Final Report for Joint Project Grant BBS/B/07217 & 07276

Title: **Changes in information transmission at an auditory synapse in the binaural pathway during short-term synaptic modulation.**

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Background to the Project

The calyx of Held is a giant synapse in the auditory brainstem. It is mediated by the transmitter glutamate and provides a fast transmitting relay for information from one ear to be communicated to the auditory processing nuclei on the other side of the head. The principle of binaural comparison (comparison of sound inputs to both ears) is fundamental to the way in which the brain extracts information about the location of a sound source. There are two basic mechanisms: interaural timing differences (ITD) and interaural level (volume) differences (ILD). In animals with a sufficient distance between their ears, the sound activating the ear pointing away from the source is delayed with respect to the other ear (in man this is around 0.5 ms) so a coincidence detection and delay line mechanism can give the location (referred to as the Jeffress model). This mechanism takes place in the medial superior olive. Additionally sound must refract around the head to the down-stream ear and it also reflects off the head, so its volume in the down-stream ear is less than in the upstream ear. So again comparison of the relative volumes gives the location. This mechanism takes place in the lateral superior olive (LSO). The MNTB provides an inhibitory (glycinergic) input to both the MSO and LSO. Each MNTB neuron receives one calyx of Held; this synapse forms on the soma and is so large that it always triggers an action potential. The information about sound is encoded as trains of action potentials, so the timing of the spikes within these trains is thought to be crucial for computation of both the ITD and ILD measures of sound source. Several sources of error are obvious: 1. At high frequencies, synaptic release declines due to short-term depression. 2. A sound occurring after another loud sound, is likely to be encoded differently than a sound occurring following a quiet period. 3. Many sources of variability are known, but only a model combined with experimental validation can really start to unravel the optimal solution to the physiological problem. The idea of this grant was to have a postdoc focussed on *in silico* modelling in Stirling and a postdoc focussed on *in vitro* experiment in Leicester. We were very fortunate to find two exceptional postdocs in Matthais Hennig (Stirling) and Mike Postlethwaite (Leicester).

Progress against original objectives

The original proposal contained four stages of work:

1. Response to regular stimulation at the calyx of Held.
2. Spiking response of the MNTB neuron.
3. Information transmission.
4. Signal transmission in response to sound stimuli.

As will be described in more detail below, all four stages were largely completed as per the original proposal. The work has resulted in 6 journal papers published or in press (2 more under revision and a further 2 in preparation), plus 8 conference presentations.

Personnel

Although we have had excellent postdocs working on this project, the continuity of the grant has suffered from the promotion of these able staff into permanent posts.

Dr Matthias Hennig filled the RA post at Stirling for 2005, but left the project after one year to take up a personal MRC fellowship at the University of Edinburgh. However, he is continuing very fruitfully to collaborate on this work. A replacement, Dr Zhijun Yang, was obtained after a delay of two months. This short gap, plus the necessity to bring a second RA up to speed with the project, necessarily impacted a little

on what could be achieved in the timeframe. Dr Yang successfully completed the key work on information transmission at the calyx. He is now a postdoc at the University of Edinburgh.
Dr Margaret Barnes-Davies was the initial RA in Leicester, from 1/8/2004 until 15/10/2004. She then obtained a lectureship in the Medical Faculty at the University of Leicester. Dr Mike Postlethwaite was the major experimental RA at Leicester, from 4/4/2005 until 29/5/2007. He then obtained a permanent post in the pharmaceutical industry (Pfizer). Because of the delays in recruitment, the Leicester grant was permitted a no-cost extension and employed Dr Melissa Jordan to conduct immunohistochemical studies of NMDA receptors at the calyx (1/10/2007 until 31/3/2008).
The exchange visits between Leicester and Stirling for the postdocs and PIs to discuss data and integrate the modelling with the experimental work were very productive and made a huge contribution to the efficiency with which our staff were able to work together.

