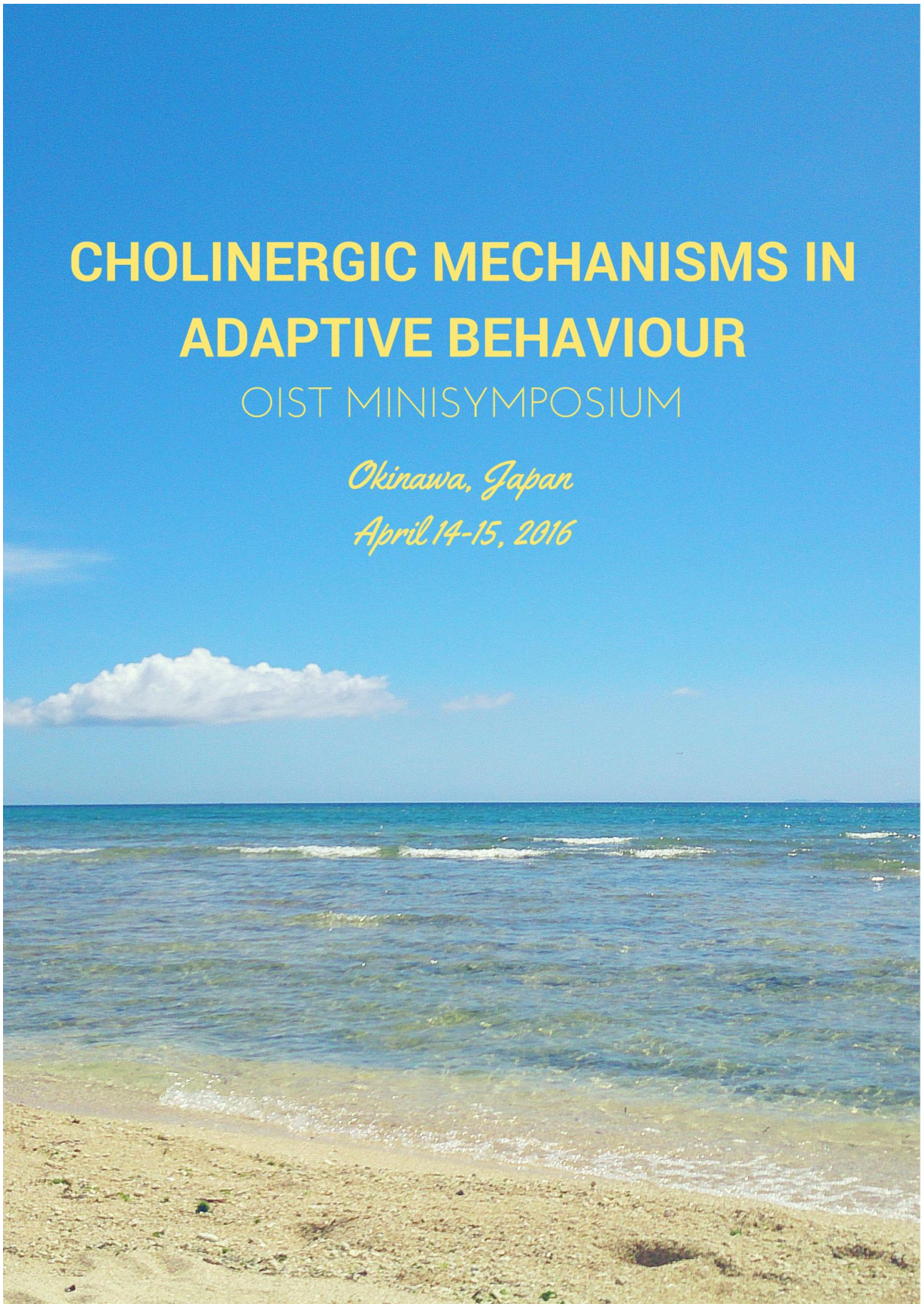
CHOLINERGIC MECHANISMS IN ADAPTIVE BEHAVIOUR
OIST MINISYMPOSIUM
Okinawa, Japan
April 14-15, 2016

Cholinergic Mechanisms in Adaptive Behaviour

OIST Minisymposium
Okinawa, Japan
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Organizers
Jeff Wickens, Aya Zucca, and Stefano Zucca

