Synapse-specific control of synaptic efficacy at the terminals of a single neuron

Thomas Lewis
Background

- Looking at homeostasis of synapses
- If homeostasis is lost it can be problematic
- NMJ is a good model synapse
- Drosophila is a good model organism
$\Delta \text{Muscle Size} = \Delta \text{Synaptic Efficacy}$

- Erik Frank hypothesized that substance may be released from the muscle fiber and interact with the synaptic efficacy.
Drosophila Anatomy

• 40 motor neurons and 30 muscle fibers per hemisegment.

Muscles 6 and 7 are both innervated by motor neuron RP3, muscle 12 by neuron RP5 and 13 by RP1. All 4 muscles are innervated by motor neurons 6/7.
Fasciclin II

- Cell adhesion molecule
- Differential expression can be disrupt
Aim

To investigate if synaptic homeostasis is maintained when innervation is altered and to elucidate what mechanisms may facilitate this maintenance.
Methods

• Genetics: Gal4-UAS system to achieve tissue specific expression.
• Immunostaining
• Physiology: intracellular recordings during nerve stimulation and of miniature excitatory junctional currents (MEJCS) using microelectrodes
• Comparisons of MEJCs in different Ca++ concentrations
Results

A - Muscles 6+7 innervated by motor neuron RP3 + 6/7

B – Muscles 12+13 innervated by motor neurons RP5 + RP1

C – Overexpression of FasII on muscle 6 = more synapse formation of muscle 6 and less on muscle 7

D - Overexpression of FasII on muscle 13 = more synapse formation of muscle 13 and less on muscle 12
Physiological Consequences

Big increase in bouton number

Small increase in quantal content

Quantal size is the same
Bouton Number $\neq$ Synaptic Output

- Either some boutons were silent or probability of transmitter release was reduced
Probability of transmitter release

(a) 

(b) 

(c) 

(d)
Increase in quantal size?

- Increased receptor sensitivity

  OR

- Increased vesicle size/filling

  OR

- Multiquantantal release
Conclusions

• When hyperinnervation occurs, synaptic homeostasis maintains efficacy by retrograde signaling that reduces probability of transmitter release
• FasII does not cause an increase in quantal size
• Increase in quantal size seen in hypoinnervated muscle could be explained by an increased sensitivity to neurotransmitter or from an increase in
Strengths and Weaknesses

• Shows two new independent mechanisms in synaptic homeostasis
• Specifically suggests where signal may originate
• Rules out one possibility of quantal size increase

• “given a 91 or 162% increase in bouton number” – poor wording
• Figure 3d is not referred to in the text
• T-test instead of ANOVA
• Figure 4c

Clinical relevance
Weaknesses - Figure 4c
Further Study

• MHC driver of UAS Gal4 too late?
• Apply small quantities neurotransmitter to see if there is an increase in sensitivity
• Gene/Protein expression comparison of WT/hypersensitive cell to find retrograde factor
• Clinical Ideas
Big Burning Questions

• Are the mechanisms activated synchronously?
• How is the set point monitored and what are the feedback signals?
• What is the relationship between muscle size, quantal size and quantal content?
Snapin is Critical for Presynaptic Homeostatic Plasticity

DK Dickman, A Tong and GW Davis, J Neurosci 2012 32:8716-8724

Kasifa Khalid
Outline

1. Background
2. Aims & Hypothesis
3. Methods
4. Results
5. Discussion
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7. Critical Appraisal
Background to Homeostasis

Method is evolutionarily conserved

Adapted from Davis. G. (2006)
Snapin-Dysbindin model (Dickman & Davis 2009)

- Mutations in 276 genes screened by forward genetics
- Characterised role of dysbindin, a schizophrenia susceptibility gene, in synaptic homeostasis
- Dysbindin and Snapin interaction – Biochemical studies
AIM
To study the unknown molecular mechanisms underlying homeostatic modulation of presynaptic neurotransmitter release

