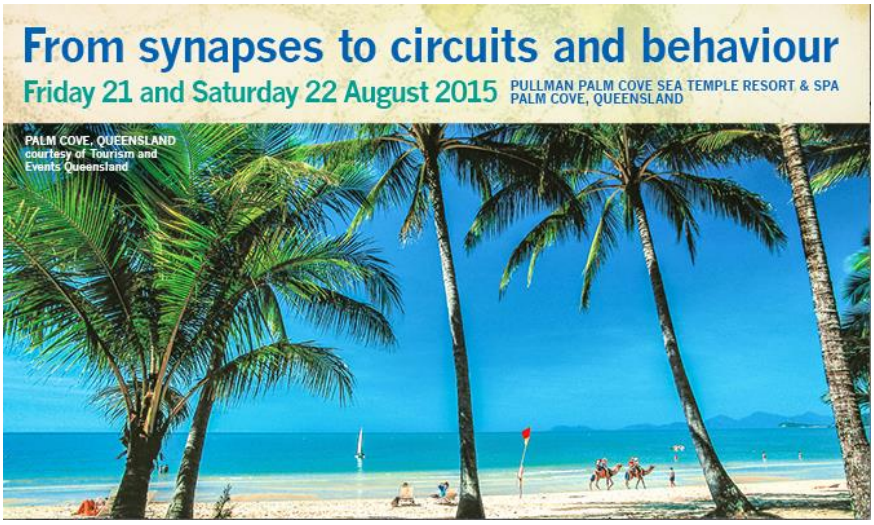

Final Report
From synapses to circuits and behavior

1. Background Details

Location: Palm Cove, Queensland, Australia
Date of Meeting: Friday 21, Saturday 22 August, 2015
Venue: Pullman Palm Cove Sea Temple Resort and Spa, Palm Cove, QLD, Australia
Organisers: Roger Nicoll, Katherine Roche and Pankaj Sah.

Perhaps no other structure is more fundamental to our understanding of the brain than the synapse. In the central nervous system, excitatory synapses represent the primary source of information communication between neurons, whether for local interactions within circuits or for linking discrete regions of the brain. Within circuits inhibitory interneurons sculpt the activity and determine the output of these circuits. One of the most extraordinary properties of synapses is the ability to undergo activity-dependent changes in synaptic strength, providing the most compelling cellular model for learning and memory.

Synapses also serve as the site of action for many commonly prescribed medications and synaptic disruption contributes to many neurological and psychiatric disorders. These include schizophrenia, autism, depression, substance abuse and addiction, Parkinson's disease, Alzheimer's disease, traumatic brain injury, stroke and epilepsy. In some cases, synaptic dysfunction is causal in disease, whereas in other cases it represents the downstream sequelae of one or more underlying molecular defects. In either case, a fundamental understanding of the formation structure, molecular organization, signaling function, and plasticity of synapses is essential to achieving progress in lessening the burden of human neurological disease and for predicting and improving mental health. In this satellite meeting we will cover recent advances in three areas; synaptic function, the role of microcircuits in behavior and finally neuropsychiatric/neurological diseases.

2. Registrations

The registration fee was set at $150 per registrant for the 2- day event.

The registration fees covered

- Morning and afternoon teas for the 2 days
- Lunches for the 2 days
- Poster session catering (drinks and canapés)

Invited speakers did not pay registration fees. Their registration fees were subsidized by ISN and other sponsorship.

3. Program

The program for the 2- day meeting included 6 sessions with the participation of 14 international invited speakers and 5 domestic invited speakers. In addition there was a poster session featuring 33 posters and a session where 6 posters were selected and their presenters were invited to give a short talk. The program was as follows:

From Synapses to circuits and behaviour
Satellite of 2015 ISN/ANS annual meeting
Pullman Palm Cove Sea Temple Resort
21-22 August, 2015

Thursday 20 August 2015

Arrival and Check in
Informal drinks at pool bar
19:00

Friday 21 August 2015

Opening Remarks and Welcome: Roger Nicoll

Session 1. Receptor/synapse complexes
Chair: Robert Malenka
8:30 - 10:30

David Bredt
"Synaptic plasticity regulated by AMPA receptor associated proteins".

Juan Lerma
"A role for kainate receptors in mood disorders"

Villu Maricq
"Molecular Machinery Underlying Motor-Mediated Transport of Synaptic AMPARs"

Katherine Roche
"Regulation of Protein Trafficking at Excitatory Synapses by Phosphorylation"

10:30-11:00
Morning Tea

Session 2. Synaptic plasticity
Chair: Pankaj Sah
11:00 - 13:00

Gina Turrigiano
"Gating of firing rate homeostasis by sleep and wake states"

Robert Malenka
"Molecular mechanisms underlying AMPA receptor delivery during LTP"

Cliff Abraham
"Astrocyte-mediated network wide metaplasticity

Roger Nicoll
"PSD-95 family MAGUKs: Key organizers of the postsynaptic density".

13:00-14:00
Lunch

Afternoon free time

17:00-19:30
Poster Session (+ beer / wine / nibbles)

Saturday 22 August 2015

Session 1. Neuronal networks
Chair: Stephen Williams
8:30 - 10:30

Jeffrey Isaacson
"Dynamic sensory representations in auditory cortex".

Bernardo Sabatini
'GABAergic identity of cholinergic neurons."

Pankaj Sah
"Prefrontal, hippocampus, amygdala networks in fear learning"
Paul Martin
"Parallel streams and parallel circuits in the primate visual system"

10:30-11:00
Morning Tea

Session 2. **(Short talks – selected from posters)**
11:00 - 13:00

13:00-14:00
Lunch

Session 3. Disorders of Neural Function
Chair: David Bredt
14:00-16:00

Richard Huganir
"Regulation of AMPA Receptors and Synaptic Plasticity in the Brain"

Jürgen Götz
"Tau and synaptic dysfunction in neurodegenerative disease"

Julie Kauer
"Glycine receptor LTP at synapses in the dorsal horn"

Thomas Kuner
"Structural plasticity of cingulate cortex neurons and their thalamic inputs in chronic pain"

16:00-16:30
Afternoon Tea

Session 4: ***Synaptic and network formation, modulation and function***
Chair: Cliff Abraham

16:00 – 18:00

Morgan Sheng
"New perspectives on NMDA receptor function"

Geoff Goodhill
"Neural coding and topographic maps"

Stephen Williams
"Cholinergic control of active dendritic integration"

Closing Remarks : Pankaj Sah

4. Speakers

Invited International Speakers

Cliff Abraham, University of Otago, New Zealand
David Bredt, Johnson & Johnson, Titusville, New Jersey, USA
Richard Huganir, Johns Hopkins School of Medicine, Boston, USA
Jeffry Isaacson, UCSD School of Medicine, La Jolla, USA
Julie Kauer, Brown University, Rhode Island, USA
Thomas Kuner, University of Heidelberg, Heidelberg, Germany
Juan Lerma, Instituto De Neurociencias, Spain
Rob Malenka, Stanford School of Medicine, Stanford, USA
Villu (Andres) Maricq, University of Utah, USA
Roger Nicoll, University of Caliifornia, San Francisco, USA
Katherine Roche, National Institutes of Helath – NINDS, USA
Bernado Sabatini, Harvard Medical School, Boston, USA
Morgan Sheng, Genentech, USA
Gina Turrigiano, Brandeis University, USA

Invited Domestic Speakers

Juergen Goetz, Queensland Brain Institute, University of Queenlsand, Brisbane, AUS
Geoff Goodhill, Queensland Brain Institute, University of Queenlsand, Brisbane, AUS
Paul Martin, University of Sydney, Sydney, AUS
Pankaj Sah, Queensland Brain Institute, University of Queenlsand, Brisbane, AUS
Stephen Williams, Queensland Brain Institute, University of Queenlsand, Brisbane, AUS

5. Highlights of the Meeting

Day 1.
The main theme of the day was the identification of molecules of different pathways influencing synaptic plasticity. Interesting was the talk by Katherine Roche about a novel and one of a kind brain specific GTPase named neorolastin which caused fewer functional synapses if deleted. This protein is a possible new target to affect learning in animals. Villu Maricq showed great C.elegands imaging data on synaptic AMPA receptor trafficking. Although the focus was on neurons the speaker Cliff Abrahams talked about astrocytes and their involvement in heterosynaptic metaplasticity.

Day 2.
The main theme of the day was neuroplasticity but at cellular and network level. Interesting was Bernado Sabatini's data showing that cholinergic neurons can be GABAergic. Highlight was the high-tech intact brain imaging of spines in relation to chronic pain over a period of months by Thomas Kuner. Julie Kauer defined a new target for blocking chronic pain.

5a. Summaries of Speakers talks

David Bredt presented data showing that PORCN protein controls the stability and

composition of hippocampal AMPA receptors. In PORCN knockout mice, AMPA receptor-mediated transmission is decreased and GluA protein levels are diminished.

Morgan Sheng used genomics and bioinformatics to assess how NMDA receptor activity controls gene networks. This analysis lead to the discovery that basal NMDA receptor activity controls astrocyte proliferation.

Julie Kauer's research defined how potentiation of glycinergic synapses is triggered and maintained, using functional studies in intact spinal cord slices. Using electrophysiological recordings they find that the inflammatory cytokine, IL-1beta, rapidly upregulates inhibitory glycine receptors on inhibitory neurons in the dorsal horn.

Roger Nicoll described a new form of synaptic plasticity. When a neuron loses a fraction of its glutamate receptors, there is a uniform decrease in the number of receptors at all synapses, as would be expected. However, over a period of 4 days most of the synapses lose all of their receptors and the few remain synapses accumulate the remaining receptors so that they have the same number of receptors as they did under control condition. A number of the mechanistic steps involved in the plasticity were described.

Villu Maricq used C. elegans to study synaptic trafficking of glutamate receptors. He found that CaMKII is critically involved in the transport of glutamate receptor containing vesicles. These vesicles are delivered to the synaptic sites and are exocytosed. I default pathway involves the insertion of receptors at the soma and the movement of these surface receptors to the synapse.

