Processing and Modulation of Sensory Signals: From the Periphery to the Cortex
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Abstracts

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Peripheral gate of somatosensory system

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Healthy peripheral nerves conduct action potentials, triggered by painful and non-painful stimulation, from their peripheral nerve endings to dorsal spinal cord. It is often assumed that the first site where peripheral somatosensory signals are integrated and analyzed is in the dorsal horn, yet, current research suggest that such processing can start earlier. The focus of this talk is at one such potential early site, the dorsal root ganglion (DRG). DRG harbors cell bodies of the pseudo-unipolar peripheral sensory neurons and resides outside of the main nerve conduction pathway. Surprisingly, these DRG-resident cell bodies express multiple receptors for CNS neurotransmitters, such as GABA, even though there is no 'classical' synapses within the ganglia. The possible roles of these somatic receptors and the source(s) of neurotransmitters that activate them are only beginning to emerge. We argue that DRG neuron cell bodies contain fully functional GABAergic system that can modulate nociceptive transmission. Thus, DRG neurons express major proteins necessary for GABA synthesis and release and, indeed can release GABA in response to depolarization. In vivo focal infusion of GABA or GABA re-uptake inhibitor directly to DRG dramatically reduces acute peripherally-induced nociception and alleviated neuropathic and inflammatory pain. In vivo focal application of GABA receptor antagonists to DRG exacerbates peripherally-induced nociception. Computer modeling identified bifurcation of peripheral axon (t-junction) as a likely site of somatic modulation of the nociceptive transmission from the periphery to the spinal cord. Direct in vivo electrophysiological recordings from the dorsal root and the distal spinal nerve (before and after the DRG), show that a GABA-ergic "filter" or "gate" within the DRG is capable of preventing propagation of pro-nociceptive action potentials through the ganglia to the spinal cord. Our findings indicate that peripheral somatosensory ganglia represent an early gate within the peripheral branch of somatosensory system, which can be therapeutically targeted.

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SA02

Differences in the neurotransmission of sensory signals between hair cell types in the mammalian vestibular system

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Balance and gaze rely on the faithful and rapid signalling of head movements by vestibular hair cells (VHCs) to primary sensory neurons. There are two types of VHCs in mammals, type-I and type-II. While type-I VHCs are contacted by a giant afferent nerve terminal, called a calyx, that encloses their basolateral membrane almost completely, type-II cells are innervated by multiple bouton afferent terminals. In both VHC types, glutamate exocytosis is triggered by Ca^{2+} influx through voltage-gated $Ca_V1.3$ Ca^{2+} channels. While signal transmission in type-I and type-II VHCs involves the Ca^{2+}-dependent quantal exocytosis of glutamate at specialised ribbon synapses, type-I cells are also believed to exhibit a non-quantal mechanism that increases the reliability and the speed of signal transmission. However, the reliance of mature type-I hair cells on non-quantal transmission remains unknown.

In this study we investigated synaptic vesicle exocytosis in mature mammalian utricular hair cells using whole cell patch-clamp recording of Ca^{2+} currents and changes in membrane capacitance (ΔC_m). Signal transfer from type-I cells to the calyceal afferent terminal was measured by cell attached recording of action potentials in the calyx in response to VHC depolarisation with an endolymphatic low Ca^{2+} solution.

We found that mature type-II hair cells responded to depolarisation with Ca^{2+}-dependent exocytosis that showed a high-order dependence on Ca^{2+}. By contrast, the Ca^{2+} current in type-I cells was approximately four times smaller and exocytosis was around ten times smaller than that observed in type-II cells. While type-II VHCs showed kinetically distinct pools of synaptic vesicles in response to increased stimulus duration, the responses of type-I cells remained comparatively small with a single pool of vesicles.

In VHCs of $Ca_V1.3$knockout mice ($Ca_V1.3^{-/-}$) both the Ca^{2+} current and exocytosis were largely absent in both type-I and type-II cells. In otoferlin knockout mice ($Otof^{-/-}$) the Ca^{2+} currents were similar to control cells but synaptic vesicle exocytosis was largely absent. Even though Ca^{2+}-dependent exocytosis was small in control type-I hair cells, or absent in $Ca_V1.3^{-/-}$ and $Otof^{-/-}$ mice, these cells were able to drive action potential activity in the postsynaptic calyces, as evident from the increase in calyceal action potential activity in response to VHC depolarisation.

These findings show that mature type-I VHCs show a much smaller Ca^{2+}-dependent exocytosis than type-II cells. The much smaller single vesicle pool in type-I cells supports a functional role for non-quantal synaptic transmission in these cells. The large vesicle pools in type-II cells would facilitate sustained transmission of tonic or low-frequency signals, whereas the restricted vesicle pool size, together with a rapid non-quantal mechanism, in type-I cells could specialise these large calyceal synapses for high-frequency phasic signal transmission.

SA03

Top-down modulation of the retinal code via histaminergic neurons in the hypothalamus

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The mammalian retina is considered an autonomous neuronal tissue, yet there is evidence that it receives inputs from the brain in the form of retinopetal axons. A sub-population of these axons was suggested to belong to histaminergic neurons located in the tuberomammillary nucleus (TMN) of the hypothalamus. We identified these retinopetal axons and found that although few in number, they extensively branch to cover a large portion of the retina. Using Ca2+ imaging and electrophysiology, we revealed how histamine application, as well as a direct activation of the retinopetal axons, modulate activity of retinal ganglion cells. Finally, we found that in humans, an antihistamine non-uniformly modulates visual sensitivity across the visual field. Since TMN activity was shown to correlate with arousal state, our data suggest that the retinal code changes with behavioral state through the release of histamine from TMN neurons.

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SA05

Detecting silence: Role of sound-offsets in auditory processing

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Natural acoustic scenes are composed of many sounds and it is behaviorally important to identify conspecific (and allospecific) communication signals within those scenes. The auditory system divides such a scene into streams corresponding to either the physical source of the sound or its temporal pattern, such as melodies or rhythms. This process of segmentation is a fundamental step in auditory scene analysis and, from a computational point of view, resembles the extensive image segmentation used in Computer Vision. While the main task in image segmentation is to find edges of visual objects caused by sudden changes in, for example luminance; in the auditory system, onsets and offsets of sounds form temporal edges that stand out against an existing background level of neuronal firing. Brain mechanisms for perception of sound onsets have been extensively investigated over the past decades, but surprisingly, mechanisms for perception of sound offsets have not (Kopp-Scheinpflug et al., 2018). In our study we aim to understand how sound-offset responses can be generated in the brain of mice, and which neuromodulatory mechanisms are in place to adapt the offset responses to changes in the acoustic environment.

