PL-1 Unexpected features of vesicle turnover in presynaptic terminals

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Synaptic vesicle fusion and recycling is critical for neuron-to-neuron communication in the brain. A typical CNS nerve terminal, merely ~1 mm in diameter, contains ~100 synaptic vesicles, only ~30 of which actively undergo exo-endocytotic recycling. Recycling speed is a critical determinant of information flow across CNS synapses, but the modes of exo/endocytosis are not completely understood. The classical cycle of full-collapse exocytosis and clathrin-mediated endocytosis, characterized at the neuromuscular junction, has also been found in CNS nerve terminals, but whether it is the sole mechanism of vesicle cycling remains controversial. The classical cycle requires several tens of seconds, thus representing a potential temporal bottleneck for steady throughput of quantal neurotransmission. One way to overcome such limitations invokes "kiss-and-run" events, wherein vesicles transiently fuse with the plasma membrane and release their contents through a partially open fusion pore, without full collapse into plasma membrane.

We have developed new fluorescence methods for detecting rapid fusion events in small central nerve terminals, building on the precedent of FM1-43 and related styryl dyes. Because the dyes undergo big increases in quantum yield when taken up into membranes, activity-dependent sequestration of dye in synaptic vesicles and release on subsequent exocytosis generate increases and decreases in nerve terminal fluorescence. In one novel approach, we monitor briefly flickering fusion pores by tracking the effect of an extracellular hydrophilic quencher, bromophenol blue (BPB), which enters vesicles and quenches the fluorescence of EGFP fused to the vesicular protein synaptobrevin-2. The quencher hastened the loss of fluorescence from vesicles stained with FM1-43 dye, indicating that the lipophilic dye was trapped in vesicles through multiple bouts of fusion-retrieval. In contrast, entry of millimolar quencher was rapid enough to allow a reliable estimation of the delay to first-fusion, a fundamental but hitherto uncharted determinant of vesicular traffic. Quenching rates fell off with reduced quencher concentration, implicating fusion pore openings lasting milliseconds. Working in conjunction with classical full-collapse fusion, the rapid fusion events widen the functional capability of synapses subjected to widely ranging input patterns.

In a second new approach, we provided the first real-time visualization of a single vesicle fusing in a small central nerve terminal. Terminals in which only one vesicle was labelled with FM1-43 were tracked with highly-intensified CCD imaging. Real-time studies of individual vesicles enabled a dissection of their first-fusion latency, second-fusion latency, and FM dye lost per fusion event. Only a small minority of terminals underwent classical full collapse. Instead, the direct finding of FM1-43 retention in synaptic vesicles after fusion demonstrated a predominance of kiss-and-run events. A single vesicle released FM dye several times during repetitive stimulation, demonstrating rapid vesicular reuse without loss of identity.

PL-2 Brain development and neural plasticity - role of IP_3 receptor/Ca^{2+} signaling

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There are many molecules involved in brain development and differentiation. Here, I focus on the role of Ca^{2+} signaling, especially on the role of Ca^{2+} release through IP_3 receptor.

IP_3 receptor is a Ca^{2+} channel responsible for releasing Ca^{2+} from internal store inside the cell.

We found that IP_3R was a developmentally regulated P400 protein, enriched in the Purkinje neuron in the cerebellum but was missing in the mutant mice where Purkinje neurons are absent or spines of Purkinje neurons are missing (Nature 342, 32-38, 1989). IP_3 receptor (IP_3R) was an IP_3 gated Ca^{2+} channel (Neuron 5, 11-18, 1990)(J. Biol. Chem. 266,1109-1116, 1991). We found that IP_3R is essential for Ca^{2+} wave and Ca^{2+} oscillation during fertilization (Science 257, 251-255, 1992). We further found that IP_3R1 is essential for determination of dorsoventral axis formation (Science 278, 1940-1943, 1997) and that the downstream target was NF-AT (nuclear factor of Activated T cell)(Nature 417 295-299 2002). Neuronal type 1 IP_3R-deficient mice generated by a gene-targeting technique exhibited a significant reduction of birth rate and abnormal behavior (ataxia and seizure) and die before 3 postnatal weeks (Nature 379, 168-171, 1996). Long-term depression of the cerebellum was blocked in the type 1 IP_3R-deficient mice (J. Neurosci. 18, 5366-5373 1998). Rota-rod test of hetero-zygous mice was impaired. Long-term potentiation (LTP) of CA1 hippocampus was enhanced but depotentiation and LTP suppression was reduced in IP_3R1-deficient mice (Learning and Memory 7 312-320 2000). These evidences suggest that IICR is involved in neuronal plasticity. We found that IP_3R is involved in determination of polarity and input specificity of activity-induced synaptic modification (Nature 408 584-588 2000). Intensive molecular and biochemical studies showed that IP_3R had unique biochemical properties. 1) IP_3R allosterically and dynamically changed its form (windmill form in the presense of Ca^{2+} and square form in the absence of Ca^{2+}). 2) IP_3R was functional (both IP_3 binding activity and Ca^{2+} releasing avtivity) even though it was fragmented by proteases into several pieces, 3) IP_3R formed a functional association with a variety of molecules: IP_3R interacted with Trp channel on the plasma membrane and 4.1N cytoskeletal protein. Overexpression of 4.1N resulted in a translocation of IP_3R1 to the plasma membrane region. CARP (carbonic anhydrase related protein) which bound to the modulatory domain of IP_3R1 lowered the affinity to IP_3.