Aim
The goal of this symposium is to bring together a group of leading researchers investigating the role of acetylcholine within brain circuits responsible for learning and behavior. Acetylcholine has long been known to play a crucial role in adaptive behavior, but the limited access to the cholinergic neurons which release acetylcholine has limited progress. The recent availability of genetic approaches to targeting cholinergic neurons for experimental study, combined with sophisticated electrophysiological, behavioral and imaging approaches, has led to an explosion of new findings. The field is on the cusp of a major advance in understanding the role of acetylcholine at cellular, circuit, and system levels. In bringing this group together we aim to take an important step toward an integrated concept of the contribution of acetylcholine to higher brain function, and the underlying neural mechanisms, by discussing the following issues:

- How to integrate the most recent results into a coherent concept of the role of acetylcholine?
- What causal and correlative evidence supports recent proposals regarding cholinergic function.
- How do cholinergic effects interact with actions of other neuromodulators such as dopamine and serotonin?
- What is the contribution of acetylcholine to overall brain function, focusing on the basal ganglia, and thalamocortical interactions?

Invited participants
Gordon Arbuthnott, Bernard Balleine, Anastasia Christakou, Stephanie Cragg, Kazuto Kobayashi, Yasushi Kobayashi, Angela Langdon, David Lovinger, Genela Morris, Margaret E. Rice, Andrew Sharott, James Surmeier

Website
https://groups.oist.jp/nru/cholinergic-mechanisms-adaptive-behaviour

Day One
Thursday, April 14, 2016
Cholinergic Mechanisms: cellular perspectives on behaviour

Morning
Coffee
9:30-12:00 pm

Welcome from Organizers

James Surmeier (Northwestern University, USA)

Striatal cholinergic interneurons and Parkinson's disease

Andrew Sharott (University of Oxford, UK)

Temporal activity signatures of striatal cholinergic interneurons are conserved across rodents and primates.

Discussion led by Stefano Zucca

Lunch and Campus Visit
12:00- 3:00 pm

Afternoon
3:00 – 7:00 pm

Stephanie Cragg (University of Oxford, UK)

Placing a brake and taking a break: How striatal cholinergic interneurons gate dopamine release and pause their own activity

Margaret Rice (New York University, USA)

Roles of acetylcholine and dopamine in motor and reward behaviors

15-minute coffee break

David Lovinger (NIH-NIAAA, USA)

Dopamine D2 receptors on striatal cholinergic neurons contribute to instrumental learning

Discussion led by Aya Zucca

Day Two
Friday, April 15, 2016
Cholinergic Mechanisms: behaviour perspectives on circuitry

Morning
9:00 - 1:00 pm

Bernard Balleine (University of Sydney, Australia)

Cholinergic modulation and choice between goal-directed actions

Angela Langdon (Princeton, USA)

A role for cholinergic interneurons in gating reward predictions in the ventral striatum

15-minute coffee break

Kazuto Kobayashi (Fukushima Medical University, Japan)

Roles of striatal cholinergic interneurons revealed by genetic cell targeting

Discussion led by Gordon Arbuthnott

Lunch
1:00- 2:00 pm

Afternoon
2:00 – 6:00 pm

Yasushi Kobayashi (Osaka University, Japan)

Cholinergic mechanism of reward prediction error computation for reinforcement learning in the pedunculopontine tegmental nucleus neurons.

Anastasia Christakou (University of Reading, UK)

Structural and neurochemical imaging of the human striatal cholinergic system

15-minute coffee break

Genela Morris (University of Haifa, Israel)

Monitoring activity of putative cholinergic interneurons (CINs) in dorsomedial striatum during learning of a multidimensional set learning task

