

LBC



Laboratório de Bioquímica Celular - UFRGS

Alternative models to study Cannabidiol and Dravet Syndrome

Prof. Fábio Klamt, PhD
Laboratory of Cellular Biochemistry/UFRGS



MJFF – RRIA 2014



INCT-TM

BOMED²¹

EMERGING TECHNOLOGY TOWARD PATHWAY-
BASED HUMAN BRAIN RESEARCH

29-30 May 2017 | D'Or Institute for Research and Education, Rio de Janeiro





Everett's story

Everett was born on January 13, 2012. He left the hospital a normal baby boy. Everett continued to develop normally and had no medical issues for approximately the first five months of his life. On June 7, 2012, Everett had a seizure in his mother's arms.



He was diagnosed with a febrile seizure and we were sent home with antibiotics for an ear infection. Later that night, Everett had another seizure lasting 30 minutes before being stopped at the emergency room by emergency anti epileptic medication.

Unfortunately, this was not an isolated incident and Everett's seizures continued. From June 2012 until November 2012, Everett had four hospital stays, five emergency room visits, underwent anesthesia four times and was placed on anti epileptic medication that intensified his daily seizures.

In November 2012, Everett experienced a status seizure lasting over one hour. He was unable to breathe on his own and was intubated and placed in ICU for a number of days and received the Dravet syndrome diagnosis.

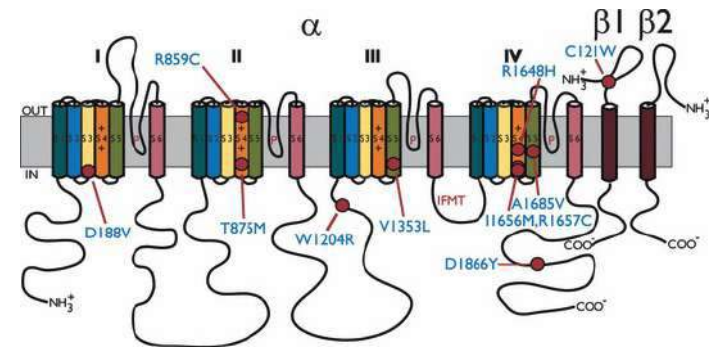
We began to deal with the reality that our son was always going to live with seizures.

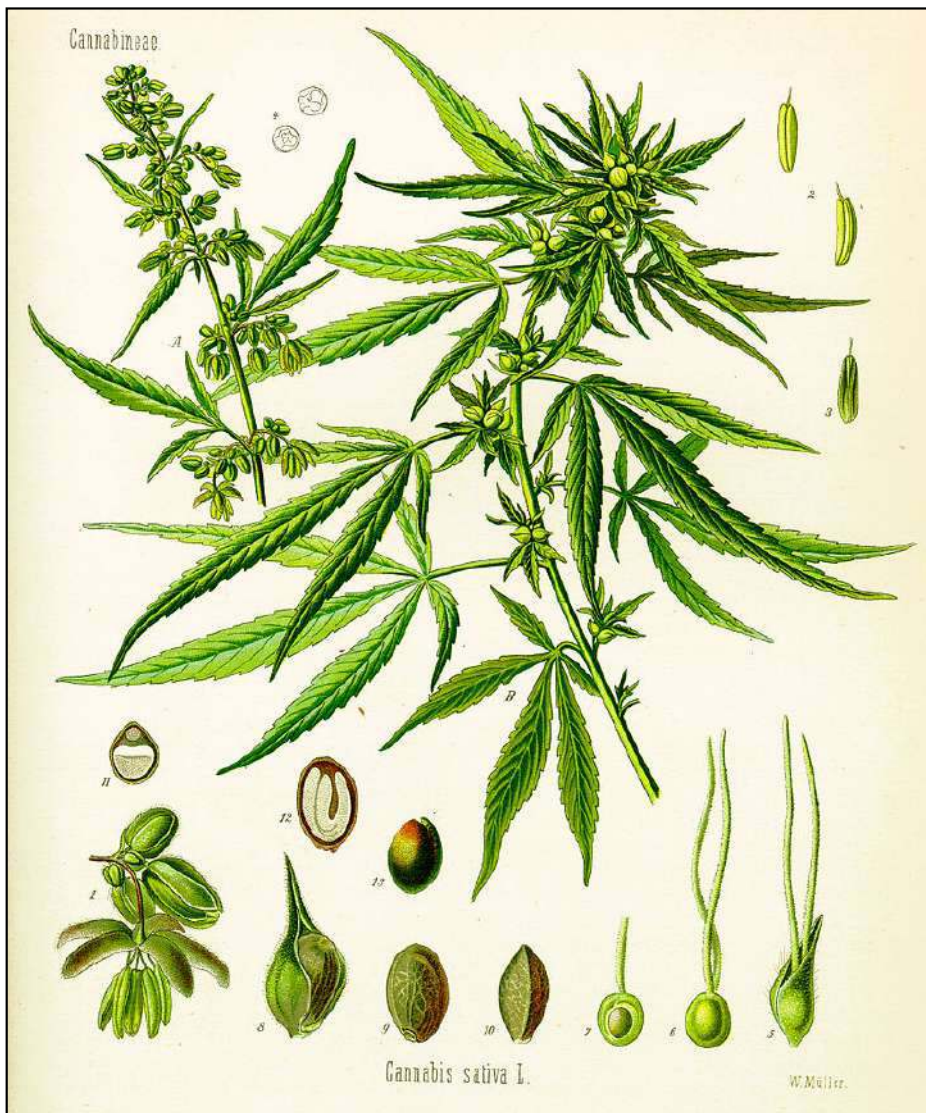
Shortly after his diagnosis, Everett was placed on clobazam, valproic acid and stiripentol. Since being placed on these drugs in November 2012, Everett has gone from having daily convulsive seizures to having rare convulsive seizures.

Today Everett attends an Early Learning Program four days per week and absolutely loves his teachers, therapists, classmates and nurse.

Dravet Syndrome

- Incidence: 1:22.000
- Seizures begin at 6 months classically with recurrent febrile status epilepticus
- Development is normal until aged 1–2 years, despite frequent, prolonged seizures
- 80% of patients have mutations of the sodium channel α -1 subunit gene, *SCN1A*, which encodes the voltage-gated channel Nav1.1.
- 30% of patients present treatment-resistant epilepsy (TRE)
- There are currently no U.S. Food and Drug Administration (FDA)-approved therapies for TRE





- *Cannabis*-based therapies have been used to treat epilepsy for millennia

- But only in the last few years we have begun to collect data adequately powered by placebo-controlled randomized trials

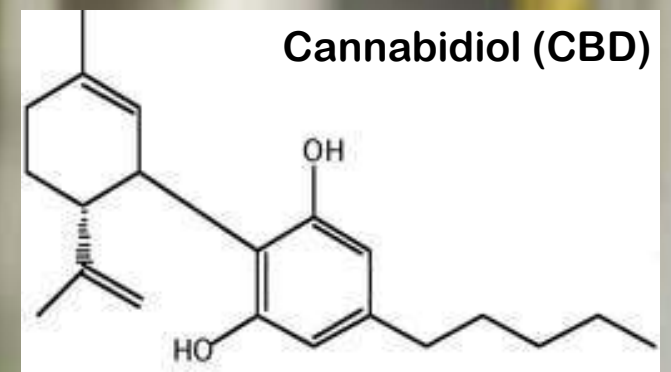




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Dr. Raphael Mechoulam



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812

MAY 25, 2017

VOL. 376 NO. 21

Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome

Orrin Devinsky, M.D., J. Helen Cross, Ph.D., F.R.C.P.C.H., Linda Laux, M.D., Eric Marsh, M.D., Ian Miller, M.D., Rima Nabhout, M.D., Ingrid E. Scheffer, M.B., B.S., Ph.D., Elizabeth A. Thiele, M.D., Ph.D., and Stephen Wright, M.D., for the Cannabidiol in Dravet Syndrome Study Group*