There is increasing evidence for the importance of sound-offset responses throughout the auditory pathway, but a circuit mechanism of generating sound-offset responses from scratch, has so far only been described for neurons of the superior paraolivary nucleus (SPN) in the auditory brainstem. Although offset responses are reliably encoded by SPN neurons, we found a discrepancy between the

percentages of SPN neurons with pure offset responses between current-clamp recordings in brain slices (87.5 ±1.5%, n= 189 neurons) and single-unit recordings in vivo (52 ±12%, n=35 neurons). While the pure offset responses can be generated from hyperpolarizing inhibition alone (Kopp-Scheinpflug et al. 2011), much of the difference between in vitro and in vivo was due to the additional influence of excitation and the occurrence of more complex firing patterns, consisting of on-off rather than off-only responses (Rajaram et al., 2019). Our data suggest that the delicate balance between excitatory and inhibitory inputs is susceptible to noise exposure and the related increase in nitric oxide (Coomber et al., 2015). Increased nitric oxide depolarized chloride reversal potentials from −83.7 ± 5.4mV to −67.3 ± 4.5mV (n = 10, p = 0.002), thus reducing the driving force for inhibition, which is a major prerequisite for generating strong offset responses. This decline in offset responses was associated with a decrease in the ability to detect short gaps in ongoing signals (Yassin et al., 2014). The activation of excitatory inputs, on the other hand, resulted in accelerated offset-response latencies from 8.84 ms (6.38/16.75 ms, n=9) to 5.55 ms (3.80/6.97 ms, n=8; p=0.018; Rajaram et al., 2019).

Taken together, we predict that changes in the acoustic environment such as noise pollution, can alter the excitation-inhibition-balance right at the start of the sound offset pathway. This may then reduce the ability to encode temporal edges and mask temporal patterns underlying vocal communication.

Reference 1 :- **Kopp-Scheinpflug, C., Sinclair, J. L. & Linden, J. F. When Sound Stops: Offset Responses in the Auditory System. *Trends Neurosci* 41, 712-728, doi:10.1016/j.tins.2018.08.009 (2018).**

Reference 2 :- **Kopp-Scheinpflug, C. *et al.* The sound of silence: ionic mechanisms encoding sound termination. *Neuron* 71, 911-925, doi:10.1016/j.neuron.2011.06.028 (2011).**

Reference 3 :- **Rajaram, E. *et al.* Slow NMDA-Mediated Excitation Accelerates Offset-Response Latencies Generated via a Post-Inhibitory Rebound Mechanism. *eNeuro* 6, doi:10.1523/ENEURO.0106-19.2019 (2019).**

Reference 4 :- Coomber, B., Kowalkowski, V. L., Berger, J. I., Palmer, A. R. & Wallace, M. N. Modulating central gain in tinnitus: changes in nitric oxide synthase in the ventral cochlear nucleus. *Front Neurol* 6, 53, doi:10.3389/fneur.2015.00053 (2015).

Reference 5 :- Yassin, L. *et al.* Nitric oxide signaling modulates synaptic inhibition in the superior paraolivary nucleus (SPN) via cGMP-dependent suppression of KCC2. *Front Neural Circuits* **8**, 65, doi:10.3389/fncir.2014.00065 (2014).

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SA06

Hearing in an acoustically complex world

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For animals to thrive in their natural environments, their sensory systems must form representations of complex objects that are invariant to changes in some physical dimensions. For example, we can recognize a friend's voice in a forest, a small office, and a cathedral, even though the acoustics of the sound reaching our ears will be very different in these 3 settings. I will discuss how neurons in auditory cortex can form stable representations of sounds in our acoustically varied world. Our 2-photon calcium imaging experiments in ferrets have highlighted the complexity of frequency receptive fields in auditory cortex, and how these are arranged along the tonotopic map. Furthermore, we have used statistical modelling and Neuropixels recordings to better understand how the brain recognizes sounds across rooms with different levels of reverberation. Our results demonstrate that the neural "de-reverberation" process is dynamic and adaptive, even in anaesthetized ferrets. Together, these frequency integration and adaptation processes allow neurons in auditory cortex to effectively represent our complex and dynamic acoustic environments. Further studies are needed to dissect these processes at the level of neural circuits.

Reference 1 :- Gaucher*, Paniello*, Ivanov, Dahmen, King & Walker (2020) Complexity of frequency receptive fields predicts tonotopic variability across species. eLife 9:e53462. https://elifesciences.org/articles/53462

Reference 2 :- Ivanov, King, Willmore*, Walker* & Harper* (in revision) Cortical adaptation to sound reverberation. https://www.biorxiv.org/content/10.1101/2021.10.28.466271v1.full

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SA07

Neural Processing of Tactile Information in the Awake Behaving State: Sensory Adaptation during Active Sensation

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The purpose of sensory systems is to drive behaviour. Yet the bulk of our knowledge of sensory systems comes from experiments on anaesthetised animals where the motor systems are disengaged. In the past, technical limitations made it difficult to study this topic in behaving animals but methodological advances are changing this. The broad aim of our research is to investigate the

neural basis of sensation in the behaving brain. In this talk, I will focus on Sensory Adaptation (SA) - exemplified by the phenomenon that the response of neurons to repeated sensory stimuli of fixed strength decreases over time. SA has classically been regarded as a fundamental aspect of how neurons respond to sensory signals. However, our knowledge of it comes almost entirely from experiments on anaesthetised animals and it is controversial whether or not SA has much influence on neurons under awake, behaving conditions. We tested whether SA occurs in awake, behaving mice in a new way, by combining in vivo electrophysiology from behaving mice with multi-camera imaging of the whiskers and machine vision. Our findings show that SA does occur in the whisker system of behaving mice and support the view that SA is indeed a fundamental aspect of sensation.

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SA08

Representation of depth from motion parallax in mouse primary visual cortex

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To guide behavior, the brain must infer the structure of the external world based on incomplete and unreliable sensory inputs. In the visual system, the signals available to the brain are limited to the two-dimensional images formed on the retinae. To reconstruct the three-dimensional location of objects in the environment, visual circuits must infer the missing depth information. Motion parallax resulting from animals' movements provides an essential and poorly understood cue of depth. I will present evidence that neurons in the mouse primary visual cortex (V1) are tuned for depth from motion parallax. Using two-photon calcium imaging in virtual reality, we show that V1 neurons integrate signals related to optic flow and the animals' locomotion to give rise to depth selective responses. These results suggest that the widespread modulation of visual responses in V1 by locomotion may function in estimation of depth of visual cues.

