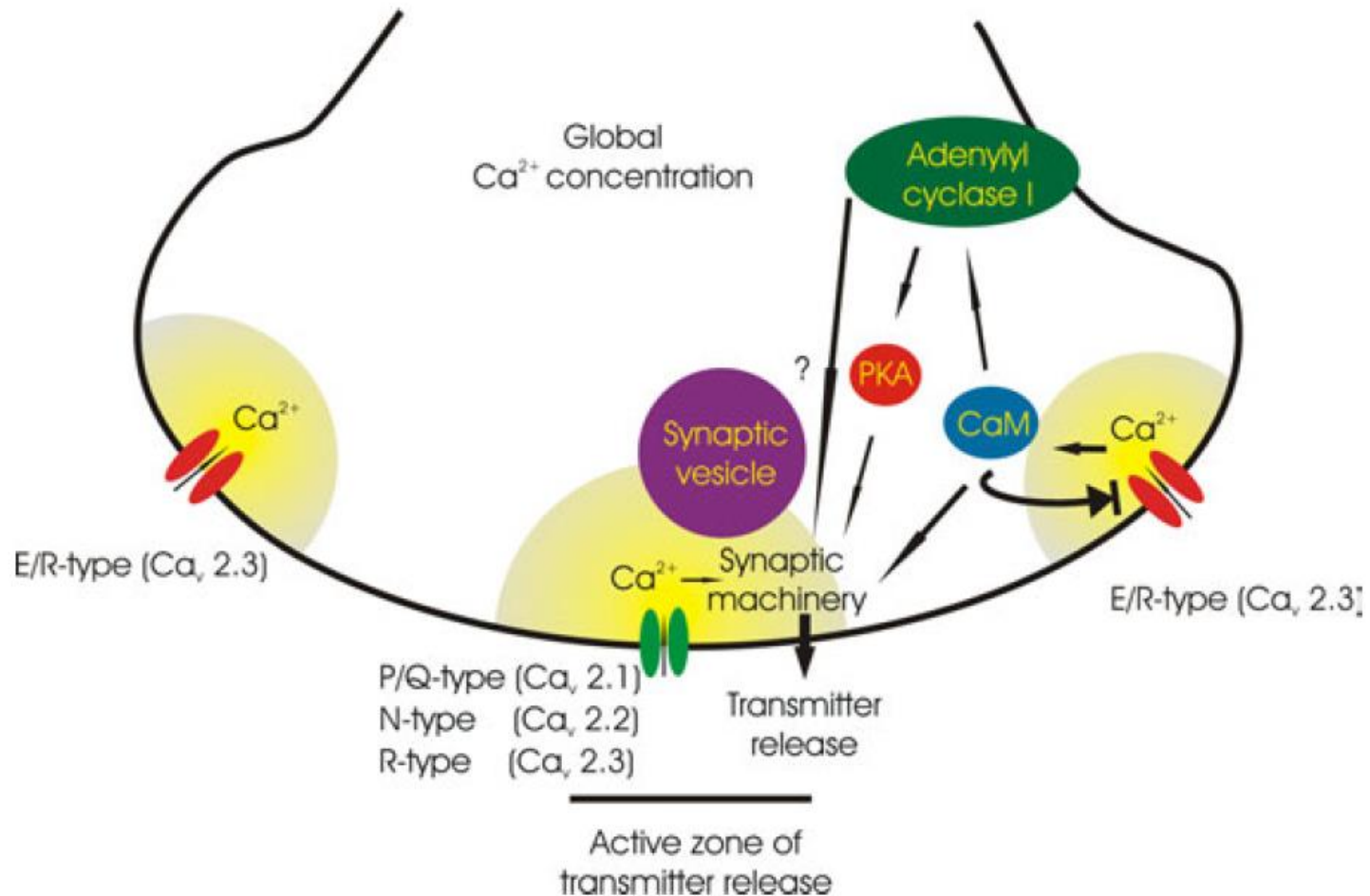


# Targeting presynaptic $\text{Ca}_v2.2$ calcium channels

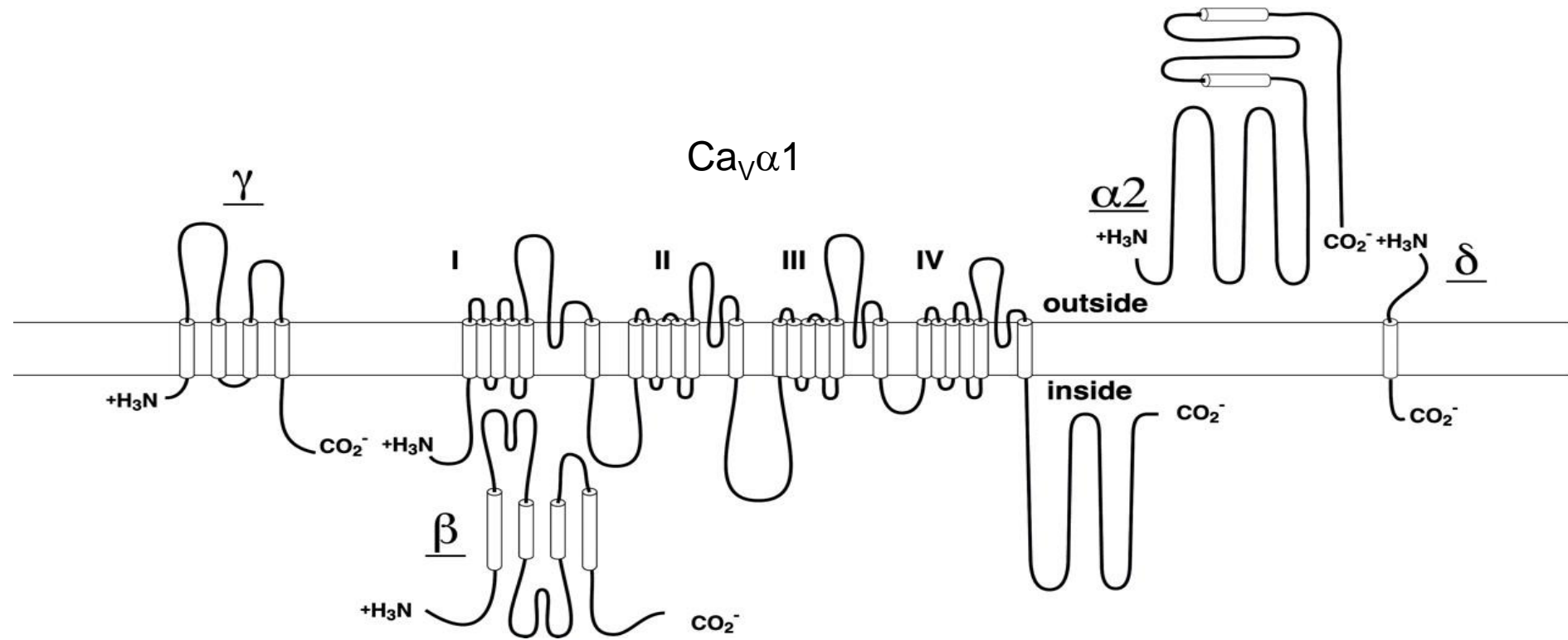
Gary Stephens

School of Pharmacy, University of  
Reading, UK

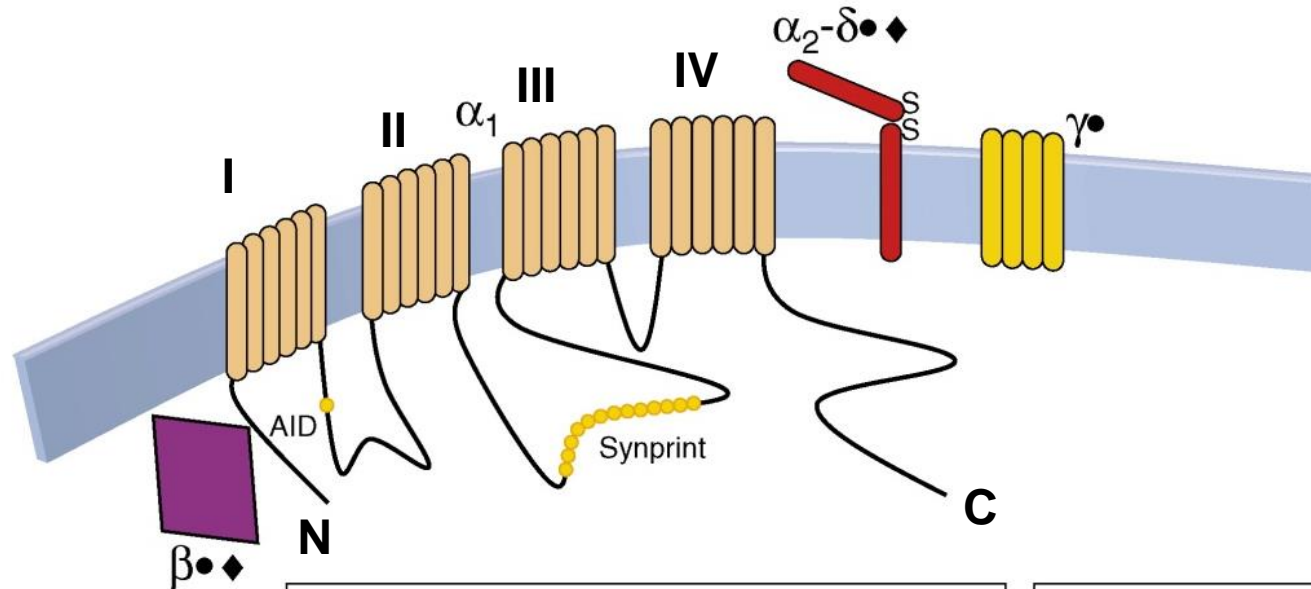
# Presynaptic $\text{Ca}^{2+}$ channels define cell excitability



# Ca<sup>2+</sup> channels are heteromultimeric proteins



$\text{Ca}^{2+}$  channels are modulatable proteins due to the presence of intracellular terminals and loops.....



| <u>Channel</u> | <u>Calcium channel <math>\alpha_1</math> subunits</u>                     |
|----------------|---|
| L-type         | $\text{Ca}_v1.1$ , $\text{Ca}_v1.2$ , $\text{Ca}_v1.3$ , $\text{Ca}_v1.4$ |
| P/Q-type       | $\text{Ca}_v2.1$  |
| N-type         | $\text{Ca}_v2.2$  |
| R-type         | $\text{Ca}_v2.3$  |
| T-type         | $\text{Ca}_v3.1$ , $\text{Ca}_v3.2$ , $\text{Ca}_v3.3$                    |

