Institute of Marine Science (IMS), University of Auckland

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ABSTRACTS

Session 1

From sensory processing to sound production - neuroethology at the Leigh Marine Laboratory

Marie Goeritz, University of Auckland

At Leigh we are very much a field based laboratory, which provides access to functioning ecosystems and natural behaviour. From a neuroethology perspective, we have a very active research program in bioacoustics.

Our work centres around how marine animals use underwater sound, and we were the first to show that larval fish and crustaceans use sound as an orientation and settlement cue.

This raised many questions: What is reef sound and how far does it propagate? What is the hearing capability of fish and crustaceans – at what distance could sound be used as an orientation cue? What is the evidence for ecologically significant orientation? Which of our local fish vocalize? What is the impact of anthropogenic noise on sound ecology? Some of these have been addressed, but many are still a work in progress and form the basis of Craig Radford's research program.

A recent focus of our research has been crustacean hearing and sound production. We have shown different sound perception abilities in a range of crustacean species and that sound might not only be used as a cue for orientation, but also for intra-specific communication. We have discovered that paddle crabs are able to produce a variety of sounds, some of which are sex-specific and associated with mating or feeding behaviors. Interestingly, some of these sounds are produced by formerly undescribed mechanisms and may involve the central pattern generating circuits of the stomatogastric nervous system.

Muscle synergies for voluntary motor control: sources of synergism and implications for stroke

Angus JC McMorland, Department of Exercise Sciences & Centre for Brain Research & Auckland Bioengineering Institute, University of Auckland.

The motor control redundancy problem is that we have many more muscles than kinematic degrees of freedom. Consequently numerous control strategies exist that could achieve any one desired movement. One solution suggested for this problem is the use of muscle synergies, common patterns of co-activation of muscles. A number of debates exist around this idea: the first of which is the mathematical formulation of synergies as either time-invariant sets of intermuscular weights being driven by time-varying activation coefficients, or as time-varying weights being driven by scale-variant impulses at particular intervals. Evidence also exists for both spinal and cortical sources for synergistic control have been identified, and a unified theory for the use of muscle synergies has not yet been developed. Where synergies are constructed within the central nervous system has significant consequences for the potential interactions between that structure and the damage caused by a stroke lesion. This talk will outline the history and current debates surrounding muscle synergy theory and report the results of several recent studies from our lab group examining the effects of task, fatigue, and stroke severity on synergy structure and recruitment.

An evolutionary perspective on pattern formation in the cerebellar granule cell layer

John Montgomery, Institute for Marine Science, University of Auckland

Cerebellar granule cells are the most numerous cell type in the brain. They provides the input to support the adaptive filter functionality of the cerebellum. Granule cells get their input from a smaller number of mossy fibres (250 times less) suggesting that one of the main functions of the granule cell layer is to provide for an expansion of the incoming signals. This would increase the predictive information available to the adaptive filter circuitry in a way that can be described as 'basis function expansion'. One form of this pattern formation is to turn a mossy fibre input into a sequence of burst firing across a group of granule cells. Across a range of vertebrate species, from sharks and weakly electric fish to mammals there is a range of mossy fibre input types spanning rich efference copy from CPGs, to pulse-like inputs and step-function activity. A burst sequence in response to a pulse input is similar to what would be achieved from a tapped delay line. But a burst sequence generated by a step input is more like an A/D conversion. The challenge in either case is to come up with a biologically plausible mechanism to generate this sequential pattern formation.

Motor pattern formation and neural circuit development during frog metamorphosis

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Frog metamorphosis constitutes one of the most dramatic developmental transformations in biology, involving the transition from a fish-like tadpole to a quadrupedal adult. We have explored the developmental plasticity of spinal locomotor networks and the motor patterns they produce as *Xenopus laevis* switches from larval tail-based undulatory swimming to propulsion by bilaterally-synchronous hindlimb kicking in the young adult. Of particular interest is the relative contributions of the spinal tail-swimming and limb kick CPG networks to behavior at key transitional metamorphic stages when functional larval and adult locomotor systems co-exist within the same animal.