Discussion led by Jeff Wickens

Closing remarks from Jeff Wickens

James Surmeier

Striatal cholinergic interneurons and Parkinson's disease

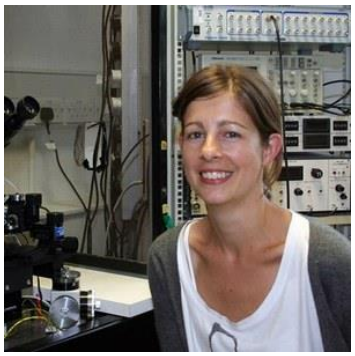
Striatal cholinergic interneurons (ChIs) have long been implicated in the network pathophysiology driving the motor symptoms of Parkinson's disease (PD). In PD models, there is clear evidence of elevated acetylcholine (ACh) release. Although some of the mechanisms underlying this change have been characterized, the role of extra-striatal circuitry in this phenomenon have gone relatively unexplored. Using a combination of anatomical, electrophysiological, optogenetic and two photon imaging approaches, we have found that in PD models the excitatory glutamatergic input to ChIs from the parafascicular nucleus (PFN) is enhanced. This enhancement leads to preferential augmentation of PFN excitation of indirect pathway spiny projection neurons (iSPNs), potentially contributing to the akinetic features of PD. In addition, new data on the influence of ChIs on network pathophysiology in levodopa-induced dyskinesia (LID) will be presented. Particular attention will be given to the role of M4 muscarinic receptors in controlling corticostriatal synaptic plasticity in direct pathway spiny projection neurons (dSPNs) and the ability of positive allosteric modulators of M4 muscarinic receptors to correct aberrant LID plasticity and behavior.

Andrew Sharott

Temporal activity signatures of striatal cholinergic interneurons are conserved across rodents and primates.

Cholinergic interneurons are prime candidates for mediating the effects of excitatory inputs to striatum and have been studied extensively in relation to reinforcement learning through their putative identification as “tonically active neurons” (TANs) in behaving animals. In primates, TANs exhibit multiphasic responses to motivationally salient stimuli that mirror those of midbrain dopamine neurons and together these two systems mediate reward-related learning in basal ganglia circuits. Here we define the range of spontaneous firing patterns displayed by identified cholinergic neurons *in vivo*, using juxtacellular labelling in rats and primates, and compare those firing patterns to other striatal interneuron populations. We demonstrate that cortical afferents evoke temporally distinct, but equally powerful, multiphasic responses as thalamic afferents in cholinergic interneurons. These electrophysiological responses are underlain by synaptic connections between cortical and thalamic afferents with both the proximal and distal dendrites of cholinergic interneurons. Finally, we show that the temporal properties of multiphasic responses to cortical and thalamic stimulation in rats, closely resemble those of TANs to motivationally relevant stimuli in the primate. Our results demonstrate that the intrinsic and extrinsically evoked electrophysiological properties of cholinergic interneurons are conserved across species and likely underlie their functional role in the striatal microcircuit.

Stephanie Cragg



Placing a brake and taking a break: How striatal cholinergic interneurons gate dopamine release and pause their own activity

Striatal cholinergic interneurons (ChIs) are anatomically and physiologically well placed to govern striatal dopamine function. Dopamine axons have high expression of particularly nicotinic receptors, and ChIs and dopamine neurons show coincident changes to their firing rates. ChIs exhibit a characteristic pause response when dopamine neurons burst fire, to signal reward prediction error-related information. I will summarize data from a body of work suggesting that ChIs govern how firing rate in dopamine neurons is translated into the release of dopamine in striatum, placing a brake on dopamine release during bursting high frequencies, until ChIs pause. I will also highlight how synchrony in a small network of ChIs can directly drive dopamine transmission, bypassing activity in dopamine neurons in midbrain, and furthermore, that ChIs can be gatekeeper to how other striatal inputs modulate dopamine. I will also present new data that provide a mechanism for the long-debated question as to how ChIs can generate pauses in their activity. In summary, we show how dynamic activity in ChIs can be generated, and discuss its outcome on dopamine function.