| <u>Ancillary subunits</u>                           |
|---|
| $\beta_{1b}$ , $\beta_{2a}$ , $\beta_3$ , $\beta_4$ |
| $\alpha_2\text{-}\delta_1$ through 4                |
| $\gamma_1$ through 8                                |

Ca<sup>2+</sup> channels are modulatable proteins due to the presence of intracellular terminals and loops.....

**Calcium channel interacting proteins** (● involved in  $\alpha_1$  regulation, ◆ involved in  $\alpha_1$  trafficking)

Ca<sub>v</sub>β  
Ca<sub>v</sub>α(1.x,2.x)  
RGK ●◆

Cavα<sub>1</sub> N-terminus

G<sub>βγ</sub> ●

Cavα<sub>1</sub> I-II linker

β subunit ●◆

G<sub>βγ</sub> ●

PKC ●

CRMP2 ●

Cavα<sub>1</sub> II-III linker

syntaxin1 ●

SNAP-25 ●

synaptotagmin ●

arrestin ◆

RGS12 ●

Cavα<sub>1</sub> C-terminus

G<sub>βγ</sub> ●

14-3-3 ●

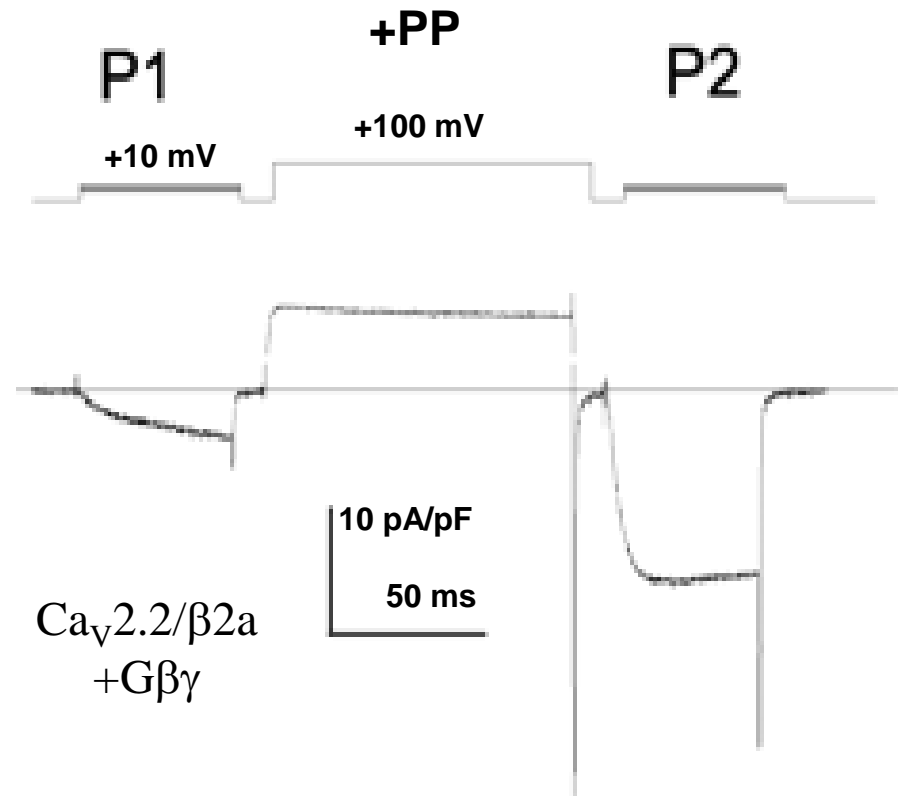
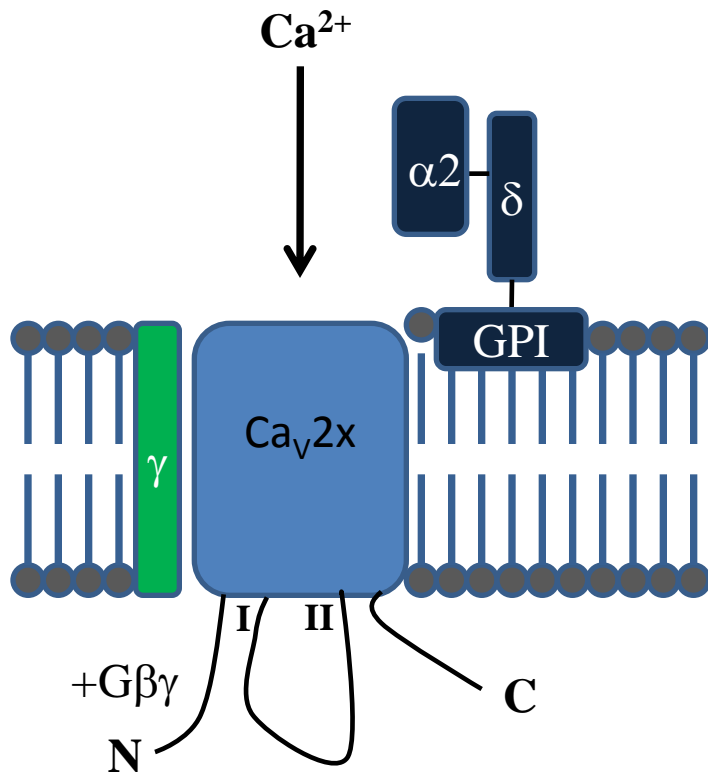
Mint1 ◆