Katherine Roche reported the identification of neurolastin, a new member of the dynamin family of GTPses. Neurolastin is a functional GTPase that also contains a functional RING domain and acts as a E3 ubiquitin ligase. Knock out mice reveal that neurolastin regulates endosomal dynamics and spine number.

Rob Malenka's talk on Molecular Mechanisms underlying AMPA receptor delivery during LTP – world leader in his field, his work involved the application of several world leading techniques to suggest a novel mechanism for channel trafficking during LTP – the putative cellular change underlying learning and memory.

Cliff Abraham's talk on Astrocyte mediated network wide metaplasticity – he's a highly regarded scientist in the field of LTP. Astrocytes are a cell type that in the past were widely dismissed as merely support cells in neuroscience, but work like this challenges that notion, and is a fantastic example of high quality experimental design and technique.

Bernado Sabatini's talk on the GABAergic properties of cholinergic neurons – in this talk, Bernado applied modern and technically challenging techniques to show that there exists a population of cells that releases two different neurotransmitters – acetylcholine as well as GABA. This finding has significant repercussions for our understanding of the role of these neurons within their circuits, and is an example of dogma breaking research, reminding students and researchers alike to always

question findings from previous work and to be wary of simplifying assumptions.

Richard L Huganir's talk on In vivo imaging of AMPA receptors – these experiments are incredibly difficult, and allow us to visualise one of the most important and illusive cellular processes in neuroscience – the dynamic movement of postsynaptic receptors in response to neural activity, over long periods of time, in an intact behaving animal. This work was incredible to hear described in person, and was a fantastic learning experience.

6. Participants

The meeting was attended 95 participants. Of these, there were 19 speakers –of which 14 were International and 5 domestic. A large proportion of the audience was from the Queensland Brain Institute, University of Queensland. There were attendees from various countries including; Australia, New Zealand, South Korea, Canada, USA, Estonia, France, Germany, Poland, Mexico, Israel and Spain. The attendees list is as follows:

7. Material Distribution

Each participant received a program and writing materials

8. Social Events

There were informal drinks on arrival for all conference attendees, held at the resorts poolside bar.

A poster session incorporating canapés and drinks was held in the afternoon of Friday 21 August.

A speaker's dinner was held on the Friday evening for all invited speakers.

All these events enabled the speakers and attendees to interact with each other.

9. Travel subsides for Invited Speakers

ISN's generous sponsorship helped to fund the registration costs and travel expenses of both our international and domestic speakers. A contribution of up to $2500 AUD was provided towards the airfares of our international speakers. Domestic speakers airfares were also fully funded by sponsorship from ISN. The remaining sponsorship from ISN was put towards the accommodation expenses of the speakers. Sponsorship from our other generous sponsors was used to fund the remainder of the accommodation and local transport costs of the speakers as well as the speaker's dinner and poster session expenses.

(Some of the speakers were self-funded – a breakdown can be seen in the attached budget in section 11).

10. Sponsorship

In addition to the generous USD $20,000 contributed by ISN, we were also able to secure funding from the following sponsors: Genentech (5000CHF), Johnson & Johnson ($7500 USD). The Queensland Brain Institute provided administration support to organise the event and venue, administer the sponsorship, manage the budget and coordinate the payment of speakers travel reimbursements. The Queensland Brain Institute will also fund any shortfall in the budget.

11. Budget

Budget for ANS-ISN Satellite Meeting in Palm Cove -21-22 August 2015	
From synapses to circuits and behaviour	**$ AUD**
Income (GST Exclusive amounts)	
Registration fee	
77 Registrations received (Registrants paid $150 - GST Free amount $136.36)	$10,499.72
Total Registrations	**$10,499.72**
Sponsorship	
ISN (Sponsoring $20,000 USD, $16000 USD has been paid upfront - AUD conversion shown)	$20,133.38
ISN ($4000 USD to be paid after event) (Approx AUD exchange conversion rate shown)	$5,673.44
Johnson & Johnson ($7500 USD - AUD conversion shown)	$9,824.47
Genentech (5000 CHF - AUD conversion shown)	$5,996.40
Queensland Brain Institute (approximate shortfall)	$557.43
Total Sponsorships	**$42,185.12**
TOTAL INCOME	***$52,684.84***
Expenses (GST exclusive amounts)	
Catering Costs	
Deposit for venue hire Pullman Palm Cove Sea Temple Spa and Resort	$1,254.55
Informal welcome drinks on arrival night	$758.64
Pre conference organisers dinner	$376.73
1st day food package @ $50 per person, based on 95 attendee	$4,184.55
2nd day food package @ $60 per person, based on 95 attendees,	$4,955.18
Poster session Drinks 95 @ $30 per head	$2,590.91
Poster session Canapes 95*$25 per head	$2,159.09
Speakers dinner (In Vivo Bar and Grill)	$1,725.73
Total Catering Costs	***$18,005.38***

Speakers Costs	
Airfares (International - Contribution towards)	
Rob Malenka	$2,349.39
Juan Lerma (Includes an extra transfer charge)	$2,567.71
Villu (Andres) Maricq	$2,500.00
Gina Turrigiano (self funded)	$0
Cliff Abraham (self funded)	$0
Jeffry Isaacson	$2,480.35
Bernardo Sabatini(Includes extra transfer charge)	$2,661.00
David Bredt (self funded)	$0
Richard Huganir	$2,500.00
Julie Kauer	$2,500.00
Thomas Kuner (Includes accomm as he stayed outside the resort)	$2,938.01
Morgan Sheng (self funded)	$0
Roger Nicholl	$2,500.00
Katherine Roche (self funded)	$0
Total Airfares (International Speakers)	***$22,996.46***
Airfares (Domestic - Contribution towards)	
Stephen Williams	$382.73
Paul Martin	$387.00
Geoff Goodhill	$322.00
Juergen Goetz (Funded by his organisation)	$0
Pankaj Sah	$210.73
Total Airfares (Domestic Speakers)	***$1,302.46***
Total Airfares	***$24,298.92***
Accommodation for Speakers and Organisers	
Rob Malenka	$398.18
Juan Lerma	$560.95
Villu (Andres) Maricq	$398.18
Gina Turrigiano	$398.18
Cliff Abraham	$796.36
Jeffry Isaacson	$398.18
Bernardo Sabatini	$398.18
David Bredt	$398.18
Richard Huganir	$398.18
Julie Kauer (Breakfasts only as she shared with Rob Malenka)	$36.36
Morgan Sheng	$398.18
Juergen Goetz	$398.18
Pankaj Sah	$597.27
Geoff Goodhill	$398.18
Stephen Williams	$597.27
Paul Martin	$597.27
Katherine Roche	$796.36
Roger Nicoll	$796.36
Total Accommodation	***$8,760.00***
Other speaker costs	
Shuttle bus transfers for speakers to and from airport $22 per transfer and 26 speakers	$538.18
Total Other Speaker Costs	***$538.18***
Other expenses	
Poster boards for poster session -10 ordered. Cost includes setup and removal	$1,033.09
Credit card fees on venue payments	$49.27

Total Other Expenses	***$1,082.36***
TOTAL EXPENSES	***$52,684.84***
Expected profit/loss	***$0***

12. List of Participants

Country	Name	Organisation
Australia	Timothy Bredy	Queensland Brain Institute, University of QLD
Australia	Wei Wei	Queensland Brain Institute, University of QLD
Australia	Robert Hatch	Queensland Brain Institute, University of QLD
Australia	Bruno van Swinderen	Queensland Brain Institute, University of QLD
New Zealand	Shane Ohline	Department of Psychology
Australia	Ann Van der Jeugd	82 Anson Street
Australia	Sarah Hunt	Queensland Brain Institute, University of QLD
Australia	Yajie Sun	Queensland Brain Institute, University of QLD
Australia	Margreet Ridder	Queensland Brain Institute, University of QLD
New Zealand	Kristin Hillman	University of Otago, Psychology
New Zealand	Joanna Williams	University of Otago
New Zealand	Bruce Mockett	University of Otago, PO Box 56
Australia	François Windels	Queensland Brain Institute, University of QLD
Australia	Lei Qian	Queensland Brain Institute, University of QLD
Australia	Marie lou Camara	Queensland Brain Institute, University of QLD
Australia	William (Bill) Phillips	Physiology and Bosch Institute, University of Sydney
Australia	Victor Anggono	Queensland Brain Institute, University of QLD
Australia	Jenny Gunnersen	Department of Anatomy and Neuroscience, The University of Melbourne
Australia	Yossi Buskila	The MARCS institute, University of Western Sydney
New Zealand	Regina Hegemann	277 Leith Walk
Australia	Jana Vukovic	Queensland Brain Institute, University of QLD
Australia	Vikram Ratnu	Queensland Brain Institute, University of QLD
Australia	Laura Leighton	Queensland Brain Institute, University of QLD
Australia	Alexandra Suchowerska	Wallace Wurth Building, UNSW Australia
Australia	Helen Gooch	Queensland Brain Institute, University of QLD
South Korea	Seung Min Um	IBS #2209 E6-3 KAIST, Yuseong-gu
South Korea	Changuk Chung	IBS #2209 E6-3 KAIST Guseong-dong, Yuseong-gu
Australia	Thomas Fath	School of Medical Sciences, UNSW
South Korea	Ryunhee kim	Center for Synaptic Brain Dysfunctions, Institute for Basic Science (IBS) and Department of Biological Sciences
South Korea	Taesun Yoo	291 Daehak-ro, Yuseong-gu