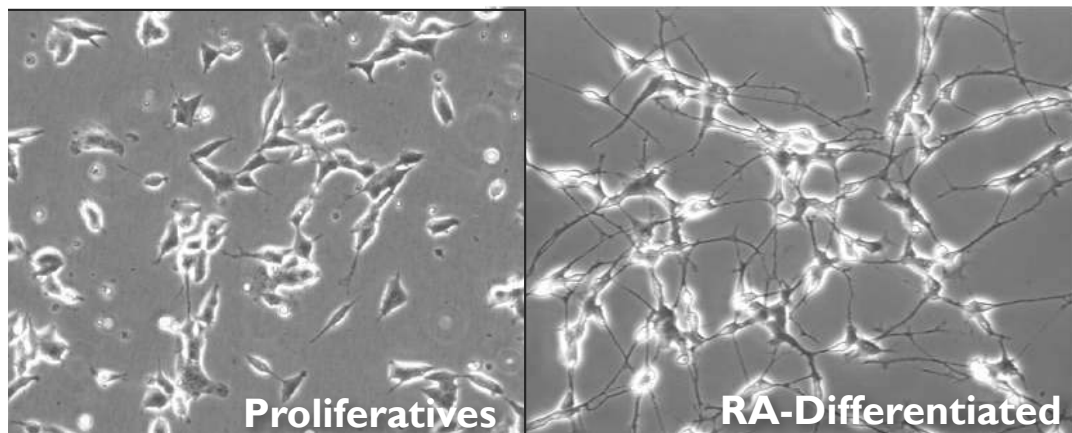
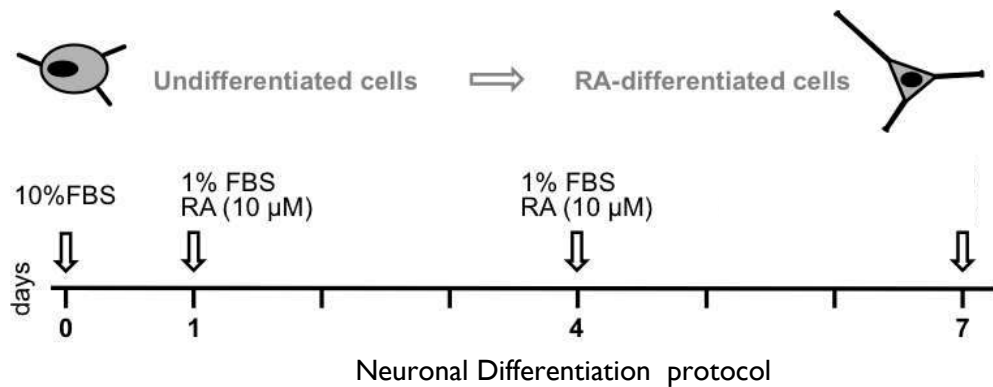
Table 2. Primary Efficacy End Point of Percentage Change in Convulsive-Seizure Frequency in Each Trial Group.*

Variable	Cannabidiol	Placebo	Adjusted Median Difference (95% CI) <i>percentage points</i>	P Value†
No. of convulsive seizures per mo — median (range)				
Baseline	12.4 (3.9 to 1717)	14.9 (3.7 to 718)		
Treatment period	5.9 (0.0 to 2159)	14.1 (0.9 to 709)		
Percentage change in seizure frequency — median (range)	-38.9 (-100 to 337)	-13.3 (-91.5 to 230)	-22.8 (-41.1 to -5.4)	0.01

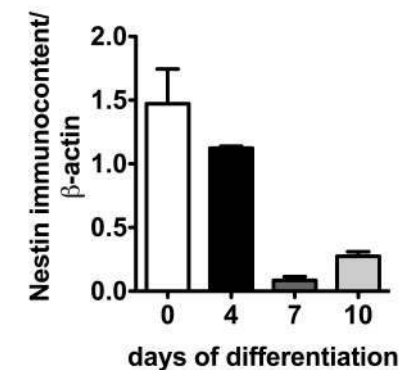
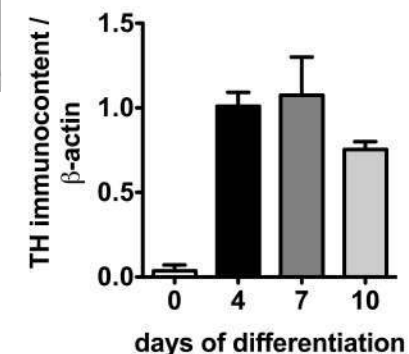
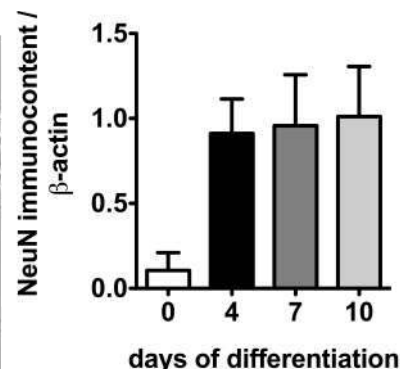
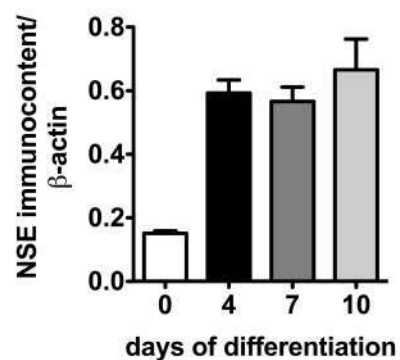
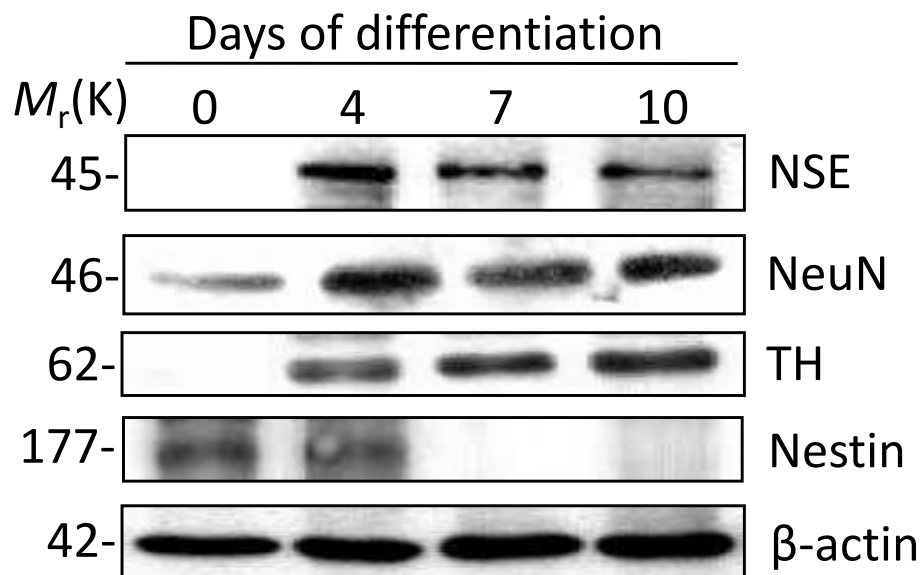
Table 4. Adverse Events Occurring with a Frequency of Greater Than 10% in Either Trial Group, According to System Organ Class and Preferred Term.*

System Organ Class and Preferred Term	Cannabidiol (N=61)	Placebo (N=59)
	<i>no. of patients (%)</i>	
Gastrointestinal		
Diarrhea	19 (31)	6 (10)
Vomiting	9 (15)	3 (5)
General		
Fatigue	12 (20)	2 (3)
Pyrexia	9 (15)	5 (8)
Infections: upper respiratory tract infection	7 (11)	5 (8)
Metabolism: decreased appetite	17 (28)	3 (5)
Nervous system		
Convulsion	7 (11)	3 (5)
Lethargy	8 (13)	3 (5)
Somnolence	22 (36)	6 (10)

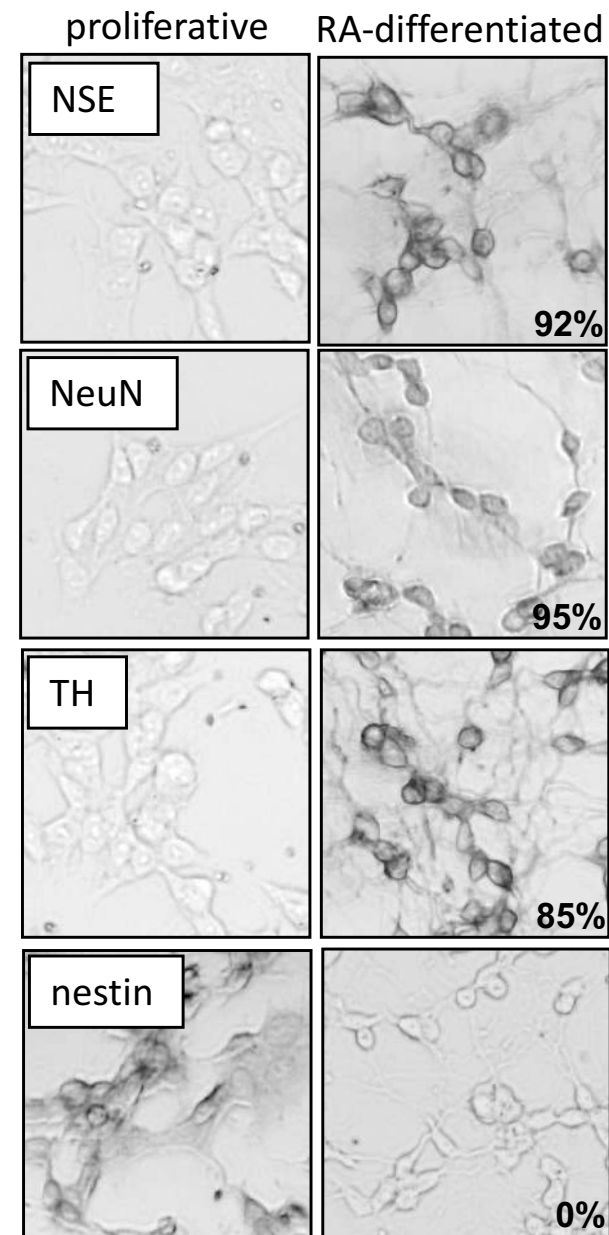
O modelo: neuroblastoma humano SH-SY5Y



Morphologies of SH-SY5Y cells; the proliferative and differentiated phenotypes.