CASK ◆

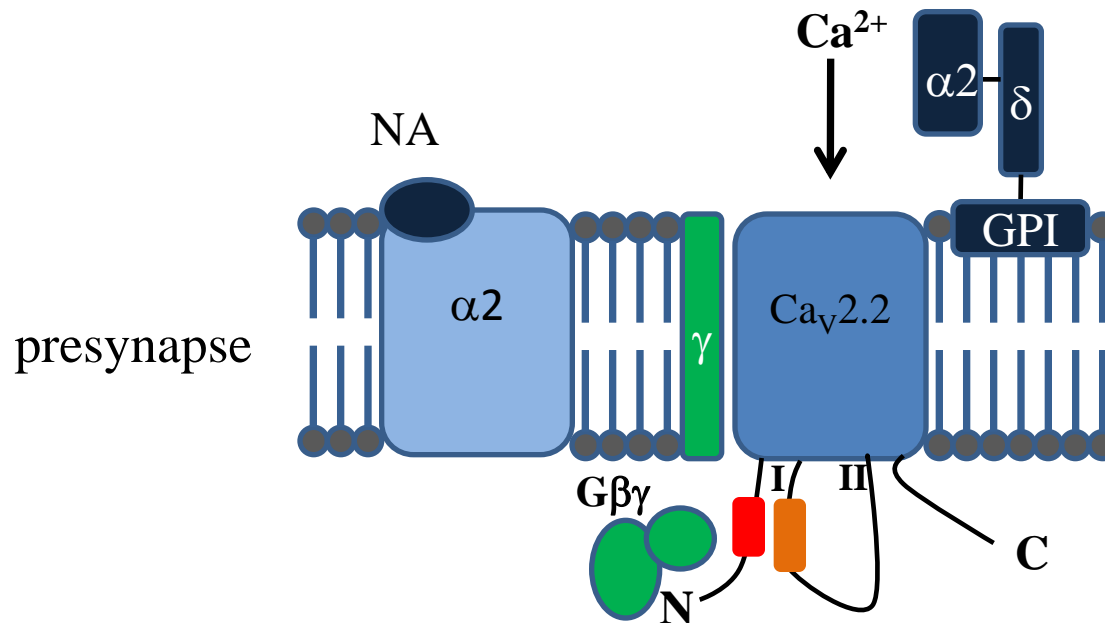
CaM ●


PKC ●


# Ca<sub>v</sub>2 subunits are subject to inhibitory Gβγ-mediated modulation



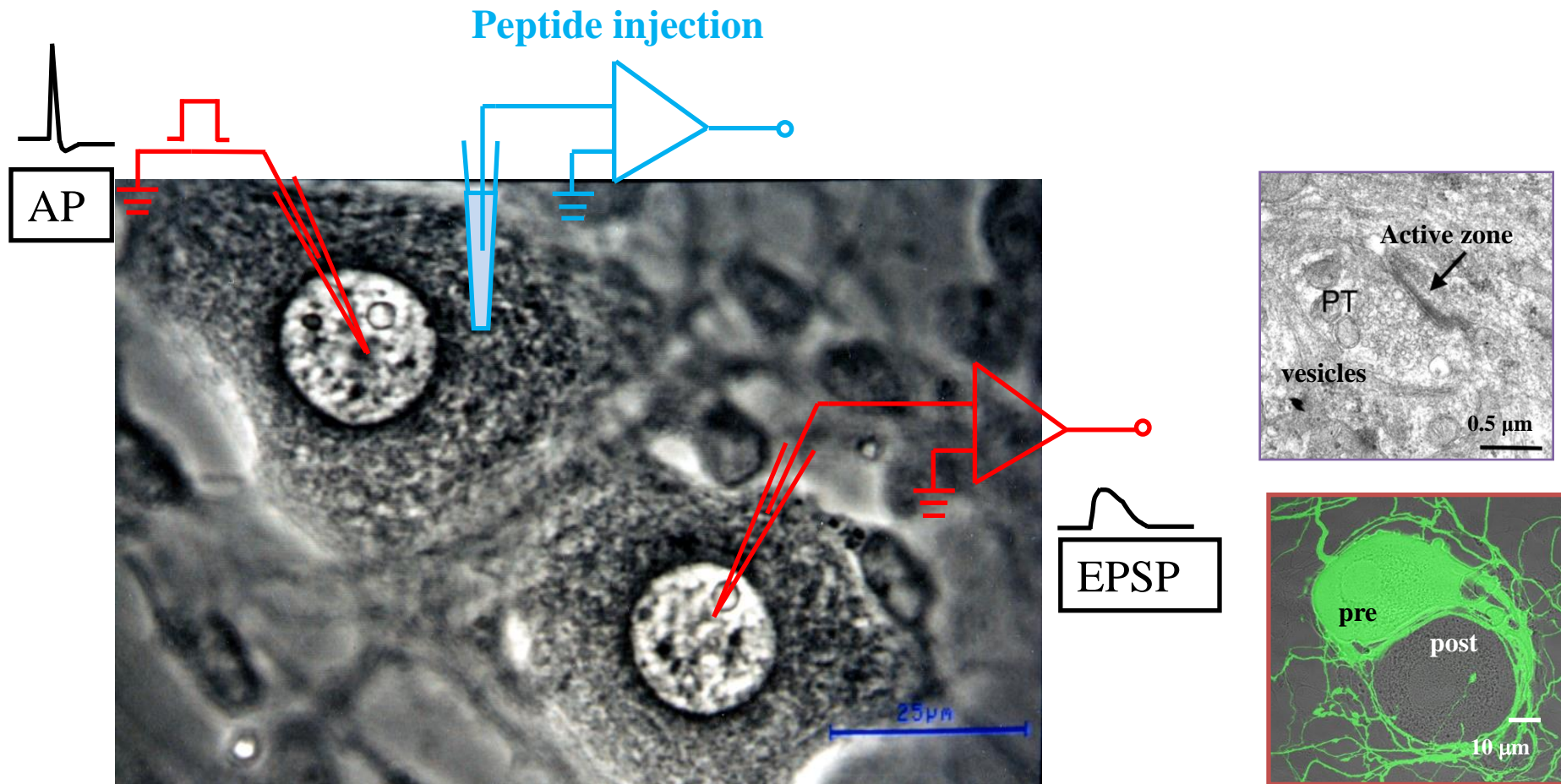
Question: Which intracellular sites are involved in transmitter release?



 = NT peptide (rat  $\text{Ca}_v2.2[45-55]$ ): YKQSIAGRART

 = AID peptide (rat  $\text{Ca}_v2.2[377-393]$ ): RQQQIERELNGYLEWIF

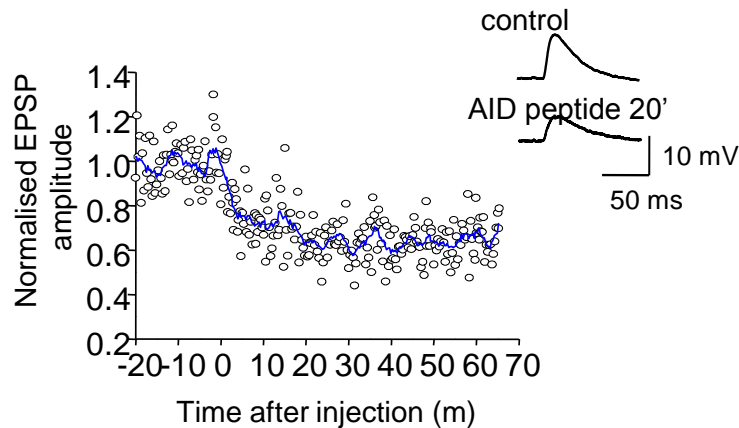
# Functional studies in rat superior cervical ganglion neuron (SCGN) model synapses



