REPORT
ISN SMALL CONFERENCES

2023 IUBMB-EMBO FOCUSED MEETING ON NEURONAL CYTOSKELETON

Name of the ISN supported event
IUBMB-EMBO Focused Meeting on Emerging Concepts of the Neuronal Cytoskeleton
https://neurocytoskeleton.com/2023/

Dates
March 26th-30th, 2023

Organizer committee
Dr. Carlos Wilson
CONICET
Centro de Investigación en Medicina Traslacional Severo R. Amuchástegui
Instituto Universitario de Ciencias Biomédicas de Córdoba
Córdoba, Argentina

Dr. Stephanie Gupton
North Carolina University at Chapell Hill
North Carolina, USA

Dr. Christophe Leterrier
Aix-Marseille University
Marseille, France

Dr. Christian González-Billault
University of Chile
Santiago, Chile

Venue
Hotel Santa Cruz Plaza
Plaza de Armas 286
Valle de Colchagua, VI Región, Chile

Registration and registration fees
The 2023 edition defined 4 fees:

- Trainees (undergraduate & PhD students, postdocs): USD 300
- Academics (young and senior PI): USD 500
- Industry : USD 800
- Speaker/attendee's guest: USD 400

Registration fees included full access to the meeting activities, accommodation, and meals (breakfast, lunch, dinner, and coffee breaks). Speakers and academics were allocated in single rooms, whilst trainees shared double rooms. Of note, all companions paid the fee with no exemption and shared the room with the speaker/attendee. All activities occurred in the conference rooms and facilities of the Hotel Santa Cruz Plaza. Every participant afforded the cost of air tickets by their own.

Since 2011, the spirit of this meeting has been to promote the participation and exchange between scientists around the globe, especially those from South America that usually need to afford expensive costs to participate in similar reunions in Europe or US. The business model of this meeting aimed to assemble a first-class program with an accessible cost for attendees. Accordingly, the registration fees partially cover the expenses per person; therefore, the participation of all attendees was subsidized by the funds obtained from sponsors, including ISN. For a detailed budget, please see section nº 11 of this report (General Budget).

Attendees registered

This year the meeting gathered 108 attendees from 18 different countries. In addition, we had 2 students working as staff supporting administrative tasks and 1 photographer fully dedicated to documenting the whole meeting. Finally, 6 speaker/attendee's guests joined us (none of them participated in the sessions, but meals and closing banquette). All companions paid the fee with no exemption.

For the complete list of attendees please see the file attached at the end of this pdf file (Attendees list)

Registration

1. Program

The 2023 edition had 30 speakers from different institutions and countries around the globe. They were distributed in 8 morning sessions from Monday 26th to Wednesday 29th (9:00-11:00; break; 11:30-13:00). Each session had 4 regular talks (20+5 min) and short talks (12+3 min) were selected from abstract submission (2 per session when applicable). Poster sessions were scheduled on Monday 27th (odd numbers) and Tuesday 28th (even numbers) from 17:00 to 20:00. Counting both poster sessions, 74 works were presented under this format.

For a detailed revision of the program, please see the meeting book attached at the end of this file.

2. Speakers

Surname	Name	Affiliation	Country
Tomas	Falzone	Universidad de Buenos Aires	AR
Sousa	Monica	IBMC/i3S University of Porto	PT
Tatyana	Svitkina	University of Pennsylvania	US
Gary	Brouhard	McGill University	CA
Kassandra	Ori-McKenney	University of California, Davis	US
Ginny	Farias	Utrecht University	NL
Nicolas	Unsain	Universidad Nacional de Córdoba	AR
Juan Salvador	Bonifacino	National Institutes of Health (NIH)	US

Katie	Baldwin	University of North Carolina at Chapel Heill	US
Frederic	Meunier	The University of Queensland	AU
Paul	Jenkins	University of Michigan Medical School	US
Lukas	Kapitein	Utrecht University	NL
Erika	Holzbaur	University of Pennsylvania	US
Rejji	Kuruvilla	Johns Hopkins University	US
Linda	Van Aelst	Cold Spring Harbor Laboratory	US
Kang	Shen	Stanford University	US
Pirta	Hotulainen	Minerva Foundation Institute for Medical Research	FI
Francisca	Bronfman	Universidad Andrés Bello	CL
Felipe	Opazo	University Medical Center Göttingen (UMG)	DE
Fernanda	Ledda	Fundación Instituto Leloir, IIBBA-CONICET	AR
Frank	Bradke	DZNE	DE
Helge	Ewers	Freie Universität Berlin	DE
Avital	Rodal	Brandeis Univeristy	US
Melissa	Rolls	Penn State	US
Shernaz	Bamji	University of British Columbia	CA
Subhojit	Roy	UC San Diego	US
Marina	Mikhaylova	Humboldt-Universität zu Berlin	DE
Matthew	Rasband	Baylor College of Medicine	US
Kristy	Welshhans	University of South Carolina	US
Thomas	Blanpied	University of Maryland School of Medicine	US

3. Plenary Speakers

Erika Holzbaur, PhD
Professor
Department of Physiology
Perelman School of Medicine, Penn State University
USA

Title of the talk: Organelle-specific mechanisms regulating the axonal dynamics of autophagosomes and mitochondria.

4. The highlight of the meeting

The neuronal cytoskeleton is integral to the form and function of the developing, mature, and aging neuron. The beautiful dynamic structures that comprise the neuronal cytoskeleton have fascinated neuroscientists and cell biologists for a century, and with new technology and capabilities, we are learning more at an astounding rate. This was the sixth version of this meeting, which has historically occurred in different cities of Chile, gathering scientists from around the world. The goal was to promote the interaction and exchange between leading scientists with the South American community of neurobiologists working in the field of the cytoskeleton, especially students, postdocs, and early-career researchers (ECR). Over the years, we noticed how important this meeting has been for those seeking positions and jobs, finding new horizons for their career development.

5. Participants

The 2023 editions counted with 118 attendees, including speakers, non-speakers attendees, speaker's guests and organizers. Participants came from 18 different countries: US (32%), Argentina (17%), Chile (13%), Germany (10%), France (4%), UK (4%), Portugal (3%), Canada (3%), The Netherlands (3%), Finland (3%), Taiwan (3%), Mexico (2%), Australia (1%), Italy (2%), India (2%), Korea (1%) Brasil (1%) and Uruguay (1%).
At this time, the meeting gathered 30 speakers, 33 academics (young and senior PIs) and 43 trainees (undergrads, PhD(c) and postdocs). Of note, 54% of attendees were women as well as 52% of speakers.
For a detailed list of participants, please see the list attached at the end of this file ("Atendees List").

6. Material distribution

At this time, the only material distributed was the program of the meeting (file attached). We did not receive ISN material to share with attendees.

7. Social events

The 2023 edition promoted socializing through activities inside the venue, including coffee breaks after talks and poster sessions. Free time after each lunch was considered (3:00-5:30 pm) to facilitate interactions between attendees, whose could use all the hotel facilities for this purpose (outdoor areas, swimming pool, business rooms, restaurant/bar). This time was especially valued by the attendees since a common critique to other events is the lack of enough free time between sessions to socialize with colleagues. The last day of the meeting (March 30th) included a closing banquette and farewell party at Hotel Santa Cruz Plaza.

8. Travel awards

In this version, we did not offer travel awards to any attendees. The reason for this decision was made on the budget and because of technical limitations to reimburse money via bank transfer or

cash. However, we used most of the funds to offer registration fee waivers (RFW) to all those attendees that requested them. For this purpose, the registration form of the website allowed each attendee to request a RFW independent of their nationality or financial status. This track for financial support was massively disseminated from October 2022 to December 2022 using different channels, including the website, email list and social media (e.g. Twitter).

9. Travel subsidies for Invited Speakers/Plenary Speakers

The registration fee for all speakers, including the plenary lecture, was entirely waived. However, all of them paid for their own air tickets with no exemption.

10. Sponsorship

The 2023 edition of the meeting had the sponsor of EMBO, IUBMB, ISN, IBRO and Nikon. Table 1 summarizes the fundraising from all sponsors. Of note, all of them transferred a part of the total before the meeting and the remaining funds will be transferred after the submission of the final report.

	€	**1 USD = 0.92€**	**1 USD = 780 CLP**
Sponsor	€	USD	CLP
EMBO	32.800 €	$35.652	$27.808.696
EMBO Waiver	4.500 €	$4.800	$3.744.000
EMBO F1	3.000 €	$3.200	$2.496.000
EMBO F2	900 €	$900	$702.000
IUBMB		$30.000	$23.400.000
ISN		$20.000	$15.600.000
IBRO		$8.480	$6.614.400
Nikon		$2.000	$1.560.000
TOTAL		$105.032	$81.925.096

Table 1. Total funding from sponsors. USD/€ to CLP conversion depends on the currency, so this is an estimated value.

In addition, this year we fundraised USD 13,221 from registrations. Therefore, total incomes are shown in table 2.

	€	**1 USD = 0.92€**	**1 USD = 780 CLP**
Sponsor	€	USD	CLP
EMBO	32.800 €	$35.652	$27.808.696
EMBO Waiver	4.500 €	$4.800	$3.744.000
EMBO F1	3.000 €	$3.200	$2.496.000
EMBO F2	900 €	$900	$702.000
IUBMB		$30.000	$23.400.000
ISN		$20.000	$15.600.000
IBRO		$8.480	$6.614.400
Nikon		$2.000	$1.560.000
Registration		$14.520	$11.325.600
TOTAL		$119.552	$93.250.696

Table 2. Total income from sponsors and registration fees. USD/€ to CLP conversion depends on the currency; accordingly, this is an estimated value.

11. General budget; detailed ISN budget, how the ISN funds were utilized

A) General budget

General budget Neurocyto 2023 Chile		
	CLP	USD
Rooms	$ 35.746.000	$ 45.828
Meals	$ 26.247.210	$ 33.650
Conference/poster rooms	$ 4.400.000	$ 5.641
Audio & Video	$ 1.756.000	$ 2.251
Transport Airport - Venue	$ 2.916.000	$ 3.738
Closing Banquet & socializin	$ 4.936.600	$ 6.329
Subtotal	$ 76.001.810	$ 97.438
VAT	$ 14.440.344	$ 18.513
TOTAL	$ 90.442.154	$ 115.951
Additionals		
Poster board rental	$2.000.000	$2.564
4iD (website & registration)	$1.013.220	$1.299
GRAND TOTAL	**$ 93.455.374**	**$ 119.815**

Table 3. Total expenses by category. Values represent the total cost for 5 days and 4 nights. 1 USD = 780 CLP.

B) ISN detailed budget

These funds were mostly allocated in supporting the participation of trainees (students and postdocs) and young PIs. As previously mentioned, the business model of this meeting aims to support the participation of all attendees independent of their financial status.

Thus, the registration fees (RF) are estimated on a base of solidarity, which means that the real cost per attendee is higher than the RF. Accordingly, every attendee received a subside to assist. In the general budget section of this report (nº 11), the grand total cost of this reunion was USD 119,815. Considering 117 attendees, the real cost per person was USD 1,024. As previously stated, the registration fees for trainees and academics were USD 300 and 500, respectively. Therefore, we used different tracks of funding (sponsors) to pay the cost by attendee.

Table 4 shows how ISN funds were allocated. Following ISN guidelines, we use more than 60% of funds to support all trainees registered for the event, representing a subtotal of USD 13,200. Remaining USD 6,800 were used to pay the cost of several services related to the meeting, such as the transport from the airport to the venue (and return) and poster board rental.

At this point we have received USD 16,000 from ISN, corresponding to the 80% of the USD 20,000 total funding. We expect to receive 20% of the remaining funds after this report to afford pending payments with the venue and services related.

Detailed ISN budget

ISN detailed budget					
A) Financial support to attendees					
Surname	**Name**	**Affiliation**	**Country**	**Category**	**Financial support (USD)**
Shivani	Bodas	IISER-Pune	IN	Trainee	300
Tsung-Yu (Chris)	Ho	UNC-Chapel Hill	TW	Trainee	300
Eissa	Alfadil	German Centre for Neurodegenerative Diseases (DZNE)	DE	Trainee	300
Agustina	Zorgniotti	Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET	AR	Trainee	300
Nahir Guadalupe	Gazal	Instituto Ferreyra (INIMEC-CONICET-UNC)	AR	Trainee	300
Nicolás Gabriel	Stuardo Castillo	Pontificia Universidad Católica De Chile	CL	Trainee	300
Cayetana	Arnaiz	IBioBA-CONICET-MPSP	PE	Trainee	300
Florencia Lucia	Merino	Biomedical Center	DE	Trainee	300
Omar Benjamin	Rivera Maya	CINVESTAV IPN	MX	Trainee	300
Laura	McCormick	UNC Chapel Hill	US	Trainee	300
Kourtney	Kroll	University of Chicago	US	Trainee	300
Joseph	Tidei	Loyola University Chicago	US	Trainee	300
Sara	Sousa	University of Porto	PT	Trainee	300
Ana Catarina	Costa	i3S	PT	Trainee	300
Juan Manuel	Bourbotte Asensio	Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC - CONICET- UNC)	AR	Trainee	300
Laura	Pulido	Purdue University	US	Trainee	300
Allison	Melton	Baylor College of Medicine	US	Trainee	300
Edoardo	Moretto	CNR	IT	Trainee	300
Julia	Bär	Humboldt University Berlin	DE	Trainee	300
Yannes	Popp	RG Optobiology, Humboldt-University Berlin	DE	Trainee	300
Carolina	Flores-Muñoz	Universidad De Valparaiso	CL	Trainee	300
Guillermina	Bruno	Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC - CONICET- UNC	AR	Trainee	300
Fabian Orlando	Ramos	INIMEC - CONICET	AR	Trainee	300
Matthew	Davies	University of Münster	DE	Trainee	300
ian	hertzler	Pennsylvania State University	US	Trainee	300
Heidi	Ulrichs	Emory University	US	Trainee	300
CLARA INÉS	CHUNGARA	Instituto de Investigación Médica Mercedes y Martín Ferreyra	AR	Trainee	300
Ernesto	Muñoz Palma	Universidad de Chile	CL	Trainee	300
Diego	Acuña	Universidad de Chile	CL	Trainee	300
Ramona	Stringhi	Università degli Studi di Milano	IT	Trainee	300
Cristopher	Villablanca	Centro de Gerociencia, Salud Mental y Metabolismo, GERO	CL	Trainee	300
Pushpa	Khanal	University of Helsinki/Minerva Foundation Institute for Medical Research	FI	Trainee	300
David	micinski	University of Helsinki	FI	Trainee	300
Milagros	Ovejero	Instituto Ferreyra (INIMEC-CONICET-UNC)	AR	Trainee	300
Erin	Fingleton	National Institutes of Health	US	Trainee	300
Adéla	Karhanová	BIOCEV	CZ	Trainee	300
Bas	van Bommel	Freie Universität Berlin	DE	Trainee	300
Sebastián	Arce Pinochet	Universidad de Chile	CL	Trainee	300
Lorena Paola	Neila	Mercedes and Martin Ferreyra Medical Research Institute	AR	Trainee	300
Camila	Gudenschwager Ruiz	Universidad de Chile	CL	Trainee	300
Kelsie	Eichel	Stanford University	US	Trainee	300
Oscar Marcelo	Lazo	University College London	GB	Trainee	300
Malina	Iwanski	Utrecht University	NL	Trainee	300
Yuli Thamires	Magalhães	University of Sao Paulo, Institute of Chemistry	BR	Trainee	300
Gaby Fabiana	Martínez	Instituto de Investigaciones Biológicas Clemente Estable / Ministerio de Educación y Cultura	UY	Young PI	500
				Subtotal	**13700**
B) Other expenses					**USD**
Poster board rental					2564
Transport from the airport to the venue					1869
Transport from the venue to the airport					1869
				Subtotal	**6302**
				GRAND TOTAL	**20002**

Table 4. Allocation of ISN funds.

12. List of participants

Please see the list of all attendees attached at the end of this file.

13. Confirmation of the used of the provided ISN slides to inform the audience on ISN and on the benefits of an ISN membership.

We did not receive ISN slides for promotion. However, we acknowledged all sponsors, including ISN, using personal slides at different times of the meeting, such as during the opening of the event (before introducing Dr. Erika Holzbaur, keynote lecture) and the business meeting scheduled on the March 30th, 2023 (please, see the program attached). The benefits of being an ISN member were advised at these times. Additionally, the ISN logo was visible in all official communications that we established with the attendees, such as the website, website registration platform, meeting book and badges.

14. Confirmation of the posting of information on the ISN supported event on the ISN Social Media channels before the event took place.

The ISN CC did not request or contact the organizing committee to post news in the ISN official social media accounts.

15. Confirmation of the posting of some concluding statements with pictures on the success of the ISN supported event on the ISN Social Media channels after the event took place.

Please click on the link to the photos in the next section (16. Photos). ISN can use them to promote the event on social media.

16. Photos

https://photos.app.goo.gl/GJ6manEAsGg5cNHY9

17. Comments of at least three attendants about the Meeting

Allison Melton, PhD student (Mathew Rasband Lab, Baylor College of Medicine, US)

"This 2023 IUBMB-EMBO Focused Meeting on Emerging Concepts of the Neuronal Cytoskeleton was a phenomenal experience for me as a trainee. The talks covered a range of exciting research on the neuronal cytoskeleton which reinforced my enthusiasm for the field and exposed me to fascinating topics that I was previously unaware of. Thanks to these talks and the feedback I received on my poster, I left Chile inspired and with lots of ideas for my own research. The meeting was also a fantastic networking opportunity. I was happy to have the chance to meet many PIs and make international friendships with other students. The meeting was wonderful all around, and I hope to be back in 2025!"

Dr. Dhanya Cheerambathur, PI (Wellcome Centre for Cell Biology, The University of Edinburgh, Scotland, UK)

"As an early-stage independent researcher I started my research group only a few years ago, I am a relative newcomer to the neuron cytoskeleton field. Nonetheless, I can confidently say that the IUMB-EMBO Neurocytoskeleton workshop was one of the best conferences that I have ever attended. The meeting was truly enriching and informative, and provided me with new ideas, insights and perspectives that will be invaluable for my lab's research. The talks were insightful and thought provoking, covering a broad range of topics. It was refreshing to see so much unpublished work presented which highlights the uniqueness of this workshop encouraging people to express their ideas and exchange information freely, without reservations. This is a testament to the organizer's vision and commitment of fostering an open and collaborative

scientific workshop. Initially, I was apprehensive about attending the workshop as a relative stranger to the field. However, I found the organizers and attendees to be extremely welcoming and engaged in valuable discussions and, which immediately made me feel at home. Overall, I cannot speak highly enough of this conference and would recommend to anyone who is getting involved in the fascinating scientific area of neuronal cytoskeleton."

Pushpa Khanal, PhD student (Pirta Hotulainen Lab, University of Helsinki, Finland)
"I am a final year PhD student at the University of Helsinki, Finland. For me, attending the neuronal cytoskeleton meeting in Chile during March 26th -30 th 2023 was a highly rewarding experience. It was my first visit to South America and the quality of scientific program, venue, food, and the people around here made it very special and memorable. I think it was very thoughtfully organized meeting in terms of the program and the number of attendants. The best part of the conference was the opportunity to meet this special group of scientist and researchers with similar research interests as mine. It felt that I as well as my research work connect well with this group. In addition, it was great to meet in person the scientists whose work I have followed during my PhD studies and getting feedback from them for my own work. Since all the attendees were staying at the same place, we had plenty of time to connect and share our professional as well as personal interests during mealtimes and breaks throughout the whole conference. I think the experiences I have had and the connections I built during this meeting will be very important for my future career."

Guadalupe Gazal, PhD student (Nicolas Unsain Lab, Instituto Ferreyra, CONICET, Universidad Nacional de Córdoba, Argentina).
"As a PhD student, attending the IUBMB-EMBO Focused Meeting on Emerging Concepts of the Neuronal Cytoskeleton (6th Edition) was an amazing experience. It was my first time in such an intense and focused meeting and was everything and more that I was expecting. The lectures were divided into sessions with different topics: all of them were astonishing. There were many great scientists, the ones that are classical references for all of us in neurons and cytoskeleton, and the new ones, the young scientists that bring such inspiring views over science, neuronal system and cytoskeleton. My favourite part of the meeting was the Poster Session: I could connect with so many PhD students, postdocs and PI's that were there. I could not be more grateful for the people that came by the day of exposing my poster and my work. I had the chance to discuss results with the very same scientists that I'm used to reading and that inspire my research with. Also this exchange helped me in so many ways: from advice to recommendations, also collaborations. I felt I was having the time of my life surrounded by such great people. I am widely thankful with the organizers for the effort and the trust of choosing my abstract for a poster presentation and for the registration waiver for attendance to the meeting."

18. The budget used for each speaker or awarded person must be specified separately.
Please see the table 4 of section nº 11-B.