Country	Name	Organisation
South Korea	Wangyong Shin	Korea Advanced Institute of Science and Technology (KAIST) 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, South Korea
Canada	Krista Mitchnick	385436 20th Sideroad
South Korea	KyungDeok Kim	IBS, #2209, E6-3, 291 Daehak-ro, Yuseong-gu
New Zealand	Megan Elder	270 Great King Street
Australia	Jocelyn Widagdo	Queensland Brain Institute, University of QLD
Australia	David Reser	Physiology Dept, Monash Uni.
Australia	Yvette Wilson	Dept. of Anatomy and Neuroscience, The University of Melbourne
Australia	Zoran Boskovic	Queensland Brain Institute, University of QLD
Australia	Emilia Lefevre	Queensland Brain Institute, University of QLD
USA	Jai Polepalli	1392 Alabama st
Australia	John Lin	University of Tasmania Private Bag 23
Estonia	Allen Kaasik	Koidula 14-4
New Zealand	Lucy Goodman	85 Park Road
New Zealand	Kevin Lee	85 Park Road, Grafton
Australia	Agnieszka Zbela	Menzies Research Institute Tasmania, Medical Sciences 2 Building
United States	Marcelo Wood	McGaugh Hall, University of California Irvine
Australia	Renata Pertile	Queensland Brain Institute, University of QLD
Estonia	Dzhamilja Safiulina	Koidula 14
France	Laura Daroles	141 bis avebue de Clichy
USA	Yi Wang	455 Main Street, Apt. 7H
New Zealand	Margaret Ryan	Department of Anatomy, University of Otago
Australia	Dario Protti	University of Sydney
Germany	Eva Mandelkow	DZNE c/o CAESAR
Germany	Eckhard Mandelkow	DZNE c/o CAESAR
New Zealand	Madeleine Kyrke-Smith	23/61 North Road
Australia	Shilpi Dixit	5/601 A
Australia	Roger Marek	Queensland Brain Institute, University of QLD
New Zealand	Johanna Montgomery	Centre for Brain Research. Dept of Physiology
Australia	Jing Zhao	Dept of Physiology, 85 Park Road
Australia	Ehsan Arabzadeh	Eccles Institute of Neuroscience
Australia	Saba Gharaei	Eccles Institute of Neuroscience
New Zealand	Chantelle Fourie	85 Park Rd
Australia	Dhanisha Jhaveri	Dept of Physiology, 85 Park Road
Australia	Phyllis Chua	Dept of Psychiatry, Monash University
USA	Basavaraj Balapal	Nathan Kline Institute, 140 Old Orangeburg Rd
Israel	Ami Citri	Silberman 2-516, Safra Campus of the Hebrew University
Australia	David Carter	U307 30 New Quay Prom
France	Mariana Ramos	INSERM U1016 Institut Cochin
Australia	Stuart McDougall	Florey Institute, University of Melbourne

Country	Name	Organisation
Poland	Ewelina Knapska	Pasteur 3
Australia	Merryn Brettle	Wallace Wurth Building, UNSW, High street
Australia	Tong Wang	1/77 Warren Street
Mexico	Perla Moreno	Instituto de Fisiología Celular, Ciudad Universitaria Cir Ext s/n
South Korea	ChiHye Chung	120 Neungdong-ro, Gwangjin-gu, Seoul, Department of Biological Sciences, Konkuk University
USA	Roger Nicoll	University of California
USA	David Bredt	Johnson and Johnson
Spain	Juan Lerma	Instituto De Neurosiensias
USA	Villu Maricq	Dept of Biology, University of Utah
USA	Katherine Roche	Porter Neuroscience Research Center
USA	Gina Turrigiano	University of California
USA	Rob Malenka	Stanford School of Medicine
New Zealand	Cliff Abraham	University of Otago
Australia	Juergen Goetz	Queensland Brain Institute, University of QLD
Australia	Stephen Williams	Queensland Brain Institute, University of QLD
USA	Jeffry Isaacson	UCSD School of Medicine
USA	Bernado Sabatini	Harvard Medical School
Australia	Paul Martin	University of Sydney
USA	Richard Huganir	Johns Hopkins school of Medicine
USA	Julie Kauer	Brown University
Germany	Thomas Kuner	University of Heidelberg
USA	Morgan Sheng	Genentech
Australia	Geoffrey Goodhill	Queensland Brain Institute, University of QLD
Australia	Pankaj Sah	Queensland Brain Institute, University of QLD

13. Abstracts for Poster session

1. The Role of Action Potential Firing in Metaplasticity

Regina Hegemann, Wickliffe C. Abraham
University of Otago, Department of Psychology and Brain Health Research Centre, Dunedin, New Zealand

Distinct patterns of inter-neuronal communication, encoded via action potential (AP) firing, can drive synaptic plasticity such as long-term potentiation (LTP), a neurobiological substrate of at least some types of memory. Recent evidence demonstrated that postsynaptic action potential firing alone facilitates subsequent LTP induction, suggesting a role of AP firing in regulating future plasticity, i.e., metaplasticity. However, cell firing has also been shown to inhibit later LTP. With the aim of unravelling this inconsistency, we recorded *in vitro* field potentials from CA1 stratum radiatum of 400 µm slices taken from 6-8 week-old male Sprague-Dawley rats. LTP was induced with 1 train (10 bursts), or a 0.5 train (5 bursts) of theta-burst stimulation (TBS). In an attempt to specifically activate multiple CA1 pyramidal cells postsynaptically without

synaptic activation, antidromic priming stimulation (3 trains of TBS, 3x3 trains of TBS, or 2 trains of 100 Hz) was delivered to the alveus, 30 min prior to LTP induction. LTP induced with 1 TBS was significantly greater in slices receiving 3x3 TBS priming stimulation ((*n*=9): 28.9 ± 3.6%, *p*=0.02) compared to unprimed control slices ((*n*=9): 17.1 ± 4.2%), measured one hour following LTP induction. Other priming protocols were not sufficient to significantly facilitate LTP (3 TBS (*n*=4): 27 ± 5.2%, *p*=0.25; 100 Hz (*n*=7): 22.6 ± 4.1%, *p*=0.48). However, slices primed with 3 trains of TBS displayed a trend towards facilitated LTP maintenance, suggesting the possibility of an enhanced late phase of LTP. Surprisingly, LTP induced with 0.5 TBS was not significantly affected by the 3x3 TBS priming protocol (Primed (*n*=7): 21.3 ± 5.9%; Control (*n*=6): 14.2 ± 4.9%, *p*=0.35. Overall, these data provide support for a role of AP firing in generating metaplasticity which appears to be highly sensitive to the pattern of cell firing and the strength of the LTP induction protocol.

2. Intrinsic circuitry of the lateral central amygdala

Authors Sarah Hunt, Pankaj Sah
Institute: Queensland Brain Institute, St Lucia, Australia

The amygdala is a region of the brain that is responsible for the processing and memory of emotions. Understanding the neural circuitry of this area is essential to grasping how it achieves this function and may aid in developing treatments for a range of anxiety disorders. Two main subregions of the amygdala, the basal and lateral nuclei (BLA), and the central nucleus (CeA), play key roles in the acquisition and expression of emotions respectively. While the BLA has been extensively studied, the CeA has only recently gained interest, and as such, its intrinsic circuitry is under intense study. Cells in the CeA are mainly inhibitory GABAergic (gamma-aminobutyric acid) cells and single unit recordings of the activity of specific cell populations suggest that these cells form strong local inhibitory connections. These local connections are central to the proposed circuitry underlying the behavioural roles of the CeA. However, our understanding of these local connections at the cellular level is in its infancy; the strength and physiological role of individual connections are largely unknown. We have made dual whole-cell recordings in the lateral CeA to determine the physiological properties and strength of local inhibitory connections. Current-clamp recordings showed three types of discharge properties: regular spiking (RS), late firing (LF) and fast spiking (FS). In paired recordings, 32 out of 94 (≈ 34%) were connected, of which 29 connected pairs were unidirectional and three were bidirectional. Connections were on average 22 ± 5 pA when voltage-clamped at -40 mV, and were inhibited by picrotoxin, indicating that these were indeed GABAergic connections. In these pairs, the presynaptic cell was RS (47%), LF (35%) or FS (18%) whereas the postsynaptic cell was mostly LF (70%). Finally, the inhibitory connection was sufficient to halt firing in the postsynaptic cell. These results confirm that cells in the lateral portion of CeA form local inhibitory connections, which are capable of silencing the postsynaptic cell.

3. Synaptic inhibition at second order neurons in the CNS.

McDougall SJ, Thek K, Guo H, Allen AM[1]Florey Institute and [1]Department of Physiology, University of Melbourne, Parkville, Victoria.

Inhibitory input at the solitary tract nucleus (NTS) modulates autonomic reflex performance. Somatostatin (SOM) neurons are a class of inhibitory interneuron within the CNS. Utilizing the SOM-Channel rhodopsin-2 mouse model we aimed to gain greater insight into synaptic inhibition at the level of second order NTS neurons. Shocks to the solitary tract evoked low jitter EPSCs that identified second order NTS neurons. LED pulses evoked consistent action potential dependent IPSCs in recorded neurons. We demonstrated that almost all second order neurons, including SOM expressing, receive inhibitory input from other, likely multiple, SOM interneurons. We investigated the neurotransmitters and post synaptic receptors involved and find LED evoked IPSCs are both GABA and glycine mediated. These findings indicate a complex inhibitory network that likely modulates reflex performance at the initial stages of central processing.