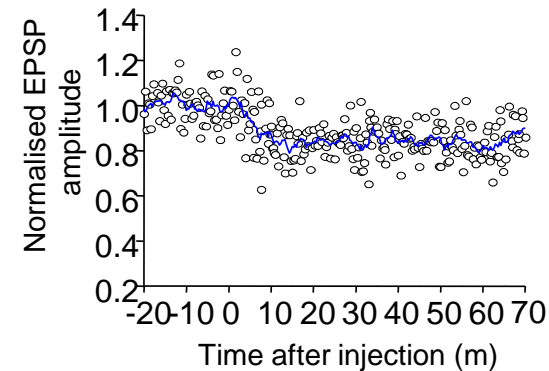


# Synthetic $\text{Ca}_v2.2$ peptides inhibit synaptic transmission in SCGN synapses: AID peptide

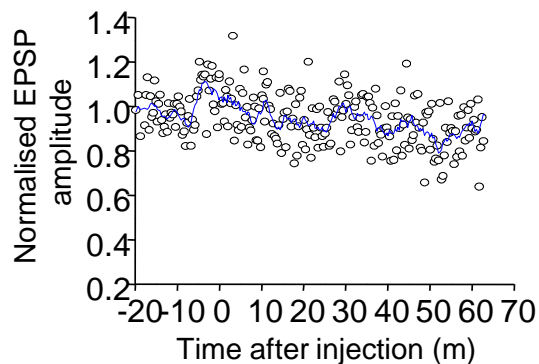
**A** 1 mM AID peptide



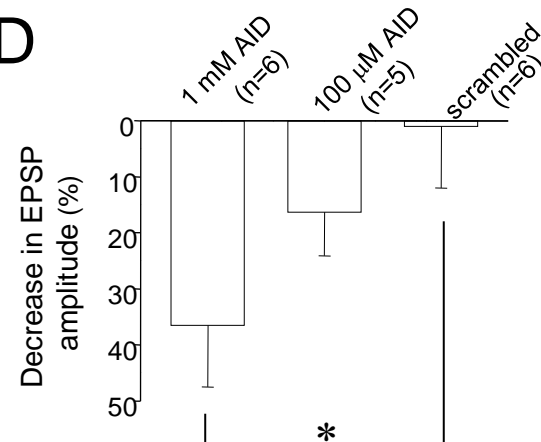
**B** 100  $\mu\text{M}$  AID peptide



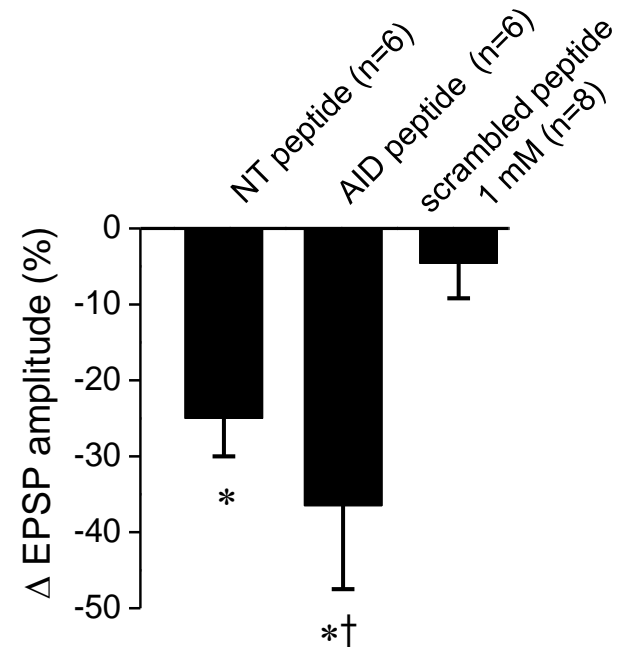
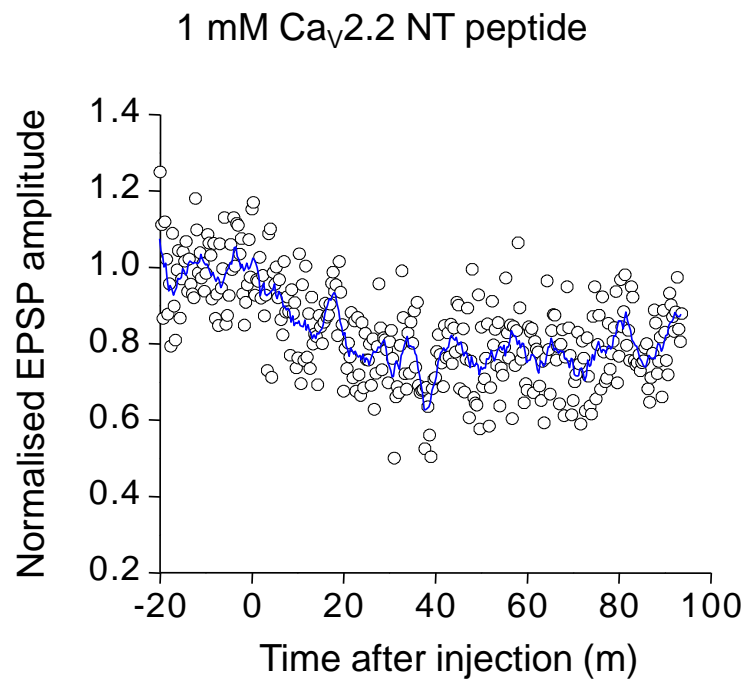
**C** scrambled AID peptide



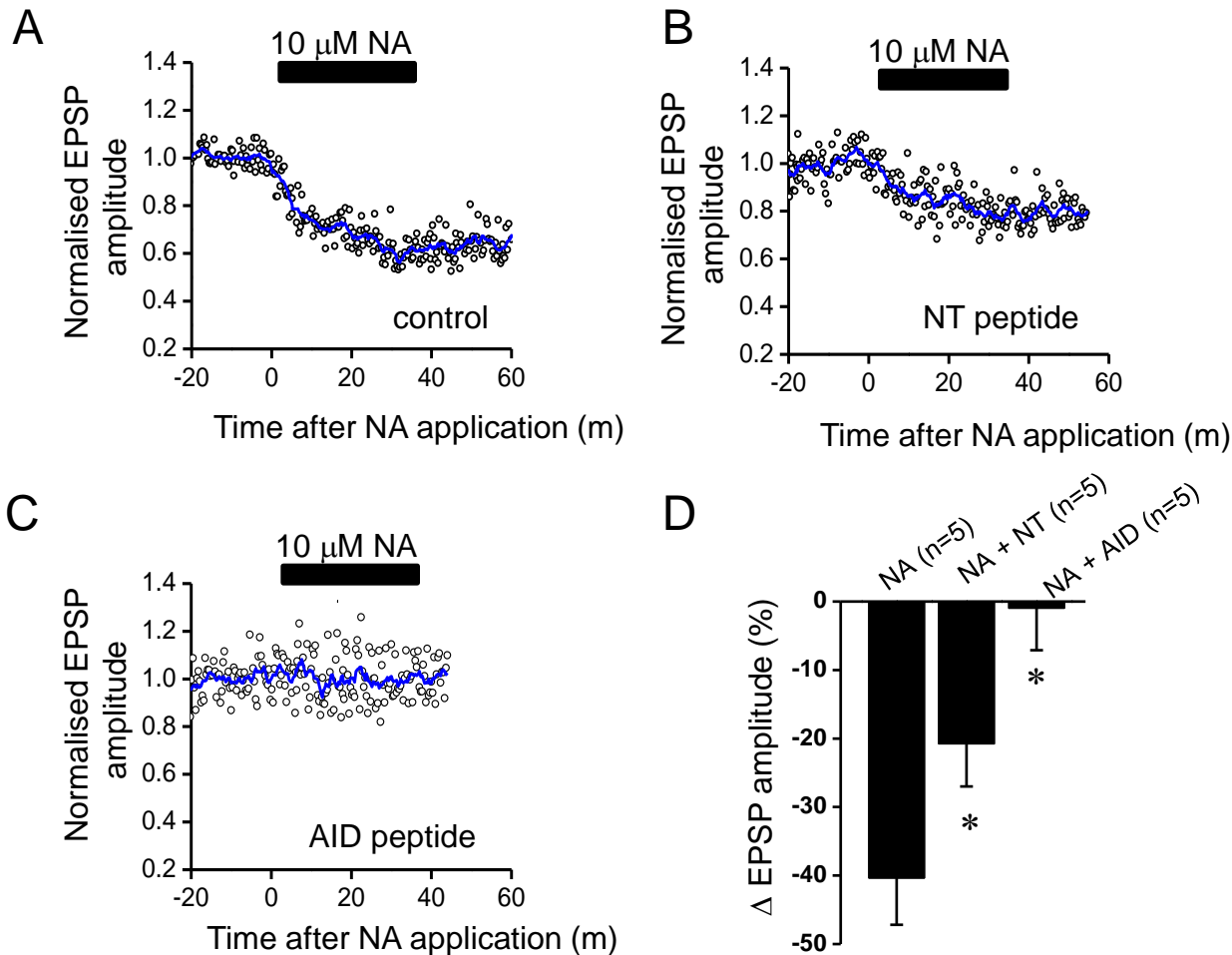
**D**



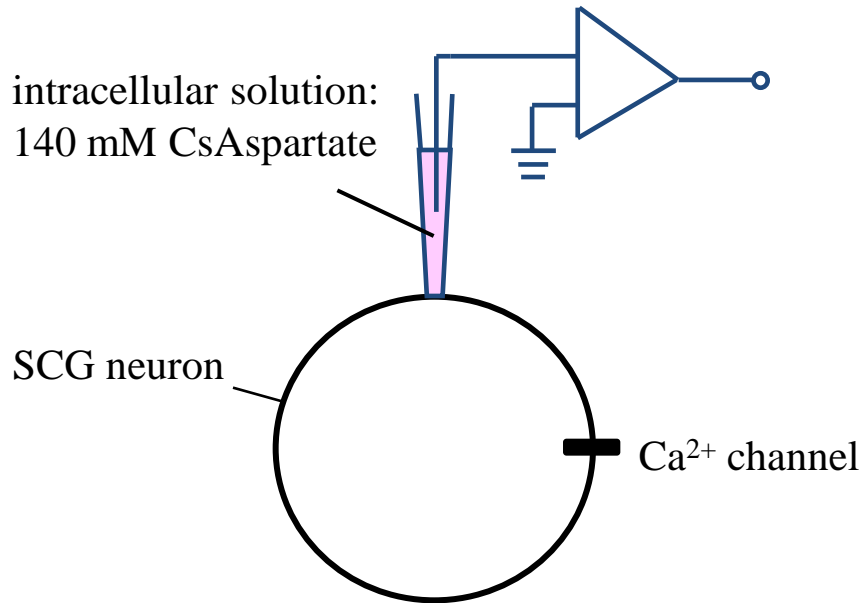
# Synthetic $\text{Ca}_v2.2$ peptides inhibit synaptic transmission in SCGN synapses: NT peptide