EMBO-IUBMB Focused meeting on Emerging Concepts of the Neuronal Cytoskeleton 6th edition

Attendees list

ORGANIZERS			
Surname	**Name**	**Affiliation**	**Country**
Carlos	Wilson	CONICET - Instituto Universitario de Ciencias Biomedicas de Cordoba	AR
Stephanie	Gupton	University of North Carolina at Chapel Hill	US
Christophe	Leterrier	Aix Marseille University	FR
Christian	Gonzalez-Billault	Universidad de Chile	CL
Speakers			
Surname	**Name**	**Affiliation**	**Country**
Tomas	Falzone	Universidad de Buenos Aires	AR
Sousa	Monica	IBMC/i3S University of Porto	PT
Tatyana	Svitkina	University of Pennsylvania	US
Gary	Brouhard	McGill University	CA
Kassandra	Ori-McKenney	University of California, Davis	US
Ginny	Farias	Utrecht University	NL
Nicolas	Unsain	Universidad Nacional de Córdoba	AR
Juan Salvador	Bonifacino	National Institutes of Health (NIH)	US
Katie	Baldwin	University of North Carolina at Chapel Heill	US
Frederic	Meunier	The University of Queensland	AU
Paul	Jenkins	University of Michigan Medical School	US
Lukas	Kapitein	Utrecht University	NL
Erika	Holzbaur	University of Pennsylvania	US
Rejji	Kuruvilla	Johns Hopkins University	US
Linda	Van Aelst	Cold Spring Harbor Laboratory	US
Kang	Shen	Stanford University	US
Pirta	Hotulainen	Minerva Foundation Institute for Medical Research	FI
Francisca	Bronfman	Universidad Andrés Bello	CL
Felipe	Opazo	University Medical Center Göttingen (UMG)	DE
Fernanda	Ledda	Fundación Instituto Leloir, IIBBA-CONICET	AR
Frank	Bradke	Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE)	DE
Helge	Ewers	Freie Universität Berlin	DE
Avital	Rodal	Brandeis Univeristy	US
Melissa	Rolls	Penn State	US
Shernaz	Bamji	University of British Columbia	CA

Subhojit	Roy	UC San Diego	US
Marina	Mikhaylova	Humboldt-Universität zu Berlin	DE
Matthew	Rasband	Baylor College of Medicine	
Kristy	Welshhans	University of South Carolina	US
Thomas	Blanpied	University of Maryland School of Medicine	US
ATTENDEES - ACADEMICS			
Surname	**Name**	**Affiliation**	**Country**
Anahi	Bignante	INIMEC-CONICET-UNC	AR
Pierre	McCrea	University of Texas MD Anderson Cancer Center	US
Mark	Dodding	University of Bristol	GB
Victoria	Rozés Salvador	CIBICI-CONICET	AR
Naoko	Kogata	Francis Crick Institute	GB
Yongcheol	Cho	DGIST	KR
Dhanya	Cheerambathur	University of Edinburgh	GB
shaul	yogev	Yale	US
Jen-Hsuan	Wei	Academia Sinica	TW
Subashchandrabose	CHINNATHAMBI	CSIR - National Chemical Laboratory	IN
Daniel	Suter	Purdue University	UM
Daniel	Hernández Baltazar	Universidad Veracruzana	MX
Ana Lis	Moyano	Instituto Universitario Ciencias Biomédicas de Córdoba (IUCBC)	AR
Cecilia	Conde	INIMEC	AR
Malina	Iwanski	Utrecht University	NL
Pei-Lin	Cheng	Institute of Molecular Biology	TW
Mariano	Bisbal	Intituto Ferreyra (INIMEC-CONICET-UNC)	AR
Gaby Fabiana	Martínez	Instituto de Investigaciones Biológicas Clemente Estable / Ministerio de Educación y Cultura	UY
Leticia	Peris	Grenoble Institut of Neurosciences	FR
Marie-Jo	Moutin	Université Grenoble Alpes	FR
James	Zheng	Emory University	US
Michael	Way	Francis Crick Institute	GB
Yuli Thamires	Magalhães	University of Sao Paulo, Institute of Chemistry	BR
Agustín	Anastasía	Instituto Ferreyra (INIMEC-CONICET-UNC)	AR
Cecilia	Alvarez	Universidad Nacional de Córdoba	AR
Shaun	Sanders	University of Guelph	CA
Shrobona	Guha	Drexel University College of Medicine	US
Marcus	Braun	Institute of Biotechnology, Czech Academy of Sciences	DE

Le	Ma	Thomas Jefferson University	US
Karin	Dumstrei	EMBO	DE
Maria-Paz	Marzolo	Pontificia Universidad Católica de Chile, Fac Ciencias Biologicas	CL
Babette	Fuss	Virginia Commonwealth University	US
Anna	Brachet	CNRS-Université de Bordeaux UMR5297	FR
ATTENDEES - TRAINEES			
Surname	**Name**	**Affiliation**	**Country**
Shivani	Bodas	IISER-Pune	IN
Tsung-Yu (Chris)	Ho	UNC-Chapel Hill	TW
Eissa	Alfadil	German Centre for Neurodegenerative Diseases (DZNE)	DE
Agustina	Zorgniotti	Centro de Investigaciones en Química Biológica de Córdoba (CIQUIBIC), CONICET	AR
Nahir Guadalupe	Gazal	Instituto Ferreyra (INIMEC-CONICET-UNC)	AR
Nicolás Gabriel	Stuardo Castillo	Pontificia Universidad Católica De Chile	CL
Cayetana	Arnaiz	IBioBA-CONICET-MPSP	PE
Florencia Lucia	Merino	Biomedical Center	DE
Omar Benjamin	Rivera Maya	CINVESTAV IPN	MX
Laura	McCormick	UNC Chapel Hill	US
Kourtney	Kroll	University of Chicago	US
Joseph	Tidei	Loyola University Chicago	US
Sara	Sousa	University of Porto	PT
Ana Catarina	Costa	i3S	PT
Juan Manuel	Bourbotte Asensio	Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC - CONICET- UNC)	AR
Laura	Pulido	Purdue University	US
Allison	Melton	Baylor College of Medicine	US
Edoardo	Moretto	CNR	IT
Julia	Bär	Humboldt University Berlin	DE
Yannes	Popp	RG Optobiology, Humboldt-University Berlin	DE
Carolina	Flores-Muñoz	Universidad De Valparaiso	CL
Guillermina	Bruno	Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC - CONICET- UNC	AR
Fabian Orlando	Ramos	INIMEC - CONICET	AR
Matthew	Davies	University of Münster	DE

ian	hertzler	Pennsylvania State University	US
Heidi	Ulrichs	Emory University	US
CLARA INÉS	CHUNGARA	Instituto de Investigación Médica Mercedes y Martín Ferreyra	AR
Ernesto	Muñoz Palma	Universidad de Chile	CL
Diego	Acuña	Universidad de Chile	CL
Ramona	Stringhi	Università degli Studi di Milano	IT
Cristopher	Villablanca	Centro de Gerociencia, Salud Mental y Metabolismo, GERO	CL
Pushpa	Khanal	University of Helsinki/Minerva Foundation Institute for Medical Research	FI
David	micinski	University of Helsinki	FI
Milagros	Ovejero	Instituto Ferreyra (INIMEC-CONICET-UNC)	AR
Erin	Fingleton	National Institutes of Health	US
Adéla	Karhanová	BIOCEV	CZ
Bas	van Bommel	Freie Universität Berlin	DE
Sebastián	Arce Pinochet	Universidad de Chile	CL
Lorena Paola	Neila	Mercedes and Martin Ferreyra Medical Research Institute	AR
Camila	Gudenschwager Ruiz	Universidad de Chile	CL
Kelsie	Eichel	Stanford University	US
Oscar Marcelo	Lazo	University College London	GB
SPEAKERS/ATTENDEES COMPANIONS			
Surname	**Name**	**Affiliation**	**Country**
Madhusree	Singh		US
Candace	Clements		US
Tom	Phan	Virginia Commonwealth University	US
David	Howland	CHDI	US
Lennart	Dreyer		DE
Evan	Blanpied		US
STAFF			
Surname	**Name**	**Affiliation**	**Country**
Jara	Michel	Universidad de Chile	CL
Chavez	Isadora	Universidad de Chile	CL
Rodrigo	Carvajal	Photograph	CL

2023 IUBMB EMBO Workshop
Emerging Concepts of the Neuronal Cytoskeleton

March 26-30th, 2023
Hotel Santa Cruz, Plaza de Armas 286
Santa Cruz, Chile,

Organizing committee
Stephanie Gupton (University of North Carolina, USA)
Christophe Leterrier (Aix-Marseille Université, France)
Carlos Wilson (Córdoba, Argentina)
Christian González-Billault (Universidad de Chile, Chile)

Program at a glance

Sunday March 26:

Registration 12:00 PM-4:00 PM Lunch Buffet 1:30 -3:00 PM

Hotel Check-in/Free Time 3:00–5:00 pm

5:00 pm: Opening Remarks

IUBMB: Alexandra Newton

Session 1 Neuronal Polarity, 5:15-6:55 Chair: Subhojit Roy

1. Kang Shen: How neuronal microtubule arrays are polarized and maintained in C. elegans neurons
2. Gary Brouhard: Doublecortin contributes to neuronal migration through suppression of neurite branching and modification of the tubulin code
3. Frank Bradke: Cytoskeletal Mechanisms of Axon Growth and Regeneration
4. Lukas Kapitein: Unraveling the organization, dynamics and structure of stable microtubules= Break

Keynote Lecture, 7:10-8:10 pm Chair: Stephanie Gupton

5. Erika Holzbaur: Organelle-specific mechanisms regulating the axonal dynamics of autophagosomes and mitochondria

Monday March 27

Breakfast Buffet 7:30-9:00 AM

Session 2: Trafficking 9:00- 11:00AM Chair: Christian Gonzalez Billault

6. Subhojit Roy: Biogenesis and Trafficking of Endocytic and Cytoskeletal proteins
7. Francisca Bronfman: Contribution of dynein-dependent transport of BDNF signaling endosomes to neuronal plasticity
8. Ginny Farias: Interactions between the ER and the cytoskeleton in neuronal polarity
9. Tomas Falzone: Human neuronal models to identify cell-intrinsic modulators of axonal polarization and transport
10. Le Ma: Regulation of Axonal Transport in Nerve Branches=

Coffee Break 11:00-11:30

Session 3: Synapses 11:30 AM-1:30 Chair: Carlos Willson

11. Avital Rodal: Dynamics and regulation of the presynaptic actin cytoskeleton by periactive zone machinery
12. Tom Blanpied: Conserved and variant aspects of synaptic nanodomain architecture
13. Frederic Meunier: Tau nanoscale biomolecular condensates at the synapse
14. Guha Shrobona: Investigating the role of microtubule-associated motor protein KIFC1 at the synapse.
15. Matthew Davies: Spectraplakin Couples Microtubule Orientation to Actin During Dendritic Pruning in Drosophila
16. Shaul Yogev: Spectrin restricts actomyosin contractility to maintain the continuity of the axonal microtubule array

Lunch 1:30-2:45

Free time 2:30- 5:30 PM

Coffee 5:00-5:30 PM

Poster Session 1 5:30-8:15 Odd Numbered Posters

Dinner Buffet 8:15 PM

Tuesday March 28

Breakfast Buffet 7:30-9:00 AM

Session 4: Signaling, local translation 9:00-11:00am Chair: Rejji Kuruvilla

17. Linda van Aelst: Shedding light on chandelier cell development and connectivity
18. Fernanda Ledda: Ligand-induced trans-synaptic adhesion in hippocampal plasticity and connectivity
19. Kristy Welshhans: Local translation of β-actin mRNA at adhesions and its role in axon guidance
20. Shaun Sanders: Regulation of fast axonal transport in neurons by protein palmitoylation
21. Laura McCormick: The E3 ubiquitin ligase TRIM9 regulates actin dynamics and synapse formation
22. Mariano Bisbal: Distinct Rho GTPase effectors inhibit axon outgrowth and promote axon elongation

Coffee Break 11:00-11:30 AM

Session 5: Axons, 11:30 am-1:15 pm Chair, Erika Holzbaur

23. Felipe Opazo: Tailored probes for the study of molecular neurophysiology
24. Paul Jenkins: Life beyond the AIS: ankyrin-dependent scaffolding of Nav1.2 in neocortical dendrites
25. Mathew Rasband: Neuronal ankyrin and spectrin cytoskeletons
26. Helge Ewers: Actin rings as ubiquitous diffusion barriers in the neuronal lineage.

Lunch 1:15-2:30
Free time 2:30- 5:30 PM

Coffee: 5:00-5:30

Poster Session 2 5:30-8:15 Even numbered Posters

Dinner Buffet 8:15 PM

Wednesday March 29

Breakfast Buffet 7:30-9:00 AM

Session 6: Dendrites, 9:00 - 11:00 am Chair: Fernanda Ledda

28. Rejji Kuruvilla: Transcytosis-mediated anterograde transport of TrkA receptors is necessary for sympathetic neuron development and function
29. Shernaz Bamji: Regulation of synapse plasticity by palmitoylating enzymes
30. Melissa Rolls: Mechanisms that maintain microtubule polarity and dynamics in dendrites
31. Kassandra Ori-McKenney: Investigating the mechanism of tau pathoconversion using an injury model.
32. Dhanya Cheerambathur: Repurposing the Chromosome-Microtubule Coupling Machinery as a “Tuner” of Actin for Dendritic Branching.=

Coffee Break 11:00-11:30 AM

Session 7: New Actin Structures 11:30 am-1:30 pm Chair:Christophe Leterrier

33. Pirta Hotulainen: From “invisible” to visible actin structures
34. Monica Sousa: Tension-driven axon growth triggers developmental stage-specific adaptations of the axonal cytoskeleton and membrane
35. Nicolas Unsain: Studying the organization of □II-spectrin within the actin/spectrin membrane-associated periodic skeleton of axons in vivo with nanometer resolution.
36. Marina Mikhaylova:Synaptic control of organelle localization: focus on the ER
37. Bodas Shivani: Mechanisms underlying the development of the axonal actin-spectrin Membrane Periodic Skeleton (MPS)

Lunch 1:30-2:45

Free time 2:30- 5:30 PM

Coffee 5:00-5:30

Session 8: Non-Neuronal cells 5:30-7:30 pm Chair: Stephanie Gupton

38. Daniel Suter: Neuronal NADPH oxidase is required for neurite regeneration
39. Juan Bonifacino: Mechanisms of axon degeneration in lysosome-transport disorders
40. Tatyana Svitkina: APC-dependent microtubule-actin cooperation in neuronal growth cones
41. Edoardo Moretto: Tau “islands” affect axonal transport in vitro and in vivo
42. Anahi Bignante: Aβ assemblies induce amyloidogenesis in recycling endosomes through a Go/Gβγ signaling.=

Business Meeting 7:30-815 Christian Gonzalez Billault

Dinner 8:15 PM

Talk Abstracts

1. Frank Bradke

Arp2/3-actomyosin antagonism establishes local-excitation global-inhibition for neuronal polarization

Tien-chen Lin1, Charlotte H. Coles1,6, Florian Fäßler2, Sebastian Dupraz1, Eissa Alfadil1, Akihiro Narita4, Max Schelski1, Kevin C. Flynn1,7, Sina Stern1, Christoph Möhl3, Brett J. Hilton1, Franz Vauti5, Hans-Henning Arnold5, Florian K.M. Schur2, Frank Bradke1

(1) Laboratory for Axon Growth and Regeneration, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. (2) Institute of Science and Technology (IST) Austria, Klosterneuburg, Austria. (3) Image and Data Analysis Facility, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany. (4) Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8601, Japan. (5) Department of Cellular and Molecular Neurobiology, Technische Universität Braunschweig, Braunschweig, Germany. (6) Current address: Biopharm Discovery, GlaxoSmithKline, Gunnels Wood Road, Stevenage SG1 2NY, United Kingdom. (7) Current address: CaseBioscience, 9252 Compass Pointe Circle, Woodbury, MN, 55129, USA

Neurons initiate their polarization with the generation of multiple neurites emanating from the cell body. We found that before one neurite becomes the axon, the neurites oscillate showing coordinated retraction and extension. Coordination is mediated through a whole-cell actin network, which ensures single axon formation. Specifically, actomyosin mediates global inhibition of neurite growth; local Arp2/3-nucleated actin branching counteracts this global anti-parallel actomyosin assembly and contraction. Actin protrusions, including waves and growth cones, are the consequence of this local relaxation of actomyosin that enable microtubule protrusion necessary for neurite extension and axon formation. This release from the actomyosin network is transient, leading subsequently to retraction of the neurite. Thus, this molecular antagonism forms the long-sought local-excitation global-inhibition (LEGI) system driving neuronal polarization.

2. Gary Brouhard

Doublecortin contributes to neuronal migration through suppression of neurite branching and modification of the tubulin code

Gary Brouhard[1], Muriel Sébastien[1], Adam Hendricks[2]

(1) McGill University, Biology, Science, 1205 Avenue Docteur Penfield, Montréal, Canada(2) McGill University, Bioengineering, Engineering, 815 Sherbrooke St W, Montréal, Canada

Doublecortin is a neuronal microtubule-associated protein (MAP) that binds directly to microtubules via two Doublecortin (DC) domains. The DC domains are very sensitive to the nucleotide state, longitudinal curvature, and protofilament number of the microtubule lattice, indicating a role in the regulation of microtubule structure in neurons. Mutations in Doublecortin cause lissencephaly and double-cortex syndrome, which are diseases caused by impaired neuronal migration. To understand the role of DCX in neuronal migration, we developed a model system based on induced pluripotent stem cells (iPSCs). We knocked out the *Dcx* gene in a male iPSC line using CRISPR/Cas9 gene editing and differentiated the cells into cortical neurons. Compared to control neurons, the DCX-KO neurons showed reduced velocities of nuclear displacements, consistent with a neuronal migration phenotype. The reduced velocities correlated with an increase in the number of branches early in the neuronal development process. Neurite branching is regulated by a host of MAPs and other protein factors, as well as by microtubule dynamics itself. Microtubule dynamics were unchanged in DCX-KO neurons, however, with similar growth rates, lifetimes, and numbers. Rather, we observed changes in microtubule post-translational modifications, also known as the tubulin code, namely a significant reduction in polyglutamylation. Polyglutamylation is usually abundant in neurons and regulates, for example, microtubule severing enzymes and intracellular trafficking by molecular motors. We propose that the reduction of polyglutamylation leads to increased neurite branching and thus reduced neuronal migration. Our results indicate an unexpected role for DCX in the homeostasis of the tubulin code.

3. Kang Shen

How neuronal microtubule arrays are polarized and maintained in C. elegans neurons

Xing Liang1, Tim Stearns1, Jessica Feldman1, Kassandra Ori-Mckenney2, Kang Shen1,3,*

1Department of Biology, Stanford University, Stanford, United States 2 College of Biological Sciences, UC Davis, United States 3Howard Hughes Medical Institute

A polarized arrangement of neuronal microtubule arrays is the foundation of membrane trafficking and subcellular compartmentalization. Conserved among both invertebrates and vertebrates, axons contain exclusively 'plus-end-out' microtubules while dendrites contain a high percentage of 'minus-end-out' microtubules, the origins of which have been a mystery. Here we show that in Caenorhabditis elegans

the dendritic growth cone contains a non-centrosomal microtubule organizing center (MTOC), which generates minus-end-out microtubules along outgrowing dendrites and plus-end-out microtubules in the growth cone. RAB-11-positive endosomes accumulate in this region and co-migrate with the microtubule nucleation complex γ-TuRC. The MTOC tracks the extending growth cone by kinesin-1/UNC-116-mediated endosome movements on distal plus-end-out microtubules and dynein clusters this advancing MTOC. Critically, perturbation of the function or localization of the MTOC causes reversed microtubule polarity in dendrites. These findings unveil the endosome-localized dendritic MTOC as a critical organelle for establishing axon-dendrite polarity. To further understand how the stability and polarity of neuronal MTs are maintained, we visualized MT arrays in vivo in C. elegans neurons with single microtubule resolution. We find that the CRMP family homolog, UNC-33, is essential for the stability and polarity of MT arrays in neurites. In unc-33 mutants, MTs exhibit dramatically reduced rescue after catastrophe, develop gaps in coverage, and lose their polarity, leading to trafficking defects. UNC-33 is stably anchored on the cortical cytoskeleton and forms patch-like structures along the dendritic shaft. Through its MT binding activity, UNC-33 promotes individual MT rescues locally. The discrete and stable UNC-33 patches create predictable MT rescue sites, preventing loss of individual MTs and maintaining the coverage and polarity of MT arrays throughout the life of neurons. The CRMPs are the longest-lived proteins in mammalian brain, which might play similar roles to maintain MT organization of neuronal MTs.

4. Lukas Kapitein

Unraveling the organization, dynamics and structure of stable microtubules

Lukas Kapitein[1]
(1) Utrecht University, Utrecht, The Netherlands

While microtubules are often highly dynamic structures that grow and shrink, it is well known that a subset of neuronal microtubules is long-lived and resist treatments that normally induce depolymerization. Given the increasing evidence that stable and dynamic microtubules have dedicated functions and facilitate transport by distinct motor proteins, it is important to better understand how these different subsets form, how they are spatially organized and how they contribute to neuronal functioning. I will present our progress in addressing these questions and demonstrate how the development of multiple new tools have enabled us to obtain new insights into the spatial organization, abundance and structural features of stable microtubules.

5. Erika Holzbaur

Organelle-specific mechanisms regulating the axonal dynamics of autophagosomes and mitochondria

Erika Holzbaur[1]
(1) University of Pennsylvania, Physiology, Perelman School of Medicine, 638A Clinical Research Building, Philadelphia, United States

Maintaining the extended axons and dendrites of neurons requires active and carefully orchestrated organelle dynamics. A limited set of dynein and kinesin motors drive organelle transport, with motor activity tightly regulated by organelle-specific scaffolding proteins and small GTPases. We are focusing on two organelles essential to maintain neuronal homeostasis – autophagosomes and mitochondria. In the axon, autophagosomes are preferentially generated distally, and then are rapidly trafficked to the soma by the dynein-dynactin complex, which interacts with proteins including JIP1, HAP1 and Huntingtin, and JIP3/4 in a sequential pathway that is regulated by the small GTPases ARF6 and RAB10. Importantly, transport is tightly linked to the maturation state of the organelle, from a newly formed autophagosome to a fully-acidified and degradatively-competent autolysosome. We have combined vitro reconstitution, live cell imaging, and computational modeling to define this pathway, and to explore the dysregulation of axonal autophagy in neurodegenerative disease, for example in response to mutations in the kinase LRRK2 that are causal for Parkinson's. In parallel, we are investigating the dynamics of mitochondria, whose motility in axons and dendrites is also driven by kinesin and dynein motors, but is differentially regulated by TRAKs and the GTP-binding protein Miro. Our data suggest that mitochondrial dynamics, including fission and fusion, are locally regulated by local translation, and that this regulation is essential for normal neuronal development.

Financing: Supported by NIH grant R35 GM126950 to E.L.F.H.

6. Subhojit Roy

Biogenesis and Trafficking of Endocytic and Cytoskeletal proteins

Subhojit Roy[1]
(1) University of California San Diego, Department of Pathology and Neurosciences

The word “trafficking” is synonymous with vesicle trafficking, but numerous non-vesicular cargoes are also conveyed and deposited in cells. In neurons, vesicular transport only accounts for a third of all the material conveyed via axonal transport, and yet, little is known about the biogenesis and trafficking of non-vesicular cargoes that move in a poorly defined rate-class called slow axonal transport. This seminar will highlight recent findings related to the biogenesis, axonal transport, and synaptic function of clathrin and actin – cargoes that are conveyed in slow axonal transport.

7.Rejji Kuruvilla

Transcytosis-mediated anterograde transport of TrkA receptors is necessary for sympathetic neuron development and function

Rejji Kuruvilla[1], Guillermo Moya-Alvarado[1], Blaine Connor[1], and Naoya Yamashita[2]

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Neuronal responsiveness to target-derived factors requires the precise axonal targeting of new receptors, synthesized in cell bodies. We discovered that TrkA receptors for the target-derived neurotrophin, Nerve Growth Factor (NGF), are delivered to axons of sympathetic neurons by a non-canonical transport mechanism called transcytosis. Transcytosis is an atypical endocytosis-based mechanism, where newly synthesized proteins are first inserted on soma surfaces, internalized, and recycled long-distance to axons. Remarkably, transcytosis of TrkA receptors is regulated by the ligand NGF itself acting on axons, suggesting a positive feedback mechanism to scale up receptor availability in axons at times of need.

Our goal is to characterize the unconventional mode of ligand-triggered transcytosis of TrkA receptors to their functional sites in axons. We are using live imaging, confocal and electron microcopy, and cell biological/biochemical analyses to monitor the dynamic behavior and transport kinetics of receptor transcytosis, uncover the identity of the organelles responsible for TrkA transcytosis, and underlying regulatory mechanisms. Recently, we found that TrkA transcytosis requires the activity of a protein tyrosine phosphatase, PTP1B, which is anchored at the endoplasmic reticulum. We generated $TrkA^{R685A}$ mice, where TrkA receptor signaling is preserved, but its PTP1B-dependent transcytosis is disrupted, to show that this mode of axonal transport is essential for sympathetic neuron development and autonomic function. Together, our findings establish TrkA transcytosis as a major pathway for supplying TrkA receptors to axons, specifically during development, and highlight the physiological relevance of this axon targeting mechanism in the nervous system.

8. Ginny Farias

Shaping axons by local ER tubule – microtubule interactions

Ginny G. Farias1

(1) Utrecht University, Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht, The Netherlands

Establishment of neuronal polarity depends on local microtubule (MT) reorganization. The endoplasmic reticulum (ER) consists of cisternae and tubules and, like MTs, forms an extensive network throughout the entire cell. How the two networks interact and control neuronal development is an outstanding question. We found that the interplay between MTs and ER tubules is essential for neuronal polarity. MTs are essential for axonal ER tubule stabilization, and, reciprocally, ER tubules are required for stabilizing and organizing axonal MTs. Recruitment of ER tubules into one minor neurite initiates axon formation, whereas local ER tubule disruption prevent neuronal polarization. The ER-resident shaping protein P180, controls axon specification by regulating local MT remodeling. In addition, P180, enriched at a somatic pre-axonal region, ensures co-stabilization of ER tubule – MT – lysosome contacts to promote kinesin-1-driven lysosome translocation into the axon. Lastly, P180 distributed along the axon promotes ER tubule – ribosome contacts for efficient local translation. Thus, neuronal ER tubule organization plays an essential role in maintaining axonal identity, by organizing MTs, and regulating both axonal lysosome availability and local axonal translation.

Financing: The work presented here has been supported by the Netherlands Organization of Scientific Research (NWO) Vidi grant (0.16.VIDI.189.019) to G.G.F, NWO-KLEIN grant (OCENW.KLEIN.236) to G.G.F. and J.K., NWO Veni grant (VI.VENI.202.113) to M.K., and the European Research Council (ERC-StG 950617) to G.G.F.