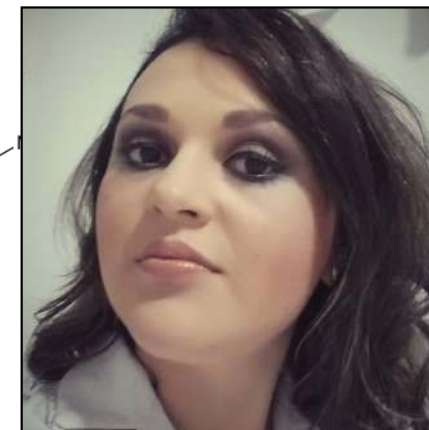
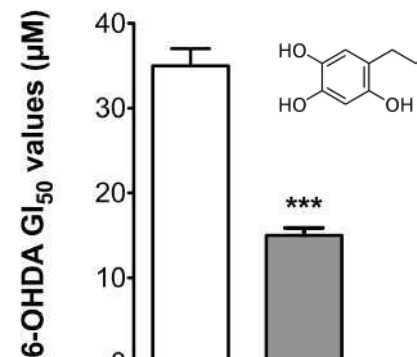
SH-SY5Y cells



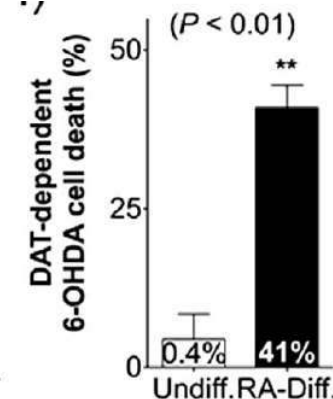
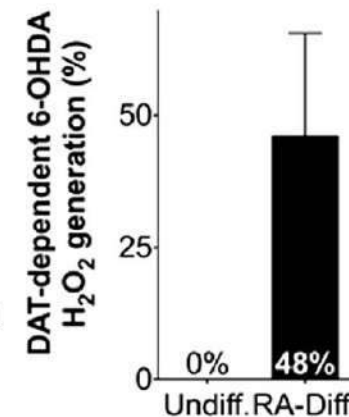
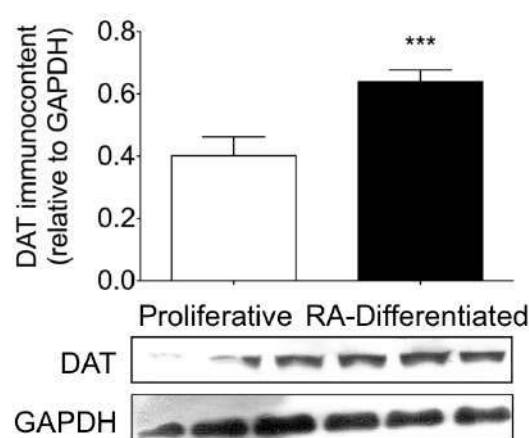
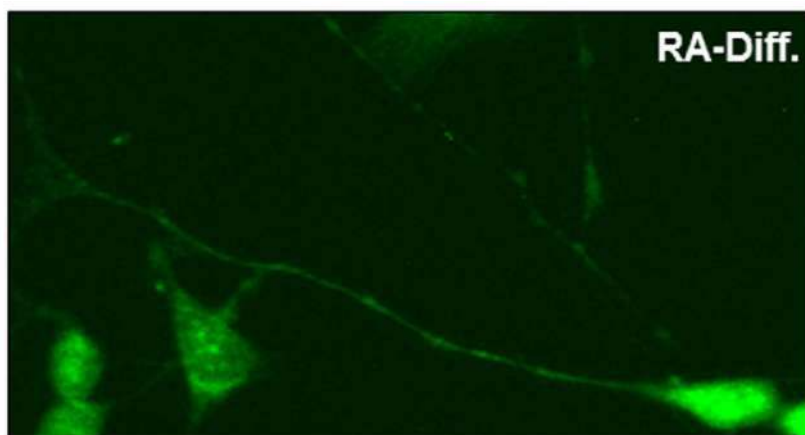
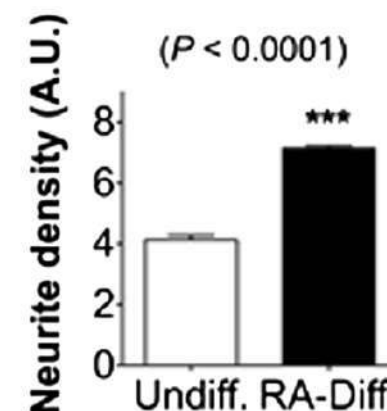
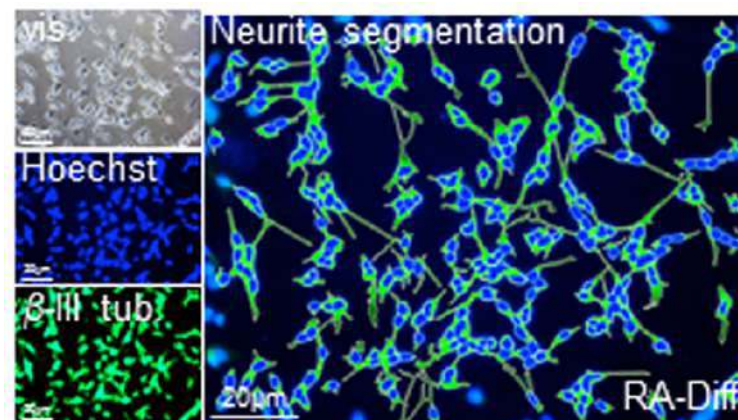
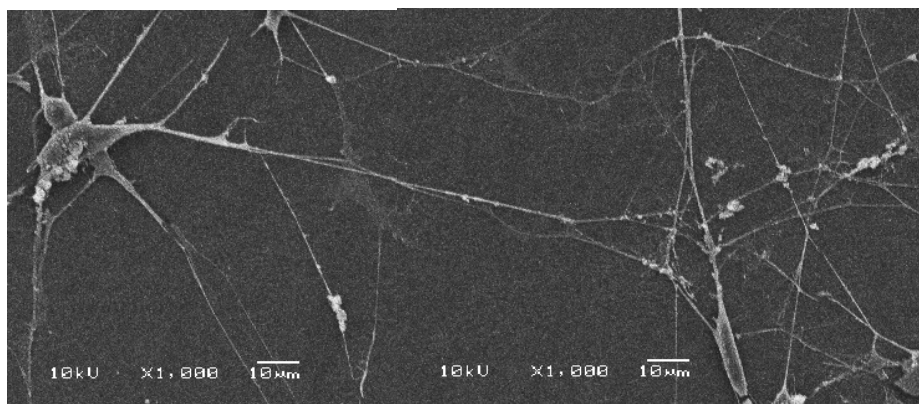
Immunohistochemistry images of neural markers in SH-SY5Y cells.