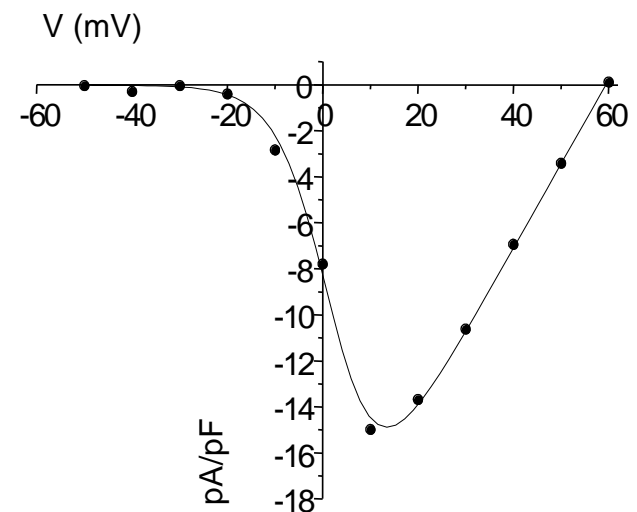
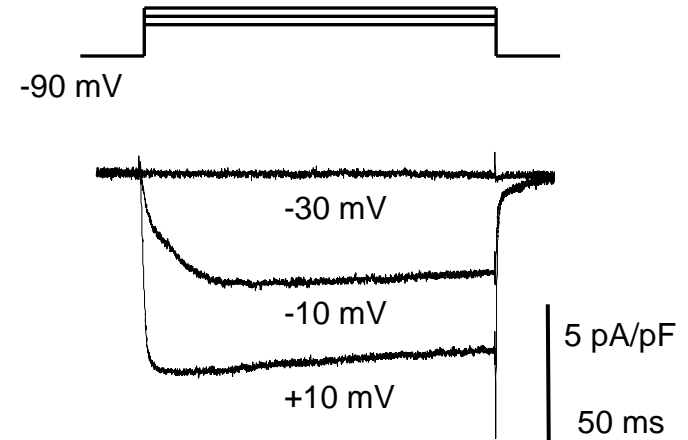
# Synthetic $Ca_v2.2$ peptides inhibit G protein modulation in SCGN synapses



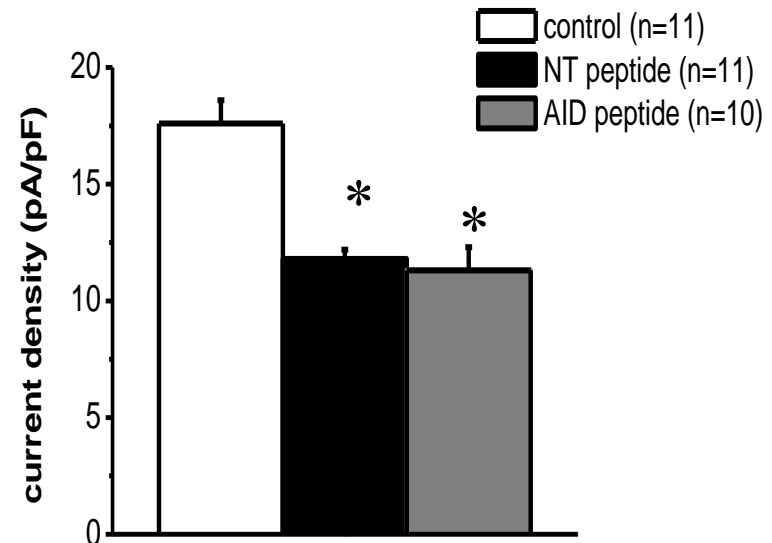
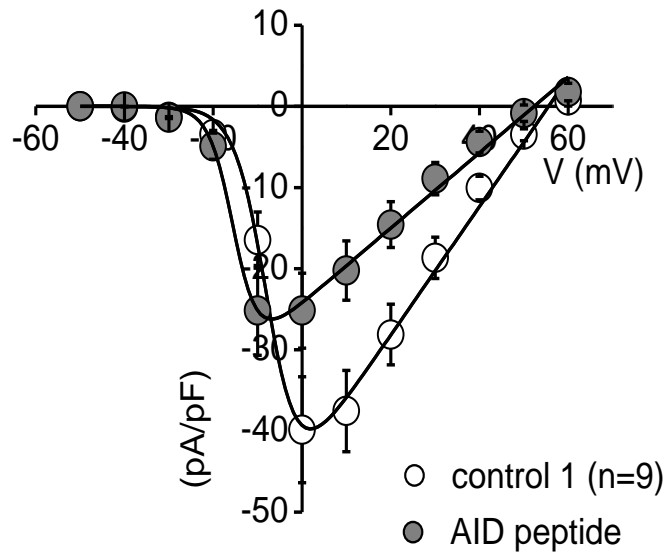
# Functional patch clamp studies in isolated rat SCGNs



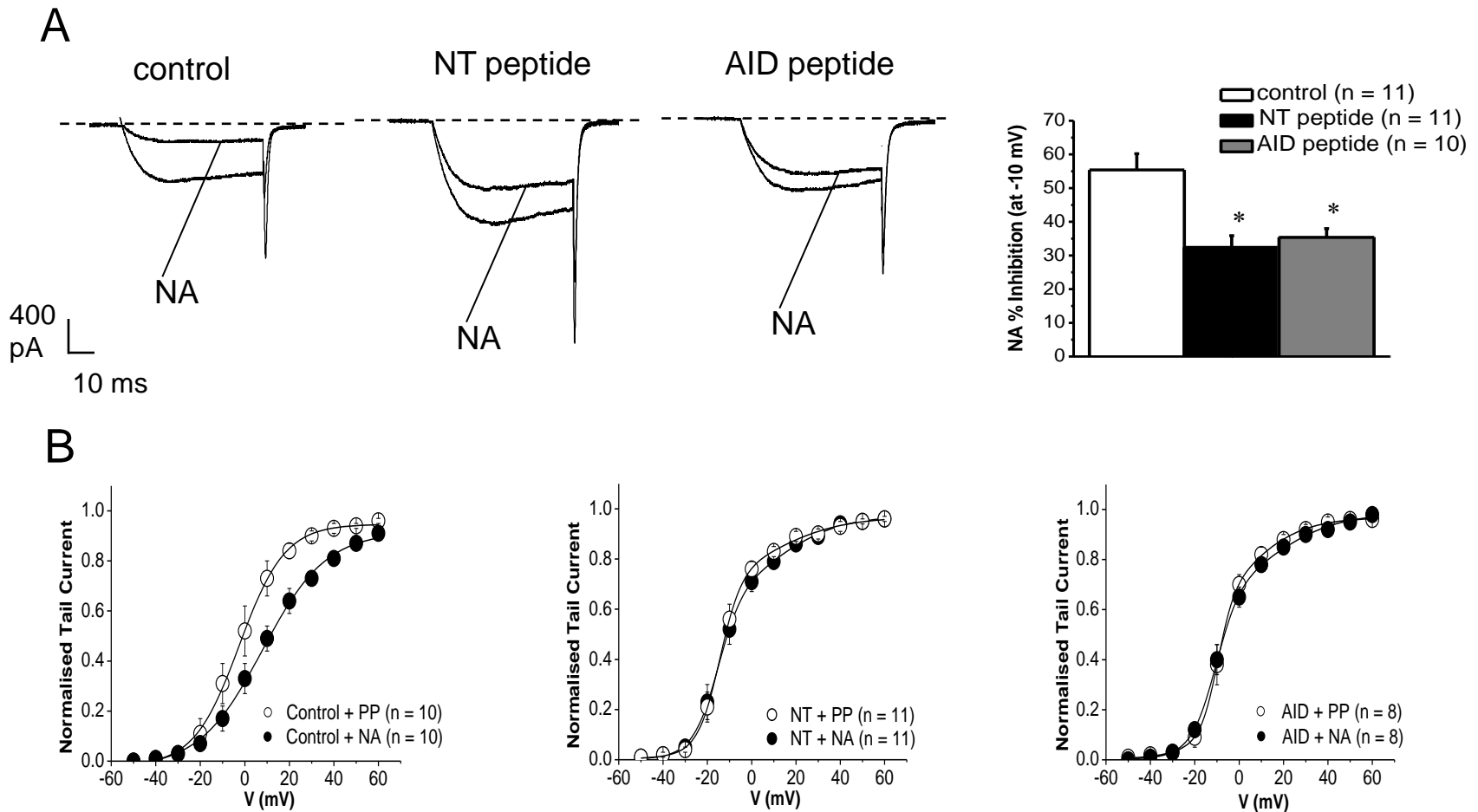
extracellular solution:  
160 mM TEABr + 10 mM Ba<sup>2+</sup>  
+ 10 μM nifedipine:  
pure Cav2.2 population



