Second part

Models of IS

- Acute models:
  - NMDA [1]
  - Prenatal Betamethasone or stress / postnatal NMDA [2]
  - Down syndrome / γ-butyrolactone (GBL) [3]

- Chronic models:
  - TTX [4]
  - ARX conditional knockout [5]
  - ARX knockin [6]
  - Multiple-hit [7]

[1] *Mares and Velisek, Dev Brain Res (1992);* [2] *Velisek et al Ann Neurol (2007);* [3] *Cortez et al Ped Res (2009);*
[4] *Lee et al Epilepsia (2008);* [5] *Marsh et al Brain (2009);* [6] *Price et al J Neurosci (2010);*
[7] *Scantlebury, Galanopoulou et al Neurobiol Dis (2010)*

Pharmacosensitivity of acute IS models (pre-treatment)

	NMDA model	Prenatal betamethasone / postnatal NMDA	Prenatal stress/postnatal NMDA	Down / GBL
$ACTH_{1-24}$	***No effect*** on latency [0.1-0.4 mg/kg i.p.]			Shortens EDR [40, 80 µg/ mouse]
$ACTH_{1-39}$ (Rat synthetic)	***No effect*** on latency [0.1 mg/kg i.p.]	Delays spasms [0.1 mg/kg i.p.]	***No effect*** with single dose. Delay and reduction of spasms after 9 doses [0.3 mg/kg/dose, 3 doses/day s.c.]	
$ACTH_{1-39}$ (porcine, natural)	Delays and reduces spasms severity [100 U/kg ACTH i.p.]			***No effect*** on EDR duration [20 – 80 µg/ mouse]
Hydrocortisone	Increases spasms [10-25 mg/kg]			
Methylprednisolone		Repetitive but not single dose reduce spasms incidence [60 mg/kg]		
Vigabatrin	Decreased spasms incidence (600-1200mg/kg)	Decreases spasms incidence (250mg/kg)		Shortens EDR (250-500 mg/kg)
Pyridoxine (B6)	Decreases spasms incidence; induces epileptiform activity (250 mg/kg)			
Clonazepam	***No effect*** (0.2-1 mg/kg)			
Valproic acid	***No effect*** (100-400 mg/kg); Modest effect			Shortens EDR (100-400 mg/kg)
Ethosuximide				Shortens EDR (25-50 mg/kg)
CGP 35348				Shortens EDR (50-200 mg/kg)
Baclofen				Prolongs EDR (0.5-2 mg/kg)
5-hydroxy-tryptophan				Prolongs EDR (100-150 mg/kg)
Rapamycin		***No acute effect*** with low doses [3 mg/kg i.p.,]		

Arx KI mouse: Early neonatal estradiol treatment prevents spasms, seizures, interneuronopathy