In *Xenopus* also, so-called 'efference copies' of the spinal motor patterns for swimming behavior drive motor output to the eye muscles in order to produce rhythmic ocular movements that compensate for the disruptive effects of self-motion on the animal's visual acuity. The change in locomotor strategy during metamorphosis results in head/body motion with entirely different dynamics, necessitating a concomitant switch in gaze-stabilizing eye movements from conjugate lateral rotations to non-conjugate convergence during the linear forward acceleration produced by each kick cycle. By using semi-intact or isolated brainstem/spinal cord preparations at pre-, intermediate and post-metamorphic stages, we monitored bilateral eye motion along with extraocular, spinal axial and limb motor nerve activity patterns during episodes of spontaneous fictive swimming. In my talk, I will describe how an adaptive interplay between locomotor and extraocular motor circuitry allows spinal CPG-derived efference copy signals to continuously satisfy gaze-stabilizing demands throughout metamorphosis as one propulsive mechanism emerges and eventually supplants the other.

Disentangling neuronal contributions in a distributed central pattern generator

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Rhythmic motor behaviors are diverse, ranging in timescales and temporal complexity. The vertebrate hindbrain contains numerous neural circuits underlying motor behaviors such as breathing, vocalization, and chewing, and is therefore well-suited for investigating the mechanisms underlying the production of complex neural patterns. Our lab investigates the central pattern generator (CPG) that produces vocalizations in the African clawed frog, *Xenopus* using a whole-cell patch-clamp technique to record activity of individual neurons during "fictive vocalizations" in the isolated brain. In male *X. laevis*, this circuit generates a biphasic pattern of nerve activity consisting of alternating slow (30 Hz) and fast (60 Hz) trills. The vocal CPG is composed of two distinct hindbrain regions: a premotor nucleus (the *Xenopus* parabrachial area) and a motor nucleus (n.IX-X). We have identified two cell types in the premotor nucleus that likely play distinct roles in vocal patterning. One of these neuronal subtypes encodes the duration, period and trill rate of calls. While these premotor neurons intrinsically encode duration and period, they require ascending inputs from the vocal motor nucleus in order to maintain normal spike rates. Therefore, we have begun recordings in n.IX-X to identify neurons that provide this ascending projection. By identifying activity patterns of local and feedback interneurons in n.IX-X, we hope to discover mechanisms by which reciprocally connected nuclei generate precisely timed motor rhythms.

Session 2

Generation of flexible rhythmic behaviors in the lobster cardiac neuromuscular system: Roles of neuromodulators, intrinsic sensory feedback, and their interactions

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Although pattern-generating networks are hard-wired, they must nonetheless generate flexible outputs. Much of the required flexibility results from the effects of modulatory neurotransmitters. Additionally, sensory inputs can modulate these pattern generating networks. Using the cardiac neuromuscular system of the American lobster, *Homarus americanus*, we are examining the roles played by sensory inputs and peptide neuromodulators in altering pattern generator output, and asking whether and how these two systems interact with one another. The neurogenic heartbeat of crustaceans is controlled by a 9-neuron pattern generator, the cardiac ganglion (CG), consisting of 5 motor and 4 premotor neurons, which fire nearly synchronous driver potentials and bursts of action potentials. Dendritic processes of CG neurons appear to provide direct stretch feedback to the CG. Cutting these processes leads to increased pattern variability in a semi-intact heart. In reduced preparations, sustained stretch of the pre-motor neuron region elicited increases in driver potential frequency and decreases in driver potential and burst duration, particularly in preparations in which baseline burst duration was long. Mechanical muscle extension caused phase delays in CG bursting while release of extension increased burst duration in neurons with long baseline durations. Interestingly, we were unable to mimic this suite of effects of stretch by injecting current into CG neurons. To examine the interactions between stretch and neuropeptides, we are applying stretches to the cardiac muscles in control saline and in saline containing the neuropeptide SGRNFLRFamide. This peptide appears to alter the response to stretch. Grant support: NSF IOS-1353023, NIH 8P20GM103423-12, Doherty Foundation/Bowdoin College.

How neurons keep their cool when it gets hot

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All physiological processes are influenced by temperature. This is a particular problem for the nervous system, as temperature changes can disrupt the well-balanced flow of ions across the cell membrane necessary for maintaining nerve cell function. Possessing compensatory mechanisms that counterbalance detrimental temperature effects is thus essential to sustain neuronal function and communication.

Maintaining the coordinated interplay between cell-intrinsic conductances is especially important for central pattern generators (CPGs) that underlie vital rhythmic behaviors such as breathing and chewing because they rely on continuous and coordinated motor activity for adequate functioning. This challenge is two-fold: creating and maintaining coordinated CPG activity, but also ensuring this activity arrives with appropriate timing at the target muscles.