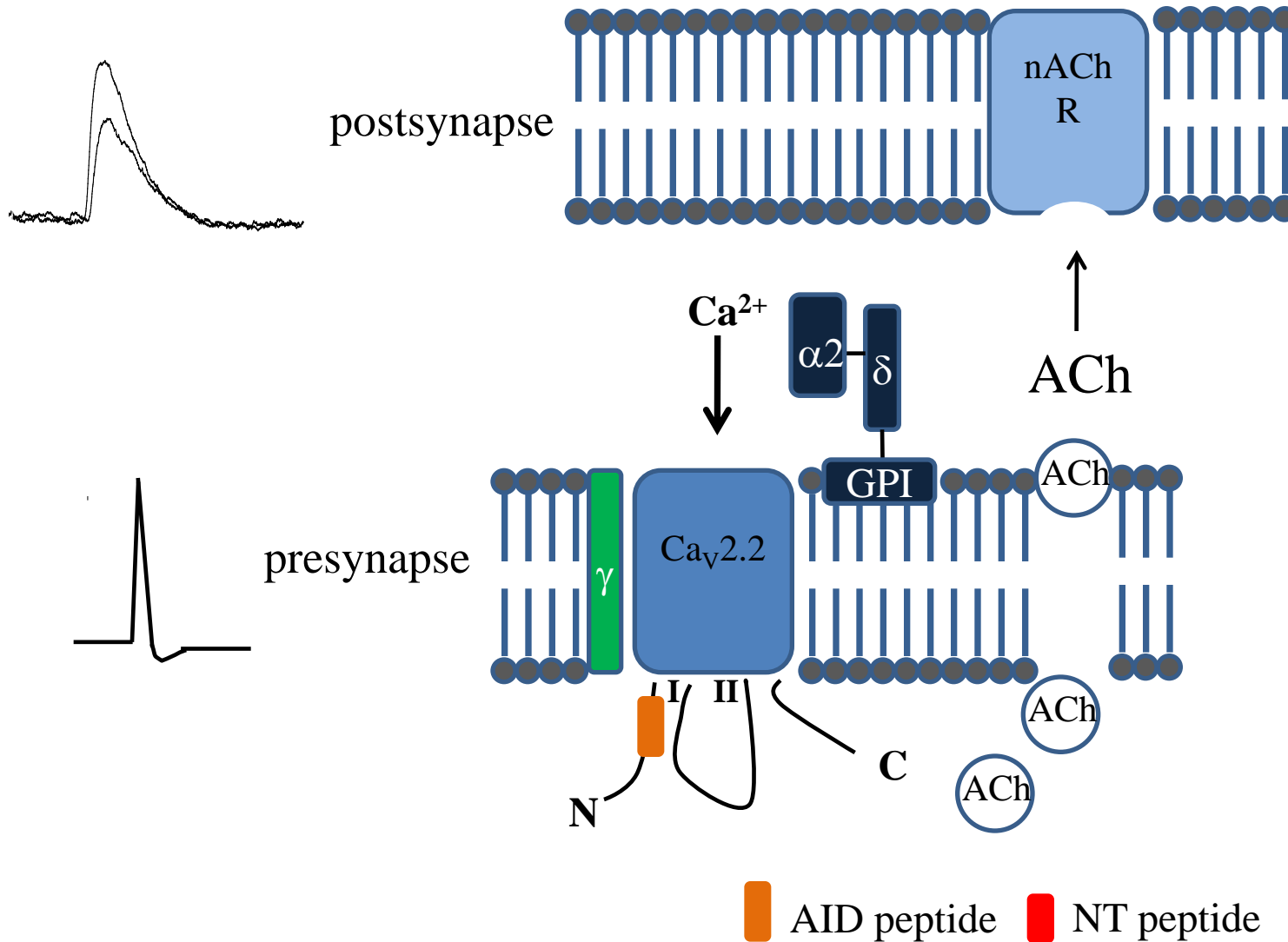
# Synthetic $\text{Ca}_v2.2$ peptides inhibit $\text{Ca}^{2+}$ current in isolated SCGNs



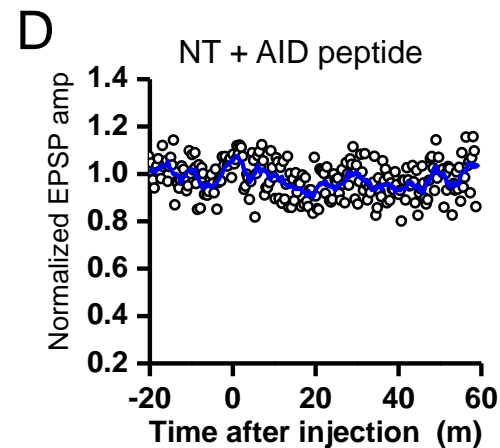
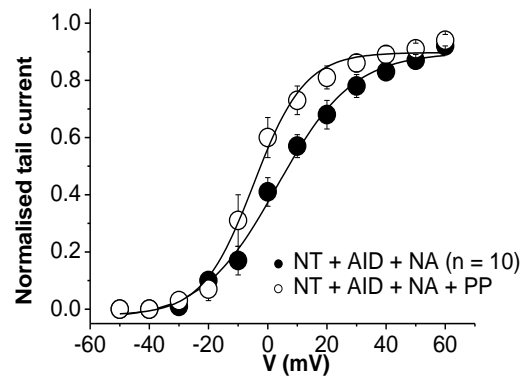
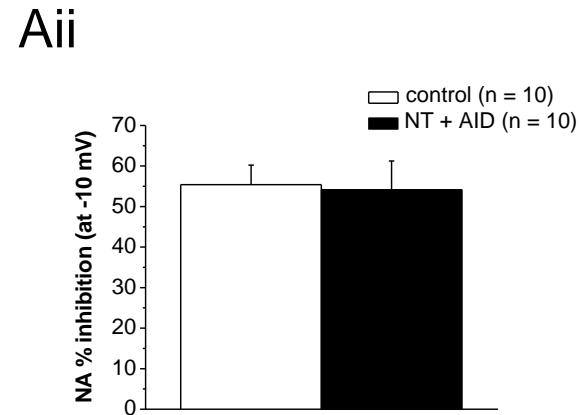
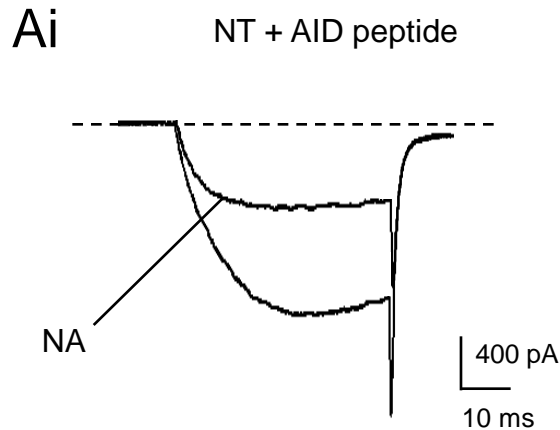
# Synthetic $Ca_v2.2$ peptides inhibit G protein modulation in isolated SCGNs



# Ca<sub>v</sub>2.2 peptides stabilise inhibitory channel conformations



# Co-application of AID and NT peptide NEGATES inhibitory effects





## Further characterization of inhibitory Ca<sub>v</sub>2.2 peptides: use of mutated peptides

### Site 1:

#### Ca<sub>v</sub>2.2 amino terminal (NT) peptides

NT peptide (rat Ca<sub>v</sub>2.2[45-55]):