SA09

Putting sounds in context: input-specific gain modulation in auditory thalamus and cortex

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Perceptual salience of sounds depends on the acoustic context in which they appear. Neural correlates of contextual sensitivity can be investigated efficiently by analysing the responses of neurons to complex sounds using nonlinear-linear "context models" (Williamson et al. 2016 *Neuron*). Context models capture the spectrotemporal sensitivity of a neuron in a principal receptive field (PRF) and its sensitivity to local acoustic context in a contextual gain field (CGF). The PRF is analogous

to a classical receptive field estimate; it describes how the firing rate of a neuron depends on particular features of the input stimulus (e.g., tone frequency and time of tone occurrence). The CGF, in contrast, describes how the *gain* of the neuron's response to a particular input depends on the context (e.g., on other sounds that occurred recently or simultaneously). Thus, this input-specific gain modulation captured in the CGF is essentially a neuronal measure of contextual sensitivity.

In this talk I will summarize both published and recent unpublished results from analysis of cortical and thalamic responses to complex sounds using context models. Previous work revealed strong input-specific gain modulation in the auditory cortex and thalamus of anaesthetized mice. CGF structure varied from neuron to neuron, but generally featured suppression of response gain by preceding sounds at similar frequencies (nonlinear forward suppression) and enhancement of response gain by simultaneous sounds at different frequencies (nonlinear off-frequency facilitation). In more recent work, we have investigated the stability of CGF structure in awake animals, using spike waveform-matching techniques to track single neurons across multiple days of recording. We find that in awake as well as in anaesthetised mice, CGFs are robust features of neuronal responses to complex sounds. Notably, CGF structure in individual neurons remains remarkably consistent across many days of recording. We conclude that contextual sensitivity is an essential and stable feature of the neural code in auditory cortex of awake animals.

Reference 1 :- Williamson RS, Ahrens MB, Linden JF* and Sahani M* (2016). Input-specific gain modulation by local sensory context shapes cortical and thalamic responses to complex sounds. *Neuron* 91:467-481. *joint corresponding authors

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SA10

Sensory and non-sensory responses induced by sequence learning in the mouse somatosensory and posterior parietal cortex

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Real-world signals such as communication sequences unfold over time with a characteristic temporal structure. Recognizing temporally ordered patterns is key to survival. To explore how cortical neuronal activity underpins this capacity, we recently developed a task in which head-fixed mice distinguish between tactile 'word' sequences constructed from distinct vibrations delivered to the whiskers, assembled in different orders. Animals lick to report the presence of the target sequence (GO/NOGO design). Mice can respond to the earliest possible cues allowing discrimination of the GO stimulus, effectively solving the task as a 'detection of change' problem, but perform better when responding later, after more evidence could be collected.

We recorded and manipulated cortical activity with two-photon imaging and optogenetics while head-fixed mice performed the task. We expected that learning the task would induce cortical neurons to refine their sensory tuning by becoming more selective to the target GO sequence. Instead, two-photon imaging showed that, upon learning, neurons in both the primary somatosensory barrel cortex (S1bf) and posterior parietal cortex (PPC) became sensitive to multiple task variables, including sensory input but also the animal's action decision (goal-directed licking) and the trial's outcome (presence or absence of a predicted reward). Optogenetic inactivation showed that while S1bf was necessary for sequence discrimination, PPC was not. Moreover, classifiers trained on the activity of S1bf neurons robustly discriminated both the sensory type of a trial (GO/NOGO) and whether the animal licked on the trial, while those trained on PPC neurons could discriminate sensory information but not the animal's actions.

Our results demonstrate that conditioning on a goal-directed sensory discrimination task results in neurons within S1bf whose activity reflects the learnt links between target stimulus and licking. They also show that PPC contains copies of task-relevant information even while playing no causal role in the animal's performance.

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SA11

Brainwide mapping of collicular circuits mediating defensive behaviours

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Modifying the feedforward transmission of information is an important mechanism by which the nervous system filters sensory inputs to preference one behaviour over another. The superior colliculus mediates visually guided behaviours via cell-type specific projections. However, how the retinal and brain-wide inputs to the superior colliculus are organized to influence visual processing and behaviour remains poorly described. Here I will discuss our labs work using large scale anatomical and functional circuit tracing techniques to dissect circuit motifs within the superior colliculus that enable the fast, reliable, yet flexible triggering of innate defensive behaviours in response to visual treats.

SA12

Kinetic features dictate sensory motor alignment in the superior colliculus

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The establishment of a meaningful alignment between sensory and motor maps is a prerequisite for the execution of all animal behaviours. A prime candidate brain region for implementing this sensory-motor transformation is the superior colliculus (SC). However, the extent to which motor and sensory representations converge in individual collicular motor neurons and what is the logic governing the presumed sensory-motor alignment remains unknown.

In order to understand the nature and logic of sensory-motor integration, in vivo intracellular and extracellular recordings were performed across the SC, in restrained and unrestrained conditions, to assess both the motor and the sensory tuning of individual neurons. Whilst traditional models of sensory-motor alignment have centred on the mapping between static spatial features, such as stimulus location and movement endpoints, we show that collicular motor units respond primarily to kinetic visual features, and reveal the existence of an alignment in vectorial space between the encoded sensory flow and movement vectors rather than between visual receptive fields and movement endpoints as previously hypothesised. Such an alignment is ideally placed to drive rapid interception of incoming targets, we show that a neural network built on these connectivity premises is able to support key aspect of SC functionality, such as visual grasping and tracking.

Overall these findings reveal a novel dimension of the sensory motor alignment. By extending the concept of spatial-motor alignment from the static to the kinetic domain this work provides a novel conceptual framework to understand the origin of sensory-motor convergence and its relevance in guiding sensory-driven goal-directed behaviour.

SA13

How does auditory cortex construct auditory space?

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Unlike vision or touch the position of a sound source is not represented at the cochlea and instead must be computed from sound localisation cues. These cues include differences in the timing and intensity between the two ears, and monaural spectral cues that result from the direction-dependent interaction of sound with the pinna. Auditory cortex is required for accurate sound localisation behaviour, yet sound localisation cues are extracted by dedicated centres in the brainstem. What role does auditory cortex play in representing sounds in space? In this talk I will review evidence from our lab that auditory cortex constructs cue-invariant representations of spatial position consistent

with a representation of perceived location. I will also consider the coordinate frame in which sounds are represented, sharing our findings from freely moving ferrets in which head-centered and world-centered reference frames could be dissociated. This approach allowed us to determine that while many cells represent sound position relative to the head, some cells represent sound location in a head-pose invariant manner, thus responding selectively to a position in the world. Finally I will share some behavioural data demonstrating that ferrets, like humans, can localise sounds in both head and world-centered reference frames.

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SA14

Perception and encoding of temporally fluctuating odour stimulus in mice

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Turbulent airflow imposes strong intensity fluctuations onto natural odour plumes. It has been hypothesised that such fluctuations could carry information about odour source composition or distance. We have recently shown that mice can indeed extract information from odour concentration fluctuations of a bandwidth up to at least 40 Hz. However, it is largely unknown how neurons in the olfactory system can encode temporal features of the odour stimulus.