9. Tomas Falzone

Human neuronal models to identify cell-intrinsic modulators of axonal polarization and transport

Tomas Falzone[1,2], Cayetana Arnaiz[1], Ivan Fernandez Bessone[2], Julieta Bianchelli[1], melina Gonzalez Prinz[1]

(1) IBioBA, MPSP-CONICET, Godoy Cruz 2390, Buenos Aires, Argentina(2) IBCN, FMED, UBA-CONICET, Cell Biology and Genetics, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires, Argentina
How extreme axonal projections from neurons deal with proteins and organelles distribution? What are the unknown regulators of this complex axonal transport system? How transport defects impact in neuronal function and lead to disease? Our work focuses on the establishment of neuronal polarity and axonal transport regulation. Polarized intracellular distribution in axons depends on proper axonal polarization and motor proteins to ensure anterograde and retrograde distribution of cargoes. This complex multistep process is highly regulated by the action of microtubule interacting proteins and different kinases. A hybrid tug-of-war/coordination model was proposed to explain bidirectional cargo transport, driven by teams of active and opposing kinesin and dynein motors. The exchange between active conformations in a cooperative multimotor arrangement paradigm determines the processive behavior and speed transitions of cargoes. The transport of APP that is necessary to maintain synaptic boutons, promote axonal growth, and react to injury is linked to its processing and vice versa. DYRK1A has been shown to phosphorylate tau, APP, microtubule-associated proteins, and proteins involved in membrane internalization. However, it is still unknown whether DYRK1A can regulate APP intracellular distribution. We show by live imaging of human-derived neurons that DYRK1A activity deferentially regulates the intracellular trafficking of the APP. Further, single-particle analysis revealed DYRK1A as a modulator of axonal transport and the configuration of active motors within the APP vesicle. Our work highlights DYRK1A as a regulator of APP axonal transport and metabolism, supporting DYRK1A inhibition as a therapeutic strategy to restore intracellular dynamics in Alzheimer's disease.
Financing: Support from Consejo Nacional de Investigaciones Científicas y Técnicas PUE Institucional IBCN2018, Universidad de Buenos Aires Grant UBACyT 2017/2019, and Agencia Nacional de Promoción Científica yTecnológica Grants PICT 2017-1648 and PICT 2019-0217.

10. Le Ma

Regulation of Axonal Transport in Nerve Branches

Le Ma1, Stephen Tymanskyj1, Bridget Curran1
(1) Thomas Jefferson University, Department of Neuroscience, 900 Walnut Street, Philadelphia, PA 19107, USA
Neurons have complex branched morphologies that pose huge challenges to microtubule-based axonal transport. To understand how such challenge is met during synaptic development and plasticity, we have investigated transport regulation at branch junctions. Using primary sensory neurons of dorsal root ganglion (DRG), we found that transport at branch junctions is not random but rather correlated with branch lengths and growth cone motility. Using an optogenetic approach, we showed that manipulating growth cone signaling could rapidly alter the preference of transport. This feature, termed transport selectivity, is seen with lysosomes and synaptic vesicles but not BDNF vesicles. It is mediated by KIF1 family of kinesin motors, as knocking down KIF1A/1B abolished signaling-dependent preference for lysosome transport. To further understand the underlying mechanism, we have developed an artificial cargo system and combine it with light-induced motor coupling to probe motor function and local environment. Using this system, we showed that MAP7, a protein that is enriched at branch junctions, reduced KIF1 mediated transport, and verified the defects of several human KIF1 mutations on transport. Currently, we are determining which kinesin motor supports transport selectivity and whether MAP7 is required for such regulation. These studies have begun to uncover molecular mechanisms that are critical to regulating axonal transport through complex branched morphologies.
Financing: Supported by R01 grants from NIH/NINDS.

11. Avital Rodal

Dynamics and regulation of the presynaptic actin cytoskeleton by periactive zone machinery

Avital Rodal[1]
(1) Brandeis University, Biology
Presynaptic nerve terminals depend heavily on endocytic membrane traffic to drive rapid recycling of neurotransmitter-containing synaptic vesicles, to regulate signaling pathways, and to enable proteostasis. Rather than being recruited to the membrane on-demand as in many non-neuronal cell types, presynaptic neuronal endocytic proteins are pre-deployed to a micron-scale mesh-like structure (the periactive zone), surrounding sites of synaptic vesicle release (the active zone). We developed new methods to measure discrete endocytic events at presynaptic terminals in *Drosophila*, by tracking endocytosis-associated actin assembly. We found that autoregulation in the pre-deployed endocytic machinery functions as a clamp to constrain actin assembly to the appropriate location and time. This may allow rapid and tunable activation of this pre-deployed machinery, when and where it is needed for membrane retrieval, over spatial and temporal scales ranging from basal endocytosis to massive

membrane uptake following strong neuronal activity. This raises the important question of how the endocytic machinery is organized within the periactive zone, which until now has only been loosely defined. Using super-resolution microscopy and new image analysis methods. we found that endocytic proteins segregate to distinct domains of periactive zone, and that their distribution correlates with release properties of nearby active zones, providing a new conceptual framework for spatial control of synaptic function and plasticity in this important but understudied region of the synapse.
Financing: This work is funded by the US National Institutes of Health NINDS R01116375

12. Thomas Blanpied

Conserved and variant aspects of synaptic nanodomain architecture

Department of Physiology, University of Maryland School of Medicine, Baltimore MD USA

Optical, chemical, and computational methods to improve the resolution of light microscopy have powered dramatic new insight to the molecular events underlying our thoughts and actions, most notably in the synapses of the brain. At synapses, organization of key proteins over nanometer scales governs nearly all aspects of neuronal information transmission: activity-release coupling in the presynaptic terminal, the net strength of postsynaptic receptor activation, and the nature and time course of intracellular signaling. Exploiting high-resolution live-cell and single-molecule localization microscopy, we have identified several elements of synaptic protein nanoarchitecture which subserve these functions. An emerging principle of this architecture is the accumulation of key proteins within nanometer-scale domains, where they localize function to discrete synaptic subregions. Critical open questions include which aspects of synaptic domain architecture are conserved or variant between synapse types, and the subsynaptic architecture of NMDA receptors. These receptors are known to be found in subsynaptic nanodomains and are thought also to help control the assembly or organization of other aspects of synaptic architecture. However, the relevance of their particular organization for synaptic function has remained unclear because the spatial relationships of these receptor nanoclusters to other functional regions of the synapse is unknown. I will discuss our recent work to elucidate the functionally relevant nanoarchitecture of NMDARs within single synapses and comparative observations in identified excitatory subtypes. Together, these experiments suggest that nanodomain organization within single synapses is broadly but noisily conserved, yet finely tuned by the molecular constituents of each synapse.

13. Frederic Meunier

Tau nanoscale biomolecular condensates at the synapse

Frederic Meunier[1]
(1) The University of Queensland, Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, QLD 4072, Brisbane, Australia

Neuronal communication relies on the release of neurotransmitters from tiny structures called synapses. These structures not only concentrate large numbers of neurotransmitter-containing synaptic vesicles but also of specialized cytosolic and membrane proteins. The mechanisms through which this robust neurotransmitter release apparatus remains concentrated within the minuscule volume of the synapse despite opposing entropic forces are mostly unknown. The ability of certain proteins to generate biomolecular condensates via liquid-liquid phase separation is an attractive process that could allow such an enrichment in both proteins and specialized organelles to remain highly concentrated and active at the synapse. However, the size of most synapses is within the micrometre range, far below the size of commonly studied biomolecular condensates. In this talk, I will discuss our attempt to identify and characterize the dynamic properties of presynaptic biomolecular condensates at the nanoscale level. We used single molecule super-resolution imaging in live hippocampal neurons together with a novel analysis pipeline to reveal that Tau forms dynamic biomolecular condensates at the synapse that are sensitive to synaptic activity and control the mobility of the recycling pool of synaptic vesicles – a critical subpopulation of synaptic vesicles responsible for the maintenance of neurotransmitter release.
Financing: This work was supported by an NHMRC grant.

14. Shrobona Guha

Investigating the role of microtubule-associated motor protein KIFC1 at the synapse.

Shrobona Guha1, Peter Baas1, Hemalatha Muralidharan1
(1) Drexel University, Neurobiology and Anatomy, 2900 Queen Lane, Philadelphia, USA

Additional to serving their necessary roles of architecture and transport in both axons and dendrites, microtubules are crucial for the maintenance of synaptic connections. On the presynaptic side, microtubules fortify the distal axon ensuring that it does not pull away. On the postsynaptic side,

microtubules fortify dendritic spines by transiently invading them. Synapse loss is downstream to many different neurodegenerative pathways. Surprisingly little has been done, to explore the mechanisms that ensure the fidelity of the synapse during health and how those mechanisms fail during disease. Here we have investigated a potential role for KIFC1, a kinesin-related motor protein known for its role in mitosis, but recently shown to also play critical roles in various aspects of the life of the neuron. In non-neuronal cells and in vitro assays, KIFC1 crosslinks microtubules to prevent them from sliding, binds to membrane proteins, and interacts with the plus end of the microtubule via EB1 and EB3 – all of which our present results demonstrate pertain to synapses. In studies on cultured rat hippocampal neurons, our results show dramatic synapse loss when KIFC1 is pharmacologically inhibited, with both sides of the synapse affected in different ways. The functional readout of this loss is reduced neuronal activity, recorded from hippocampal neurons cultured long-term on multi-electrode arrays. We posit that KIFC1 malfunction may underlie synapse loss during diseases. Moreover, new generations of drugs that strength KIFC1's attachment to microtubules may be a powerful tool for treating neurodegenerative diseases.

Financing: This work was supported by a grant to P.W.B. from the National Institute of Neurological Disorders and Stroke (NINDS) (R01 NS28785) and the Dean's Fellowship from Drexel University to S.G.

15. Matthew Davies

Spectraplakin Couples Microtubule Orientation to Actin During Dendritic Pruning in Drosophila

Matthew Davies1, Neele Wolterhoff2, Sebastian Rumpf1

(1) Institute for Neuro- and Behavioural Biology, University of Münster, Badestrasse 9, 48143, Münster, Germany(2) Division for Neurobiology, Institute for Biology, Freie Universität Berlin, Königin-Luise-Strasse 3, 14195, Berlin, Germany

Neurite pruning, the elimination of specific axonal or dendritic branches, is an essential mechanism to refine developing neural circuitry. Following local microtubule and actin disassembly, Drosophila sensory c4da neurons prune their dendrites during metamorphosis. We previously found that the uniform plus end-in orientation of dendritic microtubules is required for efficient pruning by enabling their coordinated disassembly. How dendritic microtubule organization is established is only incompletely understood. Here, we show that the spectraplakin short stop (Shot), an actin-microtubule crosslinker, is required for c4da neuron dendritic pruning. We find that Shot genetically interacts with known factors governing dendritic microtubule organization, and loss of Shot itself misorients dendritic microtubules. Forced actin depolymerization also causes dendritic microtubule orientation defects, and the actin binding ability of Shot impinges on microtubule orientation. Finally, we show that inhibition of actin depolymerization during pruning also inhibits microtubule disassembly, indicating coordination of local cytoskeleton disassembly. Our data suggest that via Shot-mediated coupling, actin is necessary for establishing plus end-in microtubule orientation in dendrites, facilitating pruning.

Financing: DFG grants to S. Rumpf and Cells in Motion Interfaculty Centre at University of Münster

16. Shaul Yogev

Spectrin restricts actomyosin contractility to maintain the continuity of the axonal microtubule array

Shaul Yogev[1]

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The neuronal axon is tiled by microtubules (MTs) that support cargo transport. While each MT polymer is much shorter than the axon, and many axons contain relatively few polymers, MTs are distributed in a way that maintains array continuity. How this continuity is maintained despite continuous forces that the cytoskeleton is subject to is unknown. Here, using single-neuron degradation and endogenous tagging of cytoskeletal proteins in vivo, we found that axonal spectrin is required for the continuity of the microtubule array. In wildtype animals, microtubules oscillated in place but remained overall immotile. Conversely, loss of spectrin or its axonal transport machinery led to processive movement of polymers, which resulted in a discontinuous array and in distal degeneration of the axon. Temporally controlled degradation of spectrin revealed excessive F-actin and non-muscle myosin II accumulation at MT ends prior to polymer movement. Pharmacological or genetic inhibition of myosin II – but not MT motors – arrested MT polymer movement and restored continuity to the MT array. Our results suggest a model in which spectrin restricts actomyosin contractility, likely by targeting it to the membrane, in order to ensure the continuity of the axonal microtubule track.

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17. Linda Van Aelst

Shedding Light on Chandelier Cell Development and Connectivity

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Among the diverse interneuron subtypes in the neocortex, chandelier cells (ChCs) are the only population that selectively innervate pyramidal neurons (PyNs) at their axon initial segment (AIS), the site of action potential initiation, allowing them to exert powerful control over PyN output. Accordingly, disruptions in ChC biology have been linked to schizophrenia, epilepsy, and ASD. Despite the importance of ChCs, little is known about (1) the molecular factors governing their selective innervation at PyN AISs and (2) extrinsic cues and/or other cell types regulating ChC axonal morphogenesis. To this end, novel, *in vivo* RNAi screening against PyN-expressed axonal cell adhesion molecules was performed, which revealed an essential role for the cell adhesion molecule L1CAM in ChC/PyN AIS innervation. Such L1CAM-dependent cell-cell contact is necessary for both the establishment and maintenance of ChC/PyN innervation and requires anchoring of L1CAM at the AIS by the ankyrin-G (AnkG)/βIV-spectrin cytoskeletal complex. In addition to PyN-expressed L1CAM, screening of other cell types/factors present in the extracellular milieu unveiled a growth-promoting role for microglia in regulating PyN AIS synapse formation by ChCs. Namely, we found that a population of microglia contacts PyN AISs and ChC cartridges and that such tripartite interactions, which rely on the AIS cytoskeleton and microglial $GABA_{B1}$ receptors, are associated with increased ChC cartridge length and bouton number and AIS synaptogenesis. Together, our studies unveil critical microglia- and L1CAM-dependent mechanisms underlying proper neocortical ChC morphogenesis and ChC/PyN AIS innervation and, as such, shed new light on the connectivity defects underlying debilitating brain disorders.

18. Fernanda Ledda
Ligand-induced trans-synaptic adhesion in hippocampal plasticity and connectivity
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The function of the nervous system critically relies on the establishment of precise synaptic contacts between neurons and specific target cells. Elucidation of the mechanism by which synapses are formed is crucial for understanding the synaptic deficits that underlie cognitive disorders. Many membrane-bound synaptic adhesion molecules (SAMs) have been involved in target recognition and synapse specification by homophilic or heterophilic trans-synaptic interactions. A third group of trans-synaptic adhesion molecules are ligand dependent adhesion molecules (LiCAMs) which combines features of both diffusible and membrane bound synaptogenic factors to develop specific neural contacts. One of these ligands is the neurotrophic factor GDNF which binds to the pre and postsynaptic receptor GFRα1. In our laboratory we have investigated the role of this system in the plasticity of adult hippocampal circuits controlling the integration of newly generated neurons. We have also analyzed the mechanisms that underlies the formation of this adhesive and transynaptic complex.

19. Kristy Welshhans
Local translation of β-actin mRNA at adhesions and its role in axon guidance
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Local translation of select mRNAs within growth cones is necessary for axon guidance; however, the complex molecular mechanisms underlying this localized translation are not well understood. We have shown that local translation of *β-actin* mRNA within growth cones is necessary for appropriate axon guidance and depends on receptor for activated C kinase 1 (RACK1), a ribosomal scaffolding protein and key member of the point contact complex. Point contacts are multi-protein adhesion sites within growth cones that regulate growth cone motility by linking the extracellular matrix (ECM) to the actin cytoskeleton. Using fluorescent translation reporters, we demonstrated that point contacts are sites of local translation of *β-actin* mRNA. The prevailing knowledge indicates that the amount of these locally translated proteins is small, leading to the hypothesis that these proteins must be strategically placed to have a maximum impact on axon guidance. Our data suggest that nascent *β-actin* may be necessary for axon guidance through its participation in point contact mechanisms. Indeed, we have followed nascent β-actin over time and find that newly translated β-actin is found at point contacts. We have also identified components of the ECM that affect intra-axonal local translation, further emphasizing the importance of point contacts in regulating local translation. Taken together, these data suggest that local translation is targeted to adhesion sites to regulate axon guidance and this involves an

ECM-initiated signaling pathway. These data provide insight into how and where local translation is regulated, thereby leading to appropriate connectivity formation in the developing nervous system.
Financing: This work is funded by the Whitehall Foundation.

20. Shaun Sanders
Regulation of fast axonal transport in neurons by protein palmitoylation
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Neurons are large, complex cells requiring efficient trafficking and delivery of neuronal proteins and organelles to specific subcellular locations. Fast, continuous transport of cargo along axonal microtubules by dynein and kinesin motors is critical for neuronal function and requires a constant source of energy. Interestingly, glycolytic enzymes were recently found tethered to fast moving vesicles to provide an 'on-board' energy supply directly to the molecular motors. The activity of motor proteins is tightly regulated, and aberrant activity can result in neurodegeneration or neurodevelopmental deficits. One important mechanism to dynamically regulate protein trafficking in neurons is the covalent addition of fatty acids to protein cysteines residues, a process known as palmitoylation. Interestingly, glycolytic enzymes as well as several kinesin and dynein motor subunits and their activators have been identified in high throughput palmitoyl-proteomic studies as potentially palmitoylated. Thus, we hypothesize that palmitoylation tethers multiple motor proteins, their activators, and glycolytic enzymes to vesicles to provide 'on-board' energy and continuous movement required for fast axonal transport. Indeed, we have recently confirmed that glycolytic enzymes as well as p150Glued, a subunit of the dynein activating complex dynactin, are palmitoylated in neurons and, interestingly, their palmitoylation regulates their vesicle association and axonal localization. These findings provide insight into the molecular mechanisms that govern fast axonal transport with implications for neurodevelopment, synaptic function, and axon degeneration.

21. Laura McCormick
The E3 ubiquitin ligase TRIM9 regulates actin dynamics and synapse formation
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In neurons, actin-rich filopodia are critical at many stages of morphogenesis, including neuritogenesis, axon guidance, and dendritic spine formation. Defects in these critical developmental processes can result in improper synaptic connectivity, neurodevelopmental disorders, and psychiatric syndromes. Previously, we demonstrated the E3 ubiquitin ligase TRIM9 localizes to growth cone filopodia and regulates axon pathfinding downstream of the guidance cue netrin. *Trim9*$^{-/-}$ mice have overt spatial learning memory deficits, yet the role of TRIM9 in synapse formation and maintenance is unknown. Here we showed TRIM9 is enriched in the post-synaptic density following differential centrifugation, suggesting a role for TRIM9 in dendritic spines. We found Netrin-dependent increases in dendritic spine number, synapse maturation, and neuronal firing in vitro, and all these responses are abrogated in *Trim9*$^{-/-}$ neurons. In vivo, we demonstrated that loss of *Trim9* alters the proteome of the postsynaptic density. In particular, we observed changes in numerous cytoskeletal proteins, including the Arp2/3 complex. While future work will focus on identifying new synaptic substrates, we have previously identified numerous neuronal targets of TRIM9. In particular, TRIM9 was required for the reversible, non-degradative ubiquitination of VASP and this modification was associated with decreased filopodia number and stability. Through mass spectrometry, we identified numerous lysine residues that are ubiquitinated in VASP, including K240 and K286. Using chemical ubiquitination, we created purified, ubiquitinated VASP to evaluate protein activity through in vitro assays. We found the actin filament elongation rate of ubiquitinated VASP was significantly reduced compared to unmodified VASP. Our results suggest VASP ubiquitination is a mechanism to negatively regulate filopodial actin dynamics.
Work was supported by R35 GM135160 (S.L.G.), and F31NS113381 (L.E.M)

22. Mariano Bisbal
Distinct Rho GTPase effectors inhibit axon outgrowth and promote axon elongation
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RhoA's crucial role in neuronal polarization, where its action restrains axon outgrowth, has been thoroughly documented. We now report that RhoA not only inhibits but also stimulates axon development depending on when and where exerts its action and identify mDia as the Rho effector of this novel stimulatory effect of RhoA. In cultured hippocampal neurons, FRET imaging revealed that RhoA activity selectively localizes in growth cones of undifferentiated neurites, while in developing axons it displays a biphasic pattern, being low in nascent axons and high in elongating ones. RhoA-Rho kinase (ROCK) signaling prevents axon initiation but has no effect on elongation, while formin inhibition reduces axon extension without significantly altering initial outgrowth.Besides, RhoA-mDia promotes axon elongation by stimulating growth cone microtubule stability and assembly, as opposed to RhoA-ROCK that restrains growth cone microtubule assembly and protrusion. Finally, we show that similar mechanisms operate during axonal regeneration, with RhoA-ROCK slowing axon regrowth after axotomy and RhoA-mDia favoring extension of regenerated axons.

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23. Felipe Opazo

Tailored probes for the study of molecular neurophysiology

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Neuronal communication relies on the precise and coordinated activity of several synaptic pathways. Their activity has long been analyzed by imaging experiments, typically by expressing a sensor molecule fused to a synaptic protein (e.g., SynaptopHluorin). During the last two decades, many variations of this approach have been employed, revealing changes in pH, calcium levels, or neurotransmitters. Unfortunately, these strategies change the delicate balance of localization and stoichiometry of synaptic molecules, thereby altering synaptic physiology. To solve this problem, we focused on developing camelid single-domain antibodies (nanobodies, Nb) tailored for live functional imaging of synapses and as ideal tools for various super-resolution microscopy techniques. Here I will introduce some of the tools we have developed during the last years to image multiple neuronal targets, as well as follow and sense different players harmonizing the molecular physiology of synapses.

24. Paul Jenkins

Life beyond the AIS: ankyrin-dependent scaffolding of Nav1.2 in neocortical dendrites

Paul Jenkins[1,3], Andrew Nelson[2], Amanda Catalfio[1], Julie Gupta[1], Lia Min[1], Rene Caballero-Floran[1], Kendall Dean[1], Carina Elvira[1], Kimberly Derderian[2], Henry Kyoung[2], Atehsa Sahagun[2], Stephan Sanders[2], Kevin Bender[2]

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Neuronal voltage-gated sodium channels (Na_Vs) play critical roles in action potential (AP) generation and propagation. *SCN2A*, which encodes $Na_V1.2$, has repeatedly emerged from genetic studies as a strong genetic risk factor for autism spectrum disorder (ASD). In developing pyramidal neurons of the medial prefrontal cortex, $Na_V1.2$ is the only Na_V detected at the axon initial segment (AIS). In mature neurons, $Na_V1.2$ is replaced by $Na_V1.6$ at the AIS and is relocalized to the dendrites. Here, $Na_V1.2$ promotes the AP backpropagation from the AIS to distal dendritic arbors, an important feature of excitable dendrites that is essential for normal synaptic function. While previous studies have shown Na_Vs are localized to the AIS by ankyrin-G (*ANK3*), the mechanisms that underlie $Na_V1.2$ localization within dendrites are unknown. We hypothesize that ankyrin-B, product of the high-confidence ASD gene *ANK2*, is the primary ankyrin that controls $Na_V1.2$ dendritic localization. Using knockout-and-rescue studies, we found that ankyrin-B knockout resulted in a significant reduction in $Na_V1.2$ levels at the dendritic membrane that was rescued by re-expression of ankyrin-B. Therefore, deletion of *Ank2* was predicted to have similar consequences on dendritic excitability as *Scn2a* loss. Consistent with this hypothesis, AP backpropagation was attenuated in *Ank2* haploinsufficient mice. Furthermore, we observed deficits in excitatory postsynaptic function identical to those previously identified in *Scn2a* haploinsufficient mice. These findings highlight a novel dendritic role for ankyrin-B in the proper localization of $Na_V1.2$ and reveal convergent dendritic function for both *Scn2a* and *Ank2*, two of the most commonly disrupted genes in ASD.

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25. Matthew Rasband

Neuronal ankyrin and spectrin cytoskeletons

Matthew Rasband[1]

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Ankyrin and spectrin cytoskeletons play multiple, and sometimes redundant, roles in the organization, function, and maintenance of neuronal membrane domains in both health and disease. Using ankyrin and spectrin conditional knockout mice, I will show how different ankyrins and spectrins are used repeatedly in different cellular contexts to organize and maintain cell-cell interactions and ion channel clustering.

