VAMP2

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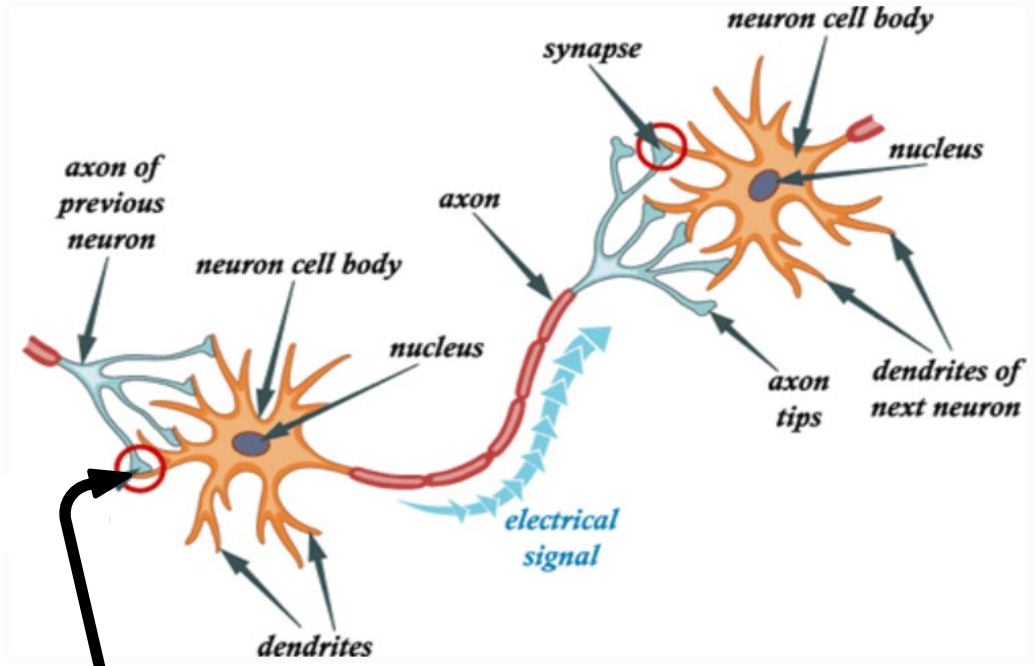
What is VAMP2?

- **V**esicle-**A**ssociated **M**embrane **P**rotein **2** (**VAMP2**) is a protein that is encoded by the *VAMP2* gene
- Expressed in the brain in nerve cells (neurons)
- Essential for synapsing of neurons (how brain cells talk to each other)
- Assists in the fusion of synapse transport vesicles to the neuron membrane allowing for neurotransmitter release
- Part of the SNARE complex which allows vesicles and membranes of neurons to fuse together for synapsing
- Most abundant synaptic vesicle protein, with approximately 70 copies per vesicle