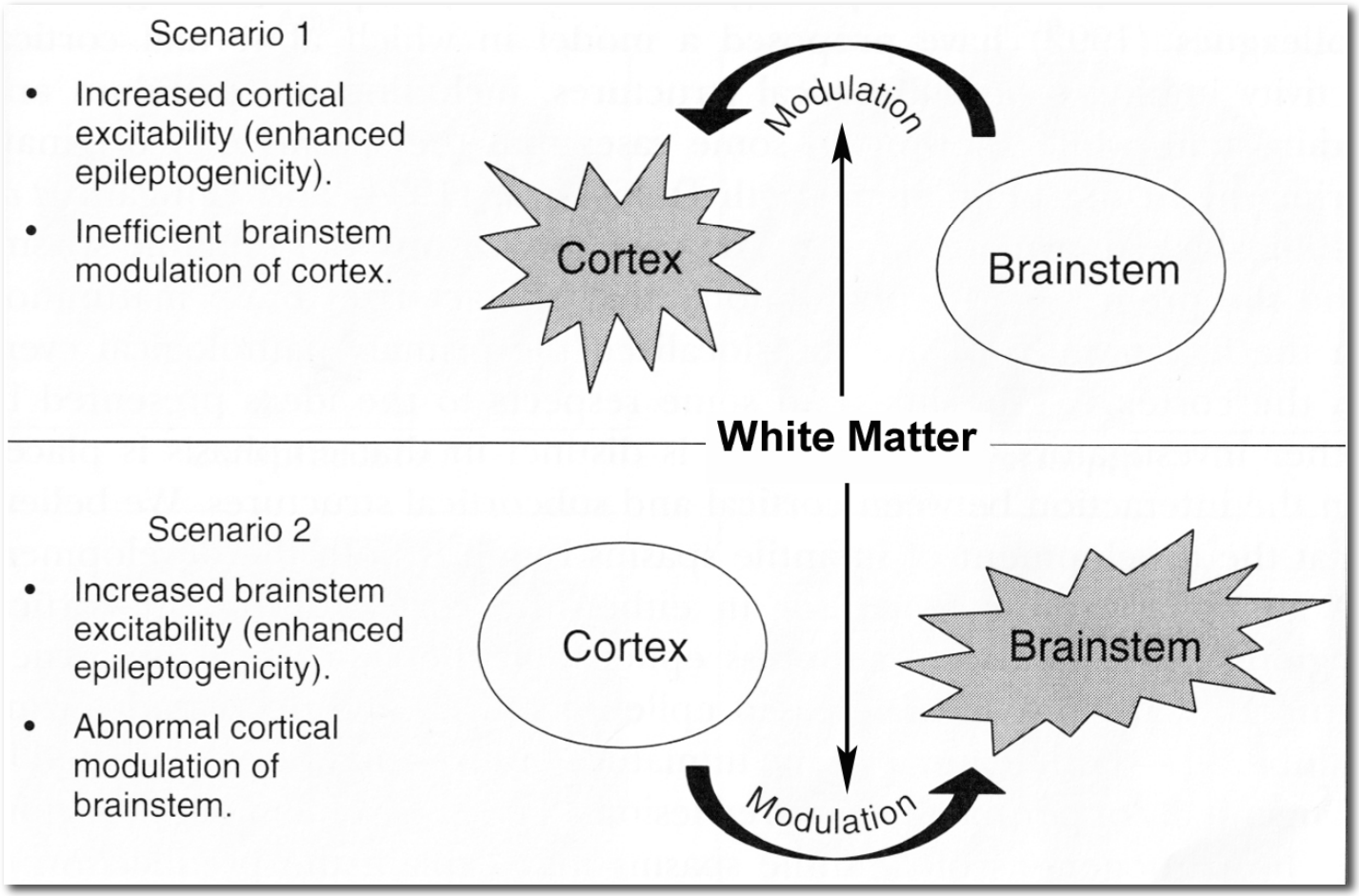
Olivetti et al (2013) Science Translational Medicine

Tetrodotoxin (TTX) model

- Chronic TTX infusion in cortex or hippocampus (PN10 till ~PN40)
- 1/3 of pups have spasms (starting at PN21)
- Later appearance of limbic seizures
- EEG recordings starting at PN42:
  - Ictal EDR
  - Hypsarrhythmia (NREM, often asymmetric)
  - Neocortical HFOs

Lee CL, Frost JD Jr, Swann JW, Hrachovy RA: Epilepsia (2008); Frost et al (Epilepsia) 2012

What predisposes children to infantile spasms?