26. Helge Ewers

Actin rings as ubiquitous diffusion barriers in the neuronal lineage.

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In the last decade since the discovery of a periodic pattern of ~ 190 nm spaced actin rings in neuronal axons, these intriguing structures have been found in a variety of neuronal cell types. We have previously shown that the actin rings are the physical location of obstacles to the lateral motion of membrane proteins in the axonal initial segment (AIS), i.e. even lipid-anchored proteins are confined between them in the AIS. We now use stem-cell derived neurons, astrocytes and oligodendrocytes to show that in these cells similar actin rings exist and that similarly actin rings act as barriers to the lateral motion of membrane proteins here. We furthermore find that disruption of actin rings reduces compartmentalisation in cells. We conclude that actin rings act as diffusion barriers in the plasma membrane across cell types in the neuronal lineage.

27. Ernesto Muñoz Palma

Glutamate and early functional NMDA Receptors contribute to hippocampal axonal elongation through Rac1 activity, which modulates both actin cytoskeleton dynamics and NOX2-mediated H2O2 production.

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NMDA Receptors (NMDARs), which are essential for maturation, neurotransmission and functionality of the nervous system, mediate Ca2+ influx following activation by the neurotransmitter glutamate. However, the role of glutamate and NMDARs during early neuronal development has not been described. Here, we found that functional NMDARs were expressed during neuronal polarity acquisition. In addition, endogenous and ectopically expressed NMDARs were distributed to the axonal compartment early in development. Interestingly, during early development neurons released glutamate. Moreover, pharmacological and genetic NMDARs loss- and gain-of-function altered neuronal polarization and axonal elongation by a mechanism that involved actin cytoskeleton rearrangement at the neuronal growth cone and regulation of the intracellular hydrogen peroxide (H2O2) content, via the Rho GTPase Rac1. In fact, the optogenetic activation of a photoactivatable Rac1 version simultaneously promotes both lamellipodia and H2O2 formation suggesting the co-occurrence of these processes. Thus, NMDARs signaling promotes dual Rac1 functions, which mediate actin cytoskeletal remodeling and H2O2 production by the NOX2 complex. Altogether, these findings suggest that early spontaneous glutamate release activates NMDARs to support neuronal development before synapse formation, indicating that glutamate is necessary for neurotransmission and also for early neuronal development and axonal growth.

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28. Francisca Bronfman

Contribution of dynein-dependent transport of BDNF signaling endosomes to neuronal plasticity

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The neurotrophic factor BDNF (brain-derived neurotrophic factor) and its receptor TrkB (tropomyosin kinase receptor B) control the connectivity of different neuronal networks in the central nervous system (CNS) by regulating the activation of signaling pathways leading to transcriptional regulation and translation of proteins. After being bound by BDNF, TrkB is endocytosed into endosomes and continues signaling within the cell soma, dendrites, and axons in signaling endosomes. Although the dynein-mediated transport of BDNF and TrkB in axons has been reported, the functional role of BDNF/TrkB axonal signaling is unknown. We will discuss recent lab findings demonstrating that dynein-dependent BDNF-TrkB-containing endosome transport is required for long-distance induction of dendritic growth. Axonal signaling endosomes increased CREB and mTOR kinase activity in the cell body, and the activity of both proteins was required for general protein translation and the expression of Arc, a plasticity-associated gene, indicating a role for BDNF-TrkB axonal signaling endosomes in coordinating the transcription and translation of genes whose products contribute to increase neuronal plasticity.
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29. Shernaz Bamji
Regulation of synapse plasticity by palmitoylating enzymes
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Palmitoylation is the most common post-translational lipid modification in the brain. It involves the addition of the fatty acid, palmitate, onto substrate proteins and is exceedingly important for protein trafficking and cell signaling. While ~20% of all proteins in the genome are substrates for palmitoylation, a striking 41% of all synaptic proteins can be palmitoylated. Using proteomic analysis, we identify a list of synaptic proteins that are differentially palmitoylated in the hippocampus of mice that have undergone fear conditioning and demonstrate that the dynamic palmitoylation of proteins is important for the establishment of long-term potentiation. This is of clinical relevance as genetic variants in palmitoylating enzymes are associated with brain disorders and as palmitoylation can be altered by environmental factors including diet.
Financing: This work was funded by the Canadian Institutes for Health Research

30. Melissa Rolls
Mechanisms that maintain microtubule polarity and dynamics in dendrites
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Neurons must maintain a stably organized array of microtubules over the lifetime of an animal using building blocks that are continually growing, shrinking, and turning over completely. This conundrum of long-term stable organization using dynamic building blocks requires tight control of microtubule behavior in neurons. One heavily regulated aspect of microtubule behavior in dendrites is nucleation. In Drosophila branched sensory dendrites, nucleation sites are housed on endosomes at branch points. Nucleation machinery is recruited to this position by many canonical Wnt signaling proteins including scaffolds Axin and dishevelled, and frizzled receptors and co-receptors. The involvement of Wnt receptors suggested that a Wnt ligand might act upstream of neuronal microtubule nucleation. We demonstrate that neighboring skin cells are the source of Wnt ligands that act upstream of dendritic microtubule nucleation. Moreover, dendrite branch points are sites of endocytosis, suggesting that internalization of the ligand determines where nucleation machinery is localized. We conclude that rather than being controlled cell-autonomously, dendritic microtubule nucleation is influenced by the surrounding cells in vivo.
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31. Kassandra Ori-McKenney
Investigating the mechanism of tau pathoconversion using an injury model.
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Traumatic brain injury (TBI) occurs when an external force causes brain damage and dysfunction. One hallmark of TBI is the presence of hyperphosphorylated tau in neurological tissues and cerebrospinal

fluid. Tau is a microtubule-associated protein that self-associates to bind cooperatively along the microtubule lattice to direct molecular motors. Under pathological conditions, tau self-assembles to form large aggregates called neurofibrillary tangles that contribute to neurodegeneration. Our work seeks to define the events that cause tau to transition from a physiological to a pathological state. We pan-neuronally expressed human 2N4R tau in Drosophila adult males and subjected them to TBI. We then performed a variety of behavioral, biochemical, and immunohistochemical assays on the adult males 24-hrs and 1-wk post-TBI. We found that pan-neuronal expression of tau caused an increase in inter-male aggression in flies subjected to TBI, both in terms of the amount of time engaged in aggressive acts and in the total number of aggressive acts. We further identified specific circuits that regulate this persistent aggressive state. We also found that both phosphorylation and oligomerization of tau contribute to this increase in aggression. In addition, we observed the presence of a tau dimer in the lysates of male flies 24 hrs post-TBI compared to controls. We performed a range of biochemical and single-molecule assays with different tau proteins to determine the molecular details underlying tau's ability to self-associate into larger oligomeric structures. Ultimately, we hope to elucidate how different states of tau contribute to alterations in brain function and behaviors.

32. Dhanya Cheerambathur

Repurposing the Chromosome-Microtubule Coupling Machinery as a "Tuner" of Actin for Dendritic Branching.

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Dendrite branching is an essential process for building complex nervous systems. A neuron's dendritic patterns govern the number, distribution, and integration of inputs. Though significant progress has been made in understanding the signalling pathways that pattern the dendrite, little is known about the intrinsic mechanisms involved in sculpting the branches. The actin & microtubule cytoskeleton are critical to provide structure and exert force during dendrite branching. Our study reveals an unexpected role for the kinetochore, the chromosome-microtubule machinery, in shaping the dendrites of the mechanosensory neuron, PVD in C. elegans. The kinetochore is a highly conserved multiprotein complex whose canonical function is to connect chromosomes to microtubules during cell division. Kinetochore proteins are enriched in the PVD dendrites where they associate with the endosomal structures and are essential for establishing the dendritic pattern of PVD independent of its cell division function. Degradation of kinetochore proteins during PVD development results in dendrite branch fusion and overexpression of kinetochore leads to hypo-branching. Surprisingly, microtubule dynamics remain unchanged in the absence of kinetochores, but F-actin dynamics is altered during dendrite branching. We show that kinetochore proteins modulate F-actin polymerization mediated by the Rac GTPases. Thus, our work suggests that kinetochore proteins act as a "tuner" of actin polymerization to control dendrite patterning. Overall, these findings reveal an unexpected architect in dendritic branching and provide insight into crosstalk between microtubules and actin-based structures that remodel dendrites.

33. Pirta Hotulainen

From "invisible" to visible actin structures

Pirta Hotulainen[1]
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We all know how the actin cytoskeleton looks in migrating cells or in neurons. There is fast polymerizing lamellipodial meshwork in the leading edge of a migrating cell and there are thicker acto-myosin bundles over the cell body. But there are many more actin structures which are less well-known. In cell line cells, different actin structures are relatively easy to highlight from other actin structures by various actin-binding-proteins. In neuronal dendrites and axons all actin looks easily "the same"; structures seem to be mainly patches or thin bundles. Super-resolution techniques have helped and even in neurons we have started to see actin structures that we could not see before, such as actin rings. In my talk, I will have a look at "invisible" actin filament structures which were done visible.

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34. Monica M Sousa

Tension-driven axon growth triggers developmental stage-specific adaptations of the axonal cytoskeleton and membrane

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During development, axon elongation is mediated by the growth cone. After synapse formation, axons continue to extend to accompany body growth. The mechanisms underlying this distinct phase of axon extension remain largely neglected. To uncover the biology of tension-driven axon growth, we designed a stretchable computer-controlled microfluidic platform, compatible with live imaging, to study dorsal root ganglia (DRG) neuron explants in different developmental stages. This allowed mirroring the conditions in which neurons undergo the strongest stretch-induced elongation: mid-gestation and the adolescent growth spurt. Using a multiomics approach, we show that genes and pathways engaged by stretch differ depending on neuronal developmental stage. Within these, membrane remodelling emerged as the only common functional category. Accordingly, stretch elicited a fast increase of plasmalemmal precursor vesicle formation and transport, especially in embryonic neurons, with membrane incorporation throughout the axon shaft culminating in increased axon length and diameter. Concurrently, whereas in embryonic DRG neurons, a rapid stabilization of the microtubule cytoskeleton occurred upon stretch, supporting increased axonal transport, in adult DRG neurons microtubule dynamics remained unaltered by tension. In summary, our work is providing important insights on the biology of axon growth post-synapse formation, and demonstrates that axon stretch results in distinct adaptations depending on developmental stage.

35. Nicolas Unsain

Studying the organization of □II-spectrin within the actin/spectrin membrane-associated periodic skeleton of axons in vivo with nanometer resolution.

Nicolas Unsain[1]

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Actin, spectrin and associated proteins form a membrane-associated periodic skeleton (MPS) that is ubiquitously present in mature axons of all neuronal types evaluated so far. The MPS is a periodic structure basically consisting of actin "rings" located transversely to the axon and separated every ~190 nm by α / β-spectrin "spacers" extended along the axon. Since its discovery, the characterization of MPS has been performed almost exclusively in cultured neurons - artificial environments in two dimensions. It is still not clear how spectrin tetramers are organized in each segment of this periodic scaffold. Because of the intimate relationship between the cortical skeleton of cells and their immediate environment, we proposed to study the spatial organization and biology of these structures in their “natural” environment, that is, in vivo in fixed tissue. We have begun by analyzing the organization principles of spectrin tetramers in MPS segments of axons within mouse sciatic nerves using 3D-STOchastic Reconstruction Microscopy (STORM). We have then used proteome reports, protein-protein interaction databases and protein folding predictors to elucidate the putative associated proteins that could explain the observed organization. This ongoing project is showing that spectrin tetramers follow a non-random distribution across a given segment of the MPS, with a characteristic distance and homogeneity in its stoichiometry. The bioinformatic analyses provided possible proteins whose presence could determine the observed distribution of spectrin.

36. Marina Mikhaylova

Synaptic control of organelle localization: focus on the ER

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The heterogeneity of the endoplasmic reticulum (ER) makes it a versatile platform for a broad range of homeostatic processes, ranging from calcium regulation to synthesis and trafficking of proteins and lipids. It is not surprising that neurons use this organelle to fine-tune synaptic properties. In this work, we address the mechanisms that enable activity dependent ER recruitment into dendritic spines, with a focus on molecular mechanisms that mediate transport and retention of the ER in spines. We investigate calcium-signalling pathways that regulate the activity-dependent recruitment of a single ER tubule into dendritic spines, as well as local formation of the spine apparatus (SA) organelle. The SA is formed by stacks of smooth ER-membrane intercalated with a dense F-actin matrix, and is continuous with the main ER network. To date, the protein synaptopodin is the only known essential component of the SA. We show that the F-actin motor myosin V plays a role in determining the localization of both synaptopodin and the SA to a subset of activated dendritic spines. This finding suggests that the presence of specific organelles at a subset of synapses may function as a ‘tag’ for synapses that are

resistant to pruning, thus providing a potential mechanism for strengthening synaptic connections over time.

37. Shivani Bodas

Mechanisms underlying the development of the axonal actin-spectrin Membrane Periodic Skeleton (MPS)

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Our previous work highlighted the importance of the Actin-spectrin Membrane Periodic Skeleton (MPS) to be crucial for buffering axonal rest tension. Stretch-induced reversible spectrin unfolding model was found to release excess mechanical stress of the system (Dubey et al., 2020). However, the mechanisms underlying MPS formation and remodeling during neuronal development remain unclear.
We showed that MPS prevalence in chick DRG axons increased with developmental stage (DIV 1-4; days in vitro), but the periodicity remained constant at ~ 190 nm. This increase in prevalence over time correlated with an increase in axonal rest tension. Perturbation of actin and microtubule dynamics affected MPS prevalence and stability and revealed the role of microtubule stability in MPS formation and maintenance. To investigate the mechanism of MPS development, we measured spectrin dynamics using FRAP. Interestingly, very little recovery was observed for spectrin even at DIV 1, when MPS was not prevalent. This suggests the recruitment of spectrin to a stable structure before the establishment of the MPS. Lat-A-treated axons revealed a faster initial recovery phase. The mobile fraction in early-stage axons (DIV1-2) was higher than that in the controls, although this was not the case for late-stage axons (DIV4). These experiments suggest the recruitment of spectrin to a stable network via a combination of actin and membrane binding which is later elaborated to form the MPS. This hypothesis is being tested using specific deletion constructs in spectrin knockout cells and is expected to reveal the sequence of interactions resulting in the development of the MPS.

38. Katie Baldwin

Molecular mechanisms of astrocyte connectivity during brain development

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Astrocytes are morphologically complex glial cells that extensively infiltrate the neuropil to directly interact with neurons and all other brain cell types. Through these interactions, astrocytes control critical aspects of brain function, including synapse formation and neurotransmission. How do astrocytes establish and balance their numerous interactions with other cells? Do defects in astrocyte connectivity drive the pathogenesis of neurological disorders, such as autism, schizophrenia, and leukodystrophy? To answer these questions, we study the function of disease-linked cell adhesion molecules in astrocytes to understand how astrocytes establish functional connections with different cell types during brain development. Furthermore, we investigate how dysfunctional astrocyte connectivity drives the pathogenesis of neurodevelopmental brain disorders. Our approach uses mouse genetics, primary cell culture, molecular and cellular biology, quantitative proteomics, and confocal and super resolution microscopy. In my talk, I will focus on recent advances in our laboratory investigating the function the astrocyte-enriched cell adhesion molecule hepaCAM and its function in regulating membrane protein stability at astrocyte-astrocyte and astrocyte-synapse contacts.

39. Juan Salvador Bonifacino

Mechanisms of axon degeneration in lysosome-transport disorders

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The functions of lysosomes are dependent on their ability to move within the cytoplasm. Previously, we described a mechanism for anterograde lysosome transport mediated by the BORC complex, the small GTPase ARL8, and kinesin-1 and -3 microtubule motors. Silencing of components of this pathway caused redistribution of lysosomes to the juxtanuclear area of the cells, as well as depletion of lysosomes from the axon in neurons. We leveraged this ability to manipulate lysosome coupling to

kinesins to address the physiological importance of lysosome transport into the axon. This is a critical question since axonal lysosomes are less acidic and less proteolytic than lysosomes in the soma, and therefore likely play additional degradation-independent roles. Indeed, recent work showed that RNA granules hitchhike on lysosomes for long-range transport in the axon. We used wild-type and BORC-KO human iPSC-derived neurons (i3Neurons) grown on coverslips or in microfluidic devices to analyze the effect of blocking anterograde lysosome transport on axonal mRNA transport. We found that BORC KO reduced transport of not only lysosomes but also specific mRNAs in the axon. Moreover, RNAseq revealed a dramatic depletion of a subset of mRNAs in the axon, including numerous mRNAs encoding ribosomal subunits and components of the mitochondrial oxidative phosphorylation reaction. In turn, this resulted in reduced protein translation ,and defects in mitochondria morphology and membrane potential in the axon. These findings thus demonstrated the critical importance of axonal lysosomes for the maintenance of axonal homeostasis, likely for repair of distal organelles through local protein synthesis.
Financing: Intramural Program of NICHD, NIH

40. Tatyana Svitkina

APC-dependent microtubule-actin cooperation in neuronal growth cones

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Precision of directional cell migration is critical for neurons, as they extend neurites toward specific targets over large distances. In this process, key cytoskeleton components – actin filaments and microtubules – perform different, but interconnected tasks. We discovered a new aspect of the actin-microtubule crosstalk during neurite extension – initiation of branched actin networks from microtubule tips in neuronal growth cones that is controlled by adenomatous polyposis coli (APC). Given that branched actin networks generate pushing force on the membrane, these findings suggest a straightforward mechanism by which microtubules can control the direction of membrane protrusion in growth cones, which is a key aspect of neuronal navigation.

41. Edoardo Moretto

Tau “islands” affect axonal transport in vitro and in vivo

Edoardo Moretto1,2, Chiara Panzi2, Skye Stuart2, Samantha De La-Rocque2, Emily Huff2, Giampietro Schiavo2,3,4
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Tau is a protein abundantly expressed in neurons where it modulates the stability of axonal microtubules, thus contributing to the regulation of axonal transport of several organelles. Tau aggregates in a group of neurodegenerative diseases named tauopathies, which include frontotemporal dementia (FTD) and Alzheimer’s disease. Recent in vitro work has uncovered the existence of tau “islands”, a microtubule-bound multimeric state of tau, which is distinct from pathological aggregates. However, whether these structures exist in intact neurons remains unclear. In cultured mouse neurons and in vivo, we found that human tau shows regions of higher density along axons, reminiscent of tau islands. FTD-linked mutations, known to increase pathological phosphorylation and aggregation of tau, induces larger islands, an effect that is reversed by inhibition of p38 MAPK, known to phosphorylate tau at multiple sites.
Functionally, we found that axonal transport of BDNF-containing secretory granules is affected by mutant tau as assessed both in vitro and in vivo by using a new assay based on two-photon microscopy. Interestingly, this impairment occurred very early on, before overt tau aggregation. Inhibition of p38 MAPK was able to partially rescue these defects in axonal transport both in vitro and in vivo. Our data suggests that tau island size regulates axonal transport, an effect dependent on tau phosphorylation. Inefficient organelles transport may have severe consequences on the activity and plasticity of neuronal circuits. The evidence that reducing tau phosphorylation by inhibiting p38 MAPK potentiated axonal transport points towards inhibition of p38 MAPK as a promising therapeutic strategy in tauopathies.

42. Anahi Bignante

Aβ assemblies induce amyloidogenesis in recycling endosomes through a Go/Gβγ signaling.

Anahi Bignante1, Magdalena Antonino1, Paula Marmo1, Romina Almirón1, Alfredo Lorenzo1
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Alzheimer´s disease (AD) is characterized by deposition of aggregated species of amyloid beta (Aβ) in the brain, which leads to progressive cognitive deficits and dementia. Aβ is generated by the successive cleavage of the amyloid precursor protein (APP), first by β-site APP cleaving enzyme 1 (BACE1) and subsequently by the γ-secretase complex. Conditions that enhance Aβ generation or reduce its clearance predispose to Aβ aggregation and the development of AD. In vitro studies have demonstrated that Aβ assemblies spark a feedforward loop heightening Aβ production. However, the underlying mechanism remains unknown. Here, we show that oligomers and fibrils of Aβ enhance colocalization and physical interaction of APP and BACE1 in recycling endosomes of human neurons derived from induced pluripotent stem cells and other cell types, which leads to exacerbated amyloidogenic processing of APP and intracellular accumulation of Aβ42. In cells overexpressing mutant forms of APP that are unable to bind Aβ or to activate Go protein, we have found that treatment with aggregated Aβ fail to increase colocalization of APP with BACE1 indicating that Aβ-APP/Go signaling is involved in this process. Moreover, inhibition of Gβγ subunit signaling with βARKct or gallein, prevent Aβ-dependent interaction of APP and BACE1 in endosomes, β-processing of APP and intracellular accumulation of Aβ42. Collectively, our findings uncover a signaling mechanism leading to a feedforward loop of amyloidogenesis that might contribute to Aβ pathology in early stages of AD and suggest that gallein could have therapeutic potential.

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Poster Abstracts

101. Ketogenic diet preserves working memory and increases dendritic arborization complexity by actin cytoskeleton dynamics regulation in aging mice.

Diego Acuña[1], Cristina Olmos[1], Gabriel Quiroz[1], Cameron Wehrfritz[2], Samah Shah[2], Birgit Schilling[2], Mitsunori Nomura[2], John C. Newman[2], Alejandro Acevedo[3], Estibaliz Ampuero[4], Úrsula Wyneken[5], Christian González-Billault[1,2,3]

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Aging is a complex process that compromises brain function progressively over time with increased risk of cognitive decline. Prefrontal cortex (PFC) is a brain region especially vulnerable to age due to significant changes in neuronal activity associated to synaptic dysfunction. Recent studies have demonstrated that nutritional interventions such as ketogenic diet (KD) preserves memory and cognition in old mice, nevertheless the molecular mechanisms specifically involved on those improvements are still unknown. Therefore, we investigated how KD administration at short-term (1 week), medium-term (6 weeks) and long- term (12 months) regulates the cortical synaptic proteome in aging mice. To this end, we performed mass spectrometry label-free data-independent acquisition by using pre-synaptic and post-synaptic enriched fractions derived from PFC of 26 months old mice. Bioinformatic analysis revealed that KD elicits discrete changes in the postsynaptic proteome at short-term administration, however, at medium and long-term administration KD preferentially modulates the presynaptic proteome through cAMP signaling pathway and actin cytoskeleton dynamics regulation. Interestingly, we observed preserved working memory and cAMP signaling pathway activation by increased expression of protein kinase A (PKA) and Brain Derived Neurotrophic Factor (BDNF) in PFC of aged mice fed with KD. In addition to these molecular changes, KD induced increased dendritic complexity in cortical neurons of layers 2/3 evidenced by morpho structural characterization in aging mice, a mechanism triggered by actin filaments stabilization. Overall, we show that KD modulates cortical proteome through key specific molecular pathways involved in synaptic connectivity and dendritic complexity preserving their function in aging mice.

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103. Axon development in the CNS tissue

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Axon development is a sophisticated process essential for neural circuit wiring. Studies over the last decade revealed new insights into this complex process. Perhaps most notable are studies addressing the effects of dimensionality on axon development. This has advanced our knowledge of axon behaviour in the presence of a third dimension. In these studies, more physiological environments exposed additional complex and dynamic aspects of axon development. However, many questions remain unanswered. During development, how do axons interact with the central nervous system (CNS) extracellular matrix? Do axonal growth cones behave differently when surrounded by such an environment? If so, what role does the growth cone cytoskeleton play during this process? Here, we aim to answer such questions by using organotypic slice cultures following *in utero* gene delivery in mouse embryos to visualise axon development *in situ*. Furthermore, we used the previously established Super-Resolution Shadow Imaging (SUSHI) method to image the interplay between axons and the extracellular environment.

104. Role of CREB3 transcription factors in neuronal differentiation models

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Neuronal processes such as growth and differentiation demand extensive membrane remodeling and protein redistribution. Formation of new neuritic processes required the adaptation of the secretory pathway, which is regulated, in multiple cell types, by members of the CREB3 family of transcription factors. CREB3 factors have emerged as signaling hubs for the regulation of Golgi homeostasis, integrating stimuli to control cellular events that impact membrane expansion and composition.