Summary of scientific results

Synaptic responses at physiological temperature (preparatory to stage 1)

Before beginning experiments and detailed modelling of short-term plasticity at the calyx of Held synapse, it was necessary to first characterize and understand the changes that take place in synaptic transmission with variation in temperature. Many of our previous studies had been conducted at room temperature, but in order to match the model and the *in vitro* experiments to published *in vivo* data we decided that all experiments would be conducted at physiological temperatures: 37^{o}C. This showed up some important gaps in our knowledge, in that very few studies had been conducted at 37^{o}C and even fewer studies had actually examined the effect of temperature on synaptic transmission. Fortuitously, Henrique von Gersdorff published a paper demonstrating that transmitter release from the calyx was not highly temperature dependent, allowing us to focus on the postsynaptic site where we demonstrated highly significant temperature effects.
In Leicester, Dr Barnes-Davies set up the calcium imaging for detection of functional synapses on the electrophysiological rig and started to conduct the synaptic experiments. Dr Mike Postlethwaite conducted the voltage clamp experiments. Experimental data of miniature and evoked excitatory postsynaptic currents (EPSCs) were obtained at a range of temperatures from 25 to 35^{o}C. Our experiments showed that both evoked and spontaneous synaptic events were about a third larger and significantly faster at 37^{o}C than at room temperature (published in the Journal of Physiology: Postlethwaite et al 2007).
In Stirling, Dr Matthias Henning undertook detailed mathematical modelling and computer simulations to show that this change may simply be due to a uniform speeding of the postsynaptic AMPA receptor kinetics (Postlethwaite et al, 2006, 2007; all modelling work was conducted by M. Hennig). This work included Monte Carlo simulations of the three dimensional diffusion of neurotransmitter in the synaptic cleft, carried out using the MCELL simulator (Bartol & Stiles), as well as deterministic modelling of the AMPA receptor response using the NEURON simulator (Carnevale & Hines). The importance of the conclusion concerning the simple change in receptor kinetics was highlighted in a subsequent commentary article in the Journal of Physiology (Plested et al, Getting hot under the calyx, *J. Physiol*. 580:13-14, 2007).

Components of short-term plasticity (stages 1 and 3)

This project proposal was partly developed from a previous collaboration between the Graham and Forsythe laboratories in which we had experimentally determined and modelled AMPA receptor desensitisation. We also had established that presynaptic metabotropic glutamate receptors were a major mechanism of presynaptic inhibition at the calyx of Held, but several groups, including us, were surprised to find so little change in transmitter release following prolonged synaptic stimulation. By combining modelling of exocytosis at the calyx of Held, with experimental verification of the model predictions and application of pharmacological tools, we were able to prove that presynaptic group III mGluRs do function as autoreceptors at this site. This was the first time that release of an endogenous transmitter (glutamate) had been shown to influence release; but the modelling also showed that the biophysics of transmitter release can largely compensate for short term changes in release probability. This work was published in the Journal of Physiology (Billups et al, 2005).

Models of short-term plasticity (stages 1, 2 and 4)

Fundamental to all stages of the proposal was the development of a mathematical model that captured key characteristics of the evident short-term plasticity (STP) at this synapse. Model formulation was based on existing and new experimental data on the EPSC amplitudes resulting from regular and random stimulation of the calyx at different frequencies, both at room temperature and latterly at physiological temperature. New voltage- and current-clamp data was collected by Mike Postlethwaite, using long trains of regular and Poisson-distributed stimuli.

The model, developed by Matthias Hennig, consists of a set of coupled differential equations that capture the magnitude and time course of identifiable biophysical mechanisms that contribute to facilitation and depression in the EPSC amplitudes (Hennig et al, 2005, 2006, 2007, 2008). Fast and slow components of STP of EPSCs is evident in the experimental recordings. These have been replicated in the model and identified as:

- facilitation of vesicle release probability
- vesicle depletion due to exocytosis
- background and activity-dependent recovery of the readily-releasable vesicle pool (RRVP)
- release-independent depression in vesicle release probability with slow recovery due to calcium channel inactivation
- release-dependent depression in vesicle release probability with slow recovery due to presynaptic mGluR activation
- AMPA receptor desensitisation

The model provides a very tight fit to experimental data from long and short stimulus trains over a range of frequencies, at room and physiological temperatures. It can accurately reproduce the time course of synaptic depression, including a very slow, long-lasting EPSC amplitude decay. The model shows that the slow decay is a consequence of release probability inhibition by multiple mechanisms, and accompanied by a partial releasable vesicle pool recovery. This prediction is supported by an analysis of patch clamp recordings with long-lasting stimuli.