To understand how neurons in the mouse olfactory bulb (OB) encode temporal features of odour stimuli, we performed *in-vivo* extracellular and patch clamp recordings while administering precisely controlled temporally fluctuating odours at multiple frequencies between 2 Hz and 20Hz. We observed that, despite the heterogeneous activity profiles, a substantial fraction of OB neurons demonstrated frequency coupling in their sub-threshold domain for 2 Hz (29/70) and 20Hz (24/70) odour stimuli. Furthermore, mitral and tufted cells showed differential coupling of their membrane potential to odour concentration fluctuations, with tufted cells showing stronger coupling for the 20Hz stimuli compared to the mitral cells. Frequency coupling was largely independent of odour quality. Upon administering odour mixtures, we observed that neurons that coupled well to the mixtures also coupled well to at least one of the individual components of the mixture. Interestingly, pharmacological blocking of the inhibitory circuitry strongly modulated frequency coupling of cell-odour pairs at both 2Hz and 20Hz.

Overall, we conclude that the mouse olfactory system has the capacity to perceive rapid changes in odour concentrations, and this challenges the typical thinking of slow sensory processing in the olfactory system. Further, projection neurons in the mouse olfactory bulb can encode aspects of the temporal structure of the odour stimulus.

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SA15

Retinal and brain circuits transmitting light intensity signals and regulating mood

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Invironmental light intensity affects the nervous system and is a powerful modulator of behavior. Light-intensity-dependent activity is observed in a subset of retinal output cells, which innervate a newly discovered nucleus of the dorsal thalamus, that in turn projects to the prefrontal cortex and striatum. Silencing the transmission along this pathway has been shown to affect mood. I will describe the retinal networks responsible for the transmission of light intensity signals, and show new results demonstrating the capacity for light-intensity encoding in diverse brain regions.

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SA16

Vestibular processing during natural self-motion: implications for perception and action

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A fundamental question in neuroscience is: How does the brain compute accurate estimates of our self-motion relative to the world in everyday life. In this talk, I will describe recent findings from my laboratory that have addressed this question and provided new insights into the processing of vestibular self-motion information to ensure stable perception and accurate motor control.

First, we have recently examined the statistics of the natural self-motion signals experienced by mice, monkeys, and humans, and then explored the neural coding strategies used by early vestibular pathways. We then found that vestibular afferents identically encode this self-motion across passive and active conditions. In contrast, the central vestibular neurons directly targeted by afferents are

markedly less sensitive to active motion. This ability to distinguish between active and passive motion is not a general feature of early vestibular processing, but rather is a characteristic of a distinct group of central vestibular neurons known to contribute to postural control and perception. To make the required distinction between passive and active stimuli, we have further shown that the cerebellum builds a dynamic prediction (e.g., internal model) of the sensory consequences of self-motion during active behaviors. Notably, when unexpected vestibular inputs become persistent during active motion, this cerebellum-based mechanism is rapidly updated to re-enable the vital distinction between active and passive vestibular inputs. Finally, we have demonstrated that ascending thalamocortical vestibular pathway even more selectively encode unexpected motion, thereby providing a neural correlate for ensuring perceptual stability during active versus externally generated motion.

Taken together, these findings have important implications for our understanding of the brain mechanisms that ensure stable perception and accurate behaviour as we move through and explore our world.

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SA17

Action, expectation, and error in mouse auditory cortex

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Many of the sensations experienced by an organism are caused by their own actions, and accurately anticipating both the sensory features and timing of self-generated stimuli is crucial to a variety of behaviors. In the auditory cortex, neural responses to self-generated sounds exhibit frequency-specific suppression, suggesting that movement-based predictions may be implemented early in sensory processing. Yet it remains unknown whether this modulation results from a behaviorally specific and temporally precise prediction, nor is it known whether corresponding expectation signals are present locally in the auditory cortex. To address these questions, we trained mice to expect the precisely timed acoustic outcome of a forelimb movement using a closed-loop sound-generating lever. Dense neuronal recordings in the auditory cortex revealed suppression of responses to self-generated sounds that was specific to the expected acoustic features, specific to a precise time within the movement, and specific to the movement that was coupled to sound during training. Predictive suppression was concentrated in L2/3 and L5, where deviations from expectation also recruited a population of prediction-error neurons that was otherwise unresponsive. Recording in the absence of sound revealed abundant movement signals in deep layers that were biased toward neurons tuned to the expected sound, as well as temporal expectation signals that were present throughout the cortex and peaked at the time of expected auditory feedback. Together, these findings reveal that predictive processing in the mouse auditory cortex is consistent with a learned

internal model linking a specific action to its temporally precise acoustic outcome, while identifying distinct populations of neurons that anticipate expected stimuli and differentially process expected versus unexpected outcomes.

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SA18

Dynamic stimulus representation in somatosensory cortex

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Sensory experience and perceptual learning alter receptive field properties of cortical pyramidal neurons, possibly mediated by long-term potentiation (LTP) of synapses. I will highlight work showing in mice that higher-order (HO) thalamic feedback to the somatosensory cortex (S1) is an important facilitator of sensory- evoked synaptic plasticity in L2/3 pyramidal neurons (PNs). HO thalamic feedback gates plasticity by promoting non- linear dendritic responses, and through interactions with a VIP-interneuron disinhibitory microcircuit motif. Its input increases upon partial sensory deprivation, and might be implicated in dynamic sensory stimulus representations in a perceptual learning paradigm. To start characterizing a possible relationship between higher-order feedback and cortical plasticity during perceptual learning, we monitor the activity of HO thalamic axons, VIP interneurons and L2/3 PNs in S1 during reward-based texture discrimination. By tracking activity patterns before and after changing reward contingencies, we find that stimulus selectivity of HO thalamic axons, L2/3 PNs and VIP interneurons is dynamic and largely relies on behavioral contingencies. A subpopulation of the neurons forecast the onset of learning by displaying a distinct and transient increase in activity, depending on past behavioral experience.