Margaret Rice

Roles of acetylcholine and dopamine in motor and reward behaviors

Striatal dopamine (DA) release is well-recognized as a key factor in motor and reward behavior. Less well appreciated is the influence of acetylcholine (ACh) in these behaviors. Mice lacking ACh synthesis in forebrain neurons, including striatal cholinergic interneurons (ChIs), are hyperactive in a novel environment. This behavior is normalized by a D2 DA receptor antagonist. Conversely, mice lacking ACh synthesis in brainstem, including pedunculopontine tegmental (PPTg) and laterodorsal tegmental (LDTg) nuclei, are hypoactive, and insensitive to D2 antagonism. Evoked DA release in *ex vivo* striatal slices from mice lacking forebrain ACh shows amplified responsiveness to burst-like stimulation frequencies, which is also seen which nicotinic ACh receptors (nAChRs) are blocked. These data implicate both striatal and brainstem cholinergic input in regulating DA-dependent motor behavior. ACh-DA interactions are also central to actions of insulin in the striatum. At physiological concentrations, insulin enhances evoked DA release in striatal slices. This enhancement requires ACh and nicotinic ACh reports (nAChRs), as the effect is lost in *ex vivo* striatal slices from mice with forebrain ACh deletion, or after pharmacological antagonism of nAChRs. Implicating ChIs in this process, insulin ACh increases ChI excitability via insulin receptors on these cells. The amplification of DA release by insulin suggests a role for this neuropeptide as a reward signal, which complements its well-established role as a satiety signal. Indeed, companion behavioral studies show that intact insulin signaling has a key role in flavor and nutrient preference. These data confirm that insulin is a reward signal that influences food choices, and provide new insight into the roles of via ACh and DA in adaptive behavior.

David Lovinger

Dopamine D2 receptors on striatal cholinergic neurons contribute to instrumental learning

Cholinergic interneurons (CINs) provide extensive innervation of other striatal neurons, and regulate their activity through ionotropic nicotinic and metabotopic muscarinic receptors. The CINs and cholinergic receptors have roles in striatal synaptic plasticity, and some behaviors involving this system have been elucidated. However, much remains to be learned about cholinergic influences on striatal physiology and behavior. Cholinergic neuronal function is regulated by dopamine, including prominent inhibitory effects of D2-like receptors on firing and ACh release. To investigate the role in synaptic plasticity and behavior of CIN D2 receptors, we generated cholinergic neuron D2 knockout mice by crossing mice in which Cre recombinase activity is driven by the Choline Acetyltransferase (ChAT) promoter with mice carrying a "floxed" D2 receptor allele (CIND2KOs). These mice are healthy, with no signs of the hypokinesia or lower body weight seen in full D2 knockouts, or the hyperactivity seen in D2 autoreceptor knockouts. In a self-paced, sucrose-rewarded instrumental fixed ratio lever-pressing-sequence task, CIND2KO mice exhibit slower acquisition and earn fewer rewards in comparison to controls. These mice also show lower breakpoints in a progressive ratio lever pressing task, but are comparable to controls in reversal learning, sucrose preference and on the rotarod skill learning task. The pressing sequence deficits are associated with a lower proportion of striatal spiny projection neurons that show lever-press related firing as measured with in vivo extracellular recording. We are also investigating the physiological consequences of CIND2KO in brain slices. Long-term depression of corticostriatal glutamatergic synaptic transmission is lost in the CIND2KO mice. In addition, D2 receptor activation causes a pause in tonic CIN firing, and CINs have been shown to pause their firing during well-learned lever pressing sequences. Thus, we will determine if this pause is lost in the CIND2KO mice. Our findings indicate that inhibitory modulation by D2Rs on CINs impairs cognition, effort and/or motivation necessary for instrumental learning. These behavioral effects may involve changes in D2-mediated firing pauses and striatal synaptic plasticity

Bernard Balleine

Cholinergic modulation and choice between goal-directed actions

For goal-directed action to remain adaptive, new strategies are required to accommodate environmental changes, a process for which cholinergic interneurons in the striatum appear critical. This learning process involves persistent changes in modulatory systems and neuronal networks in both dorsal and ventral striatum, although the neurobiological basis for these sustained effects remains unknown. Here, we describe evidence of a long-term, learning-related increase in δ-opioid receptor translocation to the somatic membrane of cholinergic interneurons specifically in the striatum. This translocation is driven by learning stimulus-outcome and action-outcome contingencies encoded during initial training, and induced firing pattern changes in cholinergic interneurons in ventral and dorsal striatum respectively. In the accumbens shell, we found that δ-opioid receptor translocation not only reflected stimulus-based predictions of reward, but was also essential to drive future stimulus-guided choice between goal-directed actions. Interestingly, similar translocation was observed in cholinergic interneurons in dorsal stratum where other evidence suggests is related to plasticity associated with the acquisition of new goal-directed actions and the interlacing of new and old learning. Our results are consistent with an emerging role for striatal cholinergic activity in decision-making and provide evidence of a long-term, experience-dependent plasticity involved in the cognitive control of action.