4. Overexpression of the actin-associated protein tropomyosin ameliorates the synaptotoxic effects of amyloid-β.

A.K. Suchowerska[1], S. Teh[1], V. Anggono[1], P. Gunning[1] and T. Fath[1]
[1] School of Medical Sciences, University of New South Wales, Sydney, Australia.
[2]Clem Jones Centre for Ageing Dementia Research and Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Regulation of the actin cytoskeleton in the postsynaptic compartment of synapses is crucial to synaptic structure, maturation and function. The postsynaptic actin cytoskeleton is regulated by a large number of actin-associated proteins including tropomyosins. Previously, we have shown a spatial segregation of different isoforms as central nervous system synapses in vitro with Tpm3 and Tpm4 tropomyosin gene products localising to the post-synaptic and Tpm1 gene products localising to the pre-synaptic compartment, suggesting isoform specific regulation of actin filament populations in these subcellular compartments. However, the role of tropomyosins in synapse loss and dysfunction in neurodegenerative disorders, such as Alzheimer's disease, is still unclear. In the current study, we analysed the effect of overexpression of the major Tpm3 isoform expressed in neurons (Tpm3.1) on amyloid-β induced cytotoxicity. We first confirmed the postsynaptic localisation of Tpm3.1 in cultured neurons and the synaptic enrichment of Tpm3.1 in C57Bl6 transgenic mice overexpressing Tpm3.1 by Western Blot analysis of hippocampal tissue from these mice. Using mixed cultures from Tpm3.1 transgenic and wild type C57Bl6 mice, we found a protective effect of Tpm3.1 overexpression, ameliorating the synaptotoxic effects of amyloid-β. Neurons from Tpm3.1 transgenic mice, treated with amyloid-β were resistant to dendritic spine loss and maintained mature morphology of dendritic spines, suggesting stabilisation of actin filaments at the synapse by Tmp3.1. Our results suggest that alterations in tropomyosin expression at the synapse have implications on the stability of the dendritic spine, affecting the susceptibility to neurodegeneration.

5. Anatomy and Physiology of the Central Extended Amygdala

Yajie Sun, Fabrice Turpin, Li Xu and Pankaj Sah
Queensland Brain Institute, University of Queensland, QLD

Anxiety is a sustained state of apprehension to distal and potential threat, which can become extremely debilitating in disease states. Anxiety disorders represent the most

common of psychiatric disorders, affecting nearly one in four adults in the population. Accumulating evidence suggests that amygdala and extended amygdala, brain regions important for emotional processing, have a central role in anxiety. In particular, the central nucleus of the amygdala (CeA), and its forebrain target the central sublenticular extended amygdala (SLEAc), two key parts of central extended amygdala, have been identified as critical elements of anxiety processing. We have studied the neuronal types and synaptic connections within this anxiety circuit using slice electrophysiological recordings in combination with tract tracing and *ex vivo* optogenetics. Our monosynaptic retrograde tracing experiments using retrograde beads or retrograde lenti-virus in C57BL6 mice revealed that the most substantial projections from the CeA to SLEAc originate from the lateral division of the CeA (CeL). We show that 78% of the retrogradely-labelled cells in CeL expressed somatostatin (SOM). To confirm the properties of this CeL to SLEAc connection, we selectively expressed Channelrhodopsin-2 (ChR2) in SOM+ neurons by injecting a Cre-dependent adeno-associated virus (AAV) encoding mCherry-tagged ChR2 into the CeL of *Som-cre* knock-in mice. Whole-cell recordings obtained from neurons in the SLEAc demonstrated that optogenetic activation of ChR2-expressing SOM+ terminals originating from the CeL evoked synaptic responses in most recorded SLEAc neurons, the majority being ChAT negative. To test reciprocal connections, we combined whole-cell patch clamp recording of retrograde labeled projection neurons to SLEAc as well as non-retrograde labeled neurons in CeL, with photo-activation of ChR2 expressing terminals originated from SLEAc in acute amygdala slices of C57BL6 mice. We show that SLEAc sends feedback projections to the CeL, which was mainly GABAergic, favoring the direct inhibition of non-retrograde labeled neurons from SLEAc. Together, these results define a reciprocal inhibitory circuit between CeL and SLEAc, two key parts of central extended amygdala, which prepare the ground to dissect the functional circuitry of CeL-SLEAc and to examine its behavioral role in anxiety.

6. A cascade linking A-beta through Tau missorting to spastin-dependent destruction of dendritic microtubules and spines

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We are interested in the physiological functions of Tau protein and its role in neurodegeneration in Alzheimer Disease, frontotemporal dementias, and other tauopathies. We are developing cell and animal models to observe the spreading of Tau pathology, the interaction with A-beta, and the effects of aggregation inhibitor compounds. This includes transgenic mice in which Tau is expressed either in a "pro-aggregant" form, or in a non-aggregating form, which can be compared with Tau knockout mice or mice expressing wildtype forms of Tau. The aberrant mislocalization and aggregation of Tau, combined with loss of synapses and microtubules are among the hallmarks of AD (review, Zempel & Mandelkow, TiNS 2014). Microtubules play essential roles in the maintenance of axons and dendrites because they provide the tracks for intracellular transport by motor proteins and the distribution of cell components. However, the causes and mechanisms of microtubule breakdown in AD are poorly understood. To elucidate the cascade of events leading to microtubule breakdown and synapse loss we exposed mature wildtype and Tau knockout neurons

to A-beta oligomers and analyzed changes in the Tau/microtubule system (Zempel et al., EMBOJ 2013). Microtubule breakdown occurs in dendrites invaded by Tau and is mediated by spastin, a microtubule-severing enzyme. Spastin is recruited to microtubules modified by polyglutamylation, mediated by translocation of TTLL6 (Tubulin-Tyrosine-Ligase-Like-6). Photoconversion of Tau labeled with Dendra2 reveals that missorted Tau in dendrites is newly synthesized and not derived from the axon. Long-term observations show that the toxic effects induced by A-beta are reversible and involve the activitation of the kinase MARK. In absence of Tau (TauKO neurons), microtubules and synapses are resistant to A-beta induced toxicity because without Tau the mislocalization of TTLL6 and the polyglutamylation of microtubules are prevented, so that there is no recruitment of spastin and no microtubule breakdown. Reintroduction of Tau re-establishes A-beta induced toxicity in TauKO neurons, which requires phosphorylation of the KXGS-motifs in the repeat domain of Tau. The results provide a rationale for microtubule stabilization as a therapeutic approach. - Funding by DZNE, MPG, WT, MetLife Foundation, Tau Consortium.

7. Tracking the Auditory Fear Engram: Discrete Populations of Neurons within Amygdala, Hypothalamus and Lateral Septum are specifically activated by Auditory Fear Conditioning

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Memory formation is thought to occur via enhanced synaptic connectivity between populations of neurons in the brain. However, it has been difficult to localise and identify the neurons which are directly involved in the formation of any specific memory. We have previously used *fos-tau-lacZ* (*FTL*) transgenic mice to identify discrete populations of neurons in amygdala and hypothalamus which were specifically activated by fear conditioning to multi-modal cues in a context fear conditioning paradigm. Here, we have extended our findings and examined neuronal activation activated by fear conditioning to a specific auditory cue in order to investigate whether the population of activated neurons in the amygdala and hypothalamus would differ if a more precise CS was used. To maximize the specificity of learning, we modified the traditional auditory fear conditioning paradigm so that association of the footshock to the context of the training chamber is minimised. Thus, the mice were exposed to long-term habituation to the training chamber and environmental enrichment prior to undergoing auditory fear conditioning. This pre-treatment ensured that the mice did not form fear conditioning responses to any non-auditory cues of the training context. We analysed the FTL expression pattern in several control groups of mice as well as mice that underwent auditory fear conditioning in order to identify populations of neurons specifically activated by fear conditioning to a discrete cue. Our analysis identified several populations of learning specific neurons activated by conditioning to a defined auditory cue. These populations of activated neurons were found in a small number of locations in the brain, including some of those previously found to be activated by context fear conditioning. These areas included the lateral septum, specific regions of the amygdalostriatal transition area, the lateral and medial amygdala and two areas in the

hypothalamus. These populations, each containing only a small number of neurons are directly implicated in fear learning and memory.

8. Neuroligin-3 on hippocampal parvalbumin interneurons facilitates contextual fear extinction

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Neuroligins (NLs) are a family of 4 postsynaptic proteins (NL1-4) that interact with presynaptic neurexins. This trans-synaptic interaction between neurexins and neuroligins has been implicated in the formation, maintenance and activity-dependent strengthening of synapses in various brain regions. Disruption of either the presynaptic neurexins or postsynaptic NLs has been implicated in autism spectrum disorders and schizophrenia. Mouse models carrying human NL autism point mutations have shown abnormal social interaction, learning and memory. The deficits in learning and memory in mice lacking NL1 and NL2 genes, as well as in mice engineered to carry the human mutations in NL3/4 have been correlated with synaptic abnormalities in either ionotropic glutamate or GABA receptors. However, the functional role of NLs at synapses, in particular NL3, requires further elucidation.

Parvalbumin (PV) interneurons have been implicated in cognition, learning and memory, as well as in the pathophysiology of various psychiatric disorders including schizophrenia and autism spectrum disorders. However, little is known about the molecular regulation of synaptic transmission onto these cells. To study the putative role of NL3 at synapses onto interneurons, we made a conditional knockout mouse for NL3 (*NL3$^{fl/fl}$*), and crossed it to a mouse line expressing cre under the parvalbumin (PV-cre) promoter to generate the NL3$^{Pvcre-/-}$ mice. These mice showed reduced reversal learning as assayed by contextual fear extinction, while contextual fear conditioning was unaltered. This reduction in extinction learning was rescued by a region and cell type specific expression of NL3 in the hippocampal PV interneurons. To identify the synaptic and circuit deficits underlying this behaviour, we made whole cell recordings from genetically identified PV cells in the hippocampal CA1 region from acute slices. In the NL3$^{Pvcre-/-}$ mice, a decrease in the paired pulse facilitation of excitatory inputs onto PV cells was observed suggesting an increase in release probability. This suggestion was further supported by an enhanced run down of NMDA receptor (NMDAR)-mediated EPSCs in NL3$^{Pvcre-/-}$ mice when MK-801 was applied. This increase in release probability correlated with a specific lack of sensitivity of the EPSC amplitude and paired pulse ratio to agonists or antagonists to Group-III mGluRs, which control release at these synapses in wild type animals. This lack of Group-III mGluR mediated control of release at these synapses renders a low pass filter at these synapses, summating incoming excitatory inputs only at frequencies lower than 10 Hz. These results demonstrate that postsynaptic NL3 plays an important role in regulating synaptic transmission at excitatory synapses onto PV interneurons by modulating presynaptic glutamate release onto these cells. The synaptic and behavioural deficits

observed as a consequence of NL3 deletion in PV cells are likely to play an important role in the pathophysiology underlying a plethora of complex neuropsychiatric disorders caused by NL3 mutations.