Although recent studies have focused on their role in the central nervous system, little is known about CREB3 factors in neuronal differentiation. Here, we analyze the expression of CREB3 family members using two models: i) NGF-induced PC12 cell differentiation and ii) differentiation of embryonic rat hippocampal neurons. The results show that NGF treatment or developing hippocampal neurons increases the expression of some proteins necessary for membrane transport (transport factors). In addition, a significant increase in CREB3L2 mRNA and protein levels is detected in response to NGF, and expression of both CREB3L1 and CREB3L2 increase in hippocampal neurons (1-7DIV). Interestingly, CREB3L2-overexpression hampers NGF-induced neurite outgrowth, while its inhibition enhances it. Consistent with this, CREB3L2-overexpressing PC12 cells show higher expression of GTPase Rab5 (a negative regulator of PC12 differentiation) than control cells. In contrast, in hippocampal neurons, CREB3L1 expression is higher than CREB3L2, and overexpression of a CREB3L1 dominant negative construct reduces axonal growth and increases Golgi fragmentation, compared to controls. Taken together, our findings strongly suggest that CREB3 factors are involved in neuronal differentiation by modulating the adaptation of the secretory pathway.

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105. The role of the BDNF Val66Met polymorphism on dopaminergic neurons structure and function.

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A SNP in the BDNF gene is present in approximately 25% of the world population. The SNP results in a valine for methionine substitution at position 66 (Val66Met) within the BDNF prodomain (pBDNF) sequence. This SNP is highly associated with increase occurrence of certain CNS disorders that include hippocampal dysfunction. We have proposed as a potential mechanism, that the pBDNF Met interacts with a complex of SorCS2 and p75NTR receptors, which results in a decrease of Rac1 activity, growth cone collapse and dendritic spine remodelling in hippocampal neurons. Interestingly, this SNP is also associated with disorders that involve CNS dopaminergic systems dysfunction (i.e. anxiety, addictions, and cognitive deficit and progression in Parkinson's disease). Therefore, we hypothesize that the Met variant of pBDNF alters the structure and function of dopaminergic neurons as it does in the hippocampus. To our surprise, only the endogenous expression of pBDNF Met (neurons cultured from BDNFMet/Met knock-in mice) reduces dopaminergic neurons axonal length, compared to the ectopic administration that was inert. Moreover, the presence of at least one Met allele has dopamine-related behavioural consequences. As zinc induces structural changes on the Met pBDNF (while the impact of this metal on the Val variant conformation is moderate) and free zinc is present in exocytic pathway organelles, we suggest that the Met pBDNF might be affecting the intracellular protein trafficking resulting in dopaminergic neuron dysfunction. The Val66Met SNP might have different mechanisms to alter hippocampal and dopaminergic circuits.

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106. Actin Flashes on microglial phagosomes are dependent on RhoA activity

Los destellos de actina en el fagosoma microglial son dependientes de la actividad de RhoA

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Microglia, the resident macrophages of the brain parenchyma, have gained attention during recent years for their implications in neurodegenerative diseases and the discovery of the TREM2-mediated disease associated microglial (DAM) phenotype in a variety of neuropathologies. Microglia are mesodermal derived cells from early yolk-sac myeloid precursors that have surveillance, neurotrophic and phagocytic functions in the CNS. Nevertheless, microglia phagocytic dynamics has been poorly studied, especially nascent phagosomal actin dynamics and reactive oxygen species (ROS) production. Nascent phagosomes are characterized by an abrupt NADPH Oxidase Complex 2 (NOX2) dependent increase in ROS production called *Oxidative Burst*. They are also characterized by a RhoGTPase dependent fine-tuning of actin dynamics mediated by -but not restricted to- RhoA and Rac1 activity. It has been previously reported that nascent to early phagosomes develop *actin flashes*, which are cyclic

bursts of actin polymerization surrounding the phagosome which may reclute actomyosin, mediating its contraction for efficient particle digestion. Using RhoA-2G-FRET in combination with actin-polymerized LifeAct-Ruby genetically encoded biosensors, we we studied the co-ocurrance of actin flashes and RhoA activation surrounding the microglial phagosome during nascent to early phagocytosis of *Saccharomyces cerevisiae* inactivated yeast particles. Interestingly, NOX2 dependent *oxidative burst* is time correlated with this phenomenon, giving interesting insights about possible signaling crosstalks between these two processes. In summary, these results may shed light on the understanding of microglial phagocytic dynamics involving the actin cytoskeleton and ROS production, which may be useful for the comprehension of disease-related phagocytic aberrations which are characteristic of numerous neurological diseases.

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107. 4R-tau induces AIS establishment in human neurons

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The axon initial segment (AIS) regulates action potential initiation and acts as a selective barrier for axonal cargo. This region presents string-like microtubule (MT) fascicles, Ankyrin-G (AnkG) and Spectrin linkers, and enrichment of ion channels. Tau, a MT-associated protein, is developmentally regulated to express isoforms with 3 or 4 MT binding domains (3R or 4R). Tau functions as a MT stabilizer and an axonal transport regulator, though recently, tau mutations have been associated with AIS defects. However, the molecular mechanisms by which tau modulates the AIS remain unknown.

We developed human neurons from iPSCs to perform tau conditional knockdowns or regulate tau isoform production. We also used murine hippocampal neurons from hTau mice to elucidate how tau regulates AIS positioning. We determined by immunofluorescence staining that tau isoforms can affect AIS maturation, positioning, and AnkG enrichment in murine hippocampal neurons. Furthermore, we characterized the maturation and positioning of the AIS in human neurons, and showed that the proportion of neurons with AIS as well as the intensity of AnkG staining increases over time. Additionally, we confirmed that 37 days-old human neurons produce 4R-tau *in vitro*. Interestingly, inducing endogenous 4R-tau production by *transplicing* significantly increased the percentage of human neurons with AIS. Finally, tau's effect on transport within the AIS was evaluated by live-imaging. Preliminarily, we showed that tau reduction selectively affects the anterograde transport of APP within the AIS. This work will reveal how tau modulates the AIS, which is essential for understanding the pathophysiological tau-derived effects found in tauopathies.

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108. Aβ assemblies induce amyloidogenesis in recycling endosomes through a Go/Gβγ signaling.

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Alzheimer´s disease (AD) is characterized by deposition of aggregated species of amyloid beta (Aβ) in the brain, which leads to progressive cognitive deficits and dementia. Aβ is generated by the successive cleavage of the amyloid precursor protein (APP), first by β-site APP cleaving enzyme 1 (BACE1) and subsequently by the γ-secretase complex. Conditions that enhance Aβ generation or reduce its clearance predispose to Aβ aggregation and the development of AD. In vitro studies have demonstrated that Aβ assemblies spark a feedforward loop heightening Aβ production. However, the underlying mechanism remains unknown. Here, we show that oligomers and fibrils of Aβ enhance colocalization and physical interaction of APP and BACE1 in recycling endosomes of human neurons derived from induced pluripotent stem cells and other cell types, which leads to exacerbated amyloidogenic processing of APP and intracellular accumulation of Aβ42. In cells overexpressing mutant forms of APP that are unable to bind Aβ or to activate Go protein, we have found that treatment with aggregated Aβ fail to increase colocalization of APP with BACE1 indicating that Aβ-APP/Go signaling is involved in this process. Moreover, inhibition of Gβγ subunit signaling with βARKct or gallein, prevent Aβ-dependent interaction of APP and BACE1 in endosomes, β-processing of APP and intracellular accumulation of Aβ42. Collectively, our findings uncover a signaling mechanism leading to a feedforward loop of amyloidogenesis that might contribute to Aβ pathology in early stages of AD and suggest that gallein could have therapeutic potential.

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109. SARA regulates TGFβ signalling during axonal development and regrowth of DRG neurons

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SARA (Smad Anchor for Receptor Activation) is a scaffold protein localized in the early endosomes (EEs) with trafficking and signaling functions. Transforming Growth Factor Beta (TGFβ) is one of the signaling pathway in which SARA is involved. Recently, in central nervous system, we have identified SARA as a negative regulator of the TGFβ pathway. In this work, we focus on the biological role of SARA and its potential association with the TGFβ pathway, during the development and regeneration of sensory neurons (peripheral nervous system). Our results shows that SARA and TβRI are endogenously expressed in embryonic dorsal root ganglion (DRG) neurons cultures. We found that SARA and TGFβ receptor type I interacts in the early endosomes (EEs) and the external addition of TGFβ potentiates this interaction. In addition, by loss-of-function experiments, SARA knockdown increases axonal development of embryonic DRGs. The same effect was observed with exogenous stimulation of the TGFβ pathway, together with an increase in the phosphorylation and nuclear translocation of Smad 2/3 proteins. During axonal regrowth of postnatal DRGs cultures, TGFβ treatment promotes both neurite elongation and branching and reduced growth cone size. TGFβ stimulation increases SARA expression along with the number and distribution of SARA endosomes both at the soma and axon level. Moreover, our preliminary results shows that the addition of TGFβ post-axotomy of DRG explants increase the number and length of axons. In summary, SARA regulates TGFβ signaling during axonal growth and regrowth of sensory neurons, key physiological processes in neurodevelopment.

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110. Mechanisms underlying the development of the axonal actin-spectrin Membrane Periodic Skeleton

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Our previous work highlighted the importance of the Actin-spectrin Membrane Periodic Skeleton (MPS) to be crucial for buffering axonal rest tension. Stretch-induced reversible spectrin unfolding model was found to release excess mechanical stress of the system (Dubey *et al*., 2020). However, the mechanisms underlying MPS formation and remodeling during neuronal development remain unclear.We showed that MPS prevalence in chick DRG axons increased with developmental stage (DIV 1-4; days in vitro), but the periodicity remained constant at ~ 190 nm. This increase in prevalence over time correlated with an increase in axonal rest tension. Perturbation of actin and microtubule dynamics affected MPS prevalence and stability and revealed the role of microtubule stability in MPS formation and maintenance. To investigate the mechanism of MPS development, we measured spectrin dynamics using FRAP. Interestingly, very little recovery was observed for spectrin even at DIV 1, when MPS was not prevalent. This suggests the recruitment of spectrin to a stable structure before the establishment of the MPS. Lat-A-treated axons revealed a faster initial recovery phase. The mobile fraction in early-stage axons (DIV1-2) was higher than that in the controls, although this was not the case for late-stage axons (DIV4). These experiments suggest the recruitment of spectrin to a stable network via a combination of actin and membrane binding which is later elaborated to form the MPS. This hypothesis is being tested using specific deletion constructs in spectrin knockout cells and is expected to reveal the sequence of interactions resulting in the development of the MPS.

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111. β2 and β3 spectrins: joint regulators of dendritic spine stability

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Recent studies using super-resolution microscopy have revealed the presence of a submembraneous actin-spectrin network known as the membrane periodic skeleton (MPS) shaping the structure of axons and dendrites. β spectrins are cytoskeletal proteins fundamental for the periodic organization in the axons and the somato-dendritic compartments. Interestingly, differently to what is observed in distal unmyelinated axons, in the somato-dendritic compartment two types of spectrins have been described: β2 and β3 spectrins. Yet their specific regulation and role there have been overlooked.

In this study we characterized the nano-organization of β2 and β3 spectrins, the role of their binding partners and their specific contribution to dendritic spine morphology and function.

112. Tau phosphorylation impedes functionality of protective tau envelopes

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Tau is an axon specific microtubule-associated protein implicated in a number of pathologies, collectively termed tauopathies. Tau contains multitude of phosphorylation sites and its (hyper)phosphorylation plays an important role in tauopathies. On the microtubule surface tau molecules can cooperatively assemble into a cohesive layer termed envelope. Tau envelopes regulate the action of other microtubule-associated proteins, such as the motility of molecular motors, and protect the microtubule against degradation by microtubule-severing enzymes. How is the formation and dynamics of tau envelopes regulated is, however, unknown. Here we show that tau phosphorylation impedes the formation and functioning of protective tau envelopes. Using a combination of reconstitution experiments and live cell imaging, we found that phosphorylated tau can be involved in the formation of tau envelopes. It slows down the envelope growth and can destabilize existing envelopes leading to their disassembly. Envelopes formed by phosphorylated tau have altered functionality, which leads to a decrease of their protective function. Combined, our results provide a link between pathology-related tau phosphorylation and decreased functioning of protective tau envelopes.

113. In silico modeling and analyses of the protein-protein interaction network of the actin/spectrin membrane-associated periodic skeleton of axons: Topological features of the network predicts functional segmentation of its components.

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The actin/spectrin membrane-associated periodic skeleton (MPS) is a periodic protein structure consisting of actin "rings" located transversely to the axon and separated every 190 nm by α/β-spectrin tetramers "spacers". In mature neurons, the MPS is present along almost the entire axonal axis. Despite being evolutionarily conserved, little is known about the functions of this structure. In the present work, we predicted the protein-protein interaction network (or the interactome) of the axonal MPS from public databases. To determine the mesoscale functional organization of the MPS interactome, we identified topological modules in the axonal interactone and performed a Gene Ontology enrichment analysis of the modules in which the MPS core proteins were located. Interestingly, we found that the core components of the MPS are located in many different modules that are enriched in different functional categories. For example, module 3, where the α/β-spectrin is located, is enriched in small GTPase signaling and protein phosphorylation, while module 27, where the α-adducin is located, is enriched in positive regulation of fatty acid transport and organic hydroxy compound biosynthetic process. In addition, we predicted the MPS axonal interactome from phylogenetically distant organisms and found that this network is conserved both at the level of components and interactions. Our analyses provide new insights into the structure and organization of the axonal MPS.

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114. Presynaptic plasticity at hippocampal mossy-fiber:CA3 synapses relies on non-enzymatic functions of disintegrin/metalloproteinase ADAM10

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A Disintegrin And Metalloproteinase 10 (ADAM10) is highly expressed in the central nervous system. It regulates the activity of many membrane proteins by shedding of their extracellular domains. ADAM10 therefore plays key roles in shaping neuronal networks. To date ADAM10 functions have been mainly

linked to the postsynapse and its enzymatic activity. We found that ADAM10 is highly enriched at presynaptic vesicles of mossy fiber (MF)-CA3 synapses in the hippocampus, the area involved in encoding short-term memory. Using conditional ADAM10 knockout mice we demonstrate that although these synapses have no drastic morphological defects, they show impairments in vesicle release and short-term plasticity. This phenotype depends on the cytosolic domain of ADAM10 rather than its ectoproteolytic activity. A mass spectrometry analysis of synaptic ADAM10 interactors revealed a network of presynaptic proteins controlling vesicle release and cytoskeletal proteins involved in organisation of vesicle pools thereby pointing to new possible ADAM10-dependent regulations of exocytic routes at MF-CA3 synapses.

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115. Purinergic receptor P2Y12 involves in Tau oligomers-induced microglial phagocytosis and endocytic trafficking via filopodia-associated actin remodelling

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Tau oligomers are escaped from damaged neurons, spread through synapses and enters extracellular space via various mechanisms such as- exosomes, neurotransmitters, membrane leakage and cell-to-cell connections, etc. Here, we found that Tau oligomers directly interacted with purinergic receptor P2Y12 that lead to microglial migration, activation and phagocytosis of Tau via remodelling membrane-associated actin structures such as- filopodia, lamellipodia and podosome. Moreover, P2Y12 signalling has impacted on Tau deposits degradation by forming actin microstructures. Here we find that microglia phagocytose full-length Tau (hTau40wt) oligomers via actin remodelling. Tau oligomers interact with P2Y12 that mediates microglial migration, lamellipodia-filopodia formation and MTOC polarization. Microglia degrade extracellular Tau oligomers' deposition by P2Y12-mediated chemotaxis via forming actin microstructures. Microglia internalize Tau oligomers by P2Y12-mediated endocytosis and accumulated in Rab7+ vesicles, but monomer traffics towards lysosomal degradation.

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116. Role of fast cycling RhoD GTPase in neuronal polarity and development

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Rho GTPases are a family of monomeric small signaling G proteins involved in the regulation of many cellular processes, including cell migration, cell polarity and cell cycling progression. One of the less studied members of this family is RhoD, the only Rho GTPase to be expressed exclusively in mammals. RhoD has the particularity of having increased intrinsic GDP/GTP exchange activity. Available data suggest RhoD has unique impacts on cytoskeletal reorganization, endosome dynamics and anterograde membrane trafficking, with signaling pathways and effectors distinct from those observed for typical Rho GTPases. Although, information about the activity and function of this protein in neuronal cell biology is limited, preliminary results of our lab propose an important role of RhoD at least in neuronal differentiation. Based on the above, the aim of this study is to obtain new data of RhoD activity and function in neuronal polarity and development. In order to study spatio-temporal activation patterns of RhoD activity, we successfully develop and characterize a fluorescence resonance energy transfer (FRET) biosensor that will be used in our neuronal systems. Furthermore, expression of RhoD activity mutants alters neuritic outgrowth and development in cultured hippocampal neurons as well as neuronal migration during cortical development in situ. In addition, using plasma membrane protein fused to GTP and engineered with reversible aggregation domains, we observe that expression of dominant negative RhoD mutant delays the anterograde trafficking of post-Golgi plasma membrane protein carriers. Altogether, our data suggest that RhoD plays an important role in neuronal development and neuritic outgrowth.

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117. Neuron-specific paxillin phosphorylation modulates alternative splicing program during early postnatal brain development

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Newborn neurons follow extrinsic and intrinsic instructions to complete a multi-stage maturation process. How newborn neurons switch on/off differential sets of protein isoforms for stage progression

had been unclear. Here, we report that neuron-specific phosphorylation at Ser119 (p-paxillinS119) of nuclear paxillin modulates the alternative RNA splicing program of maturing neurons. BDNF signaling and cdk5 activation elevate p-paxillinS119, whereas PP2A dephosphorylates it. Levels of nuclear p-paxillinS119 peak on the 7th day in vitro (7 DIV), forming nuclear speckles with key alternative splicing regulator p-SR proteins until 10 DIV. Blockade of Ser119 phosphorylation or mutations in the PY-type nuclear localization signal of paxillin significantly limits its nuclear translocation and alters the structural dynamics of the axon initial segment. Our mass spectrometry-based proteomics of 4 DIV neurons reveals that neuronal paxillin associates with multiple splicing regulators upon BDNF stimulation. By deploying a minigene splicing assay and isoform-specific RT-PCR approach to assess exon skipping efficiency, we also observed that paxillin and p-paxillinS119 are critical for timely RNA isoform switching. These findings support that neuron-specific p-paxillinS119 modulates the developmental RNA splicing program of newborn neurons.

118. Repurposing the Chromosome-Microtubule Coupling Machinery as a "Tuner" of Actin for Dendritic Branching.

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Dendrite branching is an essential process for building complex nervous systems. A neuron's dendritic patterns govern the number, distribution, and integration of inputs. Though significant progress has been made in understanding the signalling pathways that pattern the dendrite, little is known about the intrinsic mechanisms involved in sculpting the branches. The actin & microtubule cytoskeleton are critical to provide structure and exert force during dendrite branching. Our study reveals an unexpected role for the kinetochore, the chromosome-microtubule machinery, in shaping the dendrites of the mechanosensory neuron, PVD in *C. elegans*. The kinetochore is a highly conserved multiprotein complex whose canonical function is to connect chromosomes to microtubules during cell division. Kinetochore proteins are enriched in the PVD dendrites where they associate with the endosomal structures and are essential for establishing the dendritic pattern of PVD independent of its cell division function. Degradation of kinetochore proteins during PVD development results in dendrite branch fusion and overexpression of kinetochore leads to hypo-branching. Surprisingly, microtubule dynamics remain unchanged in the absence of kinetochores, but F-actin dynamics is altered during dendrite branching. We show that kinetochore proteins modulate F-actin polymerization mediated by the Rac GTPases. Thus, our work suggests that kinetochore proteins act as a "tuner" of actin polymerization to control dendrite patterning. Overall, these findings reveal an unexpected architect in dendritic branching and provide insight into crosstalk between microtubules and actin-based structures that remodel dendrites.

119. Gpr151-CSDE1 is an injury-responsive regulon for axon regeneration.

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Neurons respond to an axonal injury and change their biology that controls protein synthesis, cytoskeleton remodelling and epigenetics. These changes are required for regenerating their injured axons efficiently to achieve functional recovery, which essentially requires new protein synthesis for activating regeneration process. PTEN/TSC2/mTOR is a key regulator for protein local synthesis in axonal regeneration paradigm, however it is not well understood how the ribosomal mRNA is requlated under injury condition. Here we found an injury-responsive regulon of Gpr151 mRNA and CSDE1, a RNA-binding protein to control ribosomal mRNA in response to injury. Gpr151 mRNA is highly upregulated after sciatic nerve injury in dorsal root ganglion in mice. However, instead of being directed to ribosome for its translation, Gpr151 mRNA binds to CSDE1. The association of Gpr151 mRNA to CSDE1 stabilizes the assocaition of ribosome mRNA to CSDE1 and enhance their halflife. Potentiating this process by overexpressing the engineered 5'UTR of Gpr151 mRNA promotes axon regeneration in vitro and in vivo, which enhances the motor function recovery in mice. Here we present the first regulon controlling injury-responsive ribosome mRNA stability by Gpr151 mRNA and CSDE1.

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120. Microtubule dynamics determines DRG axon asymmetry and regenerative capacity.

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In dorsal root ganglion (DRG) neurons a single axon bifurcates into a peripheral and a central axon. Whereas the peripheral axon regenerates, the central axon only gains regenerative capacity after lesion to the peripheral axon – a paradigm know as conditioning lesion. A conditioning lesion induces a global increase in axonal transport that extends to DRG central axons, supporting their regeneration. To understand the mechanisms underlying central-peripheral DRG asymmetry and the conditioning effect, we analyzed the microtubule cytoskeleton of DRG axons. By live imaging of DRG explants from Thy1-EB3-GFP mice, we observed that microtubule dynamics is asymmetric in DRGs, with central axons displaying a more dynamic cytoskeleton. This asymmetry is recapitulated *in vitro* in pseudo-unipolar DRG neurons as a thin dynamic central-like axon and a stable thick peripheral-like axon develop. Importantly, in vivo, peripheral axon injury, but not central axon injury, stabilizes microtubules in both branches facilitating axon regeneration. Molecularly, in peripheral axons, the microtubule-severing proteins katanin and spastin are markedly decreased, while Tau and CRMP5 that protect microtubules from their severing action, are increased. In vivo, in the absence of spastin, the asymmetry of DRG axons in terms of microtubule dynamics and axonal transport is abolished and the conditioning lesion effect is obliterated. In summary, our work shows that microtubule dynamics determines DRG axon asymmetry and regenerative capacity**.** Our current experiments aim at understanding the molecular mechanisms underlying the asymmetric distribution of proteins in DRG axons.

121. Spectraplakin Couples Microtubule Orientation to Actin During Dendritic Pruning in Drosophila

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Neurite pruning, the elimination of specific axonal or dendritic branches, is an essential mechanism to refine developing neural circuitry. Following local microtubule and actin disassembly, *Drosophila* sensory c4da neurons prune their dendrites during metamorphosis. We previously found that the uniform plus end-in orientation of dendritic microtubules is required for efficient pruning by enabling their coordinated disassembly. How dendritic microtubule organization is established is only incompletely understood. Here, we show that the spectraplakin *short stop* (Shot), an actin-microtubule crosslinker, is required for c4da neuron dendritic pruning. We find that Shot genetically interacts with known factors governing dendritic microtubule organization, and loss of Shot itself misorients dendritic microtubules. Forced actin depolymerization also causes dendritic microtubule orientation defects, and the actin binding ability of Shot impinges on microtubule orientation. Finally, we show that inhibition of actin depolymerization during pruning also inhibits microtubule disassembly, indicating coordination of local cytoskeleton disassembly. Our data suggest that via Shot-mediated coupling, actin is necessary for establishing plus end-in microtubule orientation in dendrites, facilitating pruning.