Angela Langdon

A role for cholinergic interneurons in gating reward predictions in the ventral striatum

Work in recent years has leveraged the computational framework of temporal-difference reinforcement learning (TDRL) to unveil the neural substrates of reward prediction and learning. Within this framework, the ventral striatum (VS) is often thought to represent reward predictions, and to provide these predictions to midbrain dopamine neurons for the computation and broadcast of reward prediction errors. To test the contribution of the VS to dopaminergic prediction error signals, our collaborators (Yuji Takahashi and Geoff Schoenbaum) recorded activity of putative dopamine neurons in the ventral tegmental area (VTA) of rats with either sham- or neurotoxic lesions of the ipsilateral VS, as they performed an odor-guided choice task in which the timing or size of rewards was manipulated. Firing patterns of these neurons in sham-lesioned animals were consistent with reward prediction error signals. However, neurons in the lesioned animals failed to signal reward prediction errors to changes in reward timing, while prediction errors to changes in reward size were intact. To account for these findings we developed a TDRL model based on a partially observable semi-Markov decision process that explicitly dissociates learning of temporal expectations from learning of expected reward magnitude. We model lesions of the ventral striatum as an inability to learn precise temporal expectations, and show how this critically changes state value estimation and thus reward prediction error signals, mimicking the experimental results. Our model requires that prediction errors be 'gated' and signaled only when cues (or the passage of time) indicate a state transition. We hypothesize that cholinergic interneurons are involved in this gating, by providing temporally precise windows during which reward predictions are expressed by the VS and learning can occur.

Kazuto Kobayashi

Roles of striatal cholinergic interneurons revealed by genetic cell targeting

Cholinergic interneurons in the striatum, known as tonically active neurons, respond to a variety of stimuli related to reward prediction, attention and context recognition during learning processes. Striatal cholinergic interneurons also influence dopamine-dependent motor behaviour and reward-related learning. Acetylcholine efflux in the striatum increases in the phase of behavioural switching. Previous pharmacological studies have suggested the role of striatal acetylcholine in the facilitation of behavioural flexibility. However, we recently found an inhibitory role of striatal cholinergic interneurons in reversal and extinction learning based on spatial discrimination. Selective elimination of striatal interneuronal type in rats was performed with immunotoxin (IT)-mediated cell targeting[30,31]. IT targeting of cholinergic interneurons from the dorsomedial striatum (DMS), but not from the dorsolateral striatum, resulted in enhanced reversal and extinction learning, normally showing the acquisition of place discrimination with a modified T maze. This enhancement was blocked by infusion of a nonselective muscarinic receptor agonist into the DMS. In addition, short hairpin RNA (shRNA)-mediated gene silencing of M_4 muscarinic receptor also promoted the place reversal learning, whereas gene silencing of M_1 receptor did not change the reversal performance. Our data, in contrast to the previous pharmacological evidence, demonstrate that the DMS cholinergic interneurons possess an inhibitory role in behavioural flexibility, predominantly through the M_4 muscarinic receptor. In addition, IT targeting of striatal cholinergic interneurons did not alter another place reversal learning task with a shorter intertrial interval, suggesting that the impact of cell targeting is task-dependent and that striatal cholinergic function may be involved in the task requiring long-term memory.

Yasushi Kobayashi

Cholinergic mechanism of reward prediction error computation for reinforcement learning in the pedunculopontine tegmental nucleus neurons.

The pedunculopontine tegmental nucleus (PPTN) contains both cholinergic and non-cholinergic neurons, and is one of the major sources of cholinergic projections in the brainstem, and is thought to be part of the ascending reticular activating system because of its ascending projections to the thalamus that modulate cortical activation. However, the basal ganglia are highly interconnected with PPTN, and the key function of PPTN is regulation and relay of basal ganglia activity. The functions of PPTN have been implicated in a learning pathway for reward and reward prediction. Thus, PPTN provides an interface for the basal ganglia circuit to influence learning, reward and other cognitive functions. We are addressing the role of neuronal activity in the pathways of the brainstem-midbrain circuit in reward and the basis for believing that this circuit provides advantages over previous reinforcement learning theory. Several lines of evidence support the reward based learning theory proposing that midbrain dopamine (DA) neurons send a teaching signal (the reward prediction error signal) to control synaptic plasticity of the projection area. However, the underling mechanism of where and how the reward prediction error signal is computed still remains unclear. Since the PPTN is one of the strongest excitatory input sources to DA neurons, we hypothesized that the PPTN may play an important role in activating DA neurons and reinforcement learning by relaying necessary signals for reward prediction error computation to DA neurons. To elucidate the functional role of the PPTN in reward-seeking behavior, we recorded single PPTN neurons of monkeys during a reward value-conditioned eye movement task. We found that two distinct groups of neurons signal predicted and actual reward values, both of which are necessary for the computation of reward prediction error as represented by DA neurons.