SA19

Modulation of the first stages of visual processing by behavioural states

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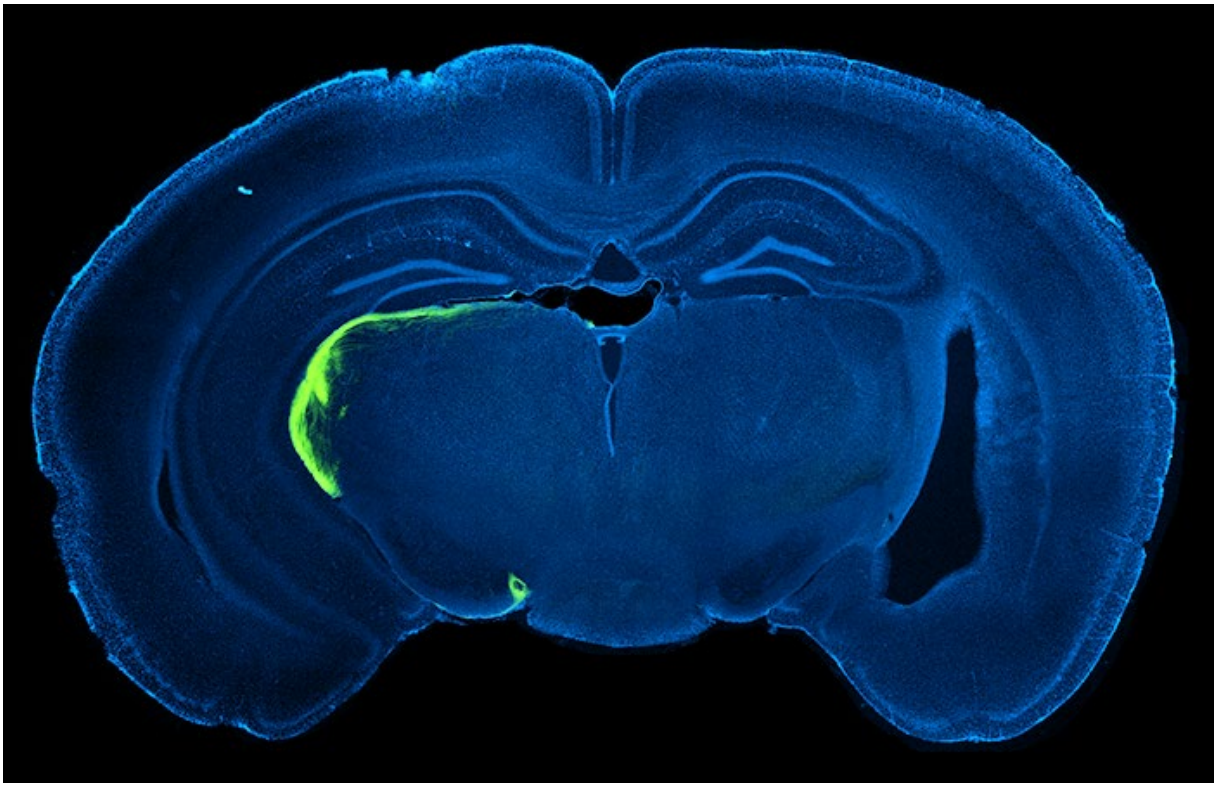
A prominent idea about the organisation of the brain is that different brain areas perform specific, largely non-overlapping functions, such as sensation, decision-making, or motor control. An increasing number of studies shows however that responses from single neurons or brain areas exhibit a mixture of functionality, e.g. neurons in primary visual cortex integrate visual information with information about locomotion speed and the subject‘s level of arousal. This integration of sensory information with information about behaviour and internal state may improve the processing of sensory input by adapting the brain to specific demands and contexts.

Behavioural modulation in the visual system has mostly been studied in cortical areas. Here I show that it is evident at even earlier stages of visual processing: in the superior colliculus, which receives the majority of retinal projections, and even in the output of the retina. We have recorded responses of large populations of neurons in the superior colliculus and of retinal axons projecting to the superior colliculus in mice that were free to run on a treadmill. Using these methods, we found that tectal neurons and their retinal inputs are modulated by running and pupil-linked arousal. To test whether further behavioural variables affect neural activity in the superior colliculus, we have trained mice to perform a visual detection task, where they were rewarded for corrected responses. We found that reward increases neural responses to successive visual stimuli and that this reward effect is independent from effects of pupil-linked arousal.

These results show that several behavioural variables impact visual processing early on in the processing hierarchy. Questions we still need to answer are: what is the purpose of this modulation and how is it achieved?



Acknowledgements :- This research was funded by the Wellcome Trust and The Royal Society (Sir Henry Dale Fellowship 220169/Z/20/Z), the BBSRC (Research Grant BB/P003273/1), and the European Commission (MCF 623872).

SA20

Olfaction and Satiety

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A feedback loop exists between the digestive and olfactory systems: Disruption of olfactory input provides protection form diet induced obesity. Conversely, the olfactory system is sensitive to metabolic signals such as insulin which, when applied exogenously, can reduce olfactory sensitivity. We have been investigating how such satiety signals modulate function within the olfactory bulb and demonstrate a key role for inhibitory periglomerular neurons using in vivo and in vitro imaging and electrophysiology. Periglomerular neurons express insulin receptors and, in a subset, insulin inhibits a voltage-gated potassium current normally active around resting membrane potentials. How this metabolic sensitivity of periglomerular neurons impacts upon the function of the olfactory bulb will be addressed.

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C01

Reward modulates visual responses in mouse superior colliculus independently of arousal

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The superior colliculus (SC) is a major recipient of visual input in the mouse and controls innate approach and avoidance behaviours. Neurons in the superficial SC (sSC), which receives direct input from the retina, do not only process visual stimuli, but are also modulated by the animal's running speed and pupil-linked arousal, like modulations observed in the primary visual cortex. While these observations were made during passive viewing of visual stimuli, a major purpose of vision is to guide behaviour. We therefore asked whether, in the context of a behavioural task, visual activity in the sSC is modulated by states or variables other than pupil-linked arousal.