Neurons and synapses

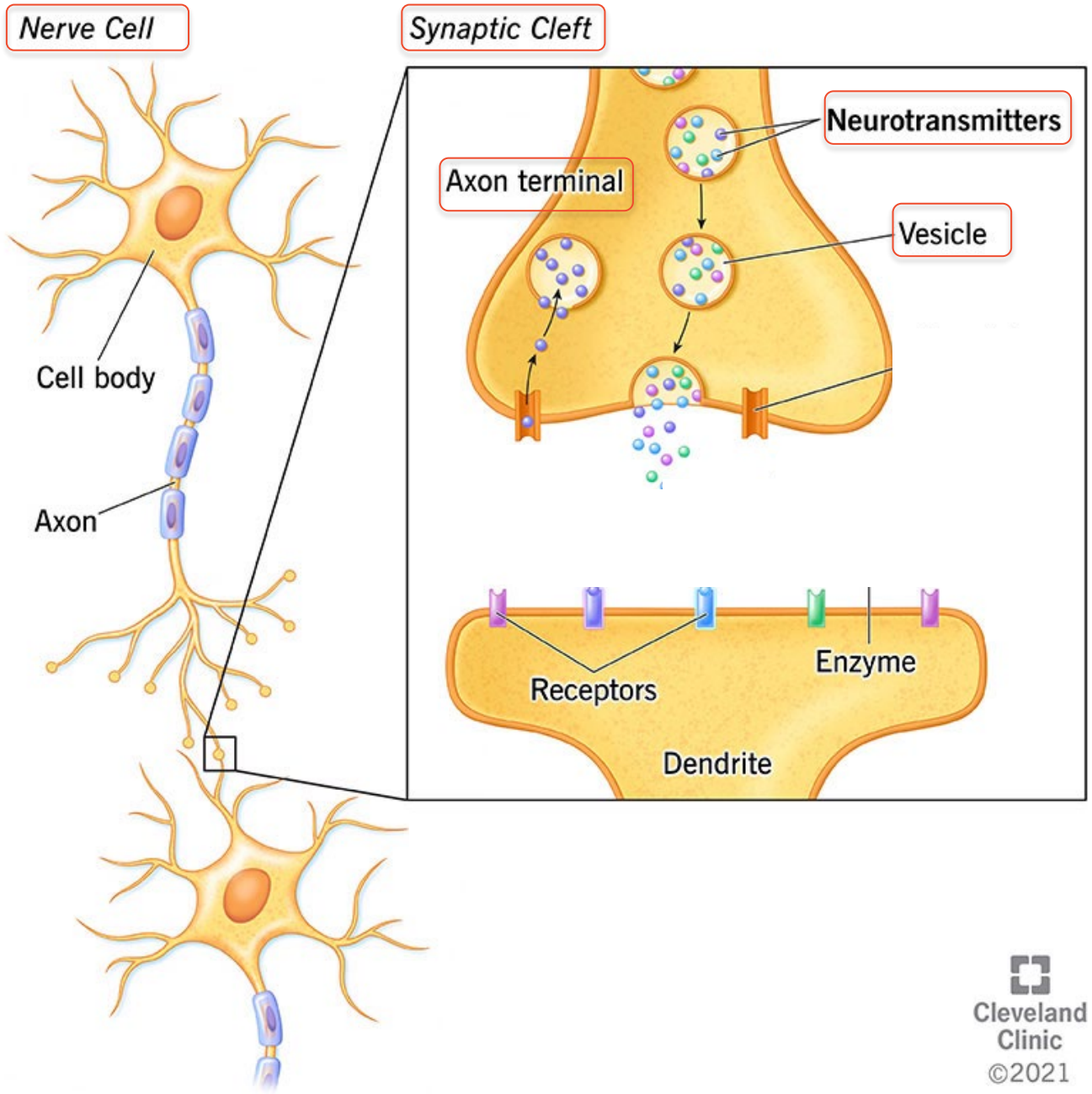
- Neurons are the fundamental units of the brain and nervous system
- They are responsible for receiving sensory input from the external world, for sending motor commands to our muscles, and for transforming and relaying the electrical signals at the steps in between.
- They relay messages at the synapse by releasing neurotransmitters