9. A syntaxin1A-mediated presynaptic mechanism of general anesthesia

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General anesthetics such as propofol and isoflurane are sedatives in addition to producing a behaviorally inert state conducive to surgery. How general anesthetics achieve this second effect – a profound loss of responsiveness – remains unclear. We have found that propofol, a commonly used post-synaptic GABA receptor agonist, reduces neurotransmitter quantal release from glutamatergic synapses in *Drosophila* larvae, suggesting a pre-synaptic mechanism of action in addition to a sleep-promoting role. Using single molecule imaging, we found that propofol restricts the mobility of membrane-bound syntaxin1A, a key SNARE protein, in neurosecretory cells. Co-expression of a truncated syntaxin1A protein lacking a trans-membrane domain completely blocked the effect of propofol. Additionally, co-expression of the same truncated syntaxin1A in *Drosophila* also produced behavioral resistance to volatile general anesthetics. Anesthesia resistance appears to be mediated by protein interactions just prior to SNARE complex formation, suggesting that general anesthetics in the membrane act on the path to SNARE assembly.

10. Secreted amyloid precursor protein alpha regulates protein synthesis in primary hippocampal neuronal cultures

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The secreted fragment of the amyloid precursor protein, sAPPα, has recently been shown to both enhance spatial memory as well as long-term potentiation, an experimental model of memory. Surprisingly little is known about how sAPPα induces changes in nerve cell activity. Since the persistence of memory and LTP is dependent on the synthesis of proteins by activated hippocampal neurons we hypothesized that sAPPα may affect protein synthesis and in early work showed that sAPPα stimulated protein synthesis in isolated nerve cell synapses. As LTP is underpinned by regulation of the AMPA-subtype of glutamate receptors, in the current study we explored whether application of sAPPα to primary hippocampal neuronal cultures regulates the synthesis of the AMPA receptor subunits GluA1 or GluA2. Following incubation with 1 nmol sAPPα, newly synthesized proteins were labelled using fluorescent non-

canonical amino acid tagging plus proximity ligation assays (PLA-FUNCAT). This method incorporates a methionine analogue bound to an azide group into newly synthesized proteins. Click chemistry attaches a biotin-bound alkyne group to the newly synthesized protein, which can then be recognized with antibodies and PLA probes to determine the identity and location of newly
synthesized proteins. Using PLA-FUNCAT, we found that sAPPα upregulated protein synthesis ($p < 0.05$, Kruskal Wallis test), and specifically, levels of newly synthesized GluR1 were increased threefold ($p < 0.0005$) while GluR2 levels were unaffected compared to baseline. By focusing specifically on newly synthesized proteins in cell soma we found a 2-fold increase in GluR1 synthesis compared to baseline ($p < 0.05$). These results indicate that a sAPPα-mediated increase in protein synthesis, including the GluR1 subunit of AMPA receptor, may be one mechanism through which it enhances LTP and memory.

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Hippocampus-driven feed-forward inhibition in the prefrontal cortex mediated by $GABA_B$ encodes task-dependent contextual information

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Purpose: It is well established that the hippocampus (HPC) plays a pivotal role in regulating contextual information. For the extinction of emotional memory, it has been shown that a disruption of HPC activity impairs context-dependent extinction of learned fear (Corcoran et al., 2005). Even though the HPC directly projects to the amygdala, which is a core region for the regulation of fear and emotions, the HPC also strongly innervates the mPFC (Parent et al., 2009), but the specific neuronal connections between these two structure, and their function is unclear. Hence, this study investigated this circuitry between the HPC and the mPFC on a neuronal level combined with pharmacological approaches *in-vitro* and *in-vivo*.
Methods: (i) To study the circuitry between the hippocampus and the mPFC, we made injections of Adeno-associated-virus (AAV) expressing the light-gated cation channel channelrhodopsin-2 (ChR2) into the ventral HPC (vHPC) in rats or the infralimbic PFC (ILPFC) in transgenic mice. Whole-cell recording were then made from principal neurons and interneurons in the ILPFC or prelimbic PFC (PLPFC) to determine synaptic properties of these connections by using receptor-specific antagonists. (ii) To investigate the physiological role of $GABA_B$-mediated synaptic transmission in the mPFC, the ILPFC of rats was infused with a $GABA_B$ antagonist in an active place avoidance task to test spatial orientation.
Results: Optical stimulation of brain sections containing the mPFC revealed that vHPC projections to the mPFC predominantly innervated interneurons in the ILPFC, which in turn caused a feed-forward inhibition onto local principal cells in layer 2/3 and layer 5/6. This feed-forward inhibition had a slow inhibitory component that was blocked by the application of the $GABA_B$-receptor antagonist CGP55845. By using transgenic mice that expressed ChR2 under a parvalbumin (Parv) promoter, we identified Parv neurons that cause the slow $GABA_B$-mediated conductance. Moreover, local infusion of CGP55845 into the ILPFC of rats significantly enhances the amount of entries into the shock zone following training in the active place avoidance task.

Conclusion: The findings of this study reveal a function of the mPFC in spacial orientation that is driven by inputs from the vHPC that spike parvalbumin-positive interneurons, which in turn cause prolonged inhibition of principal neurons to allow tuned neuronal activity.

12. Two N-terminal Isoforms of SAP97 Scaffold AMPA Receptors at the Glutamatergic Synapse

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The location and density of synaptic AMPA receptors is controlled by scaffolding proteins that lie directly beneath the postsynaptic membrane. Two N-terminal isoforms of SAP97, αSAP97 and βSAP97, are thought to play opposite and complimentary roles in regulating surface AMPA receptor location. Electrophysiology evidence suggests that αSAP97 increases synaptic strength by clustering AMPA receptors at synapses, whereas βSAP97 decreases synaptic strength by scaffolding these receptors at perisynaptic locations. However, this hypothesis has not yet been verified with imaging, as the neuronal synapse lies below the resolution limit of conventional optical microscopy.
Cultured rat hippocampal neurons were transiently transfected with α or βSAP97-eGFP, short-hairpin RNA to knock-down βSAP97 expression, or an empty eGFP control plasmid. A single molecule localisation method of super resolution imaging known as dSTORM was used to image the distribution of surface AMPA receptors on transfected neurons. Confocal microscopy was used to quantify both the size of these neurons, and the morphology and density of their synapses.
Both α and βSAP97 overexpression enhanced the clustering of GluA1-containing AMPA receptors at synapses compared to eGFP controls, whereas acute knock-down of βSAP97 relocated the receptors to perisynaptic sites ($p < 0.001$). In addition, overexpression of βSAP97 increased the density of GluA1 subunits at synapses ($p < 0.001$), and reduced the proportion of mature, mushroom-shaped dendritic spines ($p = 0.029$). Both α and βSAP97 overexpression increased the size of the presynaptic active zone via transsynaptic signalling ($p < 0.001/p = 0.024$). Compared to αSAP97, βSAP97 overexpression reduced the size of the dendritic tree ($p = 0.027$), and the proportion of postsynaptic densities with direct access to the presynaptic active zone ($p = 0.003$). The previously observed differences in synaptic strength between α and βSAP97 expressing neurons can be explained by differences in the number of synapses, rather than the location of surface AMPA receptors on the postsynaptic membrane. Both α and βSAP97 can scaffold AMPA receptors at synapses, whereas βSAP97 can actively promote synaptic incorporation of AMPA receptors by releasing them from the perisynaptic pool.

13. (Laura Daroles)

In the adult brain, structural plasticity allowing gain or loss of synapses remodels circuits to support learning. In Fragile X Syndrom (FXS), plasticity defects leading to learning deficits originates in the absence of Fragile X Mental Retardation Protein (FMRP). FMRP is a master regulator of local translation but its implication in learning-induced structural plasticity is unknown. Using an olfactory learning task requiring adult-born neurons in the olfactory bulb (OB) and cell-specific ablation of FMRP, we investigated whether learning shapes adult-born neurons morphology during their

synaptic integration into the pre-existing network and asked if these structural changes depend on FMRP. A local translation reporter was used to reveal local translation of the alpha subunit of the Calcium
Calmodulin-dependent Kinase II (**α**CaMKII) mRNA, an FMRP target, during learning in presence or absence of FMRP. Finally, we used **α**CaMKII mutant mice with altered dendritic localization of **α**CaMKII mRNA to investigate the role of locally translated **α**CaMKII in learning-dependent structural plasticity.Learning induces profound changes in dendritic architecture of adult-born neurons. Learning and associated structural changes are prevented by ablation of FMRP in adult-born neurons and rescued by an mGLUR5 antagonist, thus providing a mechanistic ground to its clinical use in FXS. Moreover, learning triggers an FMRP-dependent increase of local dendritic mRNA translation of **α**CaMKII in adult-born neurons and dendritically translated **α**CaMKII is necessary for learning and associated structural modifications.Our results strongly suggest that FMRP mediates structural plasticity of OB adult-born neurons to support olfactory learning through **α**CaMKII local translation. This reveals a new role of dendritic local translation in learning-induced structural plasticity, necessary for dendrite morphogenesis and spine production. This might be of clinical relevance for the understanding of critical periods disruption in autism spectrum disorder patients, among which FXS is the primary monogenic cause.

