The Active Zone: Structure

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Structure matters

Descartes 1695
Definition

ACTIVE ZONE
The site of the presynaptic terminal at which synaptic exocytosis occurs.

Atwood & Karuanithi 2002

Eccles 1992
The self and its brain

Eccles & Popper 1978, The Self and Its Brain

Eccles 1992
Molecular composition of active zones

Takamori et al., 2006

Eccles 1992
Textbook knowledge
Fluorescently tagged toxins

Robitaille et al., 1990 & 1993
Beams, ribs & pegs

Harlow et al., 2001
Based on the observations that the area of the presynaptic membrane directly facing the muscle fiber at mouse neuromuscular junctions is 80–100 nm (SEM, n = 25; unpubl. obs.). Thus, in terms of the average, one-half that of primary docked vesicles between the AZM orthogonal and deep to the ribs that are adjacent to the active zones (Teng et al., 1999). We show at active zones that the proportion of newly retrieved from the presynaptic membrane and destined to fuse with the membrane during such activity. This agrees with the possibility that secondary docked vesicles are attached to the nearby primary docked vesicles, and the macromolecules arise from the AZM orthogonal and deep to the ribs that are adjacent to the active zones. Moreover, the secondary docked vesicles are connected to the bands in a way similar to that of the secondary docked vesicles. Altogether, these findings are consistent with the possibility that secondary docked vesicles are manifestations of macromolecules as primary docked vesicles, the macromolecules arise from the AZM orthogonal and deep to the ribs that are adjacent to the active zones. Furthermore, the presence of secondary docked vesicles in proximity to primary docked vesicles favors subsequent docking of vesicles selectively located within 30 nm of the membrane. As the probability of a fused vesicle reattaching to the membrane is much higher than that of a primary docked vesicle, it is more likely that the secondary docked vesicles are manifestations of macromolecules attached to the nearby primary docked vesicles. Therefore, the secondary docked vesicles are connected to the bands in a way similar to that of the secondary docked vesicles.
A zoo of morphologies

A: C. elegans
B: Drosophila
C: Skate
D: Rat
E: Frog
F: Lizard
G: Thin section
H: Freeze fracture

Zhai & Bellen, 2004
Hippocampal mossy fiber boutons

Figure 1. Ramón y Cajal's Illustration of Neural Circuitry of the Hippocampus
A drawing by Ramón y Cajal based on sections of the rodent hippocampus, processed with a Golgi and Weigert stain. The drawing depicts the flow of information from the entorhinal cortex to the dentate granule cells (by means of the perforant pathway) and from the granule cells to the CA3 region (by means of the mossy fiber pathway), and from there to the CA1 region of the hippocampus (by means of the Schaeffer collateral pathway). (Based on Ramón y Cajal, 1955.)
Hippocampal mossy fiber boutons

Salin et al. 1996
Capacitance measurements and the maxipool

$\Delta C_m \sim 100$ fF

70 aF / vesicle

releasable pool

$\sim 1400$ vesicles

Hallermann, Pawlu, Jonas & Heckmann, PNAS 2003
Release per active zone

Frotscher 1996

Hagiwara et al., 2005
Fig. 6. Colocalization of the CAZ proteins CAST and Bassoon. Triple immunogold labeling for CAST, Bassoon, and VGluT1 reveals that labeling for CAST and Bassoon are colocalized on the P-face of glutamatergic terminals of the mossy fiber (A,C) and A/C fiber (B). Particles of 15 and 5 nm were used for Bassoon and CAST, respectively, in A and B, and vice versa in C. Arrows and arrowheads indicate 5-nm particles for Bassoon (C) and 10-nm particles for VGluT1 (A–C), respectively. Intensity of labeling for CAST is stronger than that for Bassoon regardless of the size of the gold particles. D: CAST labeling (5 nm) was often observed adjacent to IMP clusters on the E-face labeled for GluR1–4 (10 nm), indicating CAST localization in the AZ of glutamatergic synapses. Scale bars 200 nm in C (applies to A,C), D (applies to B,D).
Fig. 6. Colocalization of the CAZ proteins CAST and Bassoon. Triple immunogold labeling for CAST, Bassoon, and VGluT1 reveals that labeling for CAST and Bassoon are colocalized on the P-face of glutamatergic terminals of the mossy fiber (A,C) and A/C fiber (B). Particles of 15 and 5 nm were used for Bassoon and CAST, respectively, in A and B, and vice versa in C. Arrows and arrowheads indicate 5-nm particles for Bassoon (C) and 10-nm particles for VGluT1 (A–C), respectively. Intensity of labeling for CAST is stronger than that for Bassoon regardless of the size of the gold particles. D: CAST labeling (5 nm) was often observed adjacent to IMP clusters on the E-face labeled for GluR1–4 (10 nm), indicating CAST localization in the AZ of glutamatergic synapses. Scale bars 200 nm in C (applies to A,C), D (applies to B,D).
The periactive zone