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122. Structural basis of kinesin-1 autoinhibition and its dysregulation in neurological disease

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The autoinhibition of cytoskeletal motors is a fundamental regulatory mechanism that governs a vast array of cellular processes, ranging from intracellular transport to cell division and migration. These states are controlled by intramolecular or intracomplex interactions that serve to inhibit motor activity until it is required and prevent the wastage of adenosine 5′-triphosphate. We focus on the prototypic kinesin family member, kinesin-1, that is a key component of the axonal transport machinery. Over recent years, it has become clear that kinesin-1 activity is dysregulated in several different neurological and neurodegenerative diseases, including ALS. We have recently proposed a new model for assembly of the kinesin-1 heterotetramer and formation of the autoinhibited state - the lambda particle (Weijman et al. Science Advances (2022), 10.1126/sciadv.abp9660). Here I will present new data, building on this study, describing our latest insights into the mechanism of kinesin-1 autoinhibition and its pathological dysregulation by integrating of computational modelling, cryo-electron microscopy, and cell-based analyses.

124. Trio and CRMP2 interact to regulate axon morphology

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Trio is a RhoGEF critical for neurodevelopment: mutations in Trio's GEF domains are associated with profound neurodevelopmental disease, and neuronal knock-out of TRIO in mice results in gross anatomical defects and perinatal lethality. Although many studies have defined a role for Trio in neuronal processes including axon guidance and structural plasticity, how Trio is regulated in various contexts remains unclear. Through affinity purification-mass spectrometry we identified CRMP2 as a Trio-interacting protein. Western blotting reveals this interaction is likely regulated by CRMP2 phosphorylation. To understand the functional effect of the interaction, we used short hairpin RNA to knock-down Trio in primary hippocampal neurons and bath applied Semaphorin3A (Sema3A), an axon guidance cue that requires phosphorylated CRMP2 to induce growth cone collapse and axon repulsion. We observe that Trio knock-down suppresses Sema3A-induced axon repulsion. Sema3A repulsion requires phosphorylation of CRMP2 on Serine 522. To ascertain whether Trio may mediate Sema3A repulsion downstream of phosphorylated CRMP2, we expressed phosphonull CRMP2 (SA) and phosphomimetic CRMP2 S522D (SD) in hippocampal neurons and knocked-down Trio. Our results recapitulate findings that CRMP2-SA promotes axon branching, whereas CRMP2-SD suppresses axon branching. However, CRMP2-SD does not suppress axon branching in Trio knockdown neurons, suggesting a role for Trio downstream of CRMP2-SD. Our preliminary findings suggest phosphorylated CRMP2 interacts with Trio to suppress axon branching, possibly through RhoA activation. Biochemical determinants of the interaction and downstream cytoskeletal dynamics will be the subject of future experiments

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125. Pannexin-1 ablation promotes dendrite branching and dendritic spines formation in hippocampal neurons by modulating actin polymerization through Rac1/RhoA small-Rho GTPase.

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Small Rho GTPases, RhoA, Rac1, and Cdc42, play an essential role in regulating structural plasticity by controlling the assembly and stability of the actin cytoskeleton. However, the signals that control the activation or inhibition of the different small Rho GTPases in neuronal development and plasticity are relatively unknown. Pannexin-1 (Panx1) is a membrane protein that forms non-selective channels implicated in actin-dependent processes in neurons, such as cell migration and neurite extension, suggesting that Panx1 also be involved in other structural changes, such as those associated with synaptic plasticity. Here, we investigate if Panx1 channels modulate actin remodeling-dependent structural plasticity in hippocampal neurons through a mechanism that involves Rho GTPases activity. In the absence or blockade of Panx1, hippocampal neurons exhibited a higher dendritic complexity and dendritic spines density after induction of long-term chemical potentiation by glycine stimulation compared to control neurons. In addition, the absence or blockade of Panx1 channels stimulated the polymerization of F-actin and increased the expression of actin-related proteins.
Interestingly, pull-down assays revealed that the absence of Panx1 channels increased Rac1 activities, whereas inactivated RhoA under basal conditions. Nevertheless, glycine stimulation of hippocampal slices induced activation of RhoA, Cdc42, and Rac1. These modifications seem to rely on the actin–cytoskeleton dynamics as an increase in the actin polymerization and an imbalance between the Rac1/RhoA GTPase activities. Our findings highlight a novel interaction between Panx1 channels, actin, and Rho GTPases, which appear to be relevant for synapse stability.

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127. Common regulators of cell morphology in neurons and oligodendrocytes

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Process outgrowth and branching, and activity regulated structural changes are dynamic features of morphological alterations that are driven, to a large extent, by actin cytoskeletal dynamics and have been associated primarily with neuronal characteristics such as neurite outgrowth and synaptic

plasticity. However, there is increasing evidence that coordinated stoichiometries of actin filament (F-actin) assembly and disassembly originating within lamellipodial structures located at the tips of cellular processes, so called growth cones, also direct morphological changes in myelinating glia cells of the CNS, namely oligodendrocytes, thus suggesting common regulatory mechanisms and molecular players. Indeed, our previous findings point toward a role of calcium/calmodulin-dependent kinase type IIβ (CaMKIIβ) and particularly its actin binding domain in regulating the initiation of CNS myelination via a glutamate triggered mechanism that is reminiscent of the described role of CaMKIIβ in modifying actin cytoskeletal dynamics and dendritic spine morphology in response to neuronal activity and glutamate release. Furthermore, our more recent data indicate a possible involvement of the rho GTPase RhoB in modulating process outgrowth, and membrane trafficking associated with basolateral-to-apical like sorting in oligodendrocytes, and the initiation of CNS myelination in response to glutamate. Interestingly, in dendrites of layer II/III cortical neurons, RhoB has been similarly implicated in the regulation of process outgrowth and branching known to involve polarized, i.e. basolateral-to-apical like, membrane trafficking and protein sorting. Thus, taken together, our data uncover cytoskeletal regulators with potential analogous roles in neurons and oligodendrocytes; future studies will be geared toward dissecting similar and unique mechanistic features of these regulators.

128. Characterizing the nanoscale organization of the actin/spectrin membrane-associated periodic skeleton in rodent nerves using 3d-storm microscopy

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It has been recently discovered that axons and dendrites possess a particular arrangement of their cortical skeleton, referred to as the Membrane-associated actin/spectrin Periodic Skeleton (MPS). The MPS is a periodic protein structure consisting of actin “rings” located transversely to the axon and separated every 190 nm by α/β-spectrin tetramers “spacers”. The MPS can only be described using super-resolution (SR) microscopy approaches, since its spatial features lay below the diffraction limit of light. Most of published studies describe the MPS *in vitro* (in cultured neurons) and the precise organization of the spectrin “spacers” within each period has not been investigated in detail. We have thus begun a project to shed light into this in rodent nerve sections, that is, *in situ*, using 3D-STORM SR microscopy. We have first established a protocol for the examination of mouse optic and sciatic nerves preparations suitable for them by 3D-STORM. We have preliminary evidence for a model in which spectrin tetramers are arranged in each period at regular and fixed distances irrespective of axonal identity. In this sense, the number of spectrin tetramers scales with axon diameter. We have also evidenced that the localization of a spectrin tetramer in one period is correlated with the position of tetramers in neighboring periods, suggesting a structural constrain for their interaction with actin rings. We believe that describing the MPS at the nanometer scale *in situ* will provide meaningful insights into its possible structural components and its function.

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129. Characterization of the microtubule associated protein Tau and their effect in mitochondrial distribution and bioenergetics in directly induced neurons from tauopathy patients

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Millions of people worldwide live with the burden of tauopathy-associated neurodegenerative diseases including Alzheimer’s disease (AD) and Frontotemporal dementia (FTD), characterized by aberrant intracellular accumulation of hyperphosphorylated tau protein. Besides the canonical function of Tau associated to microtubule stabilization, novel insights point to a toxic gain of function and it has been proposed novel neuropathological mechanisms mediated by a toxic gain of function, associated to a deleterious effect on mitochondrial homeostatic processes, including traffic, dynamics, bioenergetics, but the underlying mechanisms are not completely understood. Directly induced neurons represent a valuable approach to study neurodegeneration in a human neuronal model, because they retain age-associated features that have been shown to be key for iNs to exhibit neurodegenerative

phenotypes. In this work, we obtained iNs from healthy subjects, sporadic AD and mutation-associated FTD patients starting from dermal fibroblasts following a direct conversion protocol. We assessed distribution and accumulation of hyperphosphorylated Tau and other cytoskeletal proteins in healthy, AD and FTD-iNs. In addition, we evaluated neuronal maturity and complexity in iNs. In parallel, we overexpressed hTau G389R in mTau-KO mouse primary neurons, a mutation associated with a severe phenotype of FTD. In both models, we assessed mitochondrial distribution and mitochondrial membrane potential to determine if there is an effect associated with Tau. Together, these results convey us novel insights of the molecular mechanisms mediating tau pathology in a human neuronal model, in sporadic and mutation-driven disease.

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130. Investigating the role of microtubule-associated motor protein KIFC1 at the synapse.

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Additional to serving their necessary roles of architecture and transport in both axons and dendrites, microtubules are crucial for the maintenance of synaptic connections. On the presynaptic side, microtubules fortify the distal axon ensuring that it does not pull away. On the postsynaptic side, microtubules fortify dendritic spines by transiently invading them. Synapse loss is downstream to many different neurodegenerative pathways. Surprisingly little has been done, to explore the mechanisms that ensure the fidelity of the synapse during health and how those mechanisms fail during disease. Here we have investigated a potential role for KIFC1, a kinesin-related motor protein known for its role in mitosis, but recently shown to also play critical roles in various aspects of the life of the neuron. In non-neuronal cells and *in vitro* assays, KIFC1 crosslinks microtubules to prevent them from sliding, binds to membrane proteins, and interacts with the plus end of the microtubule via EB1 and EB3 – all of which our present results demonstrate pertain to synapses. In studies on cultured rat hippocampal neurons, our results show dramatic synapse loss when KIFC1 is pharmacologically inhibited, with both sides of the synapse affected in different ways. The functional readout of this loss is reduced neuronal activity, recorded from hippocampal neurons cultured long-term on multi-electrode arrays. We posit that KIFC1 malfunction may underlie synapse loss during diseases. Moreover, new generations of drugs that strength KIFC1's attachment to microtubules may be a powerful tool for treating neurodegenerative diseases.

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131. Changes in nuclear shape induced by LPS in rat substantia nigra

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Introduction. In cells, the degree of spatial confinement can be determined by their largest and stiffest organelle, the nucleus. The nuclear shape result of the balance between the chromatin organization and nucleoskeleton. Although there are various immunostaining techniques for diagnostic studies using haematoxylin-eosin, the analysis of neuronal nuclear patterns has been neglected till date. **Objective.** To determine the nuclear morphometric patters in a lipopolysaccharide (LPS)-based endotoxemia rat model. **Method.** Male adult Wistar rats were used. Cellular stress was induced by LPS (500 mg/Kg, i.p.), an immune response activator. The tissue sections (5-10 µm) were immunostained with Hoechst 33342 (nuclear marker), CD11b/c-OX-42 (microglial marker) and tyrosine hydroxylase (dopaminergic phenotype marker). The evaluation of shape, size, number and hyper/hypochromic features of cell nuclei was performed by segmentation algorithm followed by an automatic thresholding analysis from ImageJ software. **Results.** During inflammation, degeneration and cell death processes in *substantia nigra*, particulars regarding the nuclear patterns were distinguished. LPS induced nuclear spatial constraints during neuronal injury. **Conclusion.** The analysis of nuclear patterns contributes to the understand of cellular process such as cell communication, cell survival, and neuronal homeostasis in stress-based animal models.

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132. Investigating Early Signaling in Axon and Dendrite Regeneration pathways

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The field of neuronal injury has focused on axons for hundreds of years, and progress has been made in understanding the physiological changes that occur in neurons during axon injury and subsequent regeneration. However, few labs have studied regeneration of dendrites. It is recognized that dendrites may be injured in the same ways as axons - during TBI, stroke, or ischemia. Without an axon, neurons cannot send signals, but without dendrites, a cell has no receptive field to integrate the information or sense the stimulus it should be sending; thus, we believe dendrite regeneration is as important as axon regeneration. We have heavily investigated Drosophila models of in vivo regeneration and recently published the first evidence of de novo dendrite regeneration in vertebrates (zebrafish). Initial observations that GCaMP lights up neurons during both axon and dendrite injury prompted us to ask: Is Ca++ influx ER/SOCE or VGCC dependent? Is it a functional signal or just a byproduct of opening the membrane? Here, we show that Cav1 and Cav3 VGCCs are the major source of Ca++ during injury and are required more strongly for dendrite than axon injury Ca++ influx. Knockdowns of Cav subunits reduce dendrite but not axon regeneration. We also found that cAMP is produced specifically after dendrite injury, and this is downstream of a Ca++ influx. We conclude that early signaling of calcium and cAMP after injury is important, and are working to understand what proteins respond to Ca++ and transduce this injury signal to initiate dendrite regeneration.

133. Coro1A role in TRIM67-regulated neuronal morphogenesis

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To establish functional neuronal circuitry, neurons progress through several developmental stages. The dramatic neuronal shape change during development depends on the cytoskeleton remodeling machinery. Previously, our lab discovered that the neuronally expressed E3 ubiquitin ligase TRIM67 is essential for appropriate neuronal morphogenesis. We found that TRIM67 localizes to the growth cone periphery and actin-rich filopodia structures, where it regulates filopodia number and stability. Interestingly, TRIM67 lacking neurons exhibit aberrant growth cone morphology, and have defects in netrin-dependent axon turning and branching. As E3 ubiquitin ligases typically have multiple substrates, we conducted an unbiased proximity-dependent biotin identification assay to identify putative TRIM67 substrates. **Coronin 1A(Coro1A)**, a conserved actin binding protein crucial for the regulation of actin dynamics, was identified as a potential TRIM67 interactor/substrate from our proteomic study. Although Coro1A is neuronally expressed, its role in neuronal development remains elusive. Through live-cell imaging and immunofluorescence staining, we discovered that Coro1A is enriched in the growth cones of developing neurons and localizes to the filopodia structures, particularly at the base region. Additionally, using neurons from *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ littermates we discovered that Coro1A is crucial for netrin-dependent growth cone morphology and axon branching, which phenocopies neurons lacking TRIM67. Here we validated Coro1A and TRIM67 are binding partners and have mapped out the domains that are required for TRIM67:Coro1A interaction. Finally, we discovered that Coro1A is ubiquitinated by immunoprecipitating Coro1A under denaturing conditions. These findings suggest a *novel role for Coronin 1A in regulating neuronal morphogenesis, and possibly functioning downstream of TRIM67-mediated pathway.*

134. A tale of two polarities: changes in microtubule polarity during the establishment of neuronal polarity

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By acting as a set of “highways”, the neuronal microtubule cytoskeleton enables the transport of cargoes such as mitochondria and synaptic proteins by kinesin and dynein motors. These motors carry cargoes along the uniformly plus-end out, stable microtubules in the axon, but also navigate the microtubules in dendrites, which are of mixed polarity and include dynamic and stable sub-populations. Interestingly however, in mature neurons, these dendritic microtubules are not randomly mixed, but are instead arranged into bundles of preferred polarity and post-translational modification (PTM) status; plus-end-in and acetylated (stable) microtubules are enriched centrally, while plus-end-out and

tyrosinated (dynamic) microtubules are enriched peripherally. However, it is not clear how or precisely when the microtubule cytoskeleton transitions from a non-polarized, centrosomal array to the polarized array with these apparent bundled sub-populations of microtubules found in mature neurons. To address this, we here use motor-PAINT, a method to map the orientations of microtubules and produce a super-resolution reconstruction of their organization. We applied this to study how the microtubule cytoskeleton changes as neurons progress through the early stages of development. Further, as the differential stability of the microtubule cytoskeleton itself has been implicated in neuronal polarization, we combined this approach with drug treatments and immunofluorescence microscopy of microtubule-associated proteins (MAPs) to elucidate the interplay between microtubule stability, bundling, positioning, and orientation during neuronal development. Our study provides insight into how the microtubule cytoskeleton rearranges during - and might actively contribute to - the different stages of neuronal development.

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135. Tau phosphorylation impedes functionality of protective tau envelopes

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Tau is an axon specific microtubule-associated protein implicated in a spectrum of neurodegenerative disorders, collectively termed tauopathies, many of which are associated with (hyper)phosphorylation of tau. Cooperatively, tau molecules can assemble into cohesive envelopes encircling the microtubule surface, regulating access and activity of other microtubule-associated proteins, for example, protecting microtubules against the activity of microtubule-severing enzymes. Phosphorylation of tau reduces the affinity of tau for the microtubule lattice, but it is unknown how it affects tau envelopes. Using a combination of reconstitution experiments and live cell imaging, here, we show that tau phosphorylation impedes the formation and function of tau envelopes. Phosphorylation of tau destabilizes tau envelopes, reducing their growth and causing their disassembly. Phosphorylated tau, nevertheless, does form envelopes and, importantly, integrates into existing envelopes assembled from non-phosphorylated tau. These envelopes, however, display a decreased protective function against the microtubule-severing enzyme katanin, indicating altered functionality of envelopes upon tau phosphorylation. Combined, our results provide a link between tau phosphorylation and the functioning of tau envelopes, suggesting that a loss of tau envelope cohesion, induced by tau phosphorylation, might contribute to neurodegeneration.

136. Gas7 links neuronal activity to the formation of new dendritic spines

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Brain stores new information by modifying connections between neurons. When new information is learnt, a group of neurons get activated and they are connected to each other via synapses. Dendritic spines are protrusions along neuronal dendrites where excitatory synapses are located. Dendritic spines are the first structures to protrude out from the dendrite to reach out to other neurons and establish a new connection. Thus, it is expected that neuronal activity enhances spine initiation. However, the molecular mechanisms linking neuronal activity to spine initiation are poorly known. Membrane binding BAR domain proteins are involved in spine initiation, but it is not known whether neuronal activity affects BAR domain proteins. Here, we used bicuculline to activate excitatory neurons in organotypic hippocampal slices. With this experimental setup we identified F-BAR domain containing Growth Arrest-Specific Protein (Gas7) as a novel spine initiation factor responding to neuron activity. Upon bicuculline addition, Gas7 clustered to create spine initiation hotspots, thus increasing the probability to form new spines in activated neurons. Gas7 clustering was dependent on PI3-kinase activity, and intact F-BAR and WW-domains. Gas7 overexpression enhanced N-WASP localization to clusters as well as it increased the clustering of actin. Arp2/3 complex was required for normal Gas7-induced actin clustering. Gas7 overexpression increased and knockdown decreased spine density in hippocampal pyramidal neurons. Taken together, we suggest that Gas7 creates platforms under the dendritic plasma membrane which facilitate spine initiation. These platforms grow upon

neuronal activation, increasing the probability of making new spines and new connections between active neurons.
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137. The Arp2/3 subunit isoform ARPC5L is required for Purkinje maintenance and motor coordination

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The Arp2/3 complex consisting of 7 evolutionary conserved subunits (ARP2, ARP3 and ARPC1-5) is unique among actin nucleators in its ability to generate branched actin filament networks. These actin networks and the force they generate are vital in maintaining the architecture of the cell and regulating numerous cellular processes including cell migration. In humans and mice, ARP3, ARPC1 and ARPC5, are encoded by two indpendent genes (ACTR3/3B, ARPC1A/B and ARPC5/5L), which leads to the formation of eight different Arp2/3 iso-complexes. We previously demonstrated that these Arp2/3 iso-complexes have different biochemical and cellular properties, but their physiological functions remain to be fully established. Previous work demonstrates that loss of function mutations in human ARPC1B lead to Wiskott-Aldrich syndrome like symptoms including severe inflammation and immunodeficiency. We have now examined the consequences of loss of ARPC5 and ARPC5L, which are 67% identical in mouse development and tissue homeostasis. Loss of ARPC5 results in embryonic lethality at E8.5. In contrast, *Arpc5l* null mice develop to full term but develop progressive gait ataxia from 2 months of age. Histological analysis reveals cerebellar atrophy concomitant with Purkinje cell death. Loss of ARPC5L specifically in Purkinje cells using the *Pcp2-cre* driver recapitulates the *Arpc5l* null mice phenotype. Our results identify an essential role Arp2/3 iso-complexes containing ARPC5L in Purkinje homeostasis. Our ongoing analysis is examining the impact of loss of ARPC5L on the actin organization in Purkinje cells in vivo and in vitro.
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138. LILAC: Enhanced actin imaging with an optogenetic Lifeact

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We have designed an improved Lifeact variant that binds to actin under the control of light using the LOV2 protein. This photoswitchable imaging reagent has two major advantages over standard actin probes. First, light control enables one to subtract the pre-illumination signal of the unbound label, yielding an enhanced view of F-actin dynamics in cells. Second, because the tool is essentially inert in the dark, it eliminates the common issue of actin network perturbations and cell sickness caused by Lifeact overexpression. We discuss factors to consider when using the probe, including a simple approach to tune the photoexcited-state lifetime in live cells.
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139. Anterograde Delivery Of Rab10-Organelles Regulates The Sorting Of Internalised Trkb For Retrograde Axonal Transport: A Matter Of Flexibility

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Neurons process real-time information coming from axon terminals to coordinate complex cellular responses, including gene expression, growth and plasticity. Input from distal axons is encoded as a stream of endocytic organelles termed signalling endosomes, which are targeted to the soma. Formation of these organelles depends on target-derived molecules, such as brain-derived neurotrophic factor (BDNF), which is recognised by TrkB receptors on the plasma membrane, endocytosed and transported to the soma along the microtubules network. Notwithstanding its physiological and neuropathological importance, the mechanism controlling the sorting of TrkB to signalling endosomes is currently unknown. In this work, we used primary mouse brain neurons and advanced confocal microscopy to uncover the small GTPase Rab10 as critical for TrkB sorting and propagation of BDNF signalling from axon terminals to the soma. We manipulated the expression and activity of Rab10 in neurons cultured in microfluidic devices, and study axonal transport, sorting of

receptors and recruitment of motor proteins. Our data demonstrate that Rab10 defines a class of axonal organelles, which are rapidly mobilised towards the axon terminal upon BDNF stimulation, enabling the axon to fine-tune retrograde signalling depending on BDNF availability at the synapse. These results suggest that Rab10 provides flexibility to the endosomal system and help to clarify the neuroprotective phenotype recently associated to Rab10 polymorphisms in Alzheimer's disease, providing novel therapeutic targets for neurodegenerative conditions.

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140: Rho GTPases as a potential chemosensitizer of GBM resistant tumor to TMZ and cisplatin in a p53-dependent mechanism

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Rho GTPases several cellular processes related to tumor progression dictating invasiveness and aggressiveness in GBM, a very aggressive tumor which effective therapies and treatments remain obscure. Here we proposed that Rho GTPase is mediating genomic stability of GBM and their resistance to TMZ and cisplatin (CP) in a p53-dependent mechanism. Therefore, we selected four GBM cell lines with different p53 status – U87-MG (p53-wt) and T98G, U251-MG and U138-MG (p53-mut) and subjected them to Rho inhibition by C3 toxin followed by DNA damage with TMZ or CP exposure. Prolonged exposure to these drugs induces the transcription of Rho GTPases regulatory genes in U87-MG cells, as confirmed by pulldown assays showing increased Rho activity. IC_{50} for TMZ and CP, as well as survival and proliferation, was decreased by Rho inhibition in wt-p53 cells. Host cell reactivation assay showed that DNA repair capacity is diminished by Rho inhibition only in wt-p53 cells exposed to TMZ and CP drugs. Then, we submitted U87-MG to p53 knockdown, which interestingly prevented Rho inhibition-induced chemosensitivity and DNA repair impairment. Next, working with subpopulation of cells resistant to these drugs, the consecutive exposition to TMZ or CP culminated in enhanced p53 phosphorylation, p53 nuclear localization and F-actin polymerization. These results suggest a role of Rho pathway and actin dynamics in mechanisms of DNA repair and acquired resistance to both genotoxic drugs, and moreover, that these via might be a fragile point of usual therapies the cause GBM resistance dependently on p53 transcriptional activity.