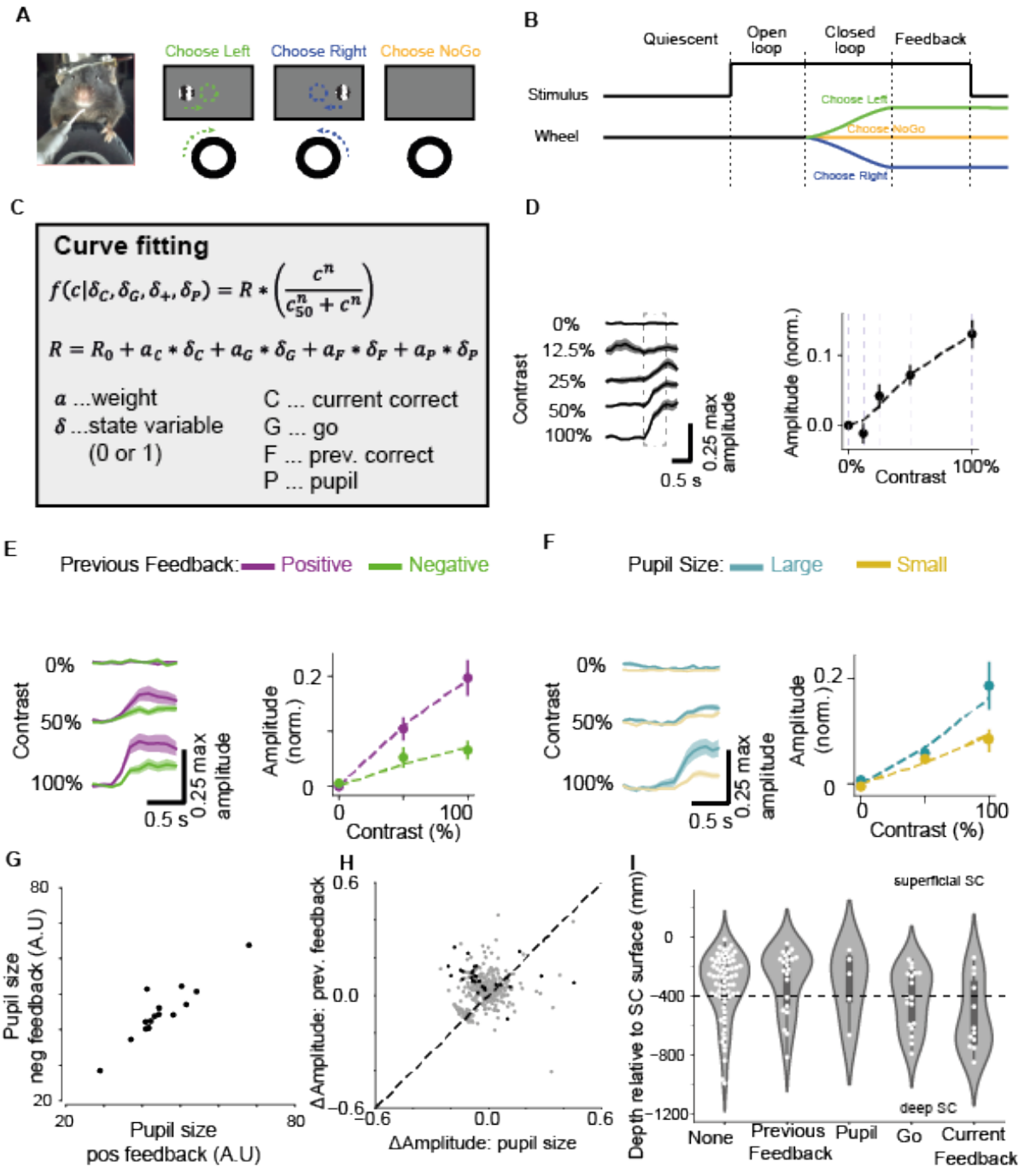
We trained mice to perform a visual detection task, where they needed to detect a stimulus of varying contrast in the left or right visual field and then interactively move the stimulus towards the centre of the visual field (Panel A). If no stimulus was presented, the mice had to refrain from moving the stimulus. Each trial was divided into distinct periods: a quiescent period with no stimulus, an open loop period, in which the mouse should hold the wheel steady, and a closed loop period, in which the mouse can move the stimulus. These were followed by positive or negative feedback (Panel B). We then imaged the sSC of 4 well-trained mice over 16 sessions, using two-photon calcium imaging.

We quantified the contribution of various task events to the neuronal visual response by their relative contribution to the gain of a hyperbolic division function ($p<0.05$, shuffle test; Panels C,D). We discovered a population of neurons that were positively modulated by previous reward (Panel E). Similarly to previous studies, we also found that pupil-linked arousal modulated the visual responses of sSC neurons, either positively or negatively (Panel F).

Our evidence shows that these two factors, pupil and feedback modulation, are independent. First, the pupil size during stimulus presentation does not change between trials following positive or negative feedback ($p=0.76$, t-test; Panel G). Second, the modulation of the gain by the two factors is independent ($p<0.001$;t-test).Panel H).

Lastly, using Neuropixels, we validated our results by recording from the entire depth of the SC. These experiments revealed that the majority of reward modulated neurons were found in the sSC rather than in deeper layers of the SC (Panel I).

Our findings show that visual responses of sSC neurons are strongly influenced by two independent state variables: pupil-linked arousal and previous reward. Future studies may reveal how these non-visual modulations help downstream processes in guiding behaviour.


A
Choose Left
Choose Right
Choose NoGo
B
Quiescent
Open loop
Closed loop
Feedback
Stimulus
Wheel
Choose Left
Choose NoGo
Choose Right
C
Curve fitting
$f(c|\delta_C, \delta_G, \delta_+, \delta_P) = R * \left(\frac{c^n}{c_{50}^n + c^n}\right)$
$R = R_0 + a_C * \delta_C + a_G * \delta_G + a_F * \delta_F + a_P * \delta_P$
a ...weight
δ ...state variable (0 or 1)
C ... current correct
G ... go
F ... prev. correct
P ... pupil
D
Contrast
0%
12.5%
25%
50%
100%
0.25 max amplitude
0.5 s
Amplitude (norm.)
0.1
0.0
0%
Contrast
100%
E
Previous Feedback: Positive Negative
Contrast
0%
50%
100%
0.25 max amplitude
0.5 s
Amplitude (norm.)
0.2
0
0
100
Contrast (%)
F
Pupil Size: Large Small
Contrast
0%
50%
100%
0.25 max amplitude
0.5 s
Amplitude (norm.)
0.2
0
0
100
Contrast (%)
G
Pupil size neg feedback (A.U)
80
20
20
80
Pupil size pos feedback (A.U)
H
ΔAmplitude: prev. feedback
0.6
0.0
−0.6
−0.6
0.0
0.6
ΔAmplitude: pupil size
I
Depth relative to SC surface (mm)
superficial SC
0
−400
−800
−1200
deep SC
None
Previous Feedback
Pupil
Go
Current Feedback

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C02

Early-life pain experience alters pain related cortical activity in adulthood

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Problem statement: Exposure to pain and injury in early-life (early-life pain, ELP) is associated with altered pain behaviour in adults. However, it is not known whether ELP has any effect upon adult cortical pain networks or upon functional connectivity between the key cortical regions involved in the sensory and emotional dimensions of pain. Here we recorded pain related neural activity in the adult rat somatosensory cortex (S1, sensory-discriminative) and the medial prefrontal cortex (mPFC, associative-affective) in a model of early-life pain. We hypothesized that early-life pain experiences in critical periods alter adult functional pain connectivity in the rat S1 and mPFC.

Methods: Ongoing wireless electrophysiological recording at S1 and mPFC was performed in two groups of awake adult male Sprague-Dawley rats before and up to 10 days after the hindpaw skin incision. One group had received the same skin incision in the first week of life (II, n=8) and the other did not (NI, n=8). The same recordings were conducted in a control group of animals which did not receive any incision (NN, n =8). Pain sensitivity (Paw withdraw threshold, PWT) was also measured using electronic von Frey stimulation on the hindpaw while recording local field potentials in S1 and mPFC.

