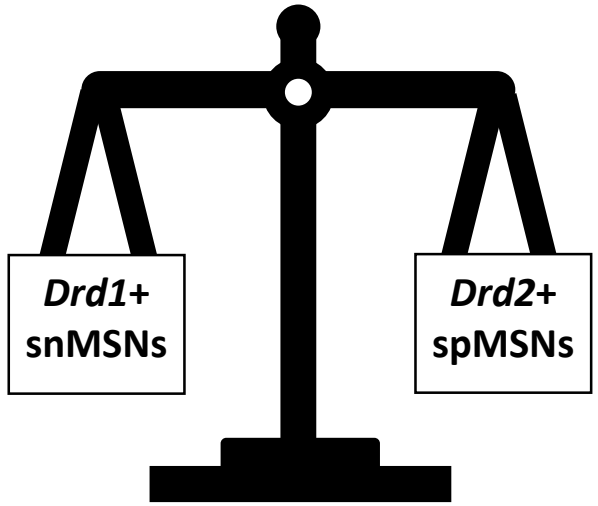


Striatum *(in situ hybridisation)*

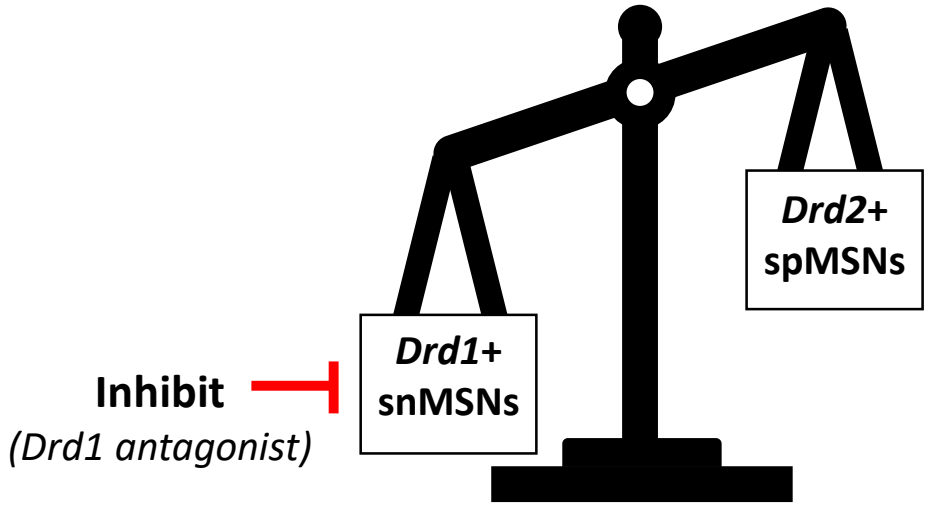




Compromised Drd2+ spMSN signaling leads to unbalanced dopamine modulation of MSNs