14. The persistence of long-term potentiation in the ventral hippocampus to medial prefrontal cortex projection in awake rats

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A potentially vital pathway in the processing of spatial memory is the ventral hippocampus to medial prefrontal cortex (vHPC-mPFC) pathway. To assess its induction and maintenance across days, long-term potentiation (LTP) was generated in this pathway of awake freely moving rats, and compared across several induction paradigms. Two different high-frequency stimulation (HFS) protocols generated LTP lasting no longer than one week. However, after delivering HFS on three consecutive days, we obtained LTP lasting an average of 20 days, due mainly to the greater initial induction after the third HFS. Thus the pathway does not require the extensive multi-day stimulation to induce LTP, as for intra-neocortical pathways, but also does not exhibit the extremely long lasting and stable LTP previously observed in area CA1 and the dentate gyrus. By using bilaterally placed stimulating and recording electrodes, we found that induction of LTP in one hemisphere also generated LTP in the same pathway in the opposite hemisphere, although it lasted less than one day. Finally, we found that repeated overnight exposure to an enriched environment transiently potentiated this vHPC-mPFC response, suggested an increased cellular excitability. However, this potentiation declined to baseline even before the two-week period of enriched environment treatment was completed. Overall, these findings are consistent with the vHPC-mPFC pathway as a key contributor to the hippocampus-mPFC interplay that, over days and weeks, leads to long-term storage of memories in the frontal cortex.

15. Activity-dependent RNA methylation in learning and memory

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Methylation of adenosine residue or **N6**-methyladenosine (m6A) is the most prevalent internal modification on eukaryotic RNA. m6A is catalysed by an RNA methyltransferase complex and is reversed by the m6A demethylating enzyme, such as FTO. In brain, the level of m6A is developmentally upregulated and peaks in adulthood, suggestive of its significant roles in adult brain function and plasticity. To date, the function of m6A in the mammalian brain is unknown. Using an antibody-based m6A capture technique followed by high throughput RNA sequencing (MeRIP-seq), we showed for the first time that m6A transcriptomic landscape was dynamically regulated in the mouse prefrontal cortex following behavioural training. A general increase in m6A stoichiometry at specific
mRNA loci was induced by novel context exposure and more intensely, by associative fear conditioning paradigm. The distribution of m6A along mRNA was highly enriched around the stop codon. Neuronal depolarization resulted in similar upregulation of locus-specific m6A. In probing the functional impact of m6A on RNA, we promoted demethylation through FTO overexpression. This led to increased stability of m6A target mRNA.
Finally, the impact of modulating m6A on learning was tested **in vivo**. Since increase in m6A stoichiometry manifested following context- and fear conditioning, we assessed the impact of pre-amplifying m6A levels on learning and memory through FTO knockdown. Basal anxiety levels were not affected in FTO knockdown mice; however, cued fear memory performance was significantly enhanced 24 hours post-fear conditioning training suggesting increased memory consolidation in this mice. Taken together, these findings provide the first demonstration of m6A role in learning and memory.

***16. Shank3*-mutant mice lacking exon 9 show altered excitation/inhibition balance, enhanced rearing, and spatial memory deficit**

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Shank3 is a postsynaptic scaffolding protein implicated in synapse development and autism spectrum disorders. The *Shank3* gene is known to produce diverse splice variants whose functions have not been fully explored. In the present study, we generated mice lacking *Shank3* exon 9 (*Shank3*$^{\Delta 9}$ mice), and thus missing 5 out of 10 known Shank3 splice variants containing the N-terminal ankyrin repeat region,

including the longest splice variant, Shank3a. Our X-gal staining results revealed that Shank3 proteins encoded by exon 9-containing splice variants are abundant in upper cortical layers, striatum, hippocampus, and thalamus, but not in the olfactory bulb or cerebellum, despite the significant Shank3 mRNA levels in these regions. The hippocampal CA1 region of *Shank3*$^{\Delta 9}$ mice exhibited reduced excitatory transmission at Schaffer collateral synapses and increased frequency of spontaneous inhibitory synaptic events in pyramidal neurons. In contrast, prelimbic layer 2/3 pyramidal neurons in the medial prefrontal cortex displayed decreased frequency of spontaneous inhibitory synaptic events, indicating alterations in the ratio of excitation/inhibition (E/I ratio) in the *Shank3*$^{\Delta 9}$ brain. These mice displayed a mild increase in rearing in a novel environment and mildly impaired spatial memory, but showed normal social interaction and repetitive behavior. These results suggest that ankyrin repeat-containing Shank3 splice variants are important for E/I balance, rearing behavior, and spatial memory.

17. Activity-dependent Ubiquitination of GluA1 and GluA2 Regulates AMPA Receptor Intracellular Sorting and Degradation

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AMPA receptors (AMPARs) have recently been shown to undergo post-translational ubiquitination in mammalian neurons. However, the underlying molecular mechanisms are poorly understood and remain controversial. Here we report that all four AMPAR subunits (GluA1-4) are rapidly ubiquitinated upon brief application of AMPA or bicuculline in cultured neurons. This process is Ca^{2+}-dependent and requires the activity of L-type voltage-gated Ca^{2+} channels and Ca^{2+}/calmodulin-dependent kinase II. The ubiquitination of all subunits occurs exclusively on AMPARs located on the plasma membrane post-endocytosis. The sites of ubiquitination were mapped to Lys-868 in GluA1 and Lys-870/Lys-882 in GluA2 carboxy-terminals. Mutation of these lysines did not affect basal surface expression or AMPA-induced internalisation of GluA1 and GluA2 subunits. Instead, it reduced the intracellular trafficking of AMPARs to the late endosomes and thus, protein degradation. These data indicate that ubiquitination is an important regulatory signal for controlling AMPAR function, which may be crucial for synaptic plasticity.

18. Sez6 binds the analgesic target α2δ and contributes to neuropathic pain plasticity

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Nerve damage or disease can trigger persistent pain, referred to as neuropathic pain, where normally non-noxious stimuli are perceived as painful (allodynia and hyperalgesia). The accompanying sensitization of primary afferent synapses in the spinal cord dorsal horn (DH) can be inhibited by the neuropathic analgesics gabapentin and pregabalin acting at their receptor, $\alpha_2\delta$. Along with the trafficking of voltage-gated calcium channel pore subunits and enhancing excitatory neurotransmitter release probability, $\alpha_2\delta$ appears to act as an adhesion molecule in synaptogenesis. Previously, we linked the seizure-related protein Sez6 to excitatory synapse development. We now present evidence for the involvement of Sez6 in structural and functional plasticity in a mouse model of neuropathic pain: i) co-immunoprecipitation of Sez6 and $\alpha_2\delta$ and co-expression in a sub-population of sensory dorsal root ganglion neurons; ii) a functional role of the Sez6-$\alpha_2\delta$ complex in excitatory synapse formation *in vitro* that is blocked by gabapentin; iii) attenuated heat hyperalgesia in Sez6 knockout (KO) mice after a peripheral nerve injury (chronic constriction injury of the sciatic nerve, or CCI); iv) reduced excitatory neurotransmission after agonist-induced transient receptor potential vanilloid 1 (TrpV1) activation in Sez6 KO CCI spinal cord slices compared to wild-type CCI controls; v) a Sez6- and CCI-dependent increase in dendritic spine density on pyramidal neurons in the medial pre-frontal cortex, a region involved in affective aspects of neuropathic pain. In conclusion, Sez6 promotes excitatory synapse formation via $\alpha_2\delta$ while a lack of Sez6 alleviates neuropathic pain states, including excitatory synapse structural and functional plasticity, along the central "pain" neuraxis.

19. A single day of 5-Azacytidine exposure induces Neurodegeneration in Neonatal Mice and Synaptic, Learning and Memory Deficits in Adult Mice

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Evidence suggests that synthetic environmental agents can have adverse effects on the structure and function of the brain and adversely affect the cognitive function. Several drugs and chemicals are known to be teratogenic when administered during early brain development leading to intellectual disabilities. However, the molecular mechanisms underlying these abnormalities are still poorly understood. The present study was undertaken to evaluate the single day exposure of 5-Azacytidine (5-AzaC), an inhibitor of DNA methyltransferase, on neurodegeneration in P7 mice and long-lasting effects on long-term potentiation (LTP), learning and memory behavior in adult mice. 5-AzaC treatment of P7 mice inhibited DNA methylation and caused neurodegeneration in a time and dose-dependent manner in the hippocampus and cortex, two brain areas that are important for memory formation and storage, respectively. 5-AzaC treatment of P7 mice induces deficits in LTP in adult hippocampal slices, impaired object recognition, spatial memory and social recognition memory in adult mice. Together, these data demonstrate that inhibition of DNA methylation by 5-AzaC treatment of P7 mice causes neurodegeneration in neonatal mice and long-lasting neurobehavioral abnormalities in adult mice. DNA methylation mediated mechanisms during development appear to be important for the normal maturation of

synaptic circuits and disruption of this process by 5-AzaC could lead to abnormal cognitive function.

20. Extending the viability of acute brain slices

Yossi Buskila

The lifespan of an acute brain slice is approximately 6–12 hours, limiting potential experimentation time. We have designed a new recovery incubation system capable of extending their lifespan to more than 36 hours. This system controls the temperature of the incubated artificial cerebral spinal fluid (aCSF) while continuously passing the fluid through a UVC filtration system and simultaneously monitoring temperature and pH. The combination of controlled temperature and UVC filtering maintains bacteria levels in the lag phase and leads to the dramatic extension of the brain slice lifespan. Brain slice viability was validated through electrophysiological recordings as well as live/dead cell assays. This system benefits researchers by monitoring incubation conditions and standardizing this artificial environment. It further provides viable tissue for two experimental days, reducing the time spent preparing brain slices and the number of animals required for research.

21. Purification of neural precursor cells reveals the presence of distinct, stimulus-specific subpopulations of quiescent precursors in the adult mouse hippocampus.