Results: Rats who had a neonatal skin incision (II) had a lower paw withdrawal threshold to mechanical stimulation following adult skin injury compared to NI (GLM; type III $\chi^2(2) = 349.86$, $p < 0.0001$). This increase in hypersensitivity was directly correlated with an increase in sensory evoked (von Frey stimulation) delta and gamma energy in S1 and delta-gamma frequency coupling in the same cortical area, which were more pronounced in II than NI rats. In addition, skin injury elicited an increase in S1-mPFC connectivity which was significantly prolonged in II rats, extending for 4 days post injury (1 way ANOVA, Day of incision: $F_{(2, 28)} = 11.75$, $p= 0.0002$; 4 days following incision: $F_{(2, 28)} = 3.84$, $p=0.03$) and also correlated to behavioural pain hypersensitivity.

Conclusion: Painful sensory experiences in early life have a significant effect on both behavioural pain sensitivity and the functional connectivity of pain-related cortical circuits. Our study elucidates an important link between persistent hypersensitivity and both local and long-range neural circuit alteration following hind-paw skin injury in adult rats, underlying the impact of early-life pain experience. The results provide novel insights into the role of S1 and mPFC in the regulation of pain sensation and affective pain.

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C03

Unravelling the role of calcium-permeable channel TRPA1 in human channelopathies and pain disorders

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Background

While voltage-gated Na^+ and Ca^{2+} human channelopathies have been extensively studied, the role of transient receptor potential (TRP) channels in human Mendelian pain disorders is less established. TRP channels are implicated in several aspects of sensation [*1*] and knockout animal models suggest that TRP channels also mediates pain perception and sensitivity [*2*]. The only human TRPA1-channelopathy so far described is associated with the gain-of-function N855S mutation, causing an autosomal dominant familial episodic pain syndrome [*3*]. We identified a novel TRPA1 variant (A172V) in a patient/study participant with an episodic somatic pain disorder. The whole study involved a cohort of 220 patients with a clinical history of neuropathic pain disorders, who underwent whole genome sequencing as part of the NIHR-Bioresource. *In silico* analysis have predicted that the A172V variant is likely to have a significant impact on the protein function, highlighting it as a promising candidate for functional studies.

Methods

In silico prediction analysis were employed to evaluate the variant position, the minor allele frequency and the concordant pathogenicity, via multiple *in silico* algorithms such as SIFT, polyphen and Align GDVD. *In vitro* functional studies involved the electrophysiological characterisation of the hTRPA1 A172V variant.

Results

The novel A172V mutation is located in the ankyrin repeat domain region of TRPA1, which appears important for the channel activation [*4*]. Our electrophysiological data have shown that heterologous over-expression of the A172V variant results in a gain of function, in response to TRPA1 agonists. In the presence of 25 µM AITC, A172V showed a pronounced linearisation of the current-voltage relationship, with a steeper activation curve observed at positive potentials compared to WT (+100mV, WT = 21.12 ± 2.46pA/pF n=13, A172V= 56.92 ± 12.52pA/pF n=9, $p<0.005$). Currents were significantly increased at positive potentials, and analysis of tail currents demonstrated a significant leftward shift of voltage dependence of channel activation ($V_{1/2}$ = 35.55 ± 5.45mV, and 59.16 ± 5.25mV, $p<0.05$, for A172V and WT, respectively). We also compared the biophysical properties of

A172V to those of N855S in response to AITC. After agonist application, N855S revealed increased outward and inward currents, and overall a more pronounced voltage-sensitivity than A172V (+100mV, N855S = 38.12 ± 10.77pA/pF; -100mV, N855S = -13.48 ± 2.40pA/pF, $V_{1/2}$ = 37.03 ± 8.28mV, n=12). Similar conclusions were observed after application of 100 µM Menthol, a non-electrophilic TRPA1 agonist ($V_{1/2}$, WT = 61.83 ± 7.37mV n = 8, A172V = 36.41 ± 7.58mV n = 9, N855S = 33.60 ± 6.67mV n = 6, $p<0.05$). These data confirm the strong phenotype previously reported for N855S, and reveal an intermediate phenotype for the A172V variant.

Conclusions

Whole genome sequencing has revealed a further human TRPA1 (A172V) pain channelopathy. This was associated with episodic truncal pain and the A172V variant resulted in gain of function of TRPA1, demonstrating the role of ankyrin repeat domain in mediating the gating properties of TRPA1. In future studies, we will be examining the role of TRPA1 variants in determining the risk and severity of neuropathic pain due to sensory neuropathy.

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C04

Manipulation of mitochondrial dynamics in astrocytes in the Nucleus of the solitary tract affects the metabolic profile of brown adipose tissue

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The prevalence of obesity worldwide is increasing sharply, and while surgical and behavioural interventions are largely employed, their efficacy is scarce on the long-term. Alternative approaches, targeting the brown adipose tissue (BAT) are being investigated due to the potent thermogenic effects of this organ. BAT activation is driven by direct adrenergic sympathetic discharge from the central nervous system (CNS) and circulating glucose and fatty acids. The nucleus of the tractus

solitarius (NTS) in the brainstem is an important relay centre for information related to nutritional status, provided by the visceral vagal afferents; astrocytes are the most abundant cells in the brain and their role in regulating systemic metabolism is of growing interest, and evidence show their involvement in BAT thermogenesis (Manaserh *et al.*, 2020).

Previous studies within our group have shown that inhibition of mitochondria fission in astrocytes-using an adenoviral vector expressing a dominant negative form of Drp1 in astrocytes of the NTS (GFAP:Drp1-K38A)-was sufficient to reduce food intake and prevent weight gain in HFD-fed rats (Patel *et al.*, 2021).

Our main aim is to investigate whether targeting NTS astrocytes with GFAP:Drp1-K38A could affect the thermogenic profile of BAT and increase adrenergic discharge to the organ, which associates with enhanced metabolic demand. The experimental protocol was approved by the ethical committee for the use of animals in research of the University of Leeds. Male SD rats were subjected to stereotactic surgery followed by an injection of either GFAP:Drp1-K38A or GFAP:GFP in the NTS. Animals were fed HFD (5.51 kcal/g) diet for 15 days and sacrificed by pentobarbital overdose (60 mg/kg).Values are mean ±SEM and analysed with one-way ANOVA.