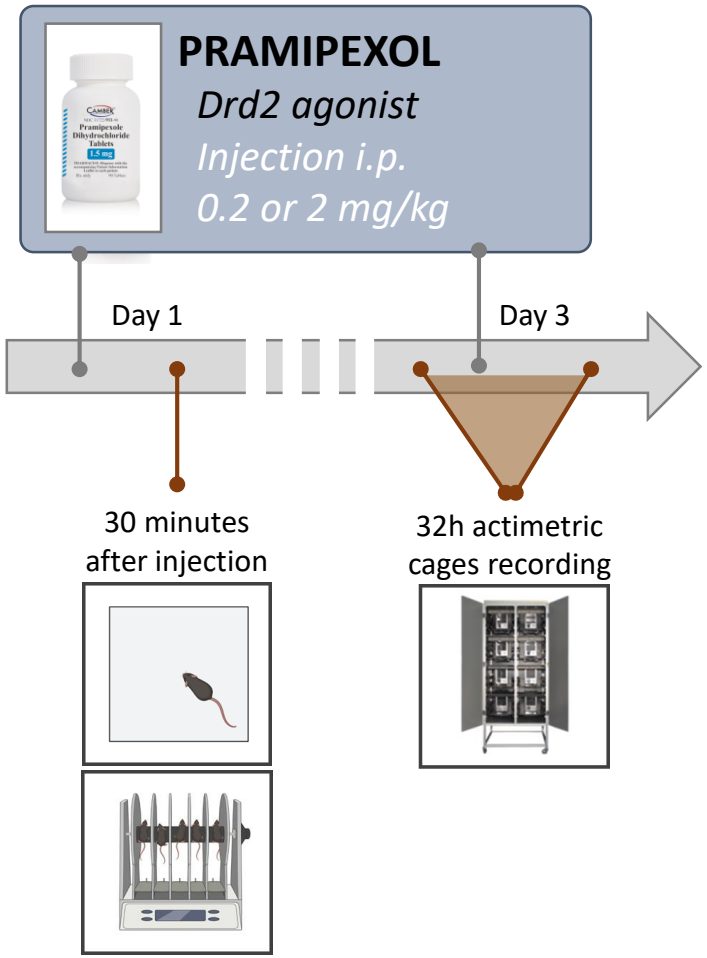


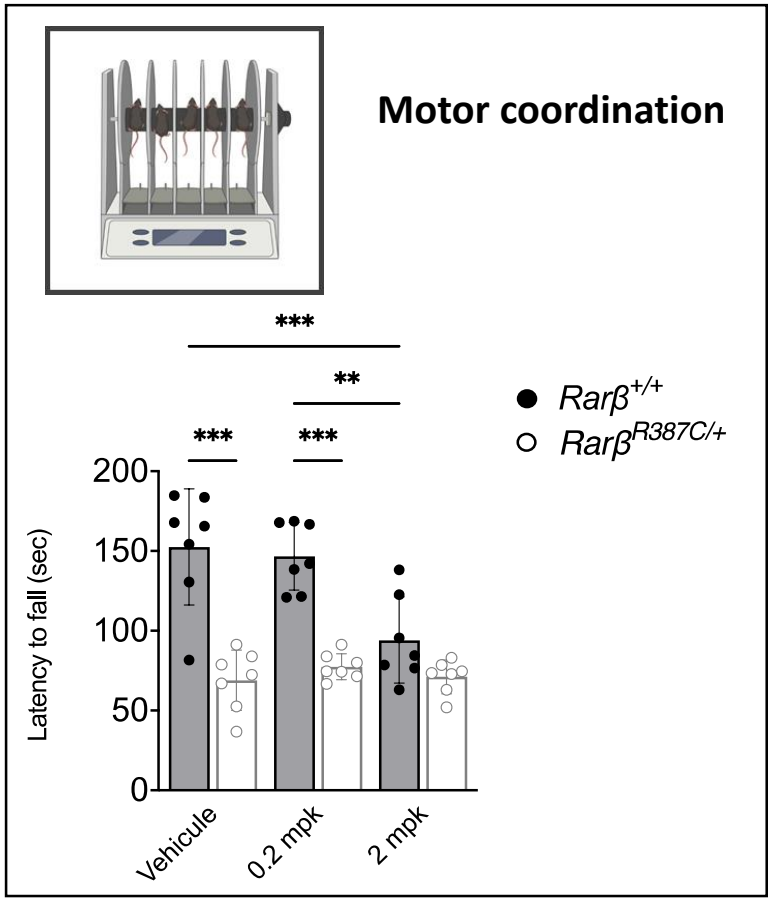
- Hyperactivity
- Coordination deficits
- Cognietive deficits