YKQSIAQRART

Ca<sub>v</sub>2.2 NT R52A,R54A peptide:

YKQSIAQ**A**A**A**T

### Site 2:

#### Ca<sub>v</sub>2.2 I-II loop alpha-interaction domain (AID) peptide

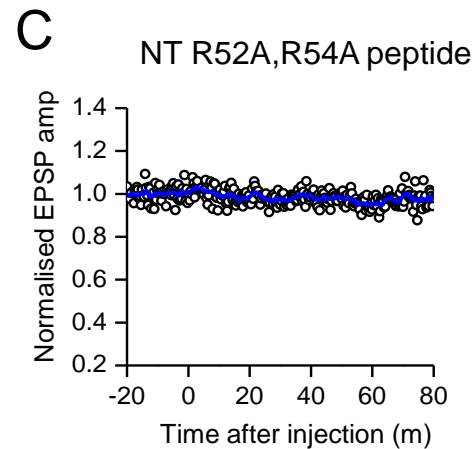
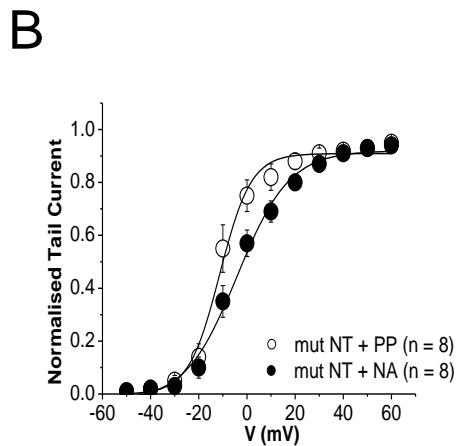
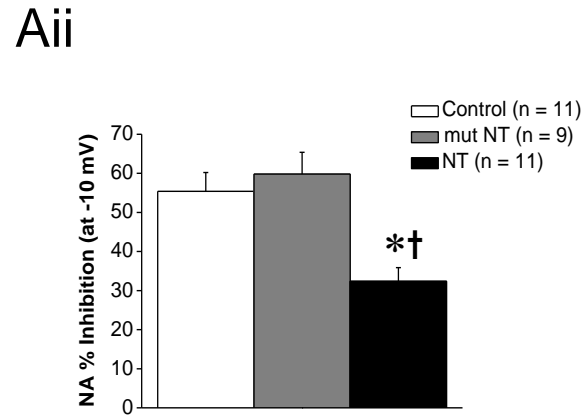
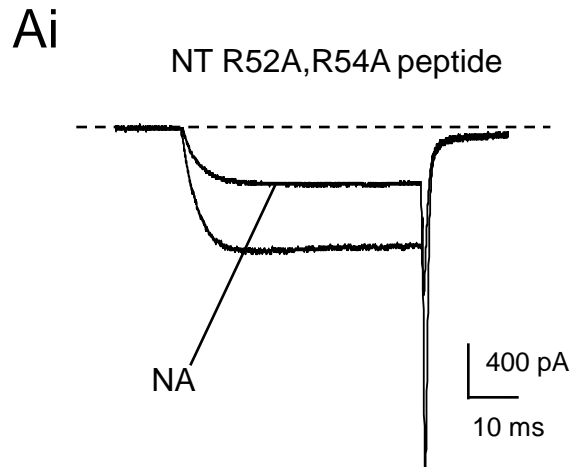
AID peptide (rat Ca<sub>v</sub>2.2[377-393]):

RQQQIERELNGYLEWIF

Ca<sub>v</sub>2.2 AID W391A peptide:

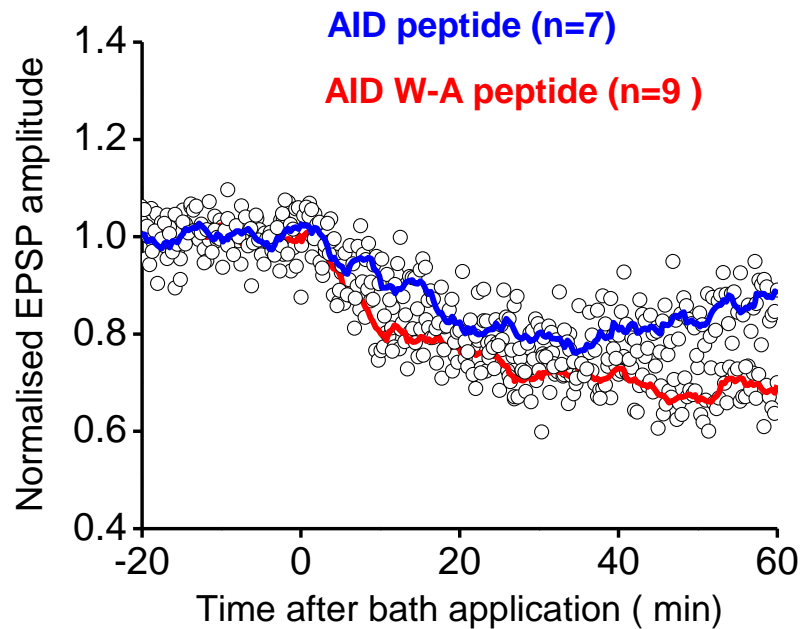
RQQQIERELNGYLE**A**IF

# Ca<sub>v</sub>2.2 NT R52A,R54A peptide LACKS inhibitory effects

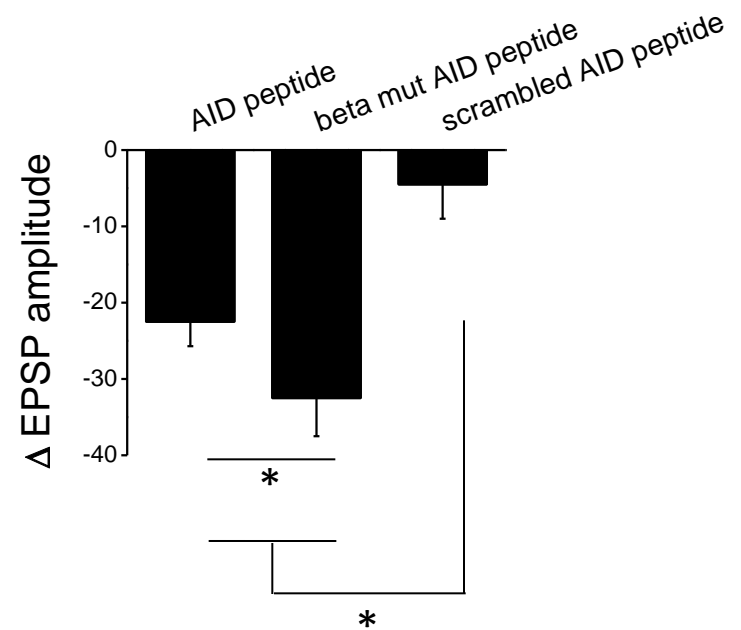


# Ca<sub>v</sub>2.2 W391A peptide has INCREASED inhibitory effects

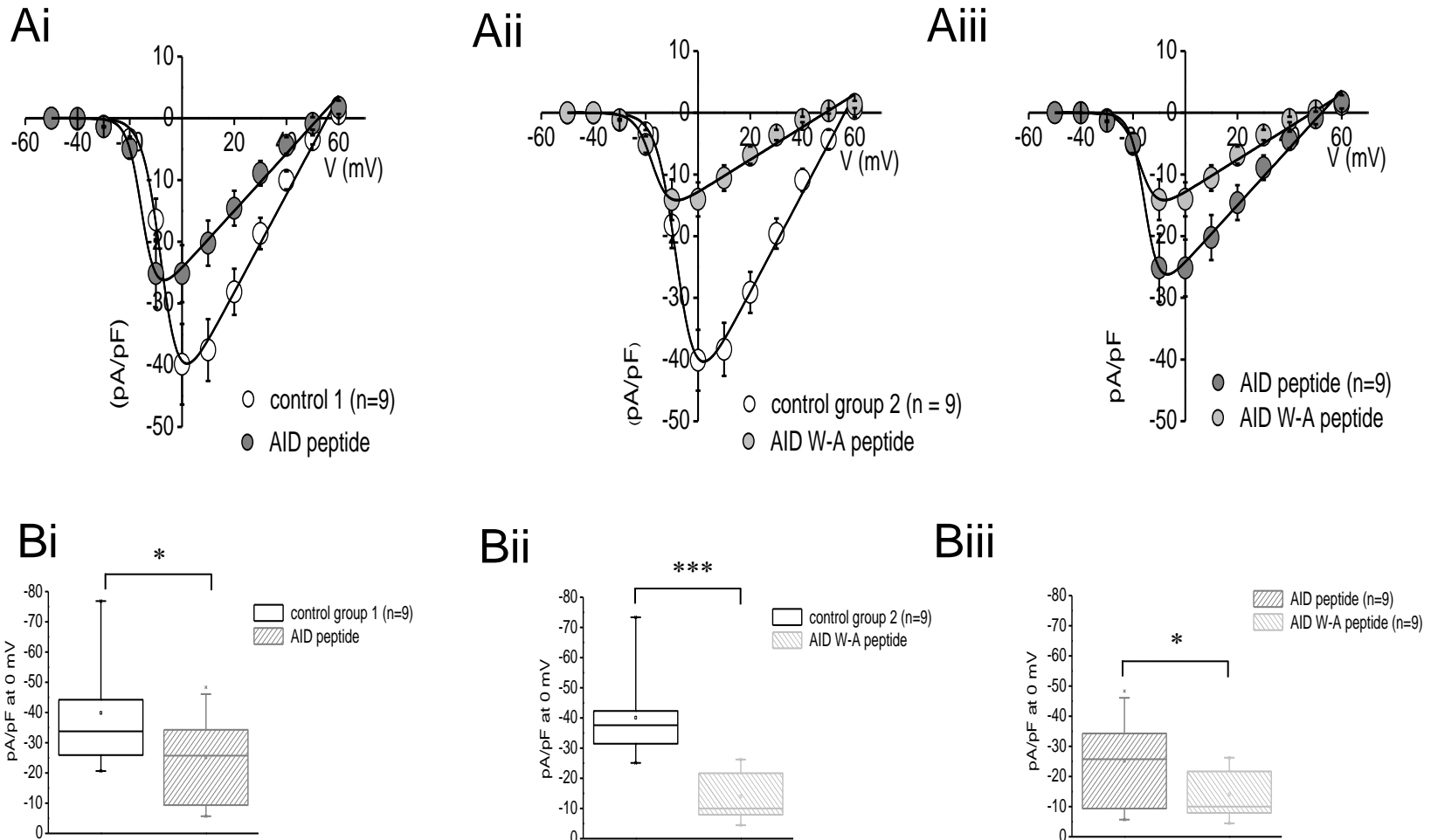
A



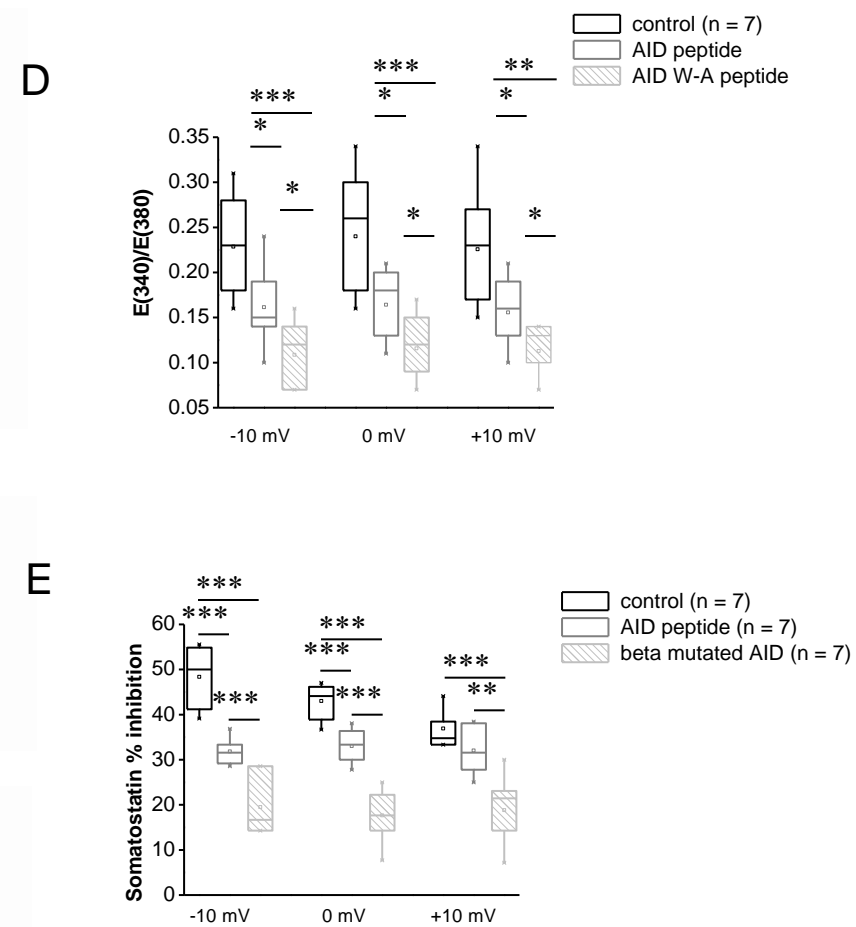
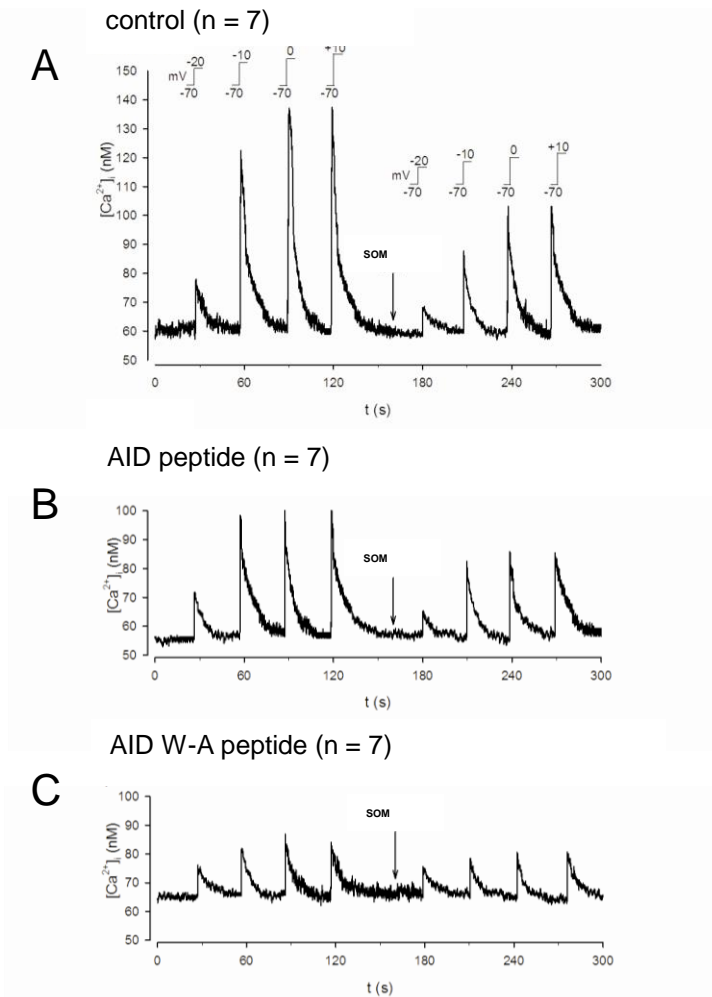
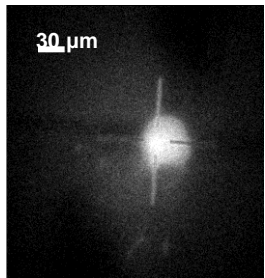
B



# Ca<sub>v</sub>2.2 W391A peptide has INCREASED inhibitory effects



# Ca<sub>v</sub>2.2 W391A peptide has INCREASED inhibitory effects



## Summary: Ca<sub>v</sub>2.2 peptides are inhibitory agents

- Synthetic Ca<sub>v</sub>2.2 peptides inhibit synaptic transmission and G protein modulation
- Ca<sub>v</sub>2.2 NT R52A,R54A peptide lacked inhibitory action: these data may implicate arginines 52 and 54 as determinants for NT-I-II loop interaction
- Ca<sub>v</sub>2.2 AID W391A peptide had increased inhibitory action: bulky tryptophan replaced by smaller alanine residue gives improved access?
- These data provide rationale for designing improved inhibitory agents

# Acknowledgements



Sumiko Mochida

Christian Vogl



Giovanna Bucci