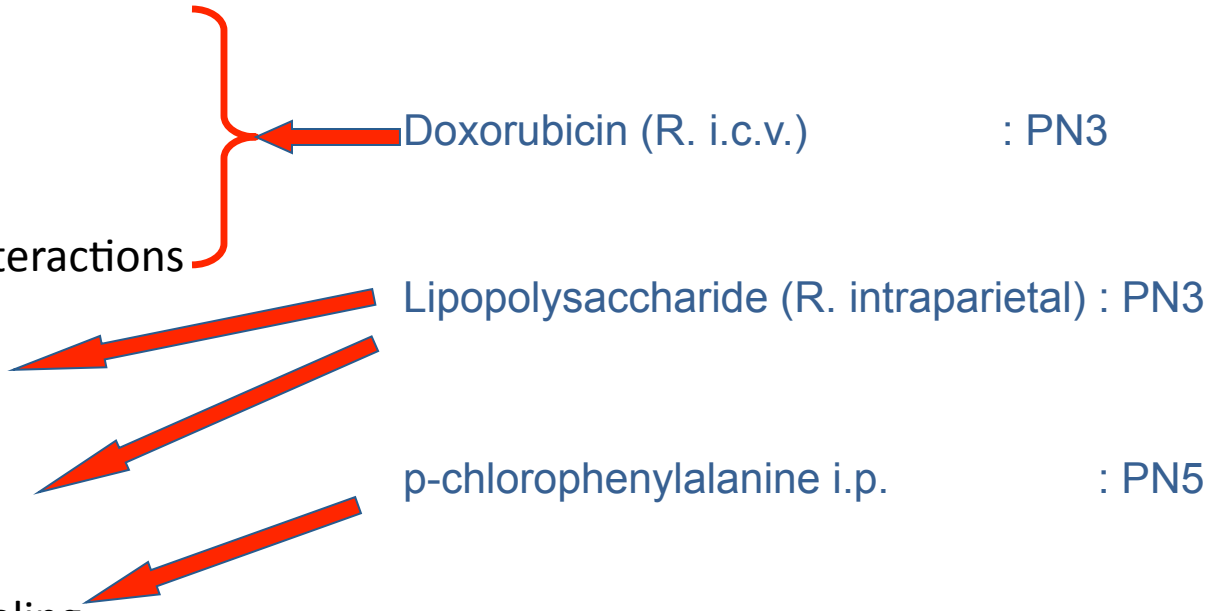


Lado and Moshé Int Rev Neurobiol. 2002;49:115-140

Infantile spasms: pathogenesis

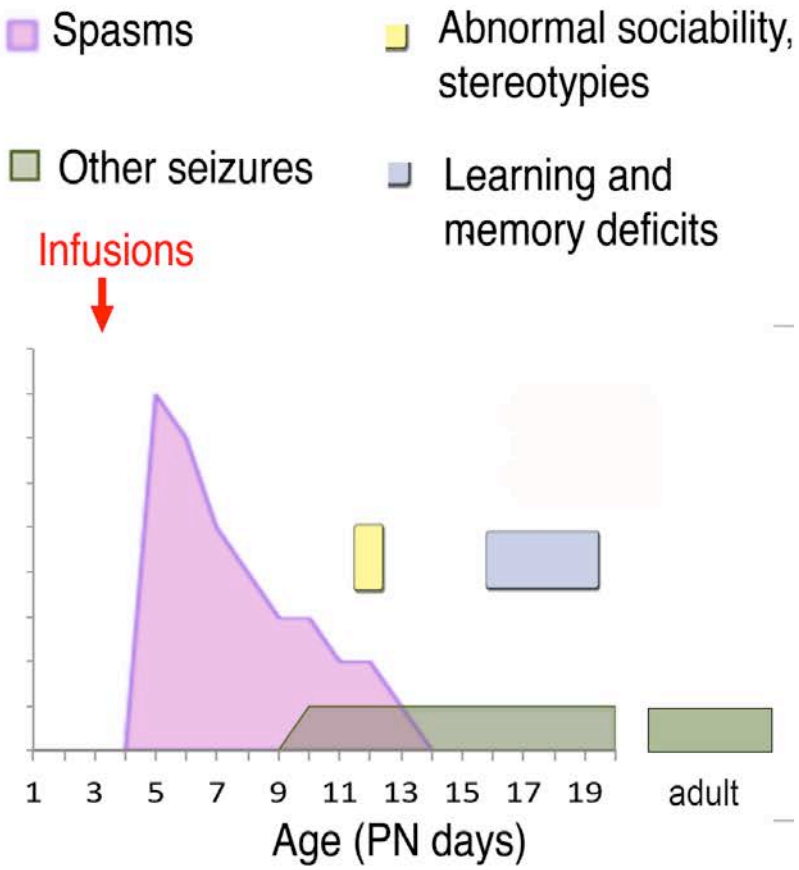
The "multiple-hit" model of symptomatic IS