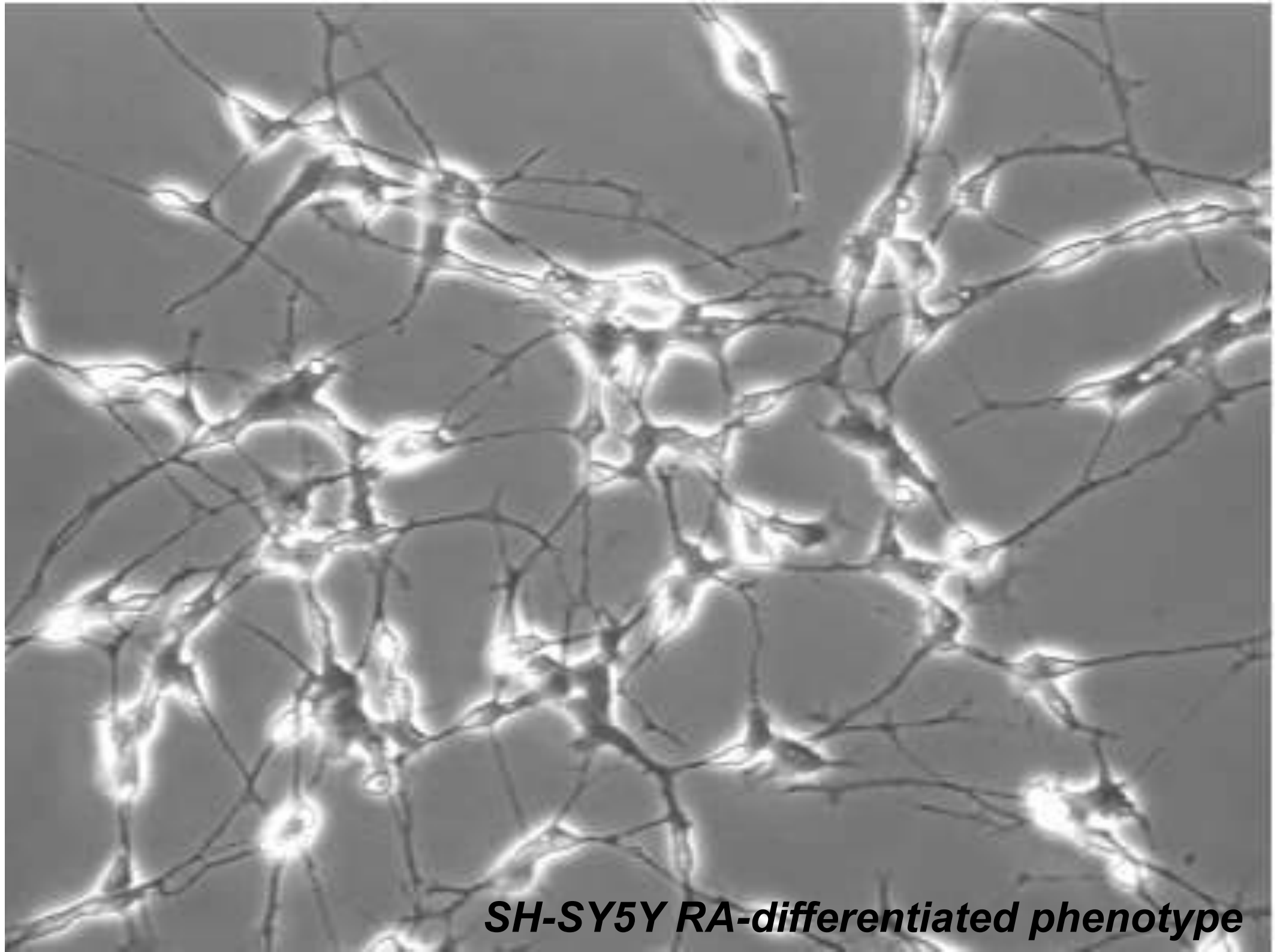
RA Differentiation Enhances Dopaminergic Features, Changes Redox Parameters, and Increases Dopamine Transporter Dependency in 6-Hydroxydopamine-Induced Neurotoxicity in SH-SY5Y Cells

Fernanda M. Lopes^{1,2} · Leonardo Lisbôa da Motta¹ · Marco A. De Bastiani¹ · Bianca Pfaffenseller¹ · Bianca W. Aguiar¹ · Luiz F. de Souza³ · Geancarlo Zanatta^{1,4} · Daiani M. Vargas¹ · Patrícia Schönhofen¹ · Giovana F. Londero¹ · Liana M. de Medeiros¹ · Valder N. Freire⁴ · Alcir L. Dafre³ · Mauro A. A. Castro⁵ · Richard B. Parsons² · Fabio Klamt¹