Using electrophysiology, optical imaging, and computational modeling in identified neurons of the crustacean stomatogastric nervous system, we have identified cellular mechanisms of temperature compensation in CPG neuron dendrites and axons. I will demonstrate that extrinsic neuromodulation counterbalances the effects of temperature changes on intrinsic cell properties and thus allows a quick and flexible compensation to temperature-induced modifications of CPG activity. Specifically, a substance-P related peptide transmitter activates a voltage-gated inward current that effectively acts as a negative leak current and compensates for a shunt of membrane potential oscillations in CPG neurons that normally occurs at elevated temperatures.

With respect to axonal propagation, I will discuss intrinsic axon properties and their changes in response to temperature. Specifically, I will focus on axons that are not expected to maintain coordinated spike and rhythm timing due to their distinct morphologies.

Rhythmic behaviour and neural mechanisms for behaviour in insect models: Deciphering the insect locomotion control network

Amir Ayali, School of Zoology, Faculty of Life Sciences, Tel-Aviv University

Motor behavior, specifically locomotion, results from the, far from fully resolved, interplay between endogenous oscillatory central pattern-generating networks (CPGs), descending inputs, and the sensory feedback that shapes the CPGs' rhythmic output. Utilizing two leading insect models, the cockroach and the locust, we monitored rhythmic activity of leg motor neurons, induced by different application procedures of pilocarpine to in-vitro isolated thoracic ganglia. We obtained insights into the leg CPGs' endogenous motor output, as well as into the above mentioned complex interactions.

In the cockroach, we found asymmetries in connectivity among the different thoracic ganglia, each controlling a pair of legs. We also report differences in the role and mode of operation of homologue network units (manifested by levator and depressor nerve activity). Many observed characteristics were similar to those exhibited by intact animals, suggesting a dominant role for feedforward control. Neuromodulation of the endogenous motor patterns by biogenic amines was also explored.

In the locust, limited pharmacological activation revealed different inherent bilateral coupling in the three thoracic ganglia. Each ganglion was capable of inflicting its own inherent pattern onto the entire system. We showed that strict synchrony among ipsilateral CPGs dominate the coupling configuration of the whole network. In contrast, the bilateral connections proved to be highly modulated. The subesophageal ganglion was found to be instrumental in activating and coordinating the leg CPGs.

We posit a connectivity scheme among components of the insect locomotion pattern generating system providing the central basis for adaptation to the environment and to higher motor commands.

The concept of central pattern generation expanded to natural behavioral sequences generally

Ari Berkowitz, Department of Biology, University of Oklahoma

Central pattern generators (CPGs) are central nervous system (CNS) networks that can generate coordinated motor outputs without movement-related sensory inputs. Historically, this concept has been applied almost exclusively to simple, rhythmic movements with essentially identical cycles that repeat continually (e.g., respiration) or episodically (e.g., locomotion). Neuroethologists study a wide range of natural behavioral sequences, many of which are complex and non-cyclical, and some of which involve learning. The concepts and experimental approaches of CPG research have not usually been applied to complex behavioral sequences, but could be. For example, it is often found for episodic CPG-driven rhythms that, once triggered, the rhythm continues for seconds in the absence of ongoing sensory activity. Similarly, fixed action patterns (Lorenz and Tinbergen, 1938), are behavioral sequences that continue for seconds following triggering by an environmental stimulus and are probably controlled by CNS programs. Also, one can demonstrate the existence, location, and components of CPGs by 1) stimulating or silencing CPG elements to alter the timing of subsequent cycles or 2) warming or cooling CPG elements, respectively, to speed up or slow down the rhythm. Similarly, the timing of some non-cyclical behavioral sequences can be altered by stimulation of CNS control elements and/or sped up or slowed down, respectively, by heating or cooling CNS control elements, as shown by such manipulations of songbird HVC nuclei to alter song motif timing. Thus, concepts and experimental approaches of CPG research might also be applied to achieve a better understanding of complex and non-cyclical natural behavioral sequences.