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141: Rho pathway: a fragile point in the GBM resistance to radiotherapy

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Glioblastoma (GBM) is the most aggressive brain tumor characterized by rapid cellular infiltration of brain tissue and is routinely treated with ionizing radiation (IR), but therapy resistance is inevitably recurring. The actin cytoskeleton of GBM cooperates with its high invasiveness but remains unclear whether Rho GTPases modulate DNA repair and sensitivity to therapies. To show that Rho GTPase pathway plays a role in the IR-resistance of GBM through modulation of DNA repair mechanisms, GBM cells with p53 wild-type (wt-p53) or p53 mutated (mut-p53) status were subjected to Rho inhibition by C3 toxin or inhibition of actin polymerization by Cytochalasin D (CytoD), followed by irradiation. Rho inhibition increases the sensitivity of gliomas to IR by increasing DNA double-strand breaks and delaying the DNA Damage Response and DNA repair by NHEJ in wt-p53 cells. p53 knockdown reverses this phenotype reducing p21 expression and Rho activity, whereas the p53 reactivation in mut-p53 cells with PRIMA-1 reverses these effects. The p53 and Rho interdependence resides on the nuclear p53 translocation facilitated by G-actin and enhanced by IR. Wt-p53 cells were subjected to rigorous cycles of low doses of IR, to obtain resistant sublines, which show altered morphology and stress fiber formation: the inhibition of Rho or actin polymerization decreases cell viability in a p53-dependent manner reversing the resistance phenotype. The p53 silencing reverses the sensitization of IR-resistant cells caused by Rho inhibition. Therefore, targeting Rho GTPase pathway components significantly diminishes GMB resistance to IR suggesting the potential value of these proteins as therapeutic targets.

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142: Effect of mechanical stiffness on axonal βII-spectrin in three dimensions.

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Introduction: β-spectrin forms a sub-membranous skeleton responsible for maintaining cell adaptation to changing environments and axon stability in response to mechanical stress. Recently, we demonstrated that substrate stiffness, a mechanical property, negatively impacts axon elongation in a three-dimensional culture (3D environment). The reduced elongation was accompanied by a strong impact on axonal F-actin cytoskeleton. Almost no F-actin rich growth cones were recognized, and F-actin staining was strongly reduced in the axonal compartment. However, the impact of this mechanical property of the stiff 3D environment on other components of the axonal cytoskeleton remains unclear. **Aims:** To evaluate the impact of a 3D stiff environment on βII-spectrin in two axonal compartments: axon and Growth Cone (GC). **Methods:** Superior cervical ganglion explants from neonatal (4-6 days old) rats were cultured in soft (G´= 18.5 Pa) and stiff (G´= 22.7 Pa) Collagen-3D matrices. At 2 days after seeding, 3D-cultures were processed to evaluate: a) the pattern of expression of βII-spectrin; b) βII-spectrin levels of fluorescence intensity through immunocytochemistry and quantitative fluorescence microscopy. **Results:** Sympathetic axons grown in a 3D stiff environment showed higher spectrin levels than those grown in soft 3D matrices. Interestingly, some axons showed regularly spaced βII-spectrin-immunoreactivity along them in both culture conditions (soft and stiff). Also, preliminary observations indicate that, in soft matrices, βII-spectrin accumulates in axonal tips (near filopodia/GC). **Conclusion:** Our results suggest that ECM stiffness increases axonal βII-spectrin, which could be indicating an adaptive behavior triggered by mechanical interactions of sympathetic axons with a stiff 3D environment.

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143. AP-4 deficiency influences ApoER2 trafficking and Reelin signaling

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Reelin is a secreted glycoprotein with several functions in neurodevelopment and the adult brain. Reelin triggers a complex signaling pathway upon binding to ApoER2 or VLDL-R receptors belonging to the Low-Density lipoprotein receptor family. This pathway has protective effects in neurodegenerative diseases such as Alzheimer's, and its reduction has been implicated in neuropsychiatric disorders, including depression, schizophrenia, and autistic spectrum disorder. Our laboratory has been working on aspects related to the regulation of ApoER2, the consequences of alterations of its trafficking on Reelin signaling, and how this signaling pathway is associated with homeostatic and neuroprotective effects. Here we present our recent and ongoing work on the role of the adaptor complex AP-4, mutated in Hereditary Spastic Paraplegia (HSP), in Reelin signaling. AP-4 binds ApoER2, regulating its polarized distribution and protein stability. Reelin effects in neuronal models, mouse hippocampal neurons, and human iPSC-derived i3 cortical neurons were affected by AP-4 deficiency. These effects are the activation of pCREB and the deployment of Golgi, which were both decreased. Moreover, our data underscored a new role of Reelin in activating autophagy by promoting ATG9 trafficking to the axon. This response was also dependent on the presence of the AP-4 adaptor complex. Overall this work reinforces the relevant role of Reelin pathway in neuronal homeostasis, showing how the condition of HSP affects relevant functions of Reelin signaling associated with neurodevelopment and learning and memory processes.

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144. Non-degradative ubiquitination of VASP regulates actin dynamics

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Filopodia are dynamic, actin-rich structures that extend outward from the cell to explore and respond to cues in the local environment. The actin polymerase VASP is a component of the filopodial tip complex, where it regulates actin polymerization and filopodial dynamics. Previously, we showed that VASP transiently co-localizes with the brain-enriched E3 ubiquitin ligase TRIM9 at the tips of neuronal filopodia. TRIM9 was required for the reversible, non-degradative ubiquitination of VASP and this modification was associated with decreased filopodia number and stability. Furthermore, the axon guidance cue netrin promoted deubiquitination of VASP. We hypothesize mono or multi-monoubiquitination of VASP is a mechanism to negatively regulate actin dynamics by blocking

VASP and actin interactions. Through mass spectrometry, we identified numerous lysine residues that are ubiquitinated in VASP, including at K240 and K286. Using chemical ubiquitination, we created purified, ubiquitinated VASP to evaluate protein activity through in vitro assays. We observe no changes in the tetramerization of ubiquitinated VASP through mass photometry. Although single ubiquitinated constructs showed minor changes in actin cosedimentation assays, we observed decreased actin bundling and binding activity of a VASP construct ubiquitinated at both K240 and K286 (2x-Ub). Furthermore, the elongation rate of 2x-Ub VASP was significantly reduced. Currently, we are visualizing VASP and actin interactions through microscopy-based assays to further characterize actin binding, bundling and elongation. Future work will explore the relationship between ubiquitinated VASP and other actin regulatory proteins.

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145. The E3 ubiquitin ligase TRIM9 regulates actin dynamics and synapse formation

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In neurons, actin-rich filopodia are critical at many stages of morphogenesis, including neuritogenesis, axon guidance, and dendritic spine formation. Defects in these critical developmental processes can result in improper synaptic connectivity, neurodevelopmental disorders, and psychiatric syndromes. Previously, we have demonstrated the E3 ubiquitin ligase TRIM9 localizes to growth cone filopodia and regulates axon pathfinding downstream of the guidance cue netrin. *Trim9*$^{-/-}$ mice have overt spatial learning memory deficits, yet the role of TRIM9 in synapse formation and maintenance is unknown. Here we show TRIM9 is enriched in the post-synaptic density following differential centrifugation, suggesting a role for TRIM9 in dendritic spines. We find Netrin-dependent increases in dendritic spine number, synapse maturation, and neuronal firing in vitro, and all these responses are abrogated in *Trim9*$^{-/-}$ neurons. Furthermore, neurons over-expressing TRIM9 show defects in spine maturation but not dendritic spine number. In vivo, we demonstrate that loss of *Trim9* alters the proteome of the postsynaptic density. In particular, we observe changes in numerous cytoskeletal proteins, including the Arp2/3 complex. Ongoing work is investigating the functional consequence of these changes to Arp2/3 accumulation in dendritic spines and neurotransmitter receptors on the neuronal surface.

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146. Mechanisms directing dendrite morphology: Pdlim5:PalmD protein complexes.

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We seek to understand cytoskeletally-based processes determining the morphology of dendrites. We are examining novel complexes of Pdlim5, a scaffold that that we earlier revealed binds to the C-terminal PDZ-binding-motif of delta-catenin and strongly promotes dendrite branching (*J Cell Biol* 2020). While Pdlim5 and delta-catenin each promote such branching, how this comes about on a mechanistic level constitutes a gap in knowledge. Our experimental findings together with use of predictive protein-folding methods leads us to propose that Pdlim5 possesses closed versus open conformations, as well as a central region with potent signaling activity involved in cell shaping. Indeed, our experimental findings support an intra-molecular binding between the N-terminal (PDZ) and C-terminal (LIM) domains of Pdlim5, with this association in turn modulated by the binding of delta-catenin (delta's PDZ-motif). In response to Pdlim5's closed versus open states, we expect that cytoskeletally-important partners of Pdlim5 have differential abilities to form a complex with Pdlim5 in executing functions relevant to dendrite shaping. In particular, we uncovered the direct association of Pdlim5 with PalmD. PalmD has reported linkages to the cytoskeleton (*e.g.*, *via* adducin/ spectrin), modulates membrane shape, and as we reveal here has notable dendrite branching activity. In parallel, other cytoskeletal regulators (e.g., cortactin & alpha-actinin) appear to be in the Pdlim5:PalmD complex, offering continuing opportunities ahead to address underlying mechanisms. In summary, using primary rat hippocampal neurons together with HT22 mouse hippocampal cells, we have revealed contributions of a novel Pdlim5:PalmD complex to the shaping of neurons.

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147. Elucidating the function of axon initial segment microtubules in vivo

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Neurons are highly polarized cells—axons and dendrites have distinct functions, morphologies, and molecular compositions. Two powerful contributors to neuronal polarity are 1) the axon initial segment (AIS), a domain at the beginning of the axon that initiates action potentials, and 2) microtubules (MTs), cytoskeletal filaments involved in axon specification and cargo trafficking. Disrupting either structure disrupts the other and causes broad downstream defects in neuronal polarity and function, suggesting that the AIS and MTs work together to control neuronal polarity. However, the interplay between the AIS and MTs remains incompletely understood. Furthermore, much of what is known comes from studies of cultured neurons. Here, I present our recent efforts to determine how the AIS and axonal MTs influence each other *in vivo*. A better understanding of this relationship will shed light on the mechanisms by which the AIS and axonal MT cytoskeleton cooperate to establish and maintain neuronal polarity.
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148. MAPping the role of Map1b in Periventricular Heterotopia: the search for a common mechanism

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Periventricular heterotopia (PH) is a cortical malformation characterized by groups of mispositioned neurons adjacent to the lateral ventricles that is commonly associated with epilepsy. It is genetically diverse, and its underlying mechanisms are not fully understood. While recent research suggests that neuronal differentiation can also be affected, it has traditionally been considered a neuronal migration disorder, supported by the identification of mutations in neuronal-enriched cytoskeletal genes. In this study, we examine the effects of PH-associated mutations in the MAP1B gene (a microtubule-associated protein gene highly expressed in developing neurons) in the mouse cortex. We used a knock-down approach to mimic the predicted loss-of-function variants, and using in utero electroporation we found that the knock-down (KD) of Map1b resulted in ectopic positioning of cells which persisted postnatally. Besides alterations in neuronal migration analyzed by live imaging of acute slices, we also observed neuronal differentiation defects both in vivo as well as in vitro. Single cell sequencing revealed a slower differentiation pace, and points towards cell division defects in progenitors and cell adhesion defects in migrating neurons. Importantly, subpopulation analysis revealed the presence of an altered neuronal population that is almost exclusively deriving from Map1b-KD cells, showing the presence of a transcriptomically different group of neurons. These findings suggest deficits in both neuronal differentiation and migration within PH, and we are further elucidating whether neurogenesis may be the major cause underlying this classic ‘migration’ disorder.
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149. Formin-mediated actin filament regulation in the axon initial segment of hippocampal neurons

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The location of the axon initial segment (AIS) at the junction between the soma and axon of neurons makes it instrumental in maintaining neural polarity and as the site for action potential generation. Research suggests actin structures are involved in AIS activity dependent structural plasticity, maintenance, and vesicle sorting. Thus, our aim is to elucidate the role of dynamic actin filaments in the AIS with regards to these processes. We utilized pharmacological treatments to inhibit the activity of actin-associated proteins or availability actin monomers. Experiments make use of a combination of widefield and super-resolution microscopy, as the specific organization of proteins at the nanoscale is crucial to understanding how relevant actin structures might contribute to AIS function. We examined the stability of the AIS after treatment with the formin-inhibiting drug SMIFH2 and showed that both the AIS and the actin rings of the periodic submembrane cytoskeleton are resistant to disruption via formin inhibition. We also showed that longitudinal actin filaments (actin trails) which are lost with this same SMIFH2 treatment colocalize with the formin Daam1, and that Daam1 is upregulated in the AIS compared to the distal axon. Actin trails are not unique to the AIS and likely have a variety of functions and regulators depending on their location. However, we have implicated them in AIS structural plasticity by showing that actin trails are upregulated under conditions which induce AIS plasticity. Further, AIS plasticity is blocked by SMIFH2 treatment.

150. Tau “islands” affect axonal transport in vitro and in vivo

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Tau is a protein abundantly expressed in neurons where it modulates the stability of axonal microtubules, thus contributing to the regulation of axonal transport of several organelles. Tau aggregates in a group of neurodegenerative diseases named tauopathies, which include frontotemporal dementia (FTD) and Alzheimer’s disease. Recent *in vitro* work has uncovered the existence of tau “islands”, a microtubule-bound multimeric state of tau, which is distinct from pathological aggregates. However, whether these structures exist in intact neurons remains unclear. In cultured mouse neurons and *in vivo*, we found that human tau shows regions of higher density along axons, reminiscent of tau islands. FTD-linked mutations, known to increase pathological phosphorylation and aggregation of tau, induces larger islands, an effect that is reversed by inhibition of p38 MAPK, known to phosphorylate tau at multiple sites. Functionally, we found that axonal transport of BDNF-containing secretory granules is affected by mutant tau as assessed both *in vitro* and *in vivo* by using a new assay based on two-photon microscopy. Interestingly, this impairment occurred very early on, before overt tau aggregation. Inhibition of p38 MAPK was able to partially rescue these defects in axonal transport both *in vitro* and *in vivo*. Our data suggests that tau island size regulates axonal transport, an effect dependent on tau phosphorylation. Inefficient organelles transport may have severe consequences on the activity and plasticity of neuronal circuits. The evidence that reducing tau phosphorylation by inhibiting p38 MAPK potentiated axonal transport points towards inhibition of p38 MAPK as a promising therapeutic strategy in tauopathies

151. Regulation of Axonal Transport in Nerve Branches

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Neurons have complex branched morphologies that pose huge challenges to microtubule-based axonal transport. To understand how such challenge is met during synaptic development and plasticity, we have investigated transport regulation at branch junctions. Using primary sensory neurons of dorsal root ganglion (DRG), we found that transport at branch junctions is not random but rather correlated with branch lengths and growth cone motility. Using an optogenetic approach, we showed that manipulating growth cone signaling could rapidly alter the preference of transport. This feature, termed transport selectivity, is seen with lysosomes and synaptic vesicles but not BDNF vesicles. It is mediated by KIF1 family of kinesin motors, as knocking down KIF1A/1B abolished signaling-dependent preference for lysosome transport. To further understand the underlying mechanism, we have developed an artificial cargo system and combine it with light-induced motor coupling to probe motor function and local environment. Using this system, we showed that MAP7, a protein that is enriched at branch junctions, reduced KIF1 mediated transport, and verified the defects of several human KIF1 mutations on transport. Currently, we are determining which kinesin motor supports transport selectivity and whether MAP7 is required for such regulation. These studies have begun to uncover molecular mechanisms that are critical to regulating axonal transport through complex branched morphologies.

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152. Microtubule modification profiles by the detyrosination enzymes

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The detyrosination/tyrosination cycle controls microtubule functions in healthy neurons and its deregulations lead to neurodevelopmental defects or neurodegeneration. This cycle is characterized by the enzymatic removal and re-addition of a gene-encoded tyrosine residue at the C-terminus of α-tubulin. It involves at least four enzymes including the tubulin tyrosine ligase (TTL) and three recently discovered detyrosinases, the enzymatic complexes composed of a vasohibin (VASH1 or VASH2) and

a small vasohibin-binding protein (SVBP), and MATCAP. SVBP acts as a chaperone and co-factor for the VASHs whose mode of action is little known.
Here, we show in reconstituted systems and cells that VASH1-SVBP and VASH2-SVBP drive the global and local detyrosination of microtubules, respectively. We solved the cryo-electron microscopy structure of VASH2-SVBP bound to microtubules, revealing a different microtubule-binding configuration of its central catalytic region compared to VASH1-SVBP. We show that the divergent mode of detyrosination between the two enzymes is correlated with the microtubule-binding properties of their disordered N- and C-terminal regions. Specifically, the N-terminal region is responsible for a significantly longer residence time of VASH2-SVBP on microtubules. We suggest that this VASH region is critical for microtubule-detachment and diffusion of VASH-SVBP enzymes on lattices.
Our results suggest a mechanism by which VASH-SVBP enzymes could generate distinct microtubule subpopulations and confined areas of detyrosinated lattices to drive various microtubule-based cellular functions. We are now examining the microtubule-binding behavior and activity of MATCAP. These different functionings may have implications on the microtubule diversity found in neurons that contain the three detyrosinating enzymes.
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153. Human neural rosettes secrete extracellular vesicles enriched in neural and glial cellular components

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Extracellular vesicles (EVs) are a heterogeneous group of membrane-enclosed nanovesicles that shuttle bioactive molecules (*e.g.* proteins, lipids and RNAs) between cells. EVs participate in stem cell renewal and differentiation during central nervous system (CNS) development. Human neural rosettes (hNRs) are radial structures of neuroepithelial cells that assemble from human induced pluripotent stem cells (hiPSCs) differentiation into neural and glial cells. hNRs are an *in vitro* model that recapitulates some stages of the neural tube morphogenesis and expresses molecular components expressed *in vivo* during CNS development. Here we showed that hiPSCs and hNRs secrete EVs (hiPSC-EVs and hNR-EVs, respectively) enriched in proteins associated with EVs and the endomembrane system. hNR-EVs are specifically enriched in cellular components from neural and glial origin. Remarkably, among glial-derived components associated with hNR-EVs we found the myelin proteolipid protein (PLP1). Although PLP1 is the major protein of myelin in the CNS it is also expressed at embryonic stages in animals, i.e., long before myelin is assembled. Through *in silico* analysis we showed that hNRs express PLP1 at various time points during development and different culture conditions. Moreover, PLP1 localizes radially along the lumen and edges of hNRs. These results indicate that EVs secreted by hNRs exhibit neural and glial cellular components and might provide an innovative approach to study human neural tube formation and EVs biological activity during CNS development.
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154. Glutamate and early functional NMDA Receptors contribute to hippocampal axonal elongation through Rac1 activity, which modulates both actin cytoskeleton dynamics and NOX2-mediated H2O2 production.

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NMDA Receptors (NMDARs), which are essential for maturation, neurotransmission and functionality of the nervous system, mediate Ca^{2+} influx following activation by the neurotransmitter glutamate. However, the role of glutamate and NMDARs during early neuronal development has not been described. Here, we found that functional NMDARs were expressed during neuronal polarity acquisition. In addition, endogenous and ectopically expressed NMDARs were distributed to the axonal compartment early in development. Interestingly, during early development neurons released glutamate. Moreover, pharmacological and genetic NMDARs loss- and gain-of-function altered

neuronal polarization and axonal elongation by a mechanism that involved actin cytoskeleton rearrangement at the neuronal growth cone and regulation of the intracellular hydrogen peroxide (H_2O_2) content, via the Rho GTPase Rac1. In fact, the optogenetic activation of a photoactivatable Rac1 version simultaneously promotes both lamellipodia and H_2O_2 formation suggesting the co-occurrence of these processes. Thus, NMDARs signaling promotes dual Rac1 functions, which mediate actin cytoskeletal remodeling and H_2O_2 production by the NOX2 complex. Altogether, these findings suggest that early spontaneous glutamate release activates NMDARs to support neuronal development before synapse formation, indicating that glutamate is necessary for neurotransmission and also for early neuronal development and axonal growth.

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155. Wnt7b stimulates axonal differentiation and development.

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In the nervous system, the establishment and maintenance of neuronal polarization is crucial for correct development and function. This asymmetry is generated in response to intrinsic and extrinsic signaling molecules. Wnts proteins are known regulators of cell polarity and has also been shown to be a symmetry-breaking factor in proliferating cells. In this study, we set out to investigate the role of Wnt7b signaling in the polarization of hippocampal neurons. We previously showed that Wnt7b affects the establishment of neuronal polarity and axonal outgrowth since Wnt7b stimulated neurons evidenced an increase in axonal length. We then focused our attention on short time Wnt7b treatment analyzing tau-1 immunoreactivity after 6 h in vitro. Surprisingly, we found that neurons exposed to Wnt7b showed higher tau-1 reactivity, a typical feature of axons, compared to controls. After that, we observed that Wnt7b stimulated neurons developed longer and more complex axon at 20 HIV. To go further, we examined the intracellular signaling pathway triggered by Wnt7b. Thus, Wnt proteins may signal through canonical or non-canonical pathway to modulate neuronal development and maturation. Pharmacological inhibition of JNK mediated non-canonical pathway abolished Wnt7b axonal effects. Consistently, we then observed that Wnt7b treatment increases the JNK activity at the axonal growth cone. Later studies evidenced that Wnt7b also increases microtubule stability around 30% compared to controls.

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156. Alpha synuclein induces differential effects in protein intracellular trafficking

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Alpha synuclein (AS) is a widely studied protein for its role in different neurodegenerative diseases such as Parkinson's disease and a group of pathologies called synucleinopathies. Despite being the focus of several studies, its normal function it is still partly unknown and the manner in which this protein is involved in neuronal death is still not fully elucidated. One of the most interesting hypothesis suggests that AS might be affecting the intracellular vesicle trafficking and consequently affecting neuronal survival (Cooper AA 2006; experiments in yeast). Our focus is to study the effects of AS on the intracellular trafficking in mammalian neurons and the impact on neuronal structure and function. For this aim, we use the FM exocytic pathway synchronization system to analyze the effect of AS on protein trafficking dynamics in cultured neurons. We found that AS reduces protein trafficking from the Golgi apparatus to neuronal processes, but not in a general way. Instead, our results show that AS affects p75NTR trafficking, while it does not have an impact on Transferrin Receptor. Interestingly, AS alters p75NTR vesicles TGN fission, but not the Transferrin Receptor. Our preliminary findings suggest that AS modifies the actin cytoskeleton dynamics, and we propose that this effect is a potential mechanism for the altered trafficking detected. These findings shed light on the mechanism by which AS may be acting in neurodegenerative diseases.

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157. Post-translational modifications of synaptic microtubules in health and disease

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Neuronal microtubules provide the architectural elements to achieve and maintain complex neuronal shapes and long distance transport. Emerging studies support the idea that microtubules also control central aspects of synaptic architecture and plasticity. Dynamic invasion of microtubules into dendritic spines (post-synaptic compartment) correlates with changes in spine morphology and synaptic transmission. Increased post-translational modifications of synaptic microtubules (detyrosination and acetylation) decreased microtubule invasion into spines and their maintenance. In the pathological context of Alzheimer's disease, decreased tubulin re-tyrosination and increased microtubule detyrosination and acetylation reduced microtubule dynamics and invasion of dendritic spines. We showed that synapses visited by dynamic microtubules were more resistant to amyloid-β oligomeric peptide toxicity. By increasing tubulin re-tyrosination, we restored microtubule entry into spines and prevented amyloid-β peptide-induced synapse loss. We also showed that an imbalanced level of microtubule tyrosination/deyrosination correlated with an imbalanced level of microtubule acetylation in Alzheimer's disease, independent of the levels of enzymes regulating tubulin acetylation. These results suggest that reduced microtubule dynamics associated with impaired tubulin re-tyrosination may contribute to the accumulation of tubulin acetylation that we detected in Alzheimer's disease. Overall, our results demonstrated that pleiotropic effects caused by a dysfunctional synaptic microtubule cytoskeleton may therefore represent a key point of vulnerability for neurons and a potential trigger for neurodegenerative disease.