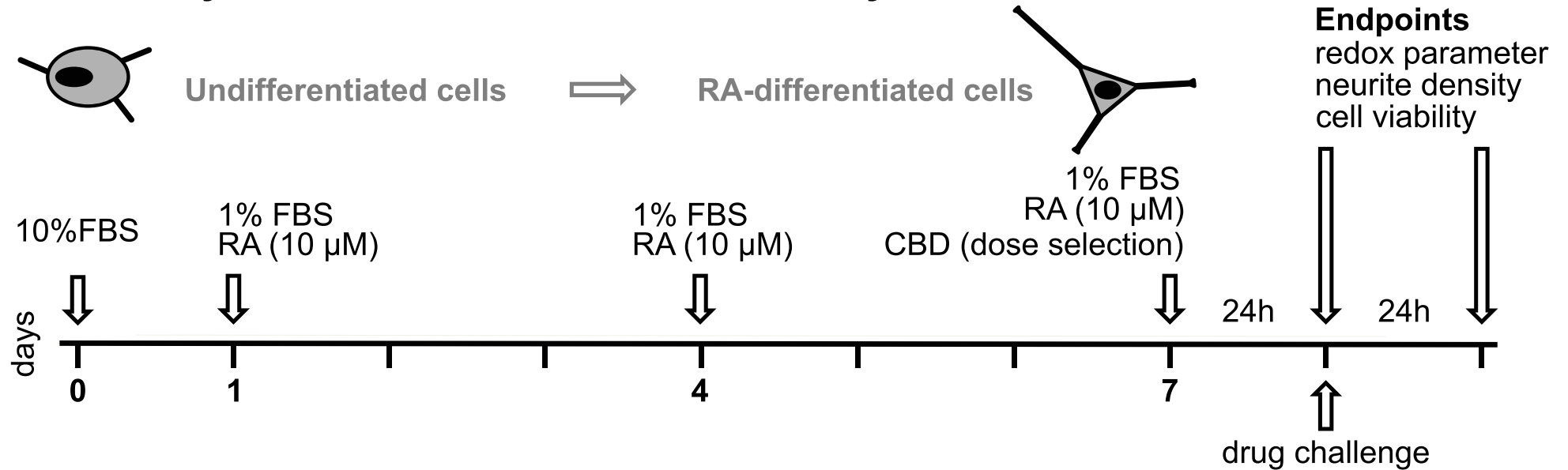
Dr. Fernanda Lopes



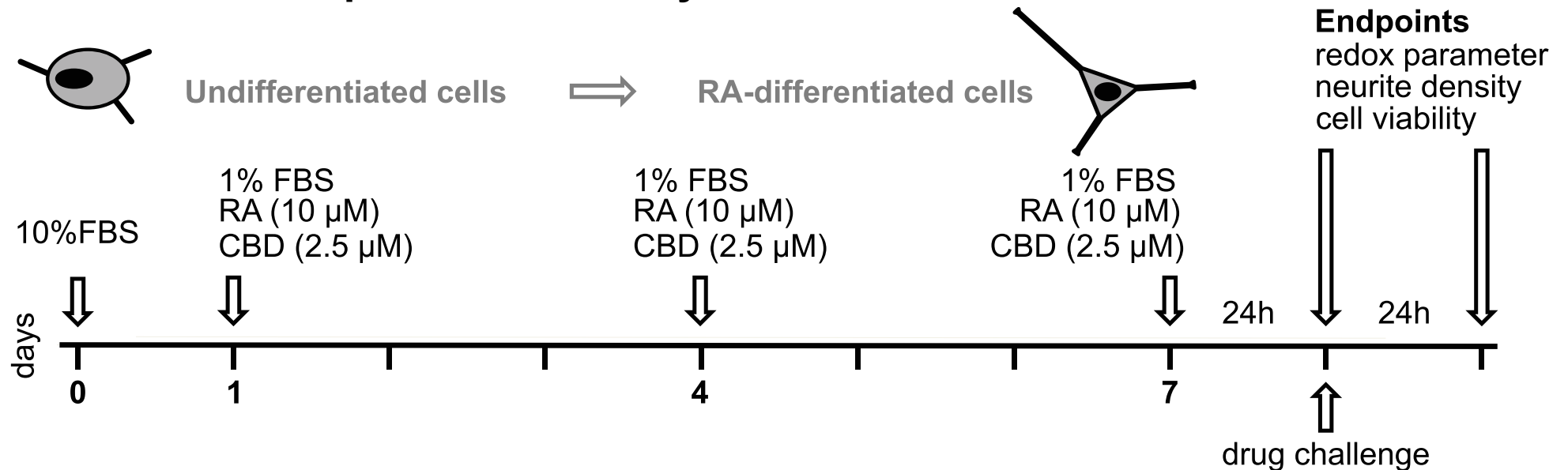


SH-SY5Y RA-differentiated phenotype

Terminally differentiated neuronal toxicity model



Neuronal developmental toxicity model

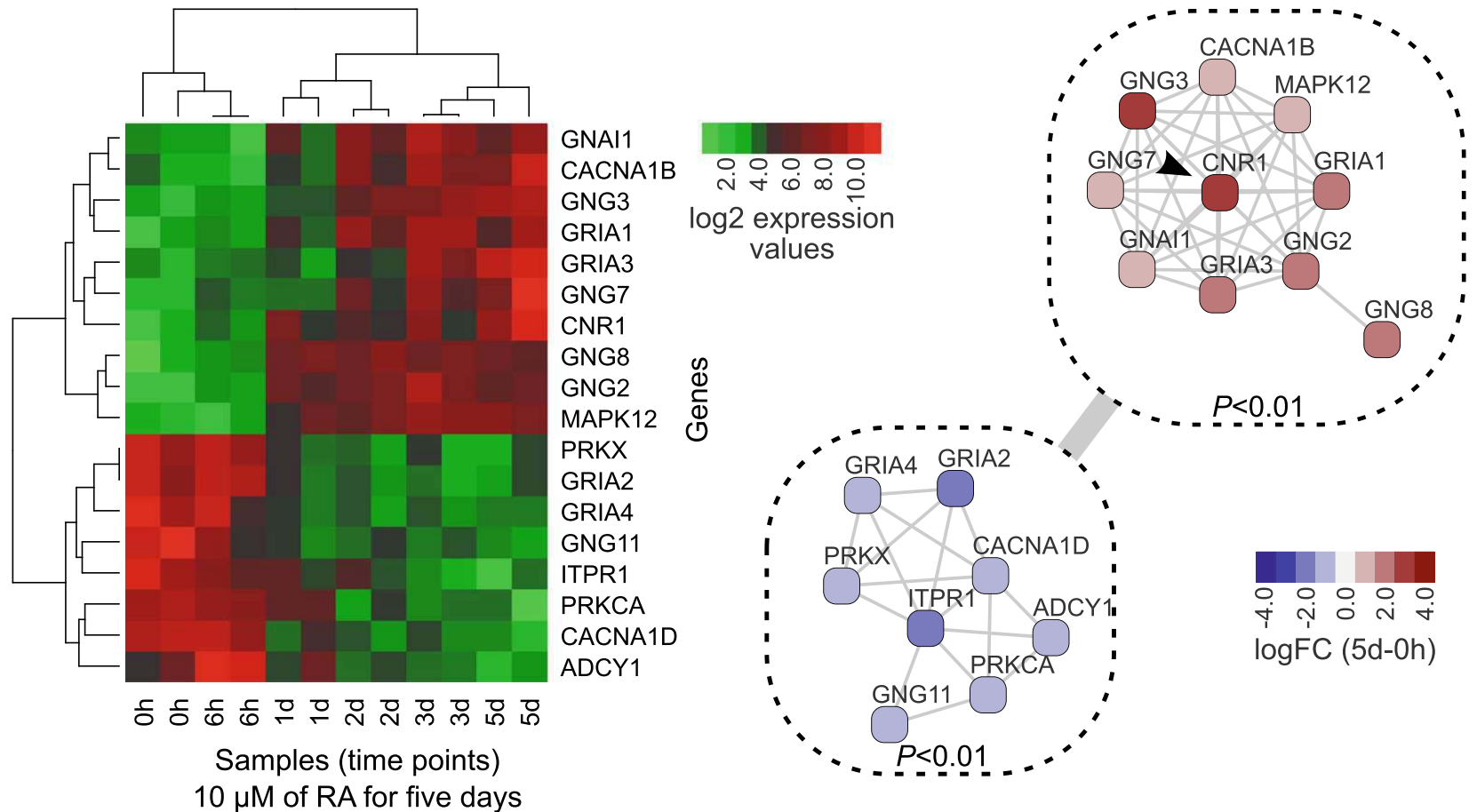


Cannabidiol Exposure During Neuronal Differentiation Sensitizes Cells Against Redox-Active Neurotoxins

Patrícia Schönhofen • Liana M. de Medeiros • Ivi Juliana Bristot • Fernanda M. Lopes • Marco A. De Bastiani • Flávio Kapczinski • José Alexandre S. Crippa • Mauro Antônio A. Castro • Richard B. Parsons • Fábio Klamt

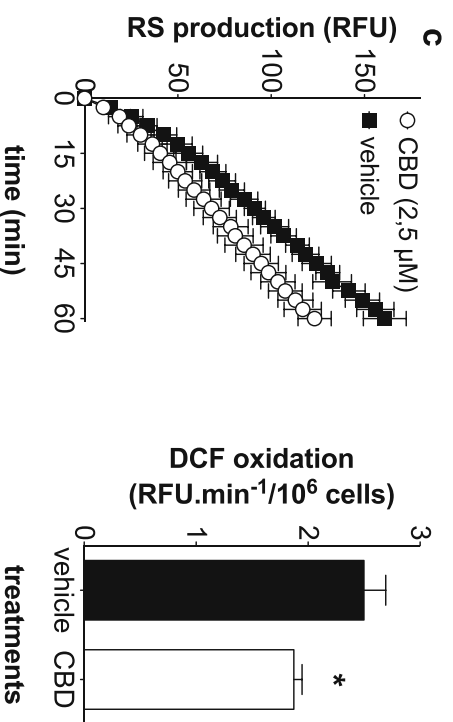
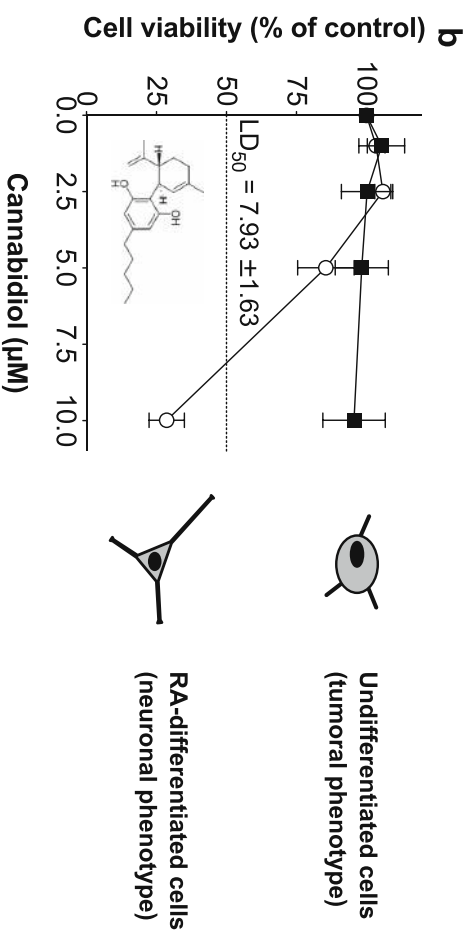
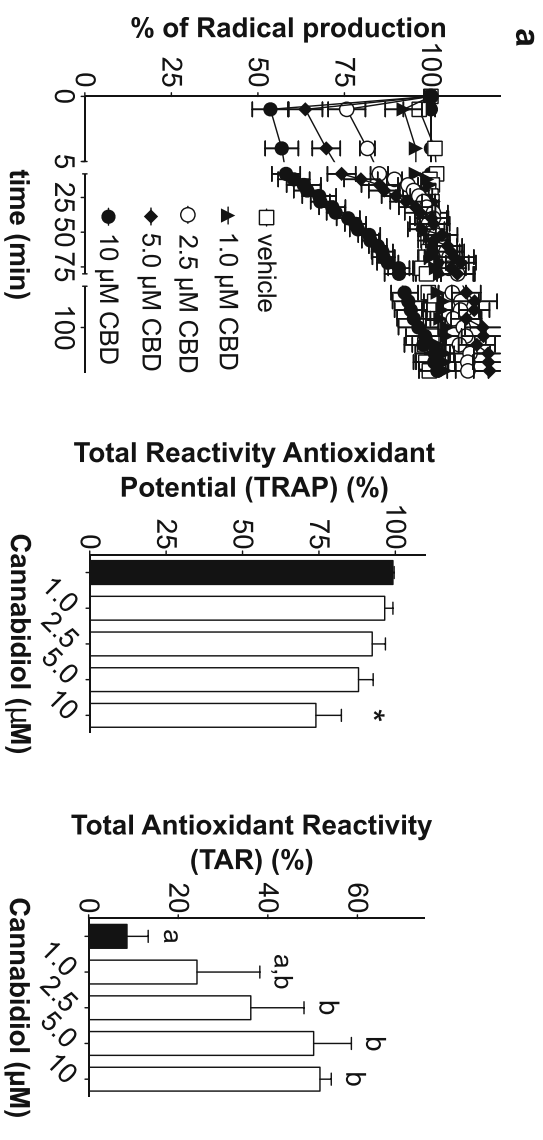