- Cortical origin
- Subcortical origin
- Cortical - subcortical interactions
- White matter lesions
- Inflammatory cascades
- Specific molecular signaling pathway abnormalities



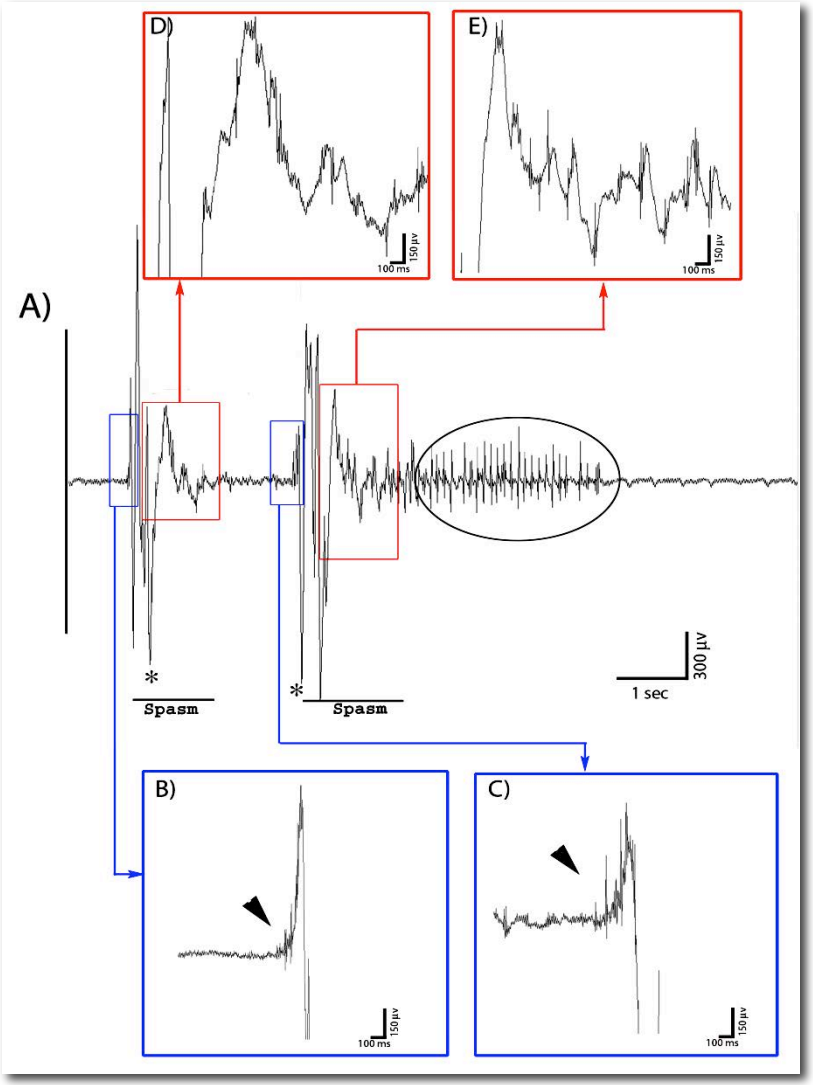
Scantlebury M, Galanopoulou AS, Chudomelova L, Raffo E, Betancourth D, Moshé SL, Neurobiol Dis (2010)

The multiple-hit model of symptomatic IS (DLP model)



Scantlebury M, Galanopoulou AS, Chudomelova L, Raffo E, Betancourth D, Moshé SL: Neurobiol Dis (2010)

EEG recording in P9 rat pup with spasms



The DLP model of symptomatic IS:
autistic features, learning and vocalization deficits