In a major outcome, the model explains stimulus-history dependent effects on recovery from depression as the result of simultaneous relaxation of multiple processes with stimulus-history dependent relative activation. This has important implications for how the synapse responds to sound-induced stimuli in the presence of ongoing spontaneous synaptic activity. Effectively the sound stimulus intensity and duration is encoded in the recovery time of the synapse, thus influencing the response to subsequent sounds. In addition, the spontaneous activity interacts with stimulus-induced activity to determine stimulus response, with potentially different responses for different spontaneous rates.

Simulations of transmission in the presence of background activity that causes chronic synaptic depression suggest that, under in-vivo-like conditions, multiple processes of release probability inhibition prevent vesicle depletion and maintain a low baseline transmission rate where recovery kinetics from depression are fast and stimulus-history dependent. The model and its implications have been published in the Journal of Physiology (Hennig et al 2008).

Information transmission (stage 3)

To investigate the transmission of information through the synapse, the deterministic model of STP was extended to a stochastic version allowing quantal recycling and release of neurotransmitter. This model was then used to explore how EPSC amplitudes may carry information about the preceding presynaptic interspike intervals (Yang et al, 2007, 2008).

STP results in the EPSC amplitude either being facilitated or depressed as a function of the time between presynaptic stimuli. In mathematical terms, the EPSC amplitude can be said to carry information about presynaptic ISIs, and the amount of information can be quantified by Shannon's measure of mutual information (MI). Computer simulations of long stimulus trains in which ISIs are Poisson-distributed around a mean frequency were used to collect data on EPSC amplitudes. These were then used to calculate the mutual information as a function of the mean stimulation frequency.

The mutual information between presynaptic spike times and the amplitude of the postsynaptic response in general decreases as the mean stimulation rate increases, but remains high even at frequencies greater than 100Hz, unlike at many neocortical synapses. The model predicts that the maintenance of information transmission across a wide frequency range is attributable to different information carriers: (1) vesicle recycling rates at low frequencies of stimulation, shifting to (2) facilitation of vesicle release probability

and (3) AMPA receptor desensitization, at high frequencies. This work is under revision for Neural Computation (Yang et al).
It remains an open question as to the significance of this information transmission for the in vivo functioning of this synaptic pathway. In vivo, the MNTB output in fact is not entirely faithful in following the calyx input, exhibiting significant failures and variation in spike onsets under high frequency stimulation (Kopp-Scheinpflug et al., JARO 4:1-23, 2002). Amongst the differences, spontaneous and sound-evoked spiking rates in the MNTB neurons are lower than presynaptically, but phase-locking to sound frequencies greater that 1kHz is higher postsynaptically. The spiking output is determined by the summation of all excitatory and inhibitory inputs and the intrinsic cellular properties, which are subject to modulation in response to cellular activity (Kaczmarek et al., Hearing Res. 206:133-145, 2005; Song et al., Nat. Neurosci. 8:1335-1342, 2005). It remains a challenge to elucidate exactly how the short term modulation of synaptic input and neuronal properties combine to determine MNTB output. It is highly likely that this pathway through the MNTB acts as rather more than an "inverting relay".

Postsynaptic mechanisms (stage 2)

The final stage of the experimental work involved exploration of the postsynaptic AMPAR and NMDAR responses in MNTB neurons. In a methodological development, we explored the problems of studying action potential generation evoked by artificial versus synaptic depolarisation. Because of the large conductance of the AMPA receptor mediated EPSC in the MNTB neuron, the overall waveform of the action potential is significantly altered by the synaptic conductance. This result is of particular use to illustrate the importance of using physiological stimuli (i.e. synaptic stimulation) in studying and modelling of synaptic transmission. This work has been presented as a poster at the British Neuroscience Association and is currently being written up as a full paper (Johnston et al).
Our studies of normal physiology highlighted the issue of NMDAR participation in synaptic transmission at the calyx of Held. This first required clarification of the developmental time-course for modelling purposes. The issue here is that NMDAR are present and activated at the calyx, but as the animal's hearing matures, the level of NMDAR-mediated current declines. Previous work had suggested that NMDAR were negligible by 18 days postnatal. Mike Postlethwaite recorded from rats ages 11, 14, 18 and 21 days old, showing that although the NMDAR declined with age, they did not 'disappear' and still made a significant contribution in mature animals. We have conducted quantitative rtPCR measurement of NMDAR mRNA levels in the rat MNTB and through the employment of Melissa Jordan we have spent 6 months testing and validating a series of NMDAR antibodies in order to assess the presence and distribution of NMDAR in the MNTB. We are currently in the process of writing this up for publication (Jordan et al).