Patrícia Schönhofen



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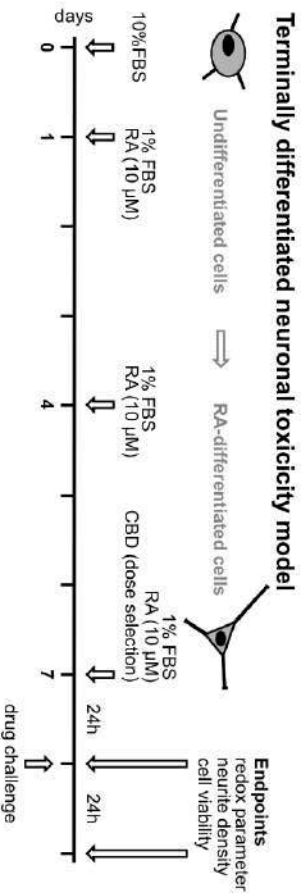
Patricia Schönhofen · Liana M. de Medeiros · Ivi Juliana Brito · Fernanda M. Lopes · Marco A. De Bastiani · Flavio Kapczinski · José Alexandre S. Crippa · Mauro Antônio A. Castro · Richard B. Parsons · Fábio Klant



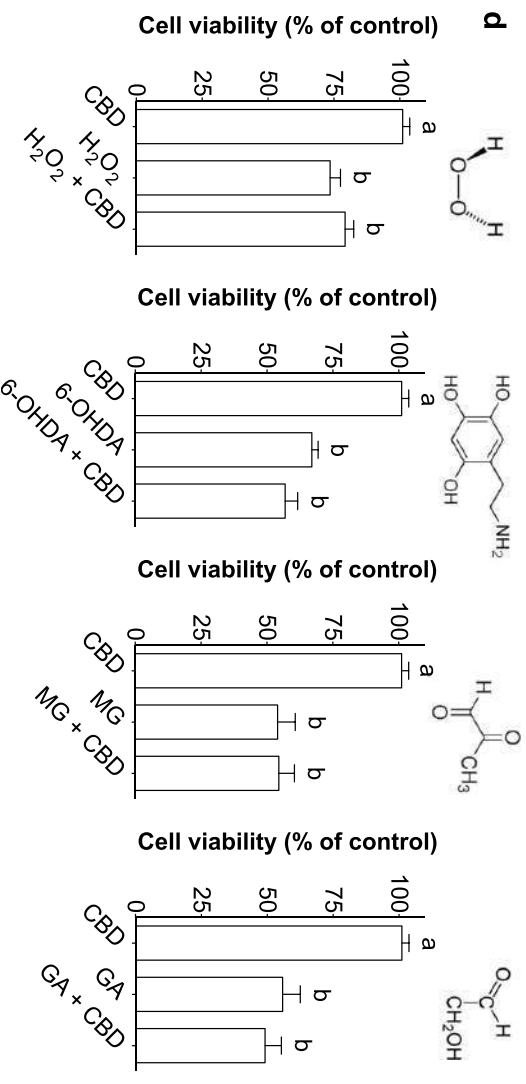
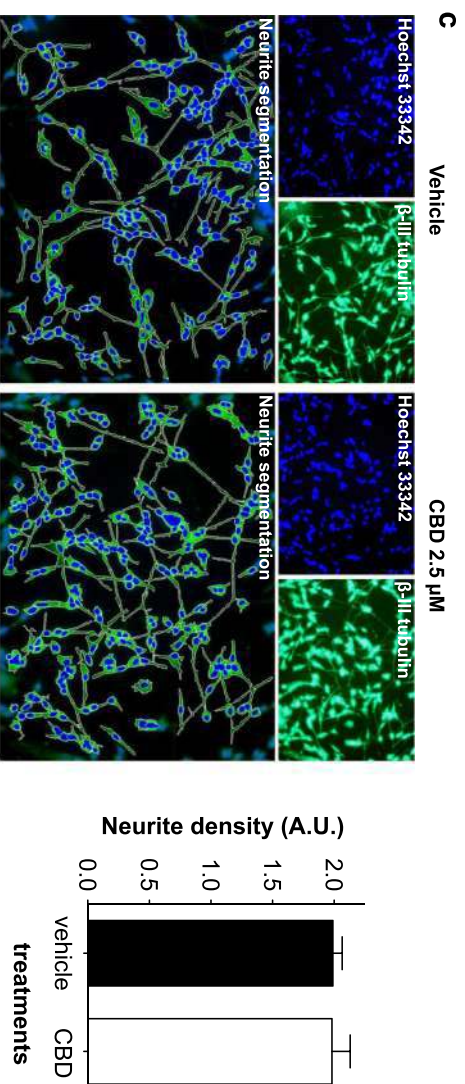
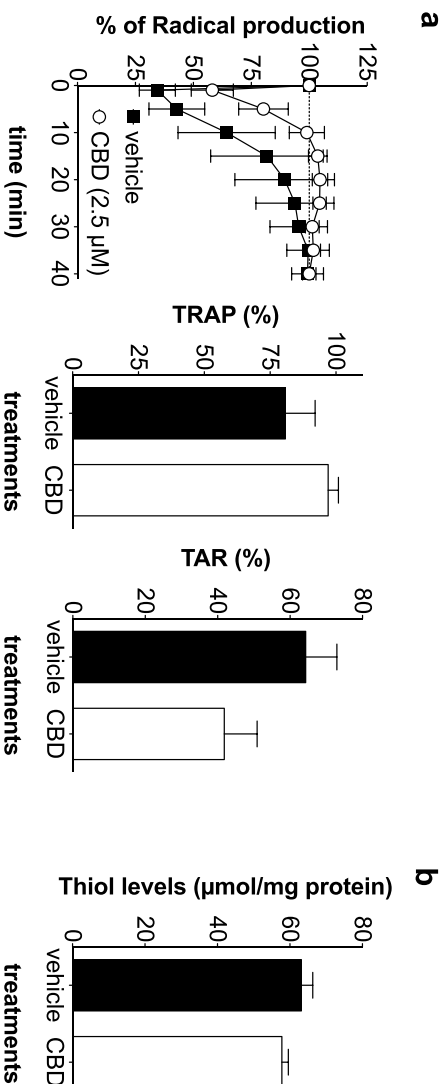
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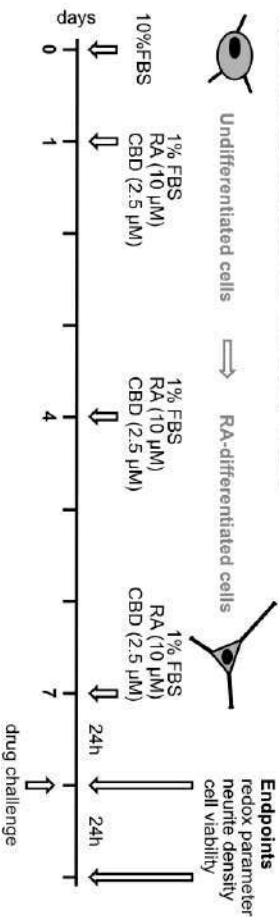
Patricia Schönhofen