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New neurons are generated from resident populations of stem and precursor cells in the adult mammalian hippocampus, and have been shown to critically contribute to the regulation of spatial learning and memory, as well as mood. The activity of these hippocampal precursors is regulated by various stimuli; however, whether these stimuli regulate the same or different precursor populations remains unknown. Here, we developed a novel cell-sorting protocol that allows the purification to homogeneity of neurosphere-forming neural precursors from the adult mouse hippocampus and examined the responsiveness of individual precursors to various stimuli using a clonal assay. We show that within the Hes5-GFP$^+$/Nestin-GFP$^+$/EGFR$^+$ cell population, which comprises the majority of neurosphere-forming precursors, there are two distinct subpopulations of quiescent precursor cells, one directly activated by high KCl depolarization, and the other activated by norepinephrine (NE). We then demonstrate that these two populations are differentially distributed along the septo-temporal axis of the hippocampus, and show that the NE-responsive precursors are selectively regulated by GABA, whereas the KCl-responsive precursors are selectively modulated by corticosterone. Finally, based on RNA-seq analysis by deep sequencing, we show that the progeny generated by activating NE- versus KCl-responsive quiescent precursors are molecularly different. These results demonstrate that the adult hippocampus contains phenotypically similar but stimulus-specific populations of quiescent precursors, which give rise to neural progeny with potentially different functional capacity.

22. Dysfunctional Synapse Plasticity Induced by Autism Spectrum Disorder Associated Shank3 Mutations

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Autism spectrum disorders (ASD) comprise a range of neurodevelopmental disorders characterised by lack of social interaction and communication, repetitive behaviours and cognitive deficits. Recent research has identified ASD-associated mutations in important synaptic proteins including Shank3, and this has prompted investigations into understanding the roles of synapse dysfunction in the pathogenesis of ASD. However, currently little is known about the effects of ASD-associated mutations in Shank3 on synapse plasticity, a mechanism which is thought to underlie cognition. Considering the pivotal role played by Shank3 in regulating both the structure and function of excitatory synapses, we hypothesised that ASD-associated mutations in Shank3 may disrupt the ability of synapses to undergo plasticity. To address this hypothesis, we induced and measured *N*-Methyl-D-aspartate receptor (NMDAR)-dependent long term potentiation (LTP), NMDAR-dependent long term depression (LTD), or metabotropic glutamate receptor (mGluR)-dependent LTD in cultured rat hippocampal neurons expressing ASD-associated mutant forms of Shank3. Neurons which either lacked Shank3 or expressed ASD-associated mutant versions of Shank3 displayed deficits in NMDAR-dependent LTP and mGluR-dependent LTD. In contrast, no effects on NMDAR-dependent LTD were observed. Moreover, these neurons expressed LTD under our NMDAR-dependent LTP-inducing paradigm. Faster NMDAR-mediated excitatory postsynaptic current (EPSC) deactivation kinetics were detected in neurons with Shank3 down-regulation, which indicated a possible change to GluN2A-containing NMDARs. This may in part contribute to the deficit in NMDAR-dependent LTP observed in neurons with loss of Shank3 by altering the threshold for LTP induction. Altogether, these results support synapse dysfunction as a major pathogenic mechanism for the development of ASD, however our data reveal that only specific forms of synaptic plasticity are affected.

23. Optogenetics reveals asymmetrical GABAergic recruitment within the lateral amygdala by auditory inputs

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The amygdala is a collection of functionally discrete, though complexly interconnected nuclei, which is critical for the acquisition and storage of emotional memory. Also known as *associative memory*, this higher-order cognitive function involves the pairing of environmentally derived sensory information, to produce learned behavioural responses. Previous investigation into associative learning is primarily centred on the behavioural learning paradigm *fear conditioning*, or its translational electrophysiological *in vitro* models. However, our present understanding of this structure is restricted by the limitations of conventional electrophysiological techniques, and the underlying neuronal circuits remain to be fully elucidated. Here, using virally targeted optogenetic stimulation with acute slice electrophysiology, we confirm that principal neurons (PN) of the lateral amygdala (LA) receive direct and robust inputs from both the auditory thalamus (AT) and auditory cortex (AC). However, in contrast to earlier findings, input specific stimulation revealed an

asymmetrical recruitment of local GABAergic circuits between the AT and AC, indicated through altered disynaptic inhibition upon LA PN. Further, discrepancies were revealed between electrical and optogenetic stimulation of AT terminals within the LA. We present evidence to suggest that electrical stimulation of the classical *cortical* and *thalamic* input sites of the LA is not input-restricted, and can instead produce the undetectable co-activation of these reciprocally connected AT and AC afferents. This study reveals input-specific circuit recruitment and information processing within the amygdala during auditory innervation, masked by conventional methods, with consequences for current models of emotional memory.

24. Neuromuscular synapse disassembly in the mouse model of anti-MuSK myasthenia gravis

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Muscle specific kinase (MuSK) is the core of a postsynaptic receptor tyrosine kinase complex at the mammalian neuromuscular junction (NMJ). The vital role of MuSK has led to the discovery of MuSK autoantibodies in myasthenia gravis (MG) patients who were previously considered sero-negative for autoantibodies. Patient anti-MuSK is largely of the IgG4 subtype, binds to the extracellular Ig domains of MuSK and disrupts the binding of MuSK to LRP4 (the neural agrin co-receptor), and to collagen Q. Repeated daily injections of IgG from MuSK MG patients into mice led to steady, proportionate declines in the postsynaptic density of acetylcholine receptor (AChR) density and endplate potential amplitudes, culminating in failure of neuromuscular transmission and muscle weakness after 14 days. The decline in AChR density was due to impaired retention of AChRs within postsynaptic membrane domains. Semi-quantitative immunofluorescence staining revealed a marked reduction in phosphorylated targets of MuSK kinase (src and the acetylcholine receptor itself) within 24 hours of the first injection of MuSK MG IgG. This was followed by the reduction in AChR, consistent with reduced MuSK kinase activity being the cause of subsequent AChR loss. During development the neural agrin/MuSK pathway functions to suppress a system, driven by synaptic acetylcholine that would otherwise disassemble AChR-rich membrane domains. This is significant because cholinesterase inhibitors such as pyridostigmine are a first-line treatment for myasthenia gravis. In the mouse model of MuSK MG (where MuSK signalling is suppressed) pyridostigmine was found to exacerbate postsynaptic AChR loss and weakness. On the other hand loss of postsynaptic AChR was substantially ameliorated by forced expression of MuSK in the tibialis anterior muscle of myasthenic mice. Our emerging understanding of the networks that regulate NMJ growth and adaptation provide new potential therapeutic targets for diverse conditions where NMJ function and survival is challenged.

25. Functional Analysis of Amyotrophic Lateral Sclerosis-associated Mutations of Profilin 1 in Primary Mouse Neurons

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease and familial ALS accounts for 10% of cases. The identification of new familial ALS mutations in the actin-binding protein profilin 1 directly implicates actin dynamics and regulation in the pathogenesis of ALS. However, how these mutations cause ALS is unknown. To help elucidate the pathomechanisms, we are studying the functional role of ALS-associated profilin1 mutations in both *in vitro* and *in vivo* models. Currently no mouse models are available for the study of the ALS-associated profilin 1 mutations. We are currently developing four new transgenic mice that overexpress profilin 1 or mutant profilin 1 with either a motor neuron specific promoter or a pan-neuronal promoter. Expression constructs for *in vitro* overexpression for functional analysis in primary mouse neurons have also been developed. Our data from studies using primary hippocampal mouse neurons, show that profilin 1 C71G expression results in increased dendritic tree length and arborisation in developing central nervous system neurons. This increase in branching is compartment specific as there was no significant change in axonal branching. Preliminary functional analysis on mature cultured neurons grown for 19 days *in vitro*, showed an increase in dendritic spine density in hippocampal neurons expressing profilin 1 C71G, compared to control vector transfected cells. Additionally, aggregations of profilin 1 have been observed in cultured hippocampal neurons transfected with profilin 1 C71G. Our study is the first to show dendrite-specific morphological changes in neurons expressing profilin 1 with an ALS-associated mutation. Studying the functional consequences of these ALS-associated profilin 1 mutations will help us to unravel some of the mysteries underlying the progression of this complex neurodegenerative disease.
Key Words: Amyotrophic Lateral Sclerosis, Profilin 1, Actin Cytoskeleton

26. "Paradoxical effect of endocannabinoids on visually-evoked responses of mouse retinal ganglion cells"

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Vision starts in the retina, a complex and highly organised part of the brain. The retina not only converts light signals into neuronal impulses, but it also carries out complex computations to allow for the extraction of complex features of the visual world. In addition, in order to remain functional under different visual conditions, different adaptation mechanisms take place in the retina. Adaptation to mean illumination and contrast adaptation are just two processes that take place in the retina. For this to occur, several mechanisms that modulate retinal signal transmission operate. The endocannabinoid (eCB) system has been characterised as a modulator of central nervous system synapses, regulating transmitter release. Recently, the eCB system has been detected in the retina, with studies indicating functional changes after application of cannabinoid receptor agonists and antagonists. Whether or not eCBs are tonically released in the retina, however, and their physiological function, are unknown. We aimed to establish whether a tonic level of eCBs exists in the retina, and if so, how it modulates retinal function and consequently visual processing.
Light-evoked responses and voltage-gated Na^+ currents were studied using single cell patch-clamp recordings of retinal ganglion cells (RGCs) of C57/BL6J mice in control conditions, and in the presence of URB597, the fatty acid amide hydrolase (eCB

degradatory enzyme) inhibitor. Addition of URB597 confirmed a tonic presence of eCB modulation: the amplitude of light-evoked responses of RGCs measured in current-clamp mode was reduced, as expected for an increase in eCB concentration. A paradoxical increase in RGC excitability, however, was also observed. This increase in RGC excitability could be explained by changes in Na^+ currents observed in voltage-clamp mode. Significant modulation of light-evoked response strength, contrast sensitivity, and short-term plasticity were observed.
These results provide evidence for a tonic role of eCBs in the retina. Implications for treatment of conditions such glaucoma with cannabinoids may result in altered visual processing.