synapse

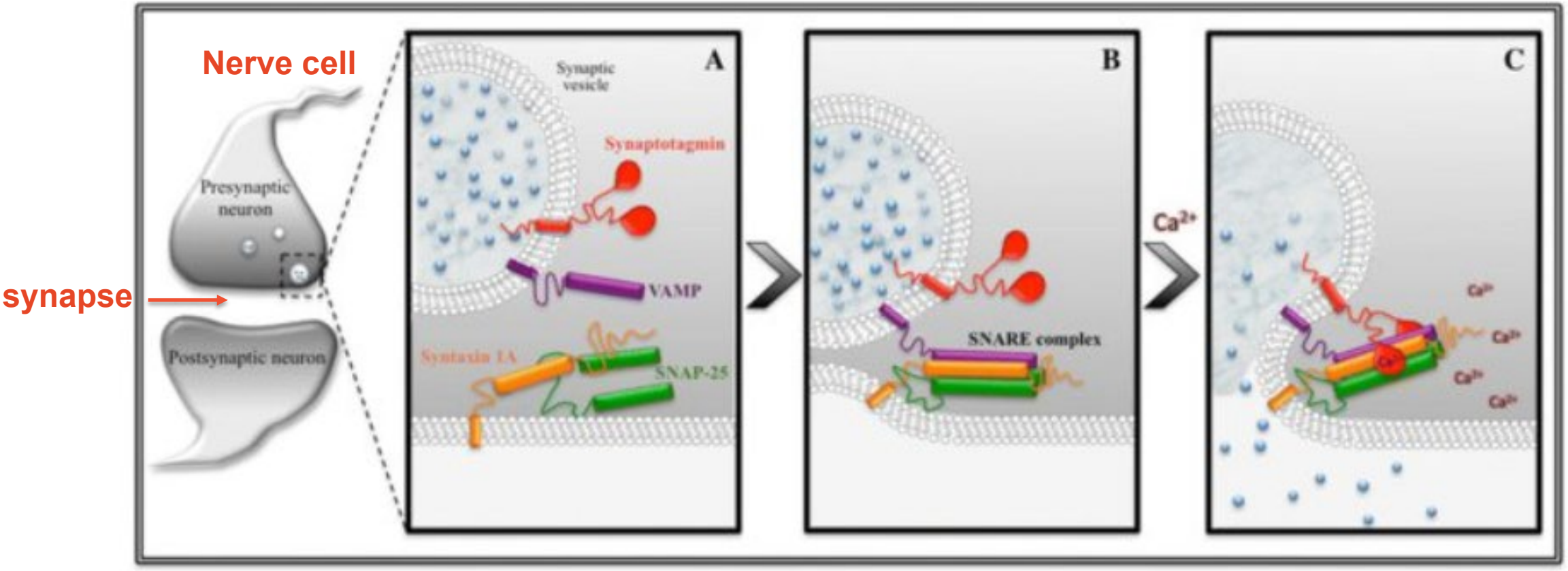
Neurotransmitters

- Chemical messengers that your body can't function without.
- Their job is to carry chemical signals ("messages") from one neuron (nerve cell) to the next target cell.
- The next target cell can be another nerve cell, a muscle cell or a gland.
- Neurotransmitters are carried by vesicles to the Axon terminal and they dock at the nerve cell membrane at the synaptic cleft.
- **VAMP2 is essential for this docking**



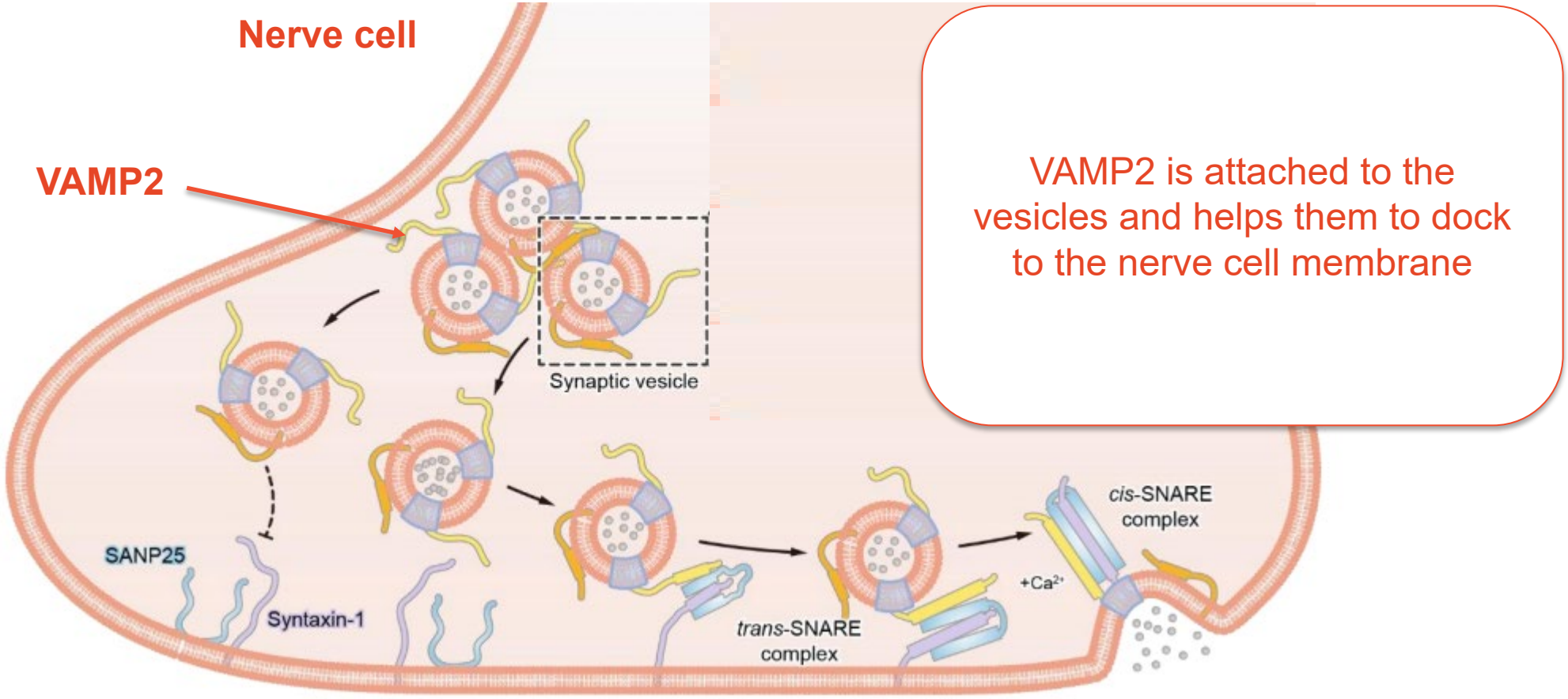
SNARE complex

– VAMP2 is part of the SNARE complex which assists in the docking of vesicles to the nerve cell membrane at the synapse