PL-3 Computations for sound localization in the owl's auditory system

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Examples of computations by neural systems are scarce, although models abound. The auditory system of barn owls performs operations similar to mathematical computations such as cross-correlation and multiplication. The anatomical and physiological bases for these processes and their behavioral significance are known. Owls use disparities in the timing of sound signals between the ears to localize sound in the horizontal plane. These cues are called interaural time differences (ITDs). Mammals including humans and birds measure ITDs by a process similar to cross-correlation. Partial decorrelation of binaural signals affects the accuracy of sound localization in both humans and owls. Addition of random noise to correlated signals reduces the degree of correlation. The amplitude difference between the correlated and random noise determines the degree of correlation. In humans, partially correlated signals produce a blurred image inside the brain and the blur increases, as the signals are further decorrelated (Blauert and Lindemann, 1986). Jeffress et al., (1962) carried out experiments in which human subjects used an electronic delay device to bring a sound image to the midpoint between the ears from an arbitrary initial locus. As the degree of correlation became smaller than about 0.2 - 0.3, the subjects' choice of delays became more variable, i. e. the standard deviations of the mean delays chosen by the subjects increased. Using partially correlated signals, we studied how owls localize such signals. We obtained both the mean localization angles and the standard deviations of the means. The standard deviations remained almost constant until the degree of correlation decreased to about 0.2 - 0.3 (Saberi et al., 1998). The curves for the standard deviations closely resemble that obtained for humans (Jeffress et al., 1962).

Cross-correlation requires means for varying the time difference (delay lines) and for measuring the degree of match (coincidence) between the two signals. In the avian auditory system, cochlear nucleus magnocellularis provides axonal delay lines and nucleus laminaris provides coincidence detectors. The auditory system performs cross-correlation in separate frequency bands. Time is encoded by phase-locked spikes, which occur at or near particular phase angles of tones or spectral components of a complex stimulus. Cross-correlation of left and right trains of phase locked spikes produces periodic changes in spike rates as a function of ITD. Thus, laminaris neurons respond to multiple ITDs that correspond to a specific interaural phase difference (i.e. ITDi $\pm$ nT, where ITDi is a frequency independent ITD and T is the period of the stimulus tone).

How does the owl's auditory system distinguish the ITD*i* from ITD*i* $\pm$ nT ? Nucleus laminaris processes ITDs in separate frequency bands, which project to high-order stations as separate parallel pathways. These bands converge on single neurons in the external nucleus of the inferior colliculus (ICx). ICx neurons are broadly tuned to frequency unlike lower-order ITD-sensitive neurons. ICx neurons respond best to a single ITD contained in a broadband stimulus. When the same neurons are stimulated with tones, they respond to the same single ITD and its phase-equivalents, which vary with frequency. The single ITD is the ITD*i*. Thus, ICx neurons can discriminate between the ITD*i* and ITD*i* $\pm$ nT, when the signal bandwidth is broad. We proposed a model in which ICx neurons receive inputs from lower-order neurons tuned to the same ITD but to different frequencies. When the ITD responses of ICx neurons for different frequencies are summed with respect to the ITD*i*, a tall peak at the ITD*i* and smaller peaks at ITD*i* $\pm$ nT result, because the peaks at ITD*i* $\pm$ nT do not match. However, the differences in peak heights are small in terms of postsynaptic potentials. Somehow, the threshold of firing is set such that the peak at the ITD*i* is above it and the peaks at ITD*i* $\pm$ nT are either below threshold or only slightly above threshold. How the threshold is adjusted in favor of the ITD*i* is not known (Peña and Konishi, 2002).