HYPOTHESIS
Snapin, via interaction with Dysbindin, is involved in the mechanism of synaptic homeostasis
Methods (1)

*Drosophila; female 3rd instar larvae*

**RNAi (RNA interference)**

**Electrophysiology**

- Use of Philanthotoxin-433 (PhTx)
- Motor axon stimulation and calculations of:
  - average excitatory junctional potential (EJP)
  - miniature EJP (mEJP)
  - quantal content (average EJP/average mEJP) + correction for non-linear summation

**Statistical Analyses**

- T-tests and 1-way or 2-way ANOVA
- Bonferroni posthoc
Methods (2)

*Immunocytochemistry*

-Markers used include: Bruchpilot Protein (BRP), Horseradish Peroxidase (HRP), DLG

-Quantification of bouton and BRP number and density

*Quantitative Real-Time PCR and Reverse Transcription PCR*
Phases of presynaptic homeostatic plasticity

1. Rapid induction by pharmacological inhibition using PhTx
2. Longer induction by manipulating expression of Glutamate receptor subunits
RESULTS
Knockdown of snapin and damt has negligible effects on baseline synaptic transmission, but completely blocks presynaptic homeostatic plasticity.
Ubiquitous damt knockdown ($\text{damt}^{\text{RNAi}}$)

Snapin is essential for rapid induction of synaptic homeostasis

$\text{damt}^{\text{RNAi}}$ now referred to as S-D$^{\text{KD}}$ (Snapin and DAMT knock-down)
Glutamate receptor expression knock-down \((Glur III^{RNAi})\)

Previous study: similar phenomena seen in animals lacking GluRIIA receptor subunit

Snapin expression is required for sustained expression of homeostatic plasticity
Synapse Morphological analysis using Immunocytochemistry

Snapin is necessary for the maintenance of synaptic homeostasis without altering NMJ morphology
Snapin and Dysbindin interaction during homeostatic plasticity

Figure 5

Loss of snapin expression is important for the observed genetic interaction with dysbindin
Dysbindin has a separable activity which alters apparent cooperativity of presynaptic release
Snapin and Dysbindin interaction to promote synaptic transmission

1. Snapin functions downstream of Dysbindin
2. Snapin functions as a co-factor necessary for Dysbindin to be fully functional
Snapin and SNAP25 interaction ($\text{dic}^{1229}$ & $\text{snap-25}^{\text{null}}$)

Snapin’s potential functions:

1. Snapin interacts with SNAP25 to synchronise calcium dependent SV release
2. Snapin interacts with DIC to promote retrograde trafficking of late endosomes from synaptic terminals to soma

**Snap25 interactions are possibly relevant to synaptic homeostasis**

Figure 7
Snapin and BLOC-1 Complex interaction

\( (blos1) \)

**BLOC-1 Complex**

- Muted
- Cnu
- Snapin
- Pallidin
- Pallidin?
- Blos1s2
- Blos1s3
- Blos1s1
- Dysbindin

**Blos1 protein not involved in homeostatic plasticity**
Summary

• Broad molecular context which potentially links homeostatic signalling to neurological disease

• Snapin promotes homeostatic modulation of presynaptic neurotransmitter release > by functioning in concert with Dysbindin and SNAP25

• Dybindin’s ability to potentiate synaptic transmission requires normal snapin expression

• Some evidence that Snapin promotes signalling between cytosolic Dysbindin and SNAP25
Big Burning Question

How does snapin regulate glutamate release?

Snapin knockdown interferes with upregulation of transmitter release when NMJ glutamate receptors were blocked. Specific mechanisms for this is not found in this paper.

Is this mechanism relevant to vertebrate NMJ?

> Gooseberry (gsb): gene implicated in a recent study on synaptic homeostasis; look at the interactions of snap25, snapin and dysbindin along with gooseberry.