Scantlebury M, Galanopoulou AS, Chudomelova L, Raffo E, Betancourth D, Moshé SL, Neurobiol Dis (2010)

Jequier Gygax M, Galanopoulou AS (2012)

Other seizure types post spasms

Generalized limbic seizures were also seen starting from P11

Hippocampal seizure recorded in P11 pup that previously had spasms

DLP model:
motor seizures in adulthood, in sleep

ELECTROGRAPHIC SEIZURE

FOCAL ONSET SEIZURE

GENERALIZED SEIZURE

Akman and Galanopoulou (2012)

DLP model:
Slow Spike and slow wave discharge (SWD) bursts

(2/7)

(2/7)

SWDs: Controls vs. DLP rats

Frequency:

Control : 7.0 ± 0 Hz

DLP : ***5.75*** ± 0.25 Hz

Age of onset:

Control : PN 153 ± 15

DLP : PN ***86.5 ± 0.5***

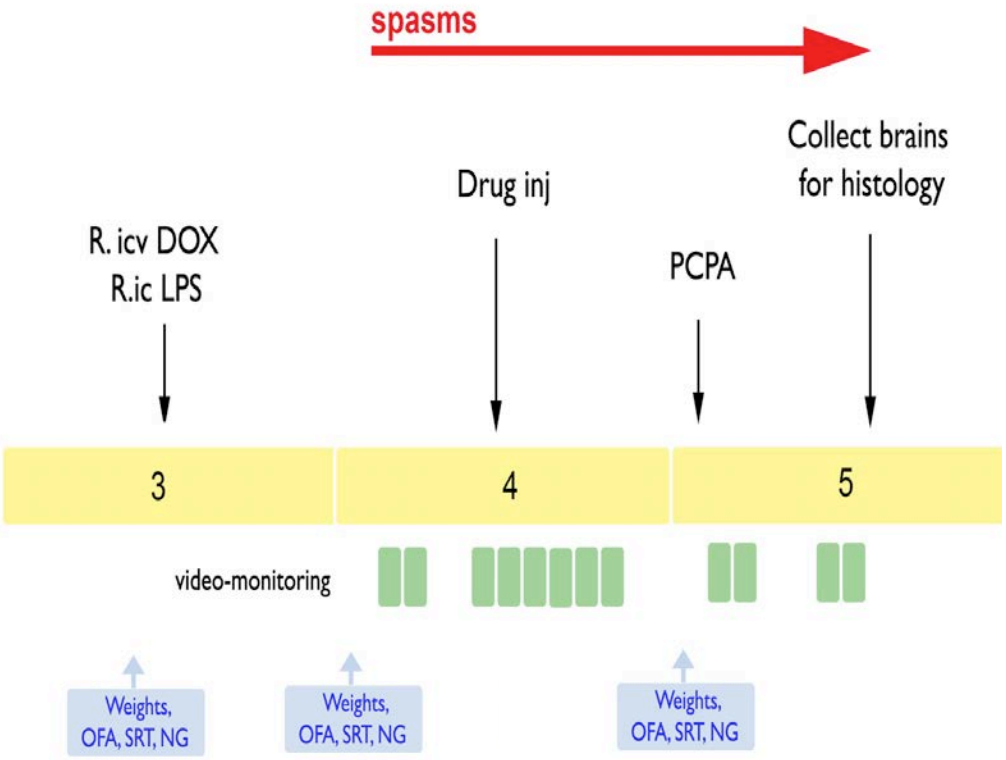
Akman, Briggs, and Galanopoulou (2012): AES annual meeting