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158. “Unroofing” cells to investigate the properties of the microtubule network.

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Investigation of microtubule (MT) properties is frequently done using cell-free reconstitution assays which are very clean systems where MTs can be polymerized from purified tubulin and subjected to manipulations using for example microtubule-associated proteins or molecular motors. However, these *in vitro* assays are also artificial systems with a non-physiological arrangement of the MT array. They are lacking the tight regulation and the multitude of interactors present in cells. Here we present an experimental model to study microtubules where HeLa cells are “unroofed” by mildly extracting the plasma membrane and cytosol, leaving cellular MTs intact without the need for fixation. This results in a microtubule network from cellular origin which can be used over prolonged periods for manipulation. Using the “unroofing”, important functions of microtubules are preserved. Motor proteins show motility on the microtubules and purified vesicles can be transported along them. This model can also be used to study post-translational modifications of microtubules, like acetylation of α-Tubulin Lysin-40 which mainly occurs on microtubules rather than on tubulin dimers. By combining the “unroofing” with a stable α-tubulin acetyltransferase 1 (αTAT1) knock-down HeLa cell line we are examining an interplay between processive cargo trafficking, microtubule damage and repair and the post-translational modifications of MTs. The benefits of such a model are, that the architecture and organisation of the microtubules are of cellular origin and therefore were built with its tight regulation but at the same time, the microtubule network is susceptible to manipulations comparable to a cell-free *in vitro* system.

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159. Neuronal growth cones prefer soft to stiff substrates

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Neuronal growth cones are highly motile structures at the tip of the axons and dendrites, which sense a variety of cues including chemical and mechanical ones to establish functional connections during nervous system development. Substrate-cytoskeletal coupling is an established model for adhesion-mediated growth cone advance through the application of traction forces. However, the detailed molecular and biophysical mechanisms underlying the mechanosensing and mechanotransduction process remain unclear. For this reason, we modified a computational motor-clutch model to better understand the changes in cytoskeletal dynamics, traction forces, and substrate deformation when the growth cone interacts with adhesion substrates of different stiffnesses. To achieve this, we have included both motor and clutch reinforcement with increasing substrate stiffness. Furthermore, we have added an actin flow threshold that indicates when the growth cone is strongly coupled to the substrate. With respect to substrate deformation behavior, our modeling results

are in good agreement with experimental data from *Aplysia* growth cones probed with force-calibrated glass microneedles. Modeling also shows that strong coupling is achieved faster on soft substrates, which is consistent with neurite outgrowth behavior on gradient polyacrylamide gels. Taken together, these results suggest that growth cones prefer soft substrates to stiff ones.
Financing: NIH R01 NS117701

160. Study of MPS in transgenic Drosophila melanogaster line using nanobodies for his detection.

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Axons and dendrites possess a particular arrangement of their cortical skeleton, referred to as the Membrane-associated actin/spectrin Periodic Skeleton (MPS). The MPS is a periodic protein structure consisting of actin “rings” located transversely to the axon and separated every 190 nm by α/β-spectrin tetramer “spacers”, making the MPS only visible using super-resolution microscopy approaches. Most studies have described the MPS in cell culture and the dynamics of the spectrin “spacers” within each period have not been investigated in detail. Our project will shed light into these aspects in the nervous system of *Drosophila melanogaster*. Since β-spectrin is expressed in all fly cells, it is necessary to “tag” β-spectrin in a cell specific manner. For this, we are using CRISPR/Cas9-mediated editing to produce a transgenic fly, in which the endogenous β-spectrin gene can be recombined in a cell-type and time-specific manner to include C-terminus tags that can then be detected by nanobodies. Thus, a specific neuronal population will recombine to include a C-terminus “ALFA-tag” and that subpopulation can then suffer a second recombination to replace “ALFA-tag” by “BC2-tag”. We are going to show advances in the molecular cloning steps towards CRISPR/Cas9-mediated editing as well as the production of the nanobodies for the detection of the tags. This transgenic fly will allow the examination of dynamical properties of the MPS in nerve tissue.
Financing: CONICET Argentina; ANPCyT-FONCYT Argentina; International Society for Neurochemistry - Career Development Grant; International Brain Research Organization - Early Career Award.

161. Exposure to lead and methylmercury affects cell migration-associated processes in human neuroblastoma cells

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During neurodevelopment, neuronal migration aims to achieve an adequate positioning for the proper functioning of the central nervous system. For that, the establishment of focal adhesion sites consisting of different molecules, including phosphorylated Focal Adhesion Kinase (p-FAK^{Tyr297}), and the generation of membrane protrusions by actin polymerization are critical processes. Previous reports have shown that exposure to lead (Pb) and methylmercury (MeHg), harms neurodevelopment; however, the differential effects of Pb, MeHg, and a mixture of both on neuronal migration-associated processes have yet to be studied. We examined the impact of separate and simultaneous exposure to Pb and MeHg (at environmentally relevant concentrations) on cell movement-associated processes in the human neuroblastoma cell line SH-SY5Y. Our results show that separately Pb (21 and 210 nM) and MeHg (1 and 100 nM) inhibited the PDGF-induced cell migration. Likewise, lower concentrations of Pb (2.1 nM) and MeHg (0.5 nM) separately did not affect the cell migration, but in co-exposure, they exerted a synergistic inhibitory effect on migration. The PDGF-induced membrane protrusions (filopodium and lamellipodium) were inhibited by simultaneous exposure to lower concentrations of Pb and MeHg, and separate exposure to 21 nM Pb, which induced the formation of aggregates of p-FAK^{Tyr297}, more prominent than those shown in cells stimulated with PDGF. The mechanisms of metal-induced abnormal lamellipodial and filopodial actin polymerization and focal adhesions remain to be further studied. Additionally, it could be necessary to consider the interactions of some environmental pollutants (such as metals) for public health and their consequences on toxicological effects
Financing: This study was funded by the Mexican Council for Science and Technology grant 32913

162. Regulation of fast axonal transport in neurons by protein palmitoylation

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Neurons are large, complex cells requiring efficient trafficking and delivery of neuronal proteins and organelles to specific subcellular locations. Fast, continuous transport of cargo along axonal microtubules by dynein and kinesin motors is critical for neuronal function and requires a constant source of energy. Interestingly, glycolytic enzymes were recently found tethered to fast moving vesicles to provide an 'on-board' energy supply directly to the molecular motors. The activity of motor proteins is tightly regulated, and aberrant activity can result in neurodegeneration or neurodevelopmental deficits. One important mechanism to dynamically regulate protein trafficking in neurons is the covalent addition of fatty acids to protein cysteines residues, a process known as palmitoylation. Interestingly, glycolytic enzymes as well as several kinesin and dynein motor subunits and their activators have been identified in high throughput palmitoyl-proteomic studies as potentially palmitoylated. Thus, we hypothesize that palmitoylation tethers multiple motor proteins, their activators, and glycolytic enzymes to vesicles to provide 'on-board' energy and continuous movement required for fast axonal transport. Indeed, we have recently confirmed that glycolytic enzymes as well as $p150^{Glued}$, a subunit of the dynein activating complex dynactin, are palmitoylated in neurons and, interestingly, their palmitoylation regulates their vesicle association and axonal localization. These findings provide insight into the molecular mechanisms that govern fast axonal transport with implications for neurodevelopment, synaptic function, and axon degeneration.[SG12]

163. Tension-driven axon elongation triggers cytoskeleton and membrane remodelling

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During development, axon elongation is mediated by the growth cone. To accompany body growth, axons continue to extend post-synapse formation. The mechanisms underlying this distinct phase of axon extension, have remained largely neglected in the field. To uncover the molecular players governing axon stretch growth, we designed a microfluidic platform where axon stretch of dorsal root ganglia (DRG) neuron explants from adult and embryonic mice was performed. This system allows mirroring two physiological conditions in which mammalian neurons undergo the strongest stretch-induced elongation: mid-gestation and the adolescent growth spurt. Our platform provides a uniformly distributed computer-controlled stretch through specifically developed firmware and software, and the growth of DRG explants on top of a transparent stretchable silicone substrate, compatible with live imaging. Transcriptomic analysis shows that axon stretch engages biological pathways related to the plasma membrane and cytoskeleton remodelling both in embryonic and adult DRG neurons. Our findings demonstrate that upon stretch, a rapid stabilization of the microtubule cytoskeleton occurs as the density of dynamic microtubules decreases and axonal microtubule growth speed increases. Changes in microtubule dynamics are accompanied by an overall increased velocity of the axonal transport of organelles. Beyond changes in cytoskeleton dynamics and trafficking, axon stretch elicits a fast increase of plasmalemmal precursor vesicles, culminating in increased axon length and enlarged axon diameter. This work will provide important insights into our understanding of the mechanisms enabling growth-cone independent axon elongation.

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164. The cyclase-associated protein 2 controls cofilin-actin rods formation in Alzheimer's Disease

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Several neurodegenerative disorders, among which Alzheimer's disease (AD), are associated with cytoskeletal abnormalities, called cofilin-actin rods. These aggregates are described as composed of

cofilin and actin and their formation occurs upon exposure to different stressors, including Aβ oligomers, the main drivers of AD synaptic failure. We have recently demonstrated that the actin-binding protein cyclase-associated protein 2 (CAP2) is a master regulator of cofilin localization and activity, through the Cys32-dependent CAP2 dimerization. Remarkably, these mechanisms are altered in AD suggesting an involvement of CAP2/cofilin pathway in AD pathogenesis. In light of these results, the aim of the study was to investigate CAP2 role in the generation of cofilin-actin rods in AD. Taking advantage of 3D confocal analysis, we found that CAP2 accumulates within rods, when specifically induced by Aβ oligomers and not by another stressor. Short-term Aβ oligomers exposure triggers the removal of CAP2 and cofilin from the postsynaptic compartment. Instead, after a long-term treatment, able to induce synaptic loss and actin rods formation, cofilin is still reduced while CAP2 dimerization impaired. To prove that CAP2 is a key element in rods formation, we tested the effects of CAP2 overexpression. In hippocampal cultures, CAP2 prevents Aβ-induced rods formation and synapses loss. Beside this, we tested the same rescue strategy in an AD mouse model: the analysis of the hippocampal region confirmed that CAP2 overexpression prevents rods formation. Overall, our data support the involvement of cofilin/CAP2 pathway in the generation of cytoskeleton abnormalities that can affect synaptic function in AD.

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165. Amyloid fibril-mediated Abl1 activation promotes axon initial segment disruption and axonal tau missorting

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The axon initial segment (AIS) is an axonal subdomain involved in neuronal compartmentalization and action potential generation. Recent studies have shown that the AIS becomes destabilized in the context of Alzheimer's disease, promoting defects in protein sorting, but the precise molecular mechanisms which promote the collapse of the AIS cytoskeleton scaffold have yet to be elucidated. Our laboratory has previously identified Abl1 kinase as a key player in the progression of Alzheimer's disease, as it becomes activated by fibrillar Aβ aggregates, promoting tau phosphorylation, dendritic spine collapse and neuronal cell death, but a possible functional interaction with the AIS hasn't been explored to date.

To evaluate a possible role of Abl1 in AIS collapse in Alzheimer's disease we evaluated AIS integrity and tau sorting in hippocampal neuron cultures treated with Aβf, Abl kinase allosteric activator DPH and Abl kinase inhibitors. Aβf treatment promoted an increase in pAbl1 foci in the AIS, statistically significant decreases in the percentage of neurons presenting an AIS, and an increase in somatodendritic tau. Abl kinase inhibition partially prevented all of these processes, and Abl1-KO neurons were protected from Aβf-promoted AIS disruption. Our results show that Abl1 kinase activation promotes a key role in axon initial segment collapse and somatodendritic invasion of axonal tau, a key hallmark of Alzheimer's disease. Axon initial segment collapse probably represents a critical step in the amyloid cascade, as this structure mediates vital processes in neuronal function which have been found to be altered in Alzheimer's disease.

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166. Neuronal NADPH oxidase is required for neurite regeneration

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NADPH oxidase (Nox), a major source of reactive oxygen species (ROS), is involved in neurodegeneration after injury and disease. Nox is expressed in both neuronal and non-neuronal cells and contributes to an elevated ROS level after injury. Contrary to the well-known damaging effect of Nox-derived ROS in neurodegeneration, recently a physiological role of Nox in nervous system development including neurogenesis, neuronal polarity, and axonal growth has been revealed. Here, we tested a role for neuronal Nox in neurite regeneration following mechanical transection in cultured neurons. Using a novel hydrogen peroxide (H_2O_2)-sensing dye, *p*-bispinacolatoboron-5'-phenylpyridylthiazole (BPPT), we found that H_2O_2 levels are elevated in regenerating growth cones following injury. Increased Nox2 co-localization with $p40^{phox}$ in the growth cone central domain suggests Nox2 activation after injury. Inhibiting Nox with celastrol or reducing ROS with the antioxidant N-acetyl-L-cysteine reduced neurite regeneration rate. Pharmacological inhibition of Nox is correlated with reduced activity of Src2 tyrosine kinase and F-actin content in the growth cone.

Taken together, these findings indicate that Nox-derived ROS regulate neurite regeneration following injury through Src2-mediated regulation of actin organization in the growth cone.

167. Actomyosin contractility in the formation and function of the axon initial segment

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In addition to electrochemical and biochemical communication, neurons are also thought to communicate via mechanotransduction. Decades of research has established that neurons sense their mechanical environment during differentiation, pathfinding, and pathological perturbations, such as traumatic brain injury or plaque deposition. However, the molecular mechanism(s) by which mechanical cues are generated, sensed, and interpreted by neurons remain incomplete. Myosin 2 is the dominant contractile motor protein in cells, and individual myosin 2 monomers assemble into filaments that pull on the actin cytoskeleton to drive contraction events. Consistent with recent literature, my preliminary data demonstrates a significant increase in active phosphorylated myosin 2 in a subcellular domain at the proximal base of axons, named the Axon Initial Segment (AIS). In addition to myosin, a mature AIS also contains the master scaffold protein Ankyrin-G that anchors voltage-gated ion channels, signaling proteins, and cell adhesion molecules to the neuronal cytoskeleton. Therefore, the AIS contains all the requisite components of adhesion-mediated mechanosensation: active actomyosin with transmembrane components that can couple the extracellular matrix to the intracellular cytoskeleton. The proximal spatial location of the AIS to the soma and nucleus makes it an opportune candidate for transducing mechanical cues into electrochemical and biochemical information that modulate neuronal decision making and behavior. We are currently using traction force microscopy (TFM) to characterize AIS mechanics. In addition, we hope to delineate the spatiotemporal regulation of myosin filament assembly more precisely in the AIS. Our overarching hypothesis is that actomyosin-generated contractility is critical for AIS maturation and neuronal mechanosensation.

168. Twinfilin, formin and capping protein form a multicomponent Ménage à Trois at the actin barbed end

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The actin cytoskeleton and its associated regulatory proteins are crucial for neuronal development. While it is well-accepted that actin dynamics are essential in growth cones, dendritic spines, and axon terminals, the underlying molecular mechanisms regulating actin dynamics within these structures have yet to be uncovered. Actin elongators (formin), blockers (capping protein), and depolymerases (twinfilin) all exist within a neuronal cell, in a shared cytoplasm. On their own these proteins have distinct activities and have long been thought to bind barbed ends in a mutually exclusive manner i.e. one at a time. However, using microfluidic assisted - total internal reflection fluorescence microscopy and multispectral single molecule imaging, we have discovered that polymerases, depolymerases and blockers can simultaneously bind the same filament barbed end. To our knowledge this is the first report of a three-protein multicomponent complex at the actin filament barbed end. We find that simultaneous presence of these proteins at the barbed end leads to much faster protein transitions at the barbed end and allows for fine control of elongation rate as well as filament lengths and might help explain how the wide diversity in size and dynamics of intracellular actin structures is achieved *in vivo*. Using separation of function mutants, we further show that twinfilin destabilizes the capping protein and stabilizes formin at the barbed end, and as a result acts as a pro-elongation factor in spite of being a barbed end depolymerase on its own.

169. Cytoskeleton remodeling in senescent astrocytes is functionally involved in the production of senescent-associated secretory phenotype (SASP)

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Cellular senescence is one of the main hallmarks of aging. The accumulation of senescent astrocytes in the central nervous system may increase the susceptibility to developing neurodegenerative diseases and aging-related dementias. Senescent cells share common features such as hypertrophy, loss of cell division, and the production of a senescent-associated secretory phenotype (SASP). Those characteristics must rely on cytoskeleton organization due to its canonical role in cell morphology and intracellular trafficking. However, the senescence-associated changes that affect the cytoskeleton are

far from being fully addressed. In this work, we analyzed a transcriptomic data set of senescent astrocytes. We identified several differences in cytoskeleton components, including cytoskeleton building blocks (i.e., actin and tubulin) and their associated molecular motors. Afterward we implemented a pharmacologically-induced senescent astrocyte model in which we observed microfilaments and microtubule reorganization, changes in microtubule dynamic, and redistribution of active Rho-GTPases in senescent astrocytes. Altogether, our data suggest that changes in the cytoskeleton may be ahead of the production of SASP in senescent astrocytes.
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170. γ-TuRC regulates radial migration and neuronal maturation during mammalian cortical development

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The γ-tubulin ring complex (γ-TuRC) is a multi-subunit protein complex composed of γ-tubulin and γ-tubulin complex proteins (GCPs, GCP2-6). γ-TuRC promotes microtubule assembly by serving as a template that allows efficient nucleation of α/β-tubulins. Therefore, γ-TuRC plays a crucial role in various cellular processes such as division, polarization, migration and differentiation. Mutations in γ-TuRC core subunits and its activator cause brain developmental disorders known as malformations of cortical development (MCDs). However, it remains elusive how γ-TuRC is involved in distinct stages of cortex formation. Here we investigate the function of γ-TuRC in key events of cortical development during mouse embryogenesis, including progenitor proliferation, multipolar-to-bipolar transition, radial migration and neuronal maturation. Knockdown of individual γ-TuRC subunits severely delays neuronal migration in the developing brain. Using live brain-slice imaging, we discovered that GCP2-depleted cells remain motile but unable to orient and migrate radially. Intriguingly, these cells also fail to differentiate into the neuronal lineage. Furthermore, analysis of human GCP2 variants from microcephaly patients demonstrated that the disease mutations exhibit migration defects similar to GCP2 loss of function. Using *in silico* molecular dynamics simulation of cryo-EM structure, *in vitro* NanoBiT-based γ-TuRC assembly assay and microtubule nucleation assay in cells, we revealed that the *in vivo* radial migration defects are attributed to compromised γ-TuRC assembly. In sum, our findings indicate that γ-TuRC is indispensable for establishing neuronal polarity that guides radial migration and neuronal maturation during cortical development. Our study thus provides novel molecular insights into how γ-TuRC controls brain development.

171. Fascin1 regulates axonal development and brain wiring

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Axon guidance depends on growth cone filopodia to sense a wide range of extracellular guidance cues. However, the molecular and cellular mechanisms that regulate filopodia dynamics and their responses to guidance cues remain unclear. Fascin1 is a ~55 kDa actin bundling protein that crosslinks actin filaments to form tight F-actin bundles in filopodia. Fascin1 is highly expressed in developing neurons and enriched in growth cone filopodia, but its role in axon guidance has not been investigated. Here, we use a combination of cell culture and *in vivo* approaches to investigate the role of Fascin1 in growth cone filopodia dynamics, motility, and axon guidance. We have developed a novel CRISPR-Cas9-mediated approach to effectively knock out Fascin1 in cultured primary cortical neurons to investigate if and how loss of Fascin1 in developing neurons affects axonal elongation, branching and guidance. To understand the *in vivo* role of Fascin1 in neuronal wiring, we examined how the loss of Singed, the *Drosophila melanogaster* ortholog of Fascin1, affects neuronal circuits and brain function in fruit flies. We found that *singed* null flies exhibit marked axonal defects in the mushroom body, a brain structure that is analogous to the mammalian hippocampus. Further examination also revealed defects in the visual circuitry. Together, our work highlights the important role of Fascin1 in actin-based axon development and brain wiring. Additionally, the use of both a cultured cell system and an *in vivo* model allows us to examine the neurodevelopmental function of Fascin1 at both the single cell and organismal levels.
Financing: The research is supported in part by funds from National Institutes of Health.

172. L-Dopa incorporation into tubulin affects microtubules dynamics, neuronal differentiation and dendritic spine density

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Microtubules are key elements of the neuronal cytoskeleton. Differential expression of tubulin isotypes and a variety of post-translational modifications constitute the code that modulates microtubule properties and functions. The reversible removal of the tyrosine residue encoded at the C-terminal end of α-tubulin involves the enzymes tubulin tyrosine ligase and the detyrosinase complexes, composed of a vasohibin (VASH) and a small vasohibin-binding protein (SVBP), and the recently described MATCAP. Previously, we demonstrated that L-3,4-dihydroxyphenylalanine (L-Dopa) is incorporated, in vitro and in living cells, into α-tubulin at the same position as tyrosine. We found, using soluble brain extracts, that L-Dopa was not released by the endogenous carboxypeptidase under the conditions that allow rapid release of tyrosine. We also reported that L-Dopa treatment impairs mitochondrial axonal transport and reduces the affinity of the motor KIF5B for L-Dopa-microtubules. Now, we have analyzed the interaction between the VASH-SVBP complex and microtubules enriched in L-Dopa-tubulin using single-molecule TIRF. We found a reduction in the complex binding to L-Dopa-microtubules and in its carboxypeptidase activity. In cultured mouse hippocampal neurons, we examined the effect of L-Dopa on neuronal differentiation and dendritic spine density. We observed a delay in the establishment of polarity and transition to stage III in WT neurons, but not in neurons without carboxypeptidase activity, and a reduction in the number of mature dendritic spines. Based on our results, we hypothesize that L-Dopa incorporation into tubulin and microtubules alters the detyrosination/tyrosination cycle and modifies microtubules with a direct implication in neuronal differentiation and synapse formation.

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173. Functions of Septin 8 palmitoylation in human IPSC derived neurons

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Septins are a family of GTPase molecules that form heteromeric complexes that further assemble into filamentous structures and associate with membranes, actin and microtubules. Septins are expressed by 13 genes, encoding for septin proteins 1 to 14 (excluding 13). In addition, septins are expressed with different splice isoforms. Isoforms of a particular septin, even though very similar in protein sequence, may have a different protein interactome and function. Consistent with this, tissue specific expression of several septins and/or splice isoforms has been observed. Several septins are strongly expressed in the brain, where they were found to regulate neurite outgrowth, dendrite branching and membrane transport in neurons. Here we focus on the neuronal function of septin 8. Septin 8 is strongly expressed in the brain and its expression increases with age. Moreover, previous studies have indicated that septin 8 is enriched in synaptic compartments. Interestingly, the brain specific Septin8 isoform seems to be palmitoylated, suggesting it may stronger affiliate with membranes. Here we investigate the role of SEPT8 in neuronal cell biology by the use of human IPSC-derived neurons.

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174. CREB3L1 transcription factor is associated with the neuronal primary cilia

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CREB3L1 belongs to the CREB3 family of transcription factors that are implicated in the ER and Golgi stress responses as regulators of the cell secretory capacity and cell specific cargos. In response to different signals, CREB3 proteins are transported from the ER to the Golgi complex where they are cleaved (activated) by S1P and S2P proteases sequentially. Although CREB3 factors have a wide range of biological functions, their role in neuronal development is not well understood. In our study, we observed that CREB3L1 localizes to the basal bodies of primary cilia during early neuronal cell development. Primary cilia are sensory organelles that project from the plasma membrane of many cell types, including neurons. They play an important role in intracellular signaling pathways and act as sensory organelle for extracellular and intracellular signals. Our preliminary results show that CREB3L1 colocalizes with γ-tubulin and inversin, a protein located in the basal body of primary cilia at early culture times (3DIV). This basal body localization is lost when CREB3L1 activation by S1P and S2P

proteases is inhibited. Our findings suggest that CREB3L1 may have a non-canonical function in primary cilia that is important for neuronal development and function. Further research on this topic could provide new insights into the mechanisms underlying neuronal function.

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