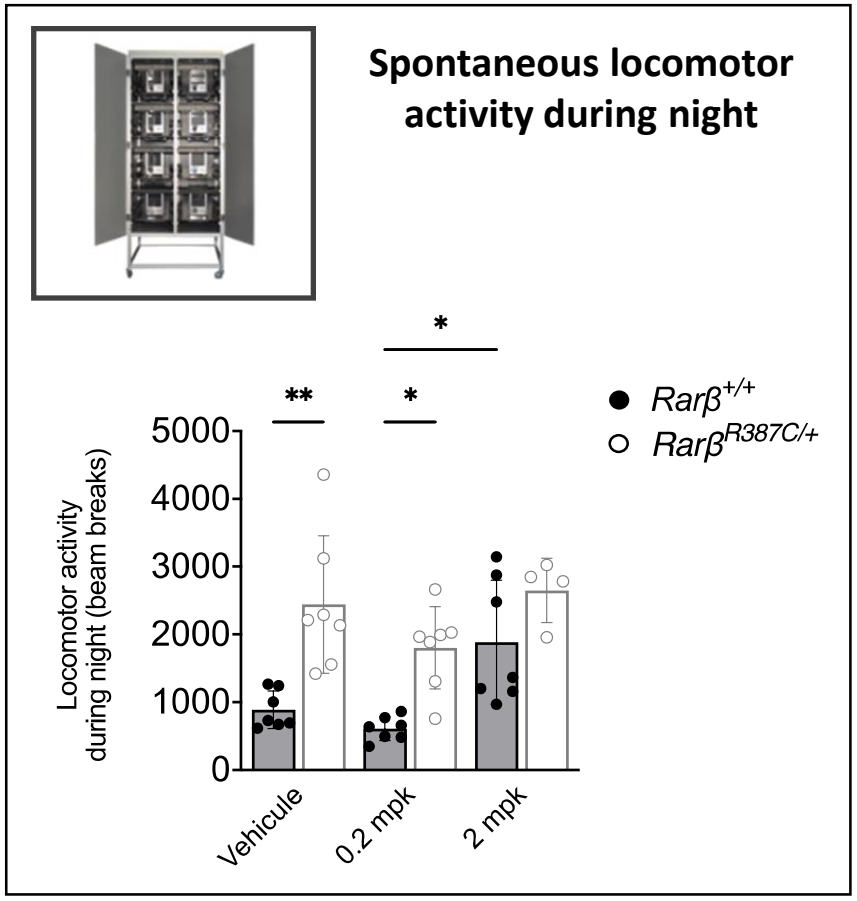


- **Reduced number of Drd2+ MSNs**
- **Reduced signaling of Drd2+ MSNs (blunted response to Drd2 antagonist, amphetamine) but not Drd1+ MSNs**
- ***Rarb*$^{Drd2-/-}$ recapitulate motor coordination deficits (Drd2-dependent ?) but not hyperactivity (Drd1-dependent ?)**

Enhancing Drd2 signaling does not correct behavioral abnormalities in *Rarb*$^{R387C/+}$ mice