Brodin & Shupliakov 2006
Drosophila melanogaster

Rubin 1988

Jan & Jan 1976

Stewart et al. 1994
Modelling glutamate release

Pawlu, DiAntonio & Heckmann, Neuron 2004
T-Bars & Shibire

PH domain of dynamin1

Pucadyil & Schmid 2009

T-Bar/Filaments

Membrane tube

Koenig & Ikeda 1998
Endophilin & Synaptojanin

Dickman et al., 2005

Verstreken et al., 2002
CacGFP

Kawasaki et al., 2004
Imaging glutamate receptors in vivo

Rasse et al., Nature Neurosci. 2005
The Wuerzburg Hybridoma Library against *Drosophila* Brain

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**Abstract:** This review describes the present state of a project to identify and characterize novel nervous system proteins by using monoclonal antibodies (mAbs) against the *Drosophila* brain. Some 1,000 hybridoma clones were generated by injection of homogenized *Drosophila* brains or heads into mice and fusion of their spleen cells with myeloma cells. Testing the mAbs secreted by these clones identified a library of about 200 mAbs, which selectively stain specific structures of the *Drosophila* brain. Using the approach “from antibody to gene”, several genes coding for novel proteins of the presynaptic terminal were cloned and characterized. These include the “cysteine string protein” gene (*Csp*, mAb ab49), the “synapse-associated protein of 47 kDa” gene (*Sap47*, mAbs nc46 and nb200), and the “Bruchpilot” gene (*brp*, mAb nc82). By a “candidate” approach, mAb nb33 was shown to recognize the pigment dispersing factor precursor protein. mAbs 3C11 and pok13 were raised against bacterially expressed *Drosophila* synapsin and calbindin-32, respectively, after the corresponding cDNAs had been isolated from an expression library by using antisera against mammalian proteins. Recently, it was shown that mAb aa2 binds the *Drosophila* homolog of “epidermal growth factor receptor pathway substrate clone 15” (*Eps15*). Identification of the targets of mAbs na21, ab52, and nb181 is presently attempted. Here, we review the available information on the function of these proteins and present staining patterns in the *Drosophila* brain for classes of mAbs that either bind differentially in the eye, in neuropil, in the cell-body layer, or in small subsets of neurons. The prospects of identifying the corresponding antigens by various approaches, including protein purification and mass spectrometry, are discussed.

**Keywords:** brain proteins, CSP, synapsin, SAP47, Bruchpilot, calbindin, PDF, Eps15
Nc82, Bruchpilot & active zones

Kittel et al., Science 2006

Kittel et al., 2006
Distribution of Calcium channels

example

average aligned with Cac
GluRIID

brp-/

control

(Cac aligned)

\( \sigma = 120 \text{ nm} \)

\( \sigma = 150 \text{ nm} \)

(GluRIID aligned)

\( \sigma = 126 \text{ nm} \)

\( \sigma = 225 \text{ nm} \)

Hallermann et al., submitted

Hell 2007
Screening Bruchpilot

A

BRP

brp<sup>nude</sup>/BSC29

brp<sup>1.3</sup>/BSC29

brp<sup>5.45</sup>/BSC29

B

Survival (%)

Walking distance (%)

time (days)

C

brp<sup>+</sup>

1724

AAA GTC AAA CAA GCA CAA ACG CAG CAA CAG CAG CAG GAT GCT GGA CCA GCT GGC TTC TTG AAG AGC TTT TTC TAA

K V K Q A Q T Q Q Q Q Q D A G P A G F L K S F F

brp<sup>nude</sup>

... AAA GTC AAA CAA GCA CAA ACG CAG TAA CAG CAA CAG CAG GAT GCT GGA CCA GCT GGC TTC TTG AAG AGC TTT TTC TAA

K V K Q A Q T Q ...
Naked dense bodies

Hallermann et al., submitted
Limitations of NMJs

- function is vital
- development
- activity dependent defects
- movement
- presynaptic voltage clamp
Front to back motion
Experimental approach

Hallermann et al., 2003

Hell 2007
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  Heisenberg

• FU Berlin: Stephan Sigrist
Thank you for your attention!

Gerolf Steiner 1967
Summary

At presynaptic active zones (AZs), the frequently observed tethering of synaptic vesicles to an electron-dense cytomatrix, represents a process of largely unknown functional significance. Here, we identified a hypomorphic allele, brpnu, lacking merely the last 1% of the C-terminal amino acids (17 of 1740) of the active zone protein Bruchpilot. In brpnu, electron dense bodies were properly shaped, though entirely bare of synaptic vesicles. While basal glutamate release was unchanged, paired-pulse stimulation provoked depression at intervals as short as 10 ms. Furthermore, rapid recovery during and following sustained release was slowed.

Our results causally link, with intra-molecular precision, the tethering of vesicles at the AZ cytomatrix to rapid synaptic depression.
first few postnatal weeks. In CaV2.1-deficient mutants, compensation by residual channels is incomplete, leading to progressive weakness and death in the third postnatal week25,27,28.

NMJs in CaV2.1-deficient muscle were topologically normal, with varicose nerve terminals fully occupying an acetylcholine receptor (AChR)-rich postsynaptic apparatus (Supplementary Fig. 6). Notably, laminin $\beta_2$ was normally concentrated in the synaptic cleft of CaV2.1-deficient NMJs. With regards to ultrastructure, CaV2.1-deficient nerve terminals bore normal concentrations of synaptic vesicles, the postsynaptic membrane was normally...