Anastasia Christakou

Structural and neurochemical imaging of the human striatal cholinergic system

The striatal cholinergic system plays a critical role in learning and behavioural flexibility. Most evidence for its function comes from animal studies, although there is some evidence from human pathology for its involvement in behavioural dysfunction. Functional neuroimaging of the striatum (e.g. functional magnetic resonance imaging (fMRI)), cannot resolve the contribution of cholinergic signaling to striatal function, even at very high resolution. On the other hand, neurochemical imaging (using magnetic resonance spectroscopy, (MRS)), cannot pick up the acetylcholine signature in the derived spectra, but it can be used to measure choline-containing compounds which are involved in the acetylcholine biochemical cascades. In our recent work we show that we can resolve choline itself from other choline-containing compounds using MRS at 3T, suggesting that choline MRS can be used as an in vivo surrogate measure of human acetylcholine function. We demonstrate dissociable contributions of trait choline levels in the dorsal and ventral striatum in learning and behavioural flexibility, and develop a functional neurochemical imaging method for monitoring the function of the system over time. We complement our neurochemical imaging findings with structural neuroimaging of the connectivity of striatal subcompartments with the primary input to the striatal cholinergic interneurons in the thalamus, and demonstrate the impact of white matter integrity of this pathway on individual differences in learning and behavioural flexibility.

Genela Morris

Monitoring activity of putative cholinergic interneurons (CINs) in dorsomedial striatum during learning of a multidimensional set learning task

Humans and animals are confronted with complex inputs from their environment, which demand a single response. Typically, chosen responses are those that yield favorable outcomes or reinforcement. This form of reinforcement learning is commonly attributed to dopamine-mediated modification of cortico-striatal connections. However, this learning crucially depends on adequate input identification. In fact, choices in real-life situations involve identification of cues that are difficult to categorize due to their multidimensional, often multimodal nature. To study the processes by which we learn the relevance of variables in a given task, we trained rats on a multisensory discrimination task, in which either visual, olfactory or spatial stimuli could be relevant to reward collection. The Animals successfully learned the discrimination task within 5-8 days. To examine whether the animals learned a simple stimulus-response association, or a strategy that involved focusing on a specific set of stimuli, we performed intra-dimensional and extra-dimensional shifts in task demand. Intra-dimensional shifts, which require forming new associations involving the same type of stimuli (odor/visual, etc.), were extremely easy to learn. By contrast, acquisition of extra-dimensional shifts (e.g., from olfactory to visual discrimination) was slow, comparable to the initial learning stage. We hypothesize that striatal representations parallel the creation of the learning-set and encode the relevant stimulus set, ignoring other (irrelevant) aspects of the maze configuration, and that activity of CINs aids this representational shift. To test this hypothesis, we recorded single-unit activity in the dorso-medial striatum of rats while they learned a version of the task in which olfactory stimuli were relevant for correct performance, while visual and spatial stimuli were incidental. We followed activity of a number of single identified putative CINs during acquisition and performance of the task. Responses of these neurons seemed to be localized to cue-sampling and reward collection sites. Preliminary results indicated that during initial stages of learning, these responses did not discriminate between different parameters of the relevant and irrelevant stimuli. However, as learning proceeded, responses of CINs differentiated between relevant stimuli but not between irrelevant ones. To study the mechanisms by which CINs acquire these differential responses, we performed in-vitro patch clamp recordings from CINs of trained and untrained rats and compared intrinsic membrane properties. We find that neurons from trained animals differ in a number of parameters that may contribute to acquisition of the typical CIN response. Specifically, medium after-hyperpolarisation (mAHP) seems to increase in the trained rats, and although sag potential is not altered significantly, latency to rebound action potential is significantly reduced.