Session 3

All together now – Ultra-precise temporal patterning in a hindbrain motor circuit depends on gap junction mediated feed-forward inhibition

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The successful execution of complex behaviours relies on central timing signals that coordinate muscle activity patterns. This includes social acoustic signalling that is widespread among several vertebrate lineages, including fishes. Using *in vivo* intracellular recordings and pharmacology in a highly vocal fish, we show that ultra-precise, synchronous firing of motoneurons depends on a gap junction mediated, feed-forward glycinergic inhibition that generates a period of reduced probability of motoneuron activation. This mechanism, together with low motoneuronal excitability, and phasic excitatory and tonic inhibitory inputs provides a novel means for achieving extreme synchronicity and temporal precision in motor patterning that, in this case, leads to rapid acoustic modulations in the millisecond range.

Ground plan for evolution of premotor control of vocal patterning

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Can we establish how the vocal-acoustic phenotypes of tetrapods were built over evolutionary time by identifying behavioral and neural characters in sound producing, vocalizing fish? We seek answers to this and related questions in a seasonally breeding group of teleost fish, the highly vocal toadfishes that are highly tractable models for establishing how the brain controls the patterning of vocal behavior. First, they have a small repertoire of calls distinguished by a limited set of acoustic parameters. Second, they have a vocal control system comparable in organization to that of birds and mammals. Third, the output of a hindbrain central pattern generator (CPG) - the vocal nerve motor volley, is readily evoked by brain stimulation and recorded in situ. We combine cellular and genetic tools with single neuron to systems level analyses, and studies of animals behaving under naturalistic conditions. Our findings demonstrate a suite of mechanisms that orchestrate the timing of vocal performances on multiple timescales ranging from milliseconds to hours and time of year, as well as the seasonal enhancement of sender-receiver coupling in this communication system. The results further reveal a foundational system, a ground plan, for neural and hormonal substrates underlying the evolution of vertebrate acoustic communication behaviors. As will be reviewed, hormonal actions in homologous forebrain and midbrain sites that sculpt social context (aggression, reproduction, affiliation) also serve homologous social communication functions, including the premotor patterning of downstream vocal CPGs. Research support from NSF (IOS1656664).

Preparatory neural activity and sensory feedback independent introductory vocalizations drive initiation of a learned motor sequence

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The learned song sequence of an adult male zebra finch is a well-studied example of a learned motor sequence. How such learned motor sequences are initiated in the brain remains poorly understood. Song bouts typically begin with a variable number of short introductory vocalizations (IVs). We have previously shown that the timing and acoustic features of these IVs reaches a consistent state each time song is about to start (Rajan and Doupe, Current Biology, 2013), suggesting the possibility that IVs serve as preparatory vocalizations that help the zebra finch brain reach a "ready" state before song initiation. However, what neural and behavioral mechanisms drive the progression of IVs towards the "ready" state remains unclear. Here, we show that the progression of IVs is independent of sensory feedback but depends on the short-term history of vocalization. Further, neural activity in premotor nucleus HVC begins to change hundreds of milliseconds before the first IV and correlates with successful song initiation. These data suggest that internal changes in neural activity coupled with sensory feedback independent repetition of a simple vocalization drive initiation of the learned song sequence. Further, the presence of neural and behavioral preparation make the zebra finch an attractive model system to study the initiation of ethologically relevant learned motor behaviors.

Neural circuit features that support species-specific vocal patterns in *Xenopus*

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Male frogs in the aquatic sub-genus *Xenopus* produce advertisement calls whose combined temporal and spectral features are species specific. Temporal features are set by a dedicated hindbrain circuit and laryngeal neuromuscular synapses. Using the isolated brain that generates fictive calling (CAPs recorded from the laryngeal nerve) in response to serotonin, we are exploring how vocal patterns are generated across species. Shared temporal patterns in genetically distant species (call convergence) are produced by divergent neural and muscular mechanisms. *X. borealis* males produce click-type calls using a female-like hindbrain circuit, laryngeal synapse and muscles. In the genetically distant species, *X. boumbaensis*, males produce click-type calls by simply reducing the duration of male-typical laryngeal motor neuron activity. Divergent temporal call patterns in related species, are produced by tuning the activity of a hindbrain vocal pattern generator; the parabrachial nucleus (PB*x*). The recently diverged species, *X. laevis* and *X. petersii*, both produce rapid (60Hz) trains of sound pulses: fast trills. Using whole cell patch clamp recordings, we show that a specific PB*x* population (FTNs) that produces a long-lasting depolarization and spikes before each fast trill CAP, exhibits intrinsic, species-specific features that correspond to fast trill period and duration. Divergently evolved differences in premotor neuron membrane properties are strong candidates for generating vocal differences between species.