Noradrenaline precursor Tyrosine Hydroxylase (TH) levels in BAT of HFD-fed animals that received GFAP:Drp1K38-A in the NTS are increased 144% when compared to GFAP:GFP controls ($p<0.001$) ($n=4$). The mRNA levels of Collagen Type I (CD36) are significantly increased, and the lipolytic markers adipose trygliceride lipase (PNPLA2) and hormone sensitive lipase (HSL) are significantly downregulated in animals that received GFAP:Drp1K38-A in the NTS when compared to controls ($p<0.0001$, $p<0.0001$ and $p<0.0001$ respectively).Uncoupling protein 1 (UCP1) mRNA transcript were unchanged between the two groups ($n=12$).

Our treatment does not seem to impact-UCP1 dependent thermogenesis, however, our results suggest that inhibition of mitochondria fission in NTS astrocytes restored TH reservoirs in BAT-essential for beta adrenergic-driven thermogenesis, and appears to induce a preferential uptake for fatty acids (FA) rather than triglycerides in BAT as suggested by increased mRNA transcripts of fatty acid transporter CD36 and decreased lipolytic markers ATGL and HSL. These findings support previous findings by (Shin *et al.*, 2018) which suggest that BAT lipolysis in brown adipose tissue is not required for non-shivering thermogenesis. and that FA are uptake in BAT following lipolysis mediated liberation of FA in white adipose tissue (WAT). In this scenario NTS astrocytes may be able to mediate a centrally driven shift in BAT metabolic demand to maximise uptake and combustion of excess nutrients derived from diet and/or WAT lipolysis during high-fat diet.

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Shin H, Shi H, Xue B & Yu L (2018). What activates thermogenesis when lipid droplet lipolysis is absent in brown adipocytes? *Adipocyte* **7,** 143–147.

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Reference 3 :- Shin H, Shi H, Xue B & Yu L (2018). What activates thermogenesis when lipid droplet lipolysis is absent in brown adipocytes? *Adipocyte* **7,** 143–147.

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Medical Rearch Foundation (MRF)

University of Leeds

C05

Two disinhibitory circuits modulate the interaction between stimulus habituation and fast contrast adaptation in primary visual cortex

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The visual scene is constantly changing, and the detection of novel stimuli among familiar ones can be crucial for survival. One strategy is to continuously adapt the sensitivity of sensory neurons to suppress responses to common stimulus features while enhancing responses to new ones. We have recently demonstrated that fast contrast adaptation of pyramidal cell (PC) responses in V1 does not simply involve a reduction in gain over seconds (depression): as many neurons increase in gain (sensitization) reflecting their control by distinct inhibitory microcircuits[1]. PC and interneuron responses also adapt over longer time-scales, for instance when the animal habituates to a neutral stimulus repeated over days[2]. We have investigated whether the local inhibitory circuits that generate fast contrast adaptation are also involved in habituation.

To answer this question, we presented mice with a 10 s visual stimulus repeatedly during 6 sessions while monitoring GCaMP6f activity. To causally test for interneuron connectivity, we expressed the inhibitory opsin ArchT. During the stimulus, we measured changes in response amplitude (ΔF/F) and adaptive properties expressed as an adaptive index (AI) positive for depressors and negative for sensitizers[1]. Experimental mice were humanely treated, and isoflurane was used as surgical anaesthesia according to UK legislation.

We found that PCs decrease their activity (ΔF/F: Session 1 [S1] 0.17 ± 0.006, n = 1195 cells; Session 6 [S6] 0.09 ± 0.005, n = 935 cells; $p < 0.001$ Mann-Whitney U test) and shift from depression to sensitization upon habituation to the stimulus (AI: S1 0.12 ± 0.009; S6 -0.11 ± 0.011; $p < 0.001$ Mann-Whitney U test). Somatostatin interneurons (SSTs) increased their activity (ΔF/F: S1 0.40 ± 0.05, n = 51 cells; S6 0.67 ± 0.09 n = 94 cells; $p < 0.01$ T-test) undergoing a shift to sensitization (AI: S1 0.23 ± 0.04; S6 -0.26 ± 0.04; $p < 0.001$ T-test). Conversely, parvalbumin (PVs) interneurons dramatically decreased their activity (ΔF/F: S1 0.25 ± 0.02, n = 226 cells; S5 0.10 ± 0.01 n = 73 cells; $p < 0.001$ Mann-Whitney U test) as did VIPs (ΔF/F: S1 0.19 ± 0.03, n = 94 cells; S6 0.07 ± 0.02 n = 12 cells; $p < 0.05$ T- test). Optogenetically silencing SSTs we did not observe differences in PC inhibition when the stimulus was novel vs familiar (Delta Amplitude with Optogenetics: S1 0.13 ± 0.005, n = 993 cells; S6 0.13 ± 0.007 n = 690 cells; $p = 0.605$ Mann-Whitney U test). Conversely, silencing VIPs and monitoring SST activity revealed that SST adaptive properties are linked to VIP inhibition which is stronger when the stimulus is novel (Delta AI: S1 -0.40 ± 0.06, n=51 cells; S6 -0.24 ± 0.05 n = 94 cells; $p < 0.001$ Mann-Whitney U test).

The increase in SST activity without increased PC inhibition suggests that disinhibition through PVs may also contribute to activity changes during habituation. Our results reveal a concerted change of cortical adaptive properties during habituation towards sensitizing adaptation, and the role of two distinct disinhibitory circuits: SST->PV->PC and VIP->SST ->PC.

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Acknowledgements :- We thank Kate Hampden-Smith for all her help with mouse husbandry and lab colleagues who discussed the project over the years. We are also grateful to our funders: this work was supported by a Wellcome Trust grant (102905/Z/13/Z and) to L.L., and S.D. received a Leverhulme Trust PhD scholarship.

C06

Widespread nociceptive maps in the human neonatal somatosensory cortex

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[1]University College London, London, United Kingdom [2]University College London Hospitals, London, United Kingdom

Topographic cortical maps are essential for spatial localisation of sensory stimulation and generation of appropriate task-related motor responses. Somatosensation and nociception are finely mapped and aligned in the adult somatosensory (S1) cortex[1]. In neonates, a whole-body map of innocuous mechanical stimulation develops in S1 over the early postnatal period[2], and although cortical responses to a noxious mechanical stimulus have been recorded from 28 weeks using functional near-infrared spectroscopy (fNIRS)[3], the topographic representation of this activity has not yet been

mapped. We propose that in infancy, when pain behaviour is disorganised and poorly directed[4], nociceptive maps are less refined compared to somatosensory maps.

We used multi-optode fNIRS to map the haemodynamic response across the sensorimotor cortex following noxious (clinically-required heel lance) and innocuous (touch of the heel and hand) mechanical stimulation in 32 newborn infants (35-42 gestational weeks at birth, 0-7 days old, 12 female). Using channel-space and source reconstruction, we assessed the latency, location, magnitude