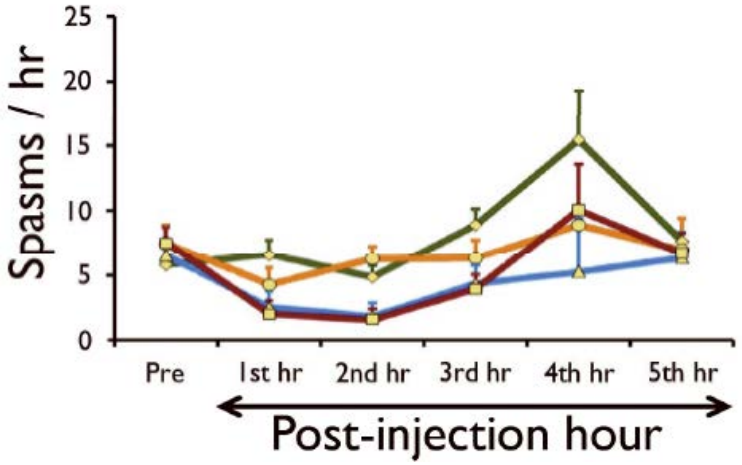
DLP model:
Loss of GABAergic neurons in layers 2/3, 6; the remaining have abnormal morphology

Altered firing patterns in substantia nigra pars compacta

Experimental design:

- blinded, randomized, vehicle-controlled, dose/time-response studies
- treatment starts after spasms onset





Multiple-Hit model: effects of drugs on spasms (given after the spasms onset)

Drug	Acute effect (latency to onset)	Sustained effect with repeat dosing		Tolerability
		Treatment period	Effect	
[1] $ACTH_{1-24}$	No effect	PN4-12	No effect	No side effects
[1] Vigabatrin	Effective with delay (within 18-24 hours)	PN4-11	1 day	High mortality (sedation, poor feeding)
* [2] CPP-115 (vigabatrin analog)	***Effective (within the first hour)***	PN4-12	2-3 days	No side effects
[3] Rapamycin	***Effective (within the first 2 hours)***	***PN4-6***	- ***suppresses spasms without rebound*** - ***Partial improvement of learning (PN16-19)***	Transient deceleration of weight growth
* [4] Carisbamate	***Effective (within the first hour)***		Not tested	No side effects
[4] Phenytoin	No effect		Not tested	No side effects
[5] NAX 5055	No effect		Not tested	No side effects

*: FDA-designated orphan drug for IS

[1] Scantlebury M, Galanopoulou AS, Chudomelova L, Raffo E, Betancourth D, Moshé SL (2010) Neurobiol Dis: 37(3): 604-12

[2] Briggs SW, Mowrey W, Hall C, Galanopoulou AS (2014) Epilepsia

[3] Raffo E, Coppola A, Ono T, Briggs SW, Galanopoulou AS (2011) Neurobiol Dis. **43**: p. 322-29.

[4] Ono T, Moshé SL, Galanopoulou AS (2011) Epilepsia. **52**(9): p. 1678-84.

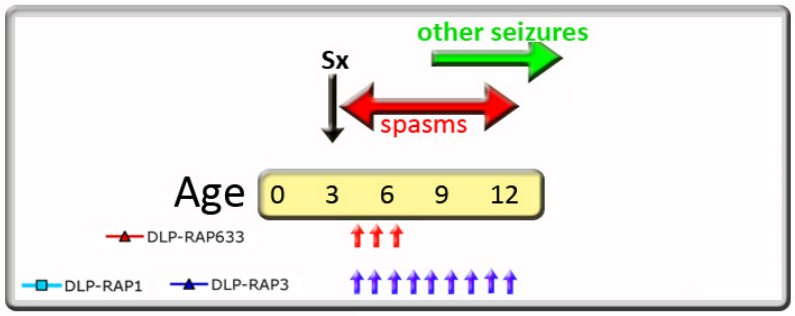
[5] Gygax M, Klein B, White S, Kim M, Galanopoulou AS (2014) Epilepsy Research

Pulse mTOR Inhibition in the DLP model of non-TSC IS

A **pulse** (3-day), **high-dose** course of rapamycin, given ***after the onset*** of spasms :