The owl can use the interaural level difference (ILD) for localization in the vertical plane, because the two ears are vertically displaced from each other. The owl interprets sound louder in the right ear as coming from above eye level and sound louder in the left ear as coming from below eye level. The owl's auditory system processes ITD and ILD in separate routes between the cochlear nucleus and the inferior colliculus. The two pathways merge in the lateral shell of the central nucleus of the inferior colliculus. This convergence provides bases for neuronal selectivity for combinations of ITD and ILD, although these neurons are still narrowly tuned to frequency. The lateral shell projects to the ICx where different frequency bands converge on single neurons. ICx neurons behave like AND gates of ITD and ILD, when the stimulus is broadband. AND gating is more likely to involve multiplication than addition. Intracellular studies show that ICx neurons multiply postsynatpic potentials for ITD and ILD. If we plot the membrane potential for each combination of ITD-ILD tested, we obtain a matrix of numbers.
An algebraic rule states that any matrix can be shown to be a sum of products of multiplications of independent vectors. The singular value decomposition uses this rule to test whether a matrix is a product of multiplication of a single or multiple pairs of vectors. Our analysis of 15 ICx neurons showed that the amplitudes of postsynaptic potentials induced by ITD-ILD pairs are due to multiplication of just two vectors. These vectors resemble the ITD and ILD response curves of the neuron (Peña and Konishi, 2001).

References:

Blauert, J. and Lindemann, W. (1986). Spatial mapping of intercranial auditory events for various degrees of interaural coherence. J. Acoust. Soc. Am., 79, 806-813.
Jeffress, L. A., Blodgett, H. C., and Deatherage, B. H. (1962). Effect of interaural correlation on the precision of centering a noise. J. Acoust. Soc. Am., 32, 1122-1123.
Peña, J. L. and Konishi, M. (2001) Auditory spatial receptive fields created by multiplication. Science 292: 249-252.
Peña, J. L. and Konish,i M. (2002) From postsynaptic potentials to spikes in the genesis of auditory spatial receptive fields. J. Neurosci. 22: 5652-5658.
Saberi, K., Takahashi, Y., Konishi, M., Albeck, Y., Arthur, B. J., and Farahbod, H. (1998) Effects of interaural decorrelato on neural and behavioral detection of spatial cues. Neuron, 21, 789 - 798.

PL-4 From stem cells to synapses in the central nervous system

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Stem cells are most often defined by their fundamental properties of self renewal and multipotentiality. The academic justification for studies of stem cells is that they provide a useful experimental system to analyze some of the basic mechanisms controlling brain development. Work from our group played an important role in the early period when stem cells of the central nervous system were first defined (McKay RDG. Science. 276:66-71, 1997). Our more recent work contributes to our fundamental understanding of three major processes in the developing nervous system: (a) cell cycle control (Tsai, R. Y. and R. McKay Genes & Dev. 16:2991-3003, 2002), (b) the control of cell fate (Panchision, D. M. et al. Genes Dev. 15: 2094-2110, 2001) and (c) the early steps in neuronal differentiation (Vicario-Abejon, C. et al. J.Neurosci. 18: 7256-7271, 1998; Vicario-Abejon, C. et al. Eur.J.Neurosci. 12: 677-688, 2000; Blondel, O., C. Collin, et al. J.Neurosci. 20: 8012-8020, 2000; Collin, C. et al. Eur J Neurosci. 13: 1273-82, 2001). Work on CNS stem cells has developed to a stage where there are also clinical implications. Recent advances in the application of stem cell biology to Parkinson's disease clearly demonstrates the potential importance of a strong scientific foundation in the use of stem cells in models of neuronal loss or injury (Studer, L. et al. Nat.Neurosci. 1: 290-295, 1998; Lee, S-H. et al. Nat.Biotechnol. 18: 675-679, 2000; Kim, J-H. et al. Nature 418: 50-6, 2002. We have also made contributions in other areas, for example in glial transplantation (Brustle, O. et al. Science 285: 754-756, 1999), adult neurogenesis (Cameron, H. A. and R. D. McKay Nat.Neurosci. 2: 894-897, 1999; Cameron, H. A. and R. D. McKay J. Comp. Neurol. 435: 406-17, 2001) and endocrine pancreatic differentiation (Lumelsky, N. et al. Science 292: 1389-1394, 2001) emphasizing the clinical importance of stem cell differentiation. These results suggest that the powerful technology developing to manipulate stem cells will allow us to analyze fate and function in many cell types.