Renata Basso Cupertino, Psychiatry and Preclinical Psychiatric Studies 2016

VAMP2 allows vesicles to dock to the neuron membrane at the axon terminal



VAMP2 is attached to the vesicles and helps them to dock to the nerve cell membrane

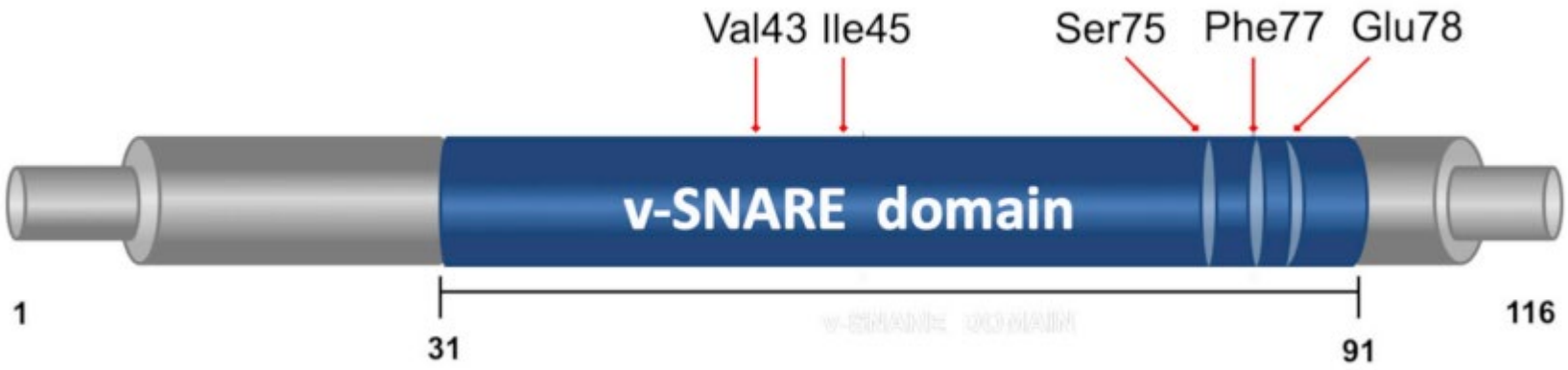
Adapted from Chuchu Wang et al Nature Communications 2020

Some reported symptoms of patients with VAMP2 mutations

- neurodevelopmental delay
- hypotonia (decreased muscle tone)
- autistic features
- hyperkinetic movements (abnormal involuntary movement)
- behavioral disturbances
  - body rocking
  - head banging
  - self-injurious behaviors
- epilepsy and/or electroencephalography (EEG) abnormalities
- variable motor stereotypies resembling Rett syndrome (absence of purposeful hand movements)
- central visual impairment

The gene and reported mutations

- The VAMP2 gene consists of one functional domain called the v-SNARE domain
- There are 22 reported mutations that are considered likely pathogenic (disease causing)
- A few reported mutations are below in the schematic



Current and future treatments

Currently, there is no effective treatment targeting the underlying impairment of neurotransmitter release

- Treatment is purely symptomatic
- Off – label treatment options have been reported testing **4-aminopyridine (4-AP)**
  - Caution: no clinical trials have been conducted and 4-AP may not be appropriate or effective for all mutations
- Gene therapy is a novel therapeutic option
  - Caution: Gene therapies may take a long to develop and safety and efficacy tests need to be rigorous.

Off label treatments

Hum Mutat. 2020 Nov;41(11):1999-2011. doi: 10.1002/humu.24109. Epub 2020 Oct 1.