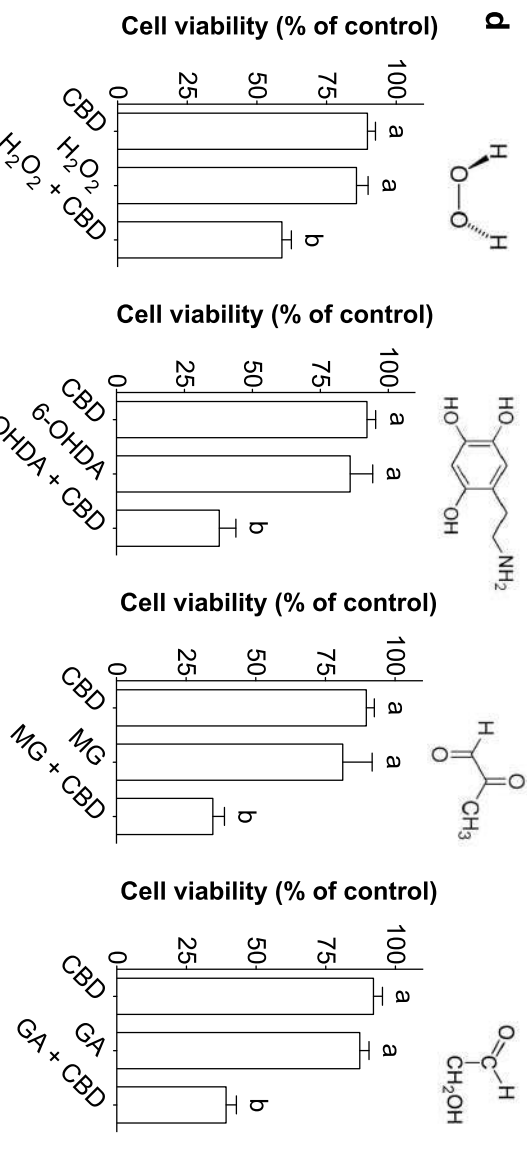
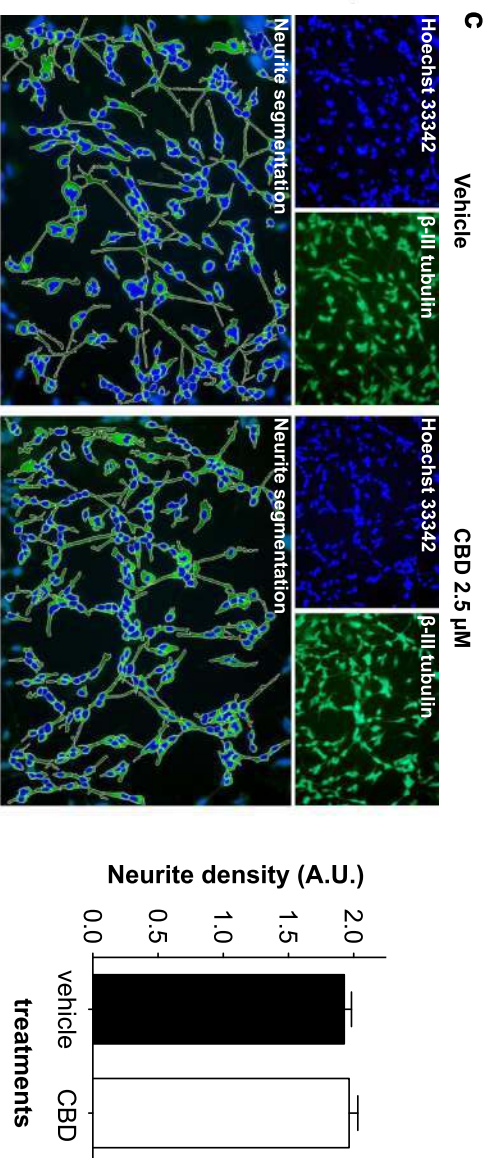
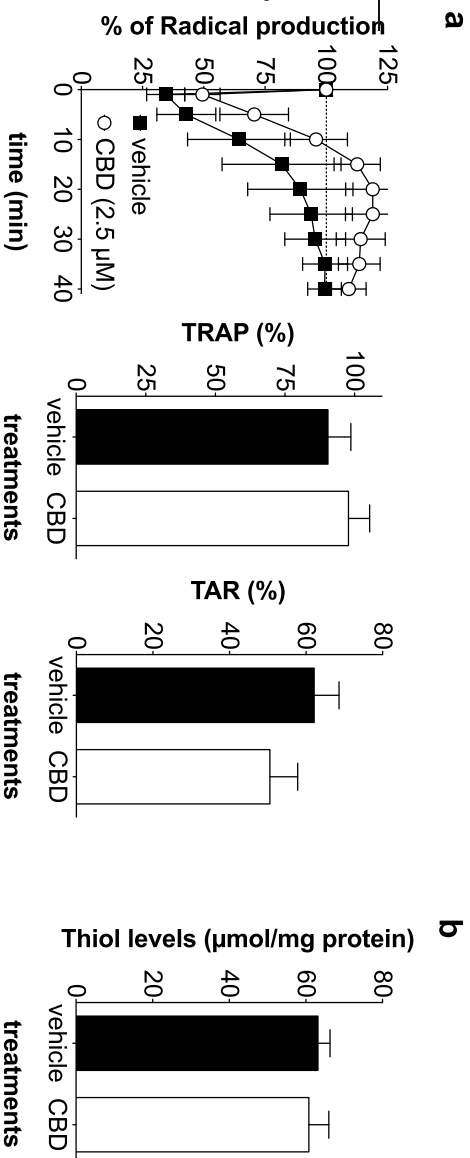
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Neuronal developmental toxicity model



Patricia Schönhofen



**Researching the potential
adverse effect of CBD
administration with iPSCs from
Dravet Syndrome (DS) patient**

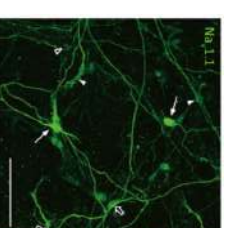
**iPS from DS (DRV1T-2):
5 years old male with nonsense mutation
c.1837 C>T on exon 11 of SCN1A gene
and mutation at splicing site IVS4-91 G>A**

**First mutation already reported to cause
severe myoclonic epilepsy and second
mutation cause febrile convulsions**

**INSTITUTO *Por* OR
PESQUISA E ENSINO**



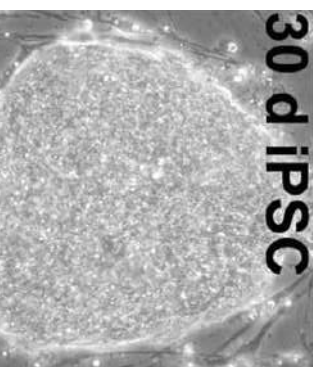
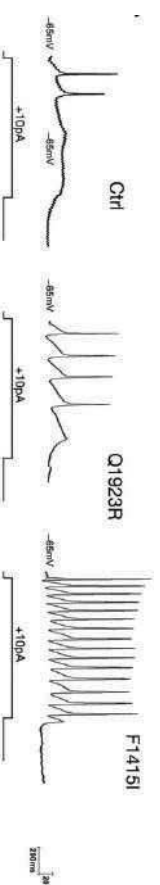
SHORT COMMUNICATION



Establishment of isogenic iPSCs from an individual with SCN1A mutation mosaicism as a model for investigating neurocognitive impairment in Dravet syndrome

Hiroshi Maeda¹, Tomohiro Chiyonobu¹, Michiko Yoshida^{1,2}, Satoshi Yamashita¹, Masashi Zaitki¹, Satoshi Kidowaki¹, Kenichi Isoda^{1,2}, Kazuhiro Yamakawa³, Masafumi Morimoto¹, Tatsutoshi Nakahata², Megumu K Saito² and Hajime Hosoi¹

Human Molecular Genetics, 2013, Vol. 22, No. 21 4241–4252
doi:10.1093/hmg/ddt275
Advance Access published on June 16, 2013



Modeling Dravet syndrome using induced pluripotent stem cells (iPSCs) and directly converted neurons

Jiao Jiao^{2,3,†}, Yanyuan Yang^{1,2,†}, Yiwu Shi⁴, Jiayu Chen², Rui Gao², Yong Fan¹, Hui Yao^{2,5}, Weiping Liao⁴, Xiao-Fang Sun^{1,*} and Shaorong Gao^{2,*}

ORIGINAL ARTICLE

Higurashi et al. *Molecular Brain* 2013, 6:19
<http://www.molecularbrain.com/content/6/1/19>



Molecular Brain

Dravet Syndrome Patient-Derived Neurons Suggest a Novel Epilepsy Mechanism

Yu Liu, MD, PhD,¹ Luis F. Lopez-Santiago, PhD,² Yukun Yuan, PhD,² Julie M. Jones, MS,³ Helen Zhang, MS,¹ Heather A. O'Malley, PhD,² Gustavo A. Patino, PhD,^{1,2,4} Janelle E. O'Brien, PhD,³ Raffaella Rusconi, PhD,² Ajay Gupta, MD,⁵ Robert C. Thompson, PhD,^{4,6} Marvin R. Natowicz, MD, PhD,^{5,7,8,9,10} Miriam H. Meisler, PhD,^{3,4} Lori L. Isom, PhD,^{2,4} and Jack M. Parent, MD^{1,4,11}

A human Dravet syndrome model from patient induced pluripotent stem cells

Norimichi Higurashi^{1,2,3}, Taku Uchida², Christoph Lossin⁴, Yoshio Misumi⁵, Yohei Okada^{6,7}, Wado Akamatsu⁶, Yoichi Imaizumi⁶, Bo Zhang⁸, Kazuki Nabeshima⁹, Masayuki X Mori¹⁰, Shutaro Katsurabayashi¹¹, Yukiyoshi Shirasaka¹², Hideyuki Okano^{6*} and Shinichi Hirose^{1,2*}