> Also gsb is the Drosophila homolog for mammalian pax3/pax7; looking at gsb interactions can help point to genes involved in synaptic homeostasis in the vertebrate NMJ.
Strengths

- Follows well from previous study
- Systematic methodology and approach
- Graphical data backed up by quantified results (Table 1)
Limitations

- 400 v/s 276 genes (Dickman & Davis, 2009)
- Inconsistent with methods applied during results, e.g: No snapin overexpression carried out in Glutamate receptor knockdown
- Early bias towards Snapin, leading to less characterisation of DAMT
- Some data is not shown (flag-tagged snapin)
Further Work (1)

- DAMT and its effects on homeostatic plasticity – DAMT effect on synaptic transmission
- Blos-1 mutation with Snapin-deficiency ?
- SNAP25 and Dysbindin inter-signalling promoted by Snapin – check via in vivo imaging
- Link mutations in cystein string protein, methusela and Hsc70 to snapin knockdown
- Combining presynaptic homeostasis to postsynaptic mechanisms
- Snapin’s function during endosomal trafficking: other BLOC1 complex proteins e.g. Palladin + Muted
- Identification of physiological or developmental conditions that promote pre-synaptic homeostatic adaptations.
- Clinical Relevance: Schizophrenia, Epilepsy and other Psychiatric disorders
Further Work (2)
References


Introduction

- Fibres of the body wall musculature (2, 4, 5, 6)

Muscle fibres receive 2 inputs: 1b and 1s
Fibre 5 receives only a single input

3rd instar larvae (wandering)
In this paper they report:

• There was (neuron specific )sexual differentiation in 3 of the 4 muscle fibres examined
• Female fibres produced larger synaptic responses (EPSPs) due to increased transmitter release
• No differences in terminal length/ of synaptic boutons

Aim?
‘Identified motor terminals in Drosophila larvae show distinct differences in morphology and physiology’
Lnenicka and Keshishian (2000)
Methods

• Examined 4 body-wall muscle fibers
• Microelectrode used to measure EPSP in muscle fibre 5.
• 2 microelectrode voltage-clamp for EPSCs muscle fibres 2 and 4 (holding potential -60mV)
• Staining with a HRP-conjugated antibody
Sexual differentiation at muscle fibre 5 (EPSPs)

- Representative EPSPs
  (averages of the first 10/20 responses during 0.1/10 Hz stimulation respectively.)

Males have smaller EPSPs: about 60% of male fibres have EPSPs under 3mV, compared to 20% in females.
Sexual differentiation at muscle fibre 5 (EPSPs)

- At 10Hz stimulation synaptic potentiation
- At 0.1Hz synaptic depression in females
Differences in mEPSPs?

- There was no significant difference in male/female muscle fiber mEPSPs
Sexual differentiation is specific to the motor axon.

- In muscle fibers 2 and 4 the Is terminal is sexually differentiated.
- Ib is not sexually differentiated.
Conclusions:

• Some neuromuscular synapses in body wall musculature of drosophila larvae are sexually differentiated (early and late third instar larvae)
• This appears to be due to increased neurotransmitter release
• No differences were found in the size of motor terminals/number of sunaptic boutons/terminal length or branching.
BBQ: What genetic factors determine transmitter release in females and how does it relate to differences in locomotion? Could the mechanism be explained by difference in active zones in male/female larvae?
Implications/Further work

• Possibility that the sexual differences could be related to male/female differences in locomotion. → Observe locomotion in larvae using DIAS. At what point does the dimorphism appear – do differences in locomotion appear concomittently?

• Is this sexual differentiation a rule or exception. If confined to specific areas what implications would this have for locomotion?

• Link to adult mating behaviour?

• Human female skeletal muscle shown to be more resistant to muscle fatigue… differences in neuromuscular synapses!?
hVAPB, the causative gene of a heterogeneous group of motor neuron diseases in humans, is functionally interchangeable with its Drosophila homologue DVAP-33A at the neuromuscular junction.