Overcoming presynaptic effects of VAMP2 mutations with 4-aminopyridine treatment

Roxanne L Simmons [1], Haiyan Li [2], Baris Alten [3], Magda S Santos [2], Ruiji Jiang [1], Brianna Paul [1], Sanam J Lalani [1], Audrey Cortesi [1], Kendall Parks [1], Nitin Khandelwal [4], Bethany Smith-Packard [5], Malay A Phoong [6], Michael Chez [7], Heather Fisher [8], Angela E Scheuerle [9], Marwan Shinawi [10], Shaun A Hussain [11], Ege T Kavalali [3], Elliott H Sherr [1], Susan M Voglmaier [2]

4-aminopyridine (4-AP) treatment

- 2020: one patient only was treated with 4-AP with a mutation in c.166C>T, p.Arg56X
- The research team first tested 4-AP in cells with different mutations in the lab and observed:
  - 2 mutants cell lines showed a decreased rate of exocytosis and the number of synaptic vesicles released from the recycling pool, compared with wild-type. One did not.
  - 4-AP treatment increased the rate and extent of exocytosis and total synaptic charge transfer
- Treated one patient for 2 years with off- label 4-AP
  - improved emotional and behavioural regulation by parental report, and objective improvement in standardized cognitive measures

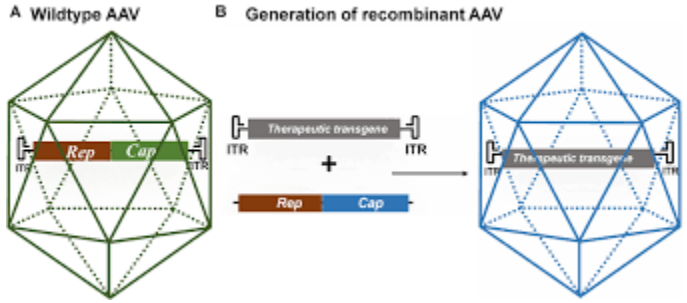
Aminopyridine treatment may extend to patients with pathogenic variants in VAMP2 and tested in vitro before treatment

VAMP2 Gene therapy

VAMP2 is a likely candidate for gene therapy

- Gene therapy is a treatment that introduces new genetic material into a person's cells to modify or manipulate the expression of a gene product or alter the biological properties of living cells to treat or cure disease.
- Gene therapy makes use of safe virus's called Adeno Associated Virus (AAV) which is used as a vehicle to transport the VAMP2 gene therapy to the patient's cells
- A number of gene therapies are now approved for other genetic disorders and available for therapeutic use eg Zolgensma® for the treatment of Spinal Muscular Atrophy.
- There are more than 4,000 gene therapy clinical trials in various phases of development

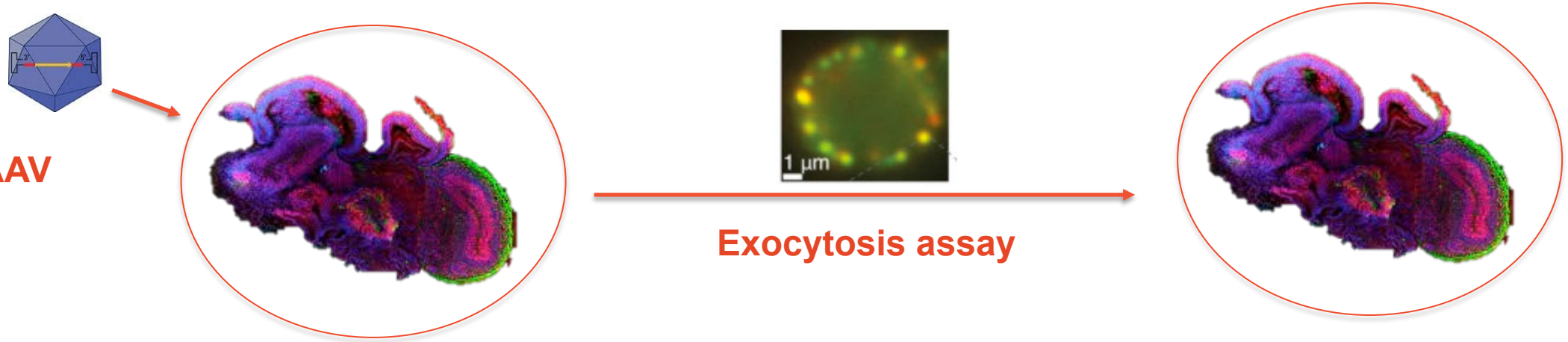
AAV



VAMP2 gene therapy research in the Gold lab

Our Gene therapy research plan:

- Grow patient cells into neuron cells and brain organoids aka ‘brains in a dish’ (relevant models to test therapies)
- Test exocytosis in neuron cells and organoids (relevant test to determine effects of VAMP2 mutations)
- Manufacture the VAMP2 gene therapy and package into AAVs
- Test VAMP2 gene therapy in neurons and organoids using exocytosis assays



About me

- I am an Associate Professor at the University of Sydney
- I lead a research group at the Childrens' hospital at Westmead and affiliated with the Children's Medical Research Institute
- My research focuses on developing curative therapies for children with rare neurodevelopmental disorders.
- Links:
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