27. Dopaminergic neurotransmission dysfunction induced by β-amyloid transforms cortical LTP into LTD and produces recognition memory impairment

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It has been proposed that amyloid beta (Aβ) impairs synaptic plasticity and memory in patients and animal models of Alzheimer's disease (AD), however, its mechanisms remain largely unknown. In this study, we show that cortical dopamine (DA) levels are decreased in a triple transgenic mouse model of AD with Aβ accumulation. We confirmed the finding by administering Aβ oligomers intracortically in WT mice. In both models, low DA converts *in vivo* cortical long-term potentiation (LTP) into long-term depression (LTD) after high frequency stimulation (HFS), which leads to impaired recognition memory. Remarkably, we found that restoring cortical DA levels, by administration of nomifensine, rescued both HFS-induced LTP and recognition memory. In WT mice the intracortical administration of 6-hydroxy-dopamine depleted DA levels and mimicked the Aβ-induced shift from LTP to LTD. Our results suggest that DA depletion by Aβ accumulation is a core mechanism underlying the early synaptopathy observed in AD models.

28. Neuronal circuits in the amygdala involved in socially transferred fear

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In its simplest form empathy can be characterized as the capacity to be affected by and/or to share the emotional state of another being (emotional contagion), which can be modelled in rodents. In our model of socially transferred fear we showed that a brief social interaction with a fearful cage mate (demonstrator) promotes aversive learning in an otherwise naïve rat (observer) and activates the amygdala of the observers, especially its central part (CeA). To elucidate the role of neuronal circuits in the central amygdala of the observers, we used two methods of functional mapping:

transgenic rats expressing, in behaviorally activated neurons, a PSD-95:Venus fusion protein and injected with anterograde tracer and a combination of retrograde tracing with c-Fos ISH. We have identified several afferent and efferent CeA projections active during socially transferred fear. We discovered strong activation especially in the periaqueductal gray (PAG) and dorsal raphe nuclei (DRN); these structures receive dense projections from the CeA and are implicated in fear and anxiety disorders. To test whether the activated circuits are similar for the socially and non-socially induced emotions, we used double immunodetection for a PSD-95:Venus construct and endogenous c-Fos. About 70% of neurons were activated by both social interaction with fear conditioned partner and subsequent fear conditioning. Moreover, using optogenetics, we showed that specific activation of CeA neurons involved during interaction with emotionally aroused partner results in a decrease of social and non-social exploratory behaviors and inhibition of ultrasonic vocalization. Together, these findings suggest adaptive role of neurons activated during socially transferred fear; they may increase vigilance/attention to external stimuli and they are, at least partially, recruited by subsequent learning of fear responses.

29. Impaired synaptic plasticity in the lateral habenula of an animal model of depression

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A number of brain areas have been suggested to undergo synaptic changes in animal models of depression. One such region is the lateral habenula (LHb) which mediates communication between forebrain and midbrain structures. Recently, it has been shown that the excitatory transmission onto LHb neurons is abnormally potentiated in learned helpless animal models of depression. In addition, a very strong correlation between the LHb activity and helplessness has been reported both in control and learned helpless animals. Therefore, it is likely that the LHb exhibit long-lasting synaptic changes; however, synaptic plasticity in LHb synapses and the effect of stress exposure has not been investigated. We hypothesized that synaptic efficacy of the LHb could undergo long-lasting changes depending on emotional valence of external cues or experiences. We found that long-term synaptic depression (LTD) occurs in the LHb in endocannabinoid (eCB) signaling dependent manner. Notably, the exposure to acute stressor impairs eCB-dependent LTD in the LHb and eCB1R activation successfully restores the LTD in the LHb of an animal model of depression. Our study revealed cellular mechanisms of LTD in the LHb and delineated its modulation by exposure to stressors.

30. Auditory Tone Representation in Amygdala of Naive Aminals

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Auditory fear conditioning is a widely used paradigm to study the physiology of associative memory. Based on this model the co-activation of aversive and sensory inputs converging onto neurons of the lateral amygdala is proposed to induce synaptic plasticity that supports fear learning. However, amygdala neurons' activity entrained

by auditory stimulation before acquisition of conditioning has not been explored. In this study, male Sprague-Dawley rats (n=5) were implanted with 6 tetrodes targeting the lateral amygdala. During a screening period, started a week after surgery, animals were presented multiple trains of tones (pips: 500ms on ; gaps: 1000ms interval off) at different frequencies (3 to 12 KHz and white noise).

We analyzed responses of single units to tones (n= 432; bins: 10ms; 30s pre-train baseline or 500ms pre pips baseline; $p<0.01$). When the pre-train baseline was used, we found that 48% of units were tone-responsive (n=209) and each of these units was responding to 2-3 frequencies. In the range tested, each frequency evoked a response from 20 to 30% of the units while white noise only evoked a response in 15% of the units.
Based on visual inspection of the peri-stimulus time histogram of units responses to auditory stimulation we defined 3 consecutive phases in the dynamic of unit activity following the onset of the tone, an early excitation (0-100ms), a late inhibition (100-500ms) and an off response (50-250ms post tone).
Unit activity was studied independently for those 3 phases, 41% of units responded during early activation 33% during late inhibition and 28% after tone. When we used the 500ms period immediately preceding tone onset as baseline, only 28% of cells responded to tones, 33% in the early activation phase, 18% during late inhibition and 12% at tone offset.
We then used pairwise correlation to study network dynamics in groups of units recorded concurrently in lateral amygdala in this different phase of responses dynamic. Significant interactions were identified, independently of firing rate, based on increased correlation between spike trains. This analysis of small networks showed that response coupling between neurons increased transiently during tone presentation independent of frequency (one-way ANOVA

31. The role of P75NTR in Cholinergic Basal Forebrain Structure

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The role of the p75 neurotrophin receptor ($p75^{NTR}$) in adult cholinergic basal forebrain (cBF) neurons is unclear due to conflicting results from previous studies and to limitations of existing $p75^{NTR}$-knock-out mouse models. In the present study we used a novel conditional knock-out line (ChAT-cre $p75^{in/in}$) to assess the role of $p75^{NTR}$ in the cBF by eliminating $p75^{NTR}$ in choline acetyl-transferase-expressing cells. We show that the absence of $p75^{NTR}$ results in a lasting increase in cBF cell number, cell size, and cholinergic innervation to the cortex. Analysis of adult ChAT-cre $p75^{in/in}$ mice revealed that mutant animals show a similar loss of cBF neurons with age to that observed in wild-type animals, indicating that $p75^{NTR}$ does not play a significant role in mediating this age-related decline in cBF neuronal number. However, the increased cholinergic axonal innervation of the cortex, but not the hippocampus, corresponded to alterations in idiothetic but not allothetic navigation as well as dysfunction in fear extinction consolidation. These findings support a role for $p75^{NTR}$-mediated regulation of cholinergic-dependent cognitive function, and suggest that the variability in previous reports of cBF neuron number may stem from limited spatial and temporal control of $p75^{NTR}$ expression in existing knockout models.

32. How does p75^{NTR} regulate memory?

Marie lou Camara

Neurotrophic growth factors essential for neuronal survival during nervous system development and regulate neuronal function in the adult brain. Brain-derived neurotrophic factor (BDNF) is a major positive modulator of cortical synaptic plasticity, underpinning long-term potentiation, whereas nerve growth factor (NGF), also expressed throughout the brain, has more restricted effects, being required for optimal function of a discrete population of neurons in the basal forebrain that provide acetylcholine to the cortex. Low levels of BDNF is associated with mood disorders and have been observed in the serum and plasma of Alzheimer's disease (AD) patients, where it is associated with faster ongoing cognitive decline. Similarly, reduced expression of NGF results in degeneration of cholinergic basal forebrain neurons, a feature of AD. Enhancing neurotrophic signaling is a validated therapeutic strategy for treating this condition.

NGF and BDNF signal via two classes of neurotrophin receptors, the p75 neurotrophin receptor (p75^{NTR}) and TrkA and TrkB respectively. Together p75^{NTR} and Trk receptors form a high-affinity receptor complex. Our lab has identified a novel signalling motif of the p75^{NTR} intracellular fragment – the 'Chopper' domain – and generated a cell-permeable peptide mimic called c29. We have established that c29 is sufficient to form the high affinity Trk-complex and furthermore can both, block p75^{NTR}-mediated cell death and promote neuronal survival and neurite outgrowth, in low neurotrophin concentrations *in vitro*. Our lab has now generated a novel transgenic mouse that can be induced to express the c29 peptide sequence and subsequently alter p75^{NTR} signalling. Preliminary findings suggest that reducing cBF p75^{NTR} signalling in the c29 transgenic mice, results in enhanced cognitive function in these mice as demonstrated by enhanced idiothetic navigation. These mice also have more robust (longer lived) contextual memories than littermates with normal p75^{NTR} function. These exciting results suggest that by altering p75^{NTR} signalling in cBF neurons it may be possible to stimulate or maintain aspects of cognition, especially those that decline in AD.

33. Cholinergic dysfunction of mesopontine tegmentum is involved in the development of Alzheimer's Disease

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Neuronal death, leading to overall brain atrophy, is one of the fundamental characteristics of Alzheimer's disease. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease, and the consequent cholinergic neurotransmitter decline affects other neurotransmitter systems. Epidemiological studies have shown that sleep apnoea is a risk factor for Alzheimer's disease. The neurons of another major cholinergic nucleus in the brain, the mesopontine tegmentum (MPT), project to upper motor neurons to control upper airway muscle tone during sleep, and are also implicated in initiating and maintaining rapid eye movement sleep, which is considered fundamental for learning consolidation and retention of memory. MPT neurons also project to the basal forebrain and produce nerve growth factor and thus may support basal forebrain neuronal survival and function throughout life. We found that lesions of MPT cholinergic neurons by

stereotaxic injection of saporin toxin conjugated to the specific urotensin II receptor peptide ligand result in altered breathing. Furthermore, the lesions produce a subsequent and selective degeneration of basal forebrain cholinergic neurons and a resultant decline in spatial memory. Loss of MPT-derived nerve growth factor due to MPT neuronal dysfunction may in turn cause dysfunction of basal forebrain neurons with negative flow-on effects on cognitive function and the development of Alzheimer's disease pathology.