Presented by Robert Lees
Contents

• Background
• Aims
• Methods
• Results
• Conclusions
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• Big Burning Question
hVAPB

- Human VAMP-associated protein B
- Expression affects the size and number of boutons at the NMJ
- Mutated in ALS type 8
- *Drosophila* VAMP-associated protein of 33kDa A (DVAP-33A) is the structural homologue
ALS8

- 2004 - P56S mutation found at 20q13.3
- Further mutations subsequently identified
- Clinical onset between third and fifth decade
- LMN symptoms
- Some patients show bulbar involvement
- Mutation also found in atypical ALS and late onset SMA
Aims

• To assess the electrophysiological and ultrastructural changes associated with under- and over-expression of DVAP-33A
• To assess the degree of homology between DVAP-33A and hVAPB
• To characterise the affects of mutations in DVAP-33A and hVAPB
Homeostatic regulation of neurotransmitter release at NMJs with altered expression of DVAP-33A

• **Underexpression** – \(\text{DVAP-33A}^{\Delta 166}\) partial loss of function mutant (null mutations are usually lethal)

• **Overexpression** – UAS/GAL4 system
  - elav-GAL4 is the pan-neural driver
  - UAS – Upstream activation sequence
1. Promoter of gene of interest is inserted upstream of GAL4
2. Therefore GAL4 protein is expressed in all cells that normally express the gene of interest
3. UAS is the promoter element recognised by GAL4

Fig. 1 Jones WD The expanding reach of the GAL4/UAS system into the behavioral neurobiology of *Drosophila* (2007) BMB reports
Staining with anti-HRP antibodies

Supplementary figure 1
Comparison of wt and DVAP-Δ166 mutants exhibit only 150 ± 7 boutons while controls contain 250 ± 8 boutons (P < 0.001, n=10 larvae)

Supplementary figure 1
Electrophysiology – DVAP-33A underexpression

- Amplitude of evoked junctional potentials (EJPs) was not significantly different to controls (P>0.05)
- Mean frequency of mEJPs was increased in mutants
- Mean mEJP amplitude was increased in mutants – increased quantal size
- 40% decrease in quantal content in mutants (44.90+0.9 in controls versus 26.91+0.5 in mutants, P<0.001)
EJP amplitude is not significantly different

Fig. 1
Mean frequency and amplitude of mEJPS was significantly increased in mutants.

Frequency: 3.80±0.24 Hz in mutants versus 2.00±0.11 Hz. P <0.001

Amplitude: 1.30±0.02 mV in mutants versus 0.80±0.01 mV in controls. P<0.001
Electrophysiology-DVAP-33A overexpression

• Amplitude of evoked junctional potentials (EJPs) was not significantly different to controls (P>0.05)
• Mean mEJP amplitude was decreased in mutants – decreased quantal size
• Increase in mutant quantal content (54.13 ± 1.5 compared to 36.66 ± 0.8 in controls. P<0.05)
EJP amplitude is not significantly different

![Graph showing EJP amplitude](image)

**Fig. 1**
Mean amplitude of mEJPS was significantly decreased in mutants

Amplitude: 0.59±0.05 mV in mutants versus 0.90±0.06 mV in controls
P<0.05
The key point:

• Changes in quantal size are maintaining normal synaptic transmission in DVAP-33A over and underexpression
Ultrastructural remodelling at NMJs lacking and overexpressing DVAP-33A

• Wanted to determine if ultrastructural remodelling at the synapse accompanies functional compensation
• Performed serial section TEM analysis of terminals lacking and overexpressing DVAP-33A
DVAP-33A$^{\Delta 166}$ mutants display an increased number of active zones

2.0 ± 0.2 Azs per bouton cross-sectional area versus 0.8 ± 0.3 in controls. Statistically significant?