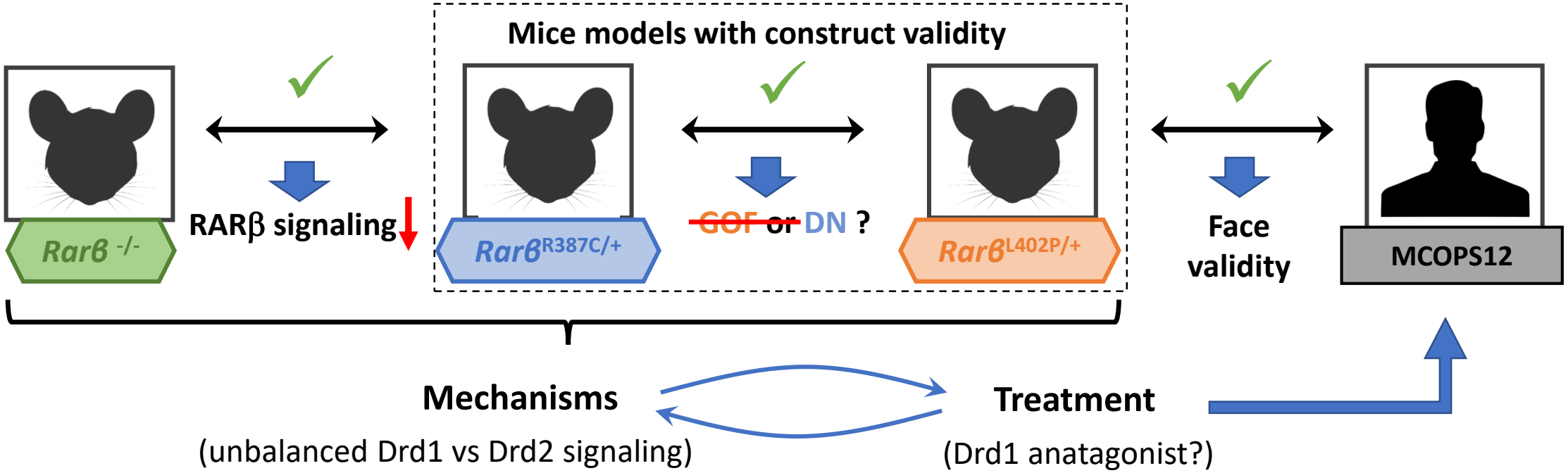




Motor coordination deficits are not rescued by pramipexol treatment.

Hyperactivity is not rescued by pramipexol treatment.

Summary and perspectives



- An overlap of behavioral and molecular phenotypes in RARb allelic series mice suggest that RARbR387C and RARbL402P variants act through dominant negative mechanism and lead to compromised RARb signaling
- An early postnatal life is an opportunity window for preventive treatments
- Unbalanced dopaminergic signaling is potential target for symptomatic treatments

➢ Targeting mutant form of RARb is a viable strategy for gene therapy approach

➢ Generation of patient-derived iPSCs for translational research

Summary and perspectives

1) One copy of RARb is sufficient to assure all essential functions of RARb -> gene therapy aiming at inactivation of mutant variant of RARb might be a valid strategy to prevent progression of the disease
2) Drug repurposing strategy to target pathophysiology of rare RARb variants

Dysfunction	Mouse Model	Treatment	Beneficial effect		
			Coordination	Hyperactivity	Memory
Unbalanced Drd1/Drd2 signaling	*RARbR* $^{387C/+}$, *RARb* $^{L402P/+}$, *RARb* $^{-/-}$	Drd2 agonist	No Effect	No Effect	?
Unbalanced Drd1/Drd2 signaling	*RARbR* $^{387C/+}$, *RARb* $^{L402P/+}$, *RARb* $^{-/-}$	Drd1 antagonist	N.D.		?
Abnormal neuronal morphology/co	*RARbR* $^{387C/+}$, *RARb* $^{L402P/+}$, *RARb* $^{-/-}$	Piracetam	No Effect	No Effect	
Mitochondria	*RARb* $^{-/-}$	Sigma 1 agonist	No Effect	No Effect	

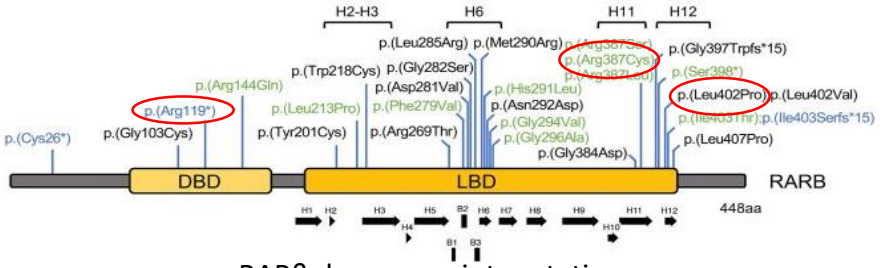
Funded by Cure MCOPS12

Summary and perspectives

1) Learn more about molecular effects of RARb mutations to guide MCOPS12 treatment strategies

Hypothesis I :