PL-5 Brain Mechanisms for the Control of Locomotion: a Multi-system Approach

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Bipedal (Bp) terrestrial locomotion is a routine, everyday activity for humans and advanced non-human primates. While its elaboration seems simple, it actually involves much skill and long-term locomotor learning, such that the CNS can achieve a seamless spatial and temporal integration of multiple motor segments. The latter operate against gravity and propel the body in various relevant directions. For this, the CNS must simultaneously control movement, appropriate postures, and balance. The CNS control features use of anticipatory and reactive processes with the latter particularly prominent after tripping and stumbling. To advance understanding of the CNS control mechanisms that operate during Bp locomotion, it seemed necessary to make use of a non-human primate model. This strategy invites the possibility of employing state-of-the-art interventional recording techniques and a cellular-to-systems level of neuroscientific analysis to the study of locomotion. To this end, we used operant conditioning to train the normally quadrupedal (Qp)-walking juvenile Japanese monkey (M. fuscata) to stand upright and walk bipedally on the surface of a moving treadmill belt. After 16-24 mos. of regular locomotor training, physical growth and further CNS maturation, M. fuscata acquired sufficient coordination of upper (head, neck, forelimb, trunk) and lower (hindlimb) motor segments to stand upright and elaborate Bp locomotion. The kinematic features of this locomotion resembled those used by the human during Bp walking. A neuroimaging study using positron emission tomography (PET) revealed that multiple brain regions (primary (M1) and supplementary (SMA) motor areas, visual cortices, cerebellum) are activated in parallel during M. fuscata's locomotion, and some intriguing differences between Qp and Bp walking. Reversible chemical inactivation of M1 vs. SMA showed cortical region-specific impairments of posture and locomotion. In summary, our M. fuscata studies have demonstrated brain mechanisms involved in the successful emergence and elaboration of Bp locomotion. We think that the study of posture and locomotion is fundamental to the understanding of basic brain-behavior relationships from the cellular to the behavioral level of analysis. Within this spectrum of experimental approaches, there is a clear and important role for approaches that feature use of the theory and techniques of systems neuroscience.

References:

Mori, S. et al. (1996). NeuroReport 7: 2277-2285.
Nakajima, K. et al. (2001). In: Sensorimotor Control. IOS Press. pp.95-102.
Mori, F. et al. (in press). In: Brain Mechanisms for the Integration of Posture and Movements. Prog. Brain Res. Elsevier.

PL-6 The role of antibodies to neuronal antigens in neurological disorders

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Myasthenia gravis (MG) is the best example of an antibody-mediated neurological disease, due to antibodies to the muscle form of the nicotinic acetylcholine receptor (AChR) in most patients. Antibodies to the receptor tyrosine kinase, MuSK, are present in some of the remaining patients [1]. These conditions are antibody-mediated because 1.there are antibodies to the extracellular surface of a functional protein, 2. there is a clinical response to plasma exchange, and 3. the disease can be transferred by injection of IgG into mice, Rarely mothers with MG give birth to babies with arthrogryposis multiplex congenital (AMC), a severe developomental condition that results from lack of fetal movement. This is associated with maternal antibodies that specifically inhibit the function of the fetal isoform, and have little or no effect on the function of the adult isoform. The condition can be reproduced in mice by maternal-to-fetal transfer of the antibodies; the mouse offspring were born with fixed joint contractures and other developmental abnormalities [2].

Other neurodevelopmental conditions could be due to maternal antibodies directed at CNS membrane proteins. Two of five sera from women who have had children with neurodevelopmental problems, including autism and special language disorders, contained antibodies binding to neurons in developing mouse cerebellum. We tested "maternal-to-fetal" transfer in one case. The mice were born at term and developed normally, but they showed significant defects in motor coordination. These preliminary results are consistent with a role for maternal antibodies in some cases of neurodevelopmental disorders [3].

Antibodies to voltage-gated potassium channels (VGKC) are found in patients with neuromyotonia, and result in motor nerve hyperexcitability with muscle cramps and twitching. Some patients develop "limbic" symptoms such as anxiety, memory loss, insomnia, seizures and hallucinations, and VGKC antibodies appear to be closely linked to the disease [4,5] and the patients respond to plasma exchange or improve spontaneously with reduction in VGKC antibodies.

References:

Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. 2001. Autoantibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med 7: 365-368.

Jacobson L, Polizzi A, Morriss-Kay GM, Vincent A 1999 An animal model of antibody-mediated neurodevelopmental disease: arythrogryposis multiplex congenita caused by antibodies to fetal acetylcholine receptor. J Clin Invest 103 1031-1038.

Dalton P, Deacon R, Blamire AM, Pike M, McKinlay I, Stein J, Styles P, Vincent A. Experimental evidence for a fetopathogenic antibody in the mother of children with autism and a severe speech disorder. Ann Neurol in press.

Buckley C, Oger J, Clover L, Tuzun E, Carpenter K, Jackson M, Vincent A. 2001. Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol 50: 74-79