Fig. 2
In DVAP-33A overexpression the density of vesicles per bouton is decreased.
• This indicates that structural remodelling can take place whereby:
• 1. active zones are concentrated in a reduced number of boutons
• 2. the pool of vesicles can be diluted in an increased number of boutons
DVAP-33A is the functional homolog of hVAPB

- It is important to assess how relevant DVAP-33A function is to hVAPB
- DVAP-33A is 62% similar to hVAPB
- Tested the ability of hVAPB to functionally substitute for the loss of DVAP-33A
- Showed that hVAPB and DVAP-33A can functionally substitute for each other's function
DVAP-33A is the functional homolog of hVAPB

- Used GAL4 drivers to express hVAPB in null (DVAP-33AΔ448, DVAP-33AΔ20) and hypomorphomic (DVAP-33AΔ166) mutants
- Morphological and physiological phenotypes were rescued with hVAPB
- Indicates that the human and Drosophila protein share a common structure and perform homologous functions
Null-DVAP-33A phenotype rescued with hVAPB

Fig. 3
Controls in blue.
Expression levels of VAP proteins affect the abundance of specific receptor subunits and the volume of post-synaptic receptor clusters

• Increase or decrease in quantal size maintains functional homeostasis
• Hypothesised that this may be due to the composition of post-synaptic glutamate receptors
• Glutamate receptor expression has previously been shown to regulate quantal size at the Drosophila NMJ (DiAntonio et al 1999)
Glutamate receptors

- Glutamate receptors cluster opposite presynaptic active zones.
- 5 receptor subunits have been identified in Drosophila: GluRIIA, GluRIIB, GluRIII, GluRIID and GluRIIE
- Used previously characterised antibodies to assess glutamate receptor abundance and distribution in synapses lacking DVAP-33A
Increase in cluster count and volume

GluRIIA significantly upregulated in response to underexpression of DVAP-33A

Fig. 4
GluIIA downregulated with hVAPB expression

Fig. 5
• These results demonstrate that VAP proteins are components of a trans-synaptic signal mechanism that regulates quantal size by shaping the post-synaptic glutamate receptor field.
hVAPB carrying the ALS8 mutation rescues the DVAP-33A mutant phenotype

- Used Gal4 drivers to express hVAPBP56S and DVAPP58S in DVAP-33A-null mutants
- Morphological and physiological phenotypes were rescued
- Indicates that the pathogenic allele retains some wt properties
hVAPB carrying the ALS8 mutation rescues the DVAP-33A mutant phenotype

Fig. 6
Transgenic expression of the Drosophila mutant protein in neurons recapitulates several hallmarks of the human disease

- Transgenic larvae expressing DVAPP58S showed locomotor deficits
- Enhanced neuronal death
- Aggregate formation and depletion of endogenous protein at the synapse
Aggregate formation

Nerve fibre of 3\textsuperscript{rd} instar larvae stained with antibodies for DVAP-33A (red) with antibodies for the neuronal cell surface marker anti-HRP (green)
Loss of endogenous protein

Fig. 9
Conclusions

• DVAP-33A plays an important role in bouton formation
• Functional homeostasis is maintained in over and underexpression of DVAP-33A by alterations in quantal size and remodelling of active zones
• DVAP-33A is the functional homologue of hVAPB
Conclusions

• Postsynaptic glutamate receptors may be the mechanism by which quantal size is modulated
• hVAPBP56S maintains some wt properties
• Transgenic expression of DVAPP58S in neurons recapitulates several major hallmarks of ALS
Strengths

• Gives us a novel insight into a homeostatic mechanism for EJP control
• Provides evidence to support the relevance of the Drosophila DVAPP58S model
• Appropriate study design
• Appropriate data collection
Limitations

• Aims and hypothesis are not clear

• Statistical analysis not always undertaken (or at least not stated)

• Interchange DVAP-33A and hVAPB in some experiments
Future work

• Could look to further characterise the mechanism behind the modulation of quantal size

• Would knockout of GluRIIA upregulate DVAP-33A?
Big Burning Question

• What does DVAP33A/hVAPB normally do and why does its mutation lead to disease?
  – Disruption of homeostatic mechanism?
  – Disruption of autophagy and/or mitophagy?
  – Affecting Ca$^{2+}$ homeostasis and disrupting kinesin 1?