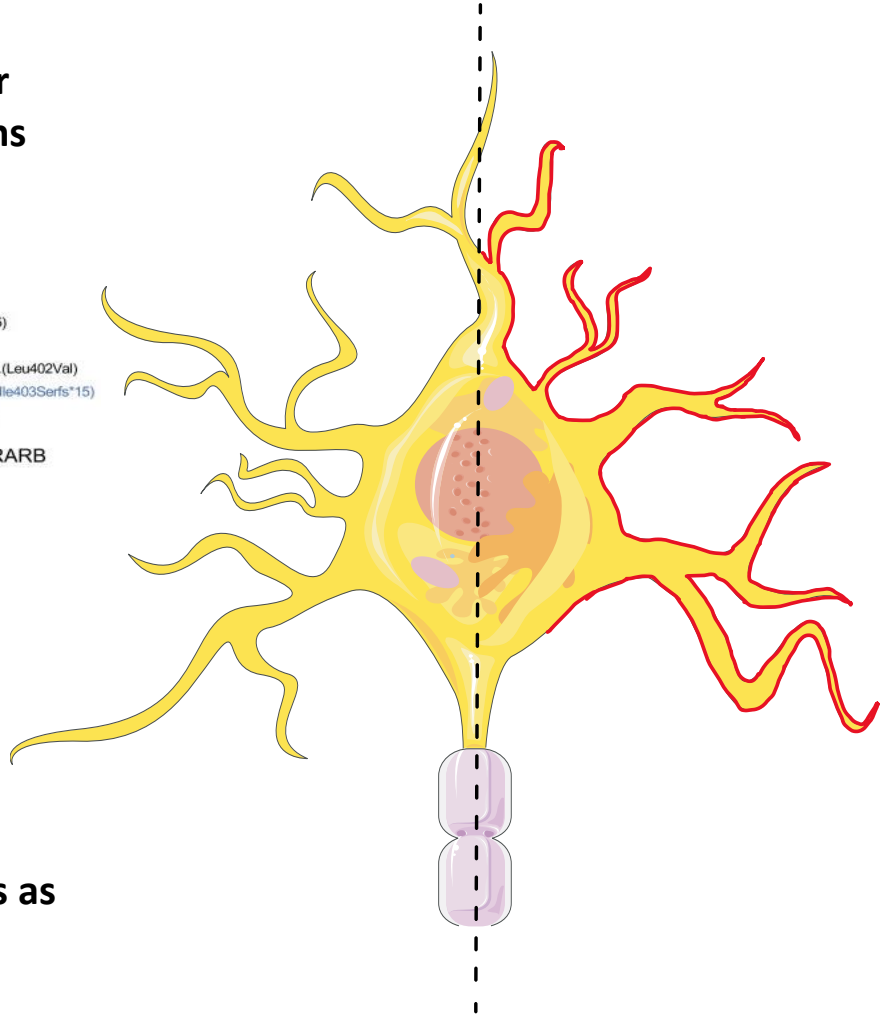
MCOPS12-causing RARβ variants alter RARβ interactions with partner proteins



RARβ de novo point mutations

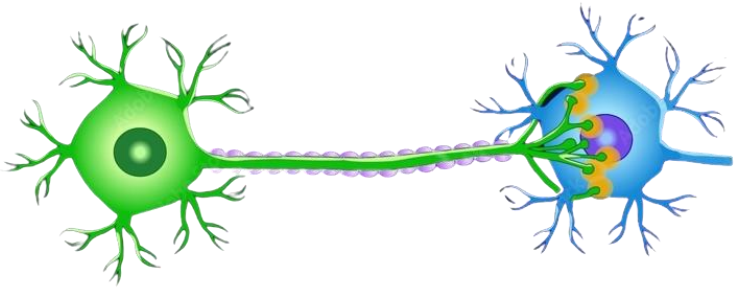
Objective I :

Identify RARβ interacting proteins as potential treatment targets



Hypothesis II :

MCOPS12-causing RARβ variants alter striatal cellular communication



Objective II :

Proteomic analyses of synaptic compartments

Acknowledgments

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