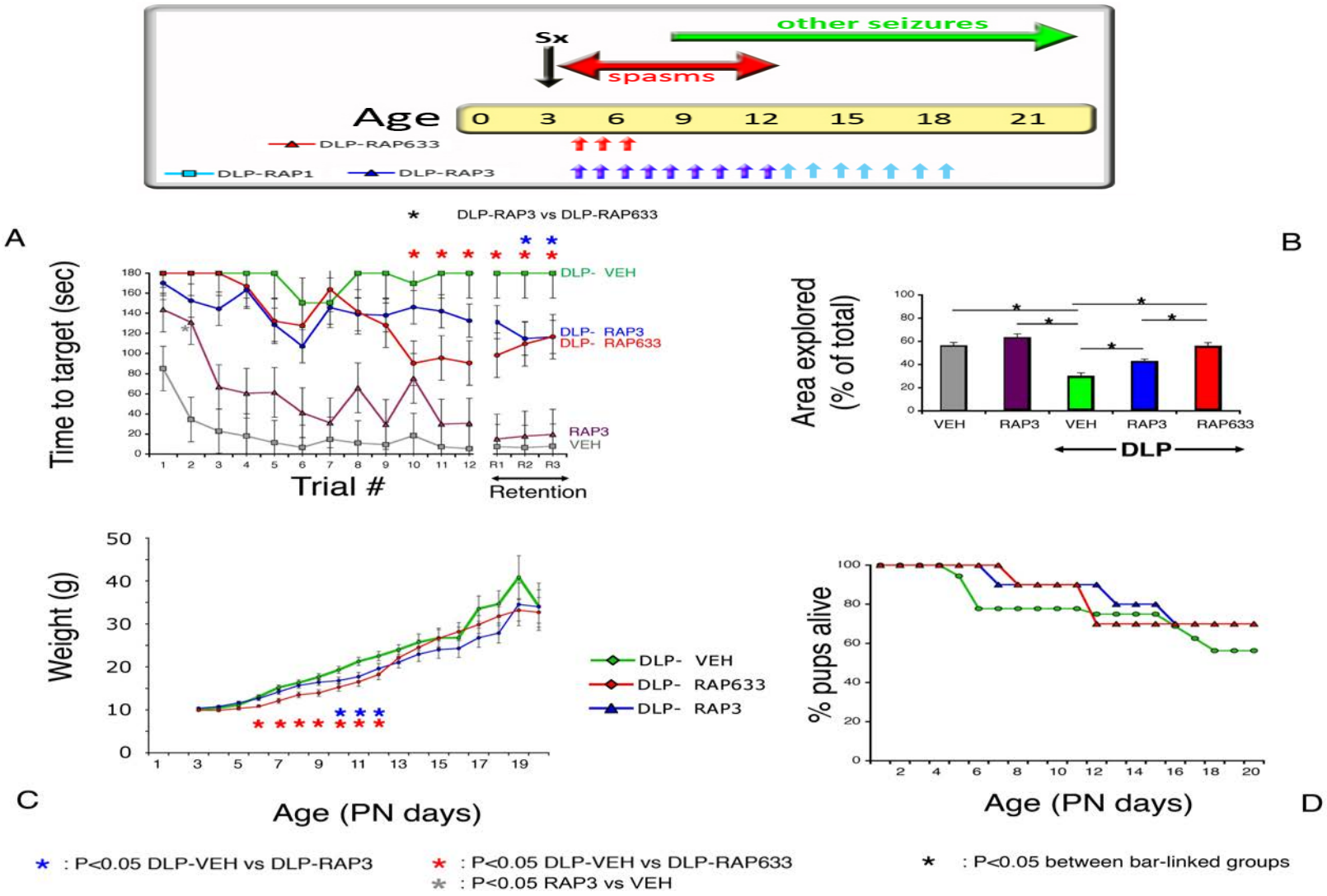
- –Normalizes mTORC1 (pS6-ir)
- –Suppresses spasms

Lower doses of rapamycin cause delayed but not acute suppression of spasms



Raffo E, Coppola A, Ono T, Briggs SW, Galanopoulou AS: Neurobiol Dis 2011;43:322-9.