Principal conclusions and opportunities arising

In summary, this project has established a state-of-the-art model, based on experimental data, of short-term plasticity at the calyx of Held synapse in the mammalian auditory system. This model predicts that the multiple time courses of STP will strongly and systematically affect the postsynaptic response of the synapse to particular temporal signals. In other words, the synapse is not simply a relay point, but acts as a complex signal filter. Specific conclusions, which are all the result of combined experimental and mathematical modelling, are:

- Synaptic function is strongly temperature dependent, with a major effect being a speeding of AMPAR kinetics with increasing temperature.
- Presynaptic mGluRs are functionally significant in modulating neurotransmitter release.
- NMDARs do contribute to the postsynaptic response, even in mature animals.
- The calyx exhibits multi-time-scale dynamics that shapes its response to sound stimuli as a function of the strength and duration of the stimuli and the rate of ongoing spontaneous background activity.
- The postsynaptic response carries information about presynaptic spike times over a wide range of spike frequencies, with different molecular pathways being the main information carriers over different specific frequency ranges.

Additional outcomes

With Ralph Scheggenburger, Ian Forsythe has written a large review on the calyx of Held (2006). Forsythe has also written a Nature News and Views (2007) covering an article on the origin of spontaneous activity in the auditory pathway.
In addition to the scientific publications, all computer code to implement the models is publicly available from the authors and through the ModelDB database (http://senselab.med.yale.edu/modeldb/). This will allow other researchers to compare and build upon our models.
Invited seminars have been given on this work by Graham (John Curtin School of Medical Research, Australian National University, Canberra: September 2006; Bionic Ear Institute, Melbourne: February 2008; Queensland Brain Institute, University of Queensland, Brisbane: February 2008) and Hennig (Max Planck Institute for Dynamics and Self-Organization, Goettingen: January 2007; Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, January 2008).
The results are relevant to other synapses in the brain, so are of interest to anyone studying chemical synaptic transmission, not just in the auditory system. The seminar given by Graham at the ANU in September 2006, resulted in a successful application for a Royal Society International Short Visit Award, enabling Graham to work with Dr Christian Stricker at the ANU during Jan/Feb 2008, to compare STP at the calyx with STP at the neocortical synapses studied by Stricker. As an outcome we are developing a canonical synapse model that can be easily configured to match the characteristics of different types of synapse. Initial work with the model highlights the significance of the synaptic configuration (numbers of release sites) and combination of STP components (particularly frequency-dependent components) in generating the postsynaptic response to temporal inputs and determining whether steady state or transient signals are best transmitted (paper accepted for oral presentation at ICANN'08).
Discovery in the Forsythe lab of a volume transmission mechanism resulting from synaptic and cellular activity prompted new modelling work by Graham on the spread of nitric oxide through the volume of MNTB tissue in response to spontaneous and sound-evoked activity (Steinert et al, manuscript resubmitted to Neuron).

Future work

A number of research avenues proceed from this work, both specific to this synaptic pathway and considering the wider implications of STP in the nervous system. Forsythe and Graham are developing a further joint grant proposal (likely to BBSRC) to explore models of sound processing across the MNTB, integrating models of STP at the calyx with models of spiking MNTB neurons, other excitatory and inhibitory inputs to principal MNTB cells and activity-dependent volume transmission mechanisms within the MNTB. This will allow explicit investigation of the role of the MNTB in sound source localization. Graham and Stricker will continue to collaborate on formulating a generic STP model and studying role of STP throughout the nervous system. Forsythe has submitted a European Science Fund Advanced Grant application to continue the work on volume transmission. This proposal includes collaboration with Graham on modelling NO diffusion and spiking responses of MNTB neurons.

Publications (Names in **bold** are authors funded by the BBSRC grant)

Papers

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Full papers in preparation

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Abstracts

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