RESEARCH

Open Access



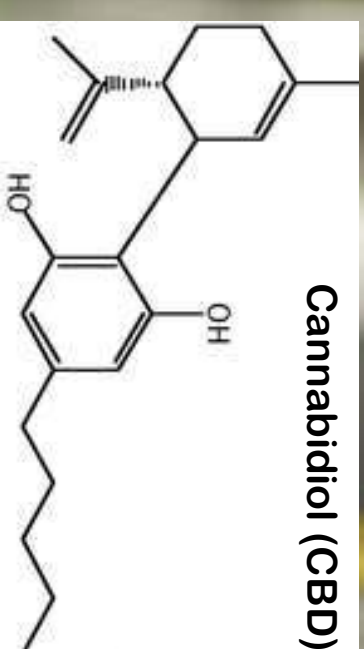
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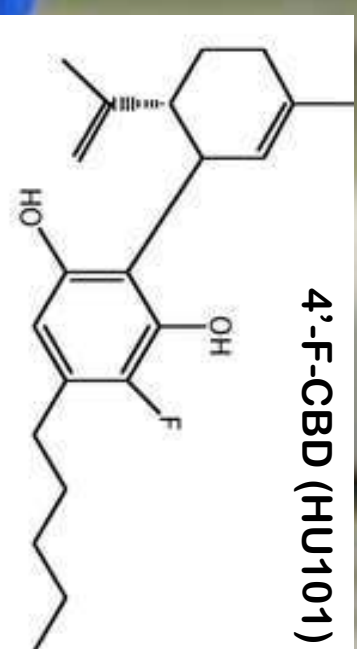
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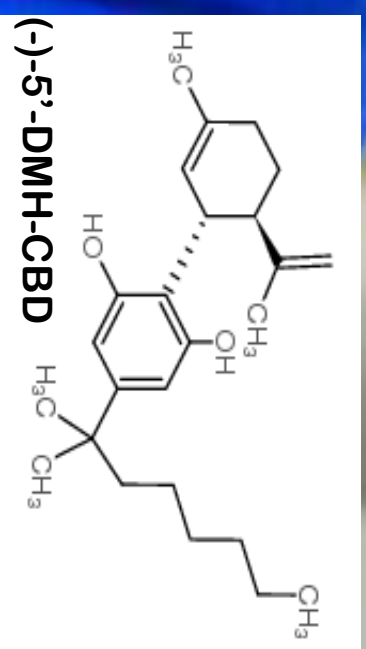
Dr. Raphael Mechoulam



Cannabidiol (CBD)

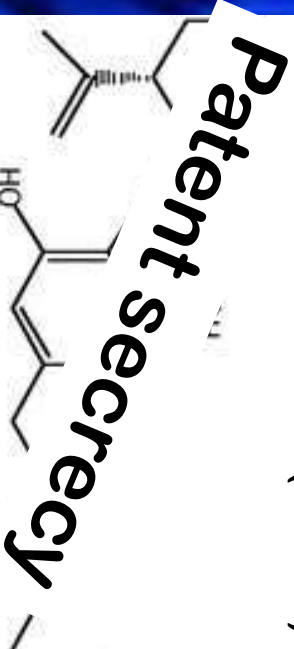


4'-F-CBD (HU101)



(-)-5'-DMH-CBD

CBD (HU556)

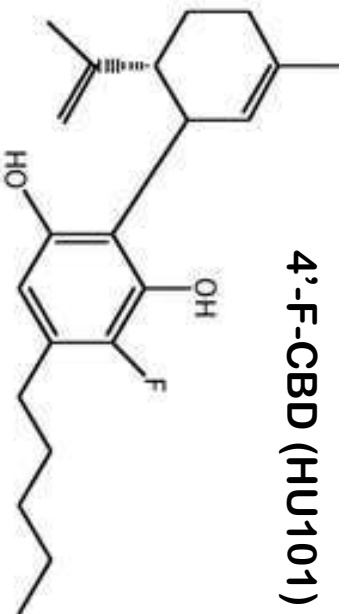


Patent *Secrecy*

RESEARCH ARTICLE

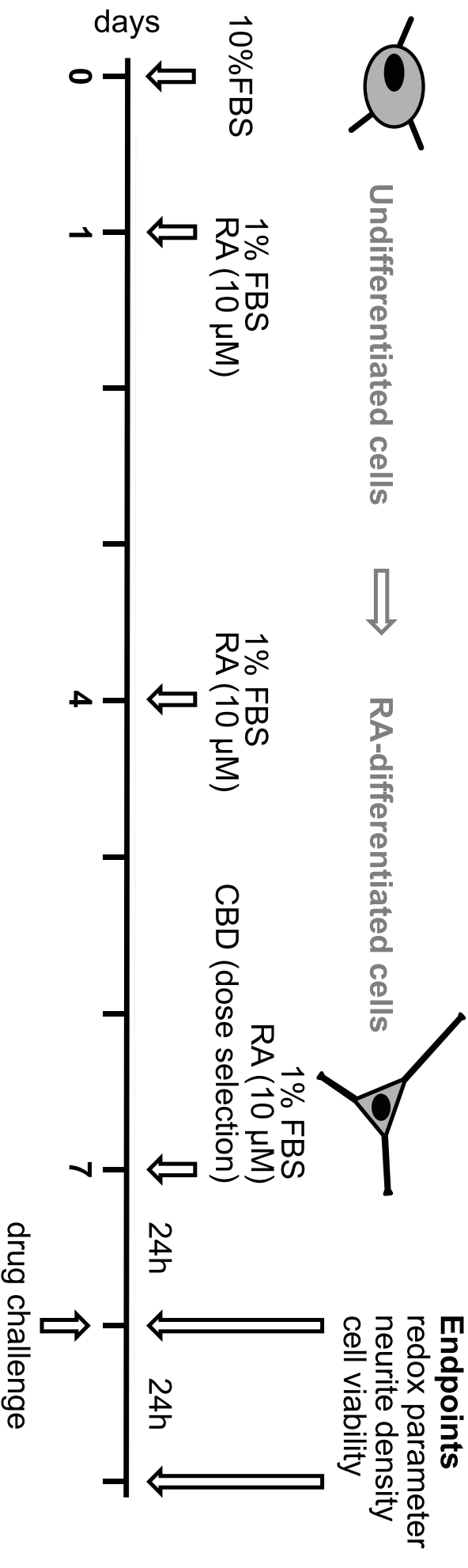
Fluorinated Cannabidiol Derivatives: Enhancement of Activity in Mice Models Predictive of Anxiolytic, Antidepressant and Antipsychotic Effects

4'-F-CBD (HU101)

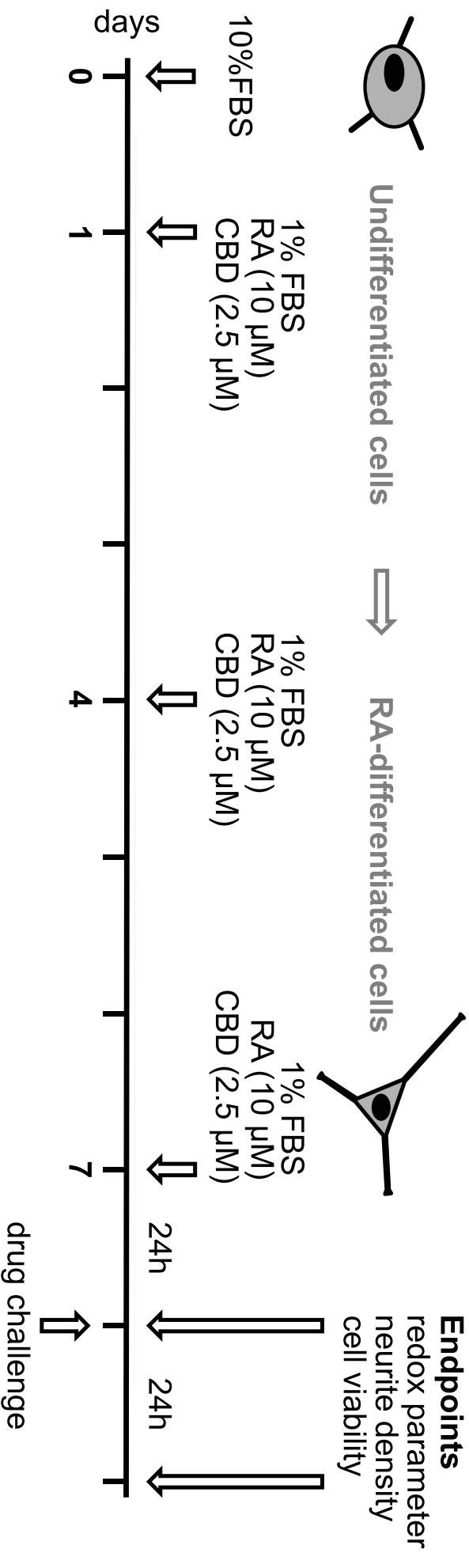


Aviva Breuer¹, Christeene G. Hai¹, Manoela V. Fogaça², Felipe V. Gomes², Nicole Rodrigues Silva², João Francisco Pedraza³, Elaine A. Del Bel⁴, Jaime C. Hallak³, José A.rippa³, Antonio W. Zuardi³, Raphael Mechoulam^{1*}, Francisco S. Guimarães^{2*}

Terminally differentiated neuronal toxicity model



Neuronal developmental toxicity model



Collaborators



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UFRJ
Dr. Marcelo Reis
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Dra. Sylvie Devalle
Gabriela Vitoria
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Prof. Geancarlo Zanatta
Physics Department
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CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico
Doenças Neurodegenerativas
(# 46698/2014-8)


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