High dose pulse rapamycin improves cognitive outcome (Barnes maze, DLP model)



Raffo E, Coppola A, Ono T, Briggs SW, Galanopoulou AS (2011) Neurobiol Dis

mTOR pathway and IS

Tuberous sclerosis (TSC) and IS:

- 38% of patients with TSC develop IS
- 5-10% of IS patients have TSC

"*Toropathies*" and IS:

- focal cortical dysplasias type II
- Hemimegalencephaly (including PTEN mutations)
- STRADalpha mutations (PMSE: polyhydramnios, megalencephaly and symptomatic epilepsy)

Chu-Shore et al, 2010; Crino 2007; Miyata et al 2004; Ljungberg et al, 2006; Orlova et al 2010

CPP-115, a vigabatrin analogue, decreases spasms in the multiple-hit rat model of infantile spasms

(drug given after the onset of spasms; blinded, randomized design)

- CPP-115 is a new high affinity GABA aminotransferase inhibitor with lower than vigabatrin reported risk for retinal toxicity.

- In the multiple-hit rat model of symptomatic infantile spasms, CPP-115 given after the onset of spasms:
  - reduced behavioral and electroclinical spasms at 400-times lower doses than vigabatrin
  - was well tolerated, without impairing survival, growth, neurodevelopmental reflexes
  - did not affect learning

- CPP-115 is currently on phase I trials and has been granted orphan drug status with indication for infantile spasms in the USA.

Briggs, Mowrey, Hall, Galanopoulou (2014) Epilepsia, in press

CPP-115 was provided by Dr Steven Miller (Catalyst Pharmaceuticals Partners Inc, Coral Gables FL)

Why IL-1ra?

- Evidence for IL-1β involvement in the pathogenesis of IS
  - Clinical studies showing abnormal IL-1β (high) and/or IL-1ra (low) levels in infants with IS [1]
  - Our preliminary studies showing:
    - increased IL-1β expression in the DLP model, peri-infusionally
    - that inflammatory insults that can induce IL-1β may trigger spasms (LPS)

–IL-1ra has entered clinical use for other indications (Kineret: Rheumatoid arthritis) and similar drugs are under clinical testing for seizures (not IS).

–Experimental evidence that:

- IL-1ra may have therapeutic effects on other seizure models [2]
- Systemic use of IL-1ra may benefit other CNS diseases (i.e. stroke) [3]

[1] Shiihara et al (2010) Brain & Dev; Haginoya et al (2009) Epilepsy Res; Yamanaka et al (2010) J Neurol Sci.
[2] Vezzani group (multiple refs); Marchi et al (2009)
[3] Greenhalgh et al (2010); Clark et al (2008); Girard et al (2012)

Kineret: prevents spasms progression in DLP rats

(each rat's response to the drug is compared using frequencies normalized to its own pre-treatment baseline)

Tufikameni Brima, PhD

Lessons from the animal models

- Common pathogenic mechanisms are:
  - Interneuronopathies: deficit in GABAergic interneurons
  - Focal lesions can generate spasms
  - Cortical-subcortical lesions
  - mTOR overactivation/role of interleukins
- New therapies may be in the horizon and they could offer new insight into mechanisms controlling IS.
  - Carisbamate: acute effects on spasms (single injection experiments)
  - Pulse mTOR inhibition: acute suppression of spasms (at high doses) with possible subsequent cognitive improvement
  - Vigabatrin analog: reduces spasms with better tolerability profile
  - Estrogen pretreatment at the appropriate developmental level may prevent

There is hope

Translational research is important to identify etiology-specific differences in the pathogenesis of ‘catastrophic’ epilepsies as studies in West syndrome suggest

This will lead to new treatments that stop the seizures, modify the underlying disease and improve outcome

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- Advisory Board: Lundbeck, UCB
- Patent# 200802116183;

  Assignee: Einstein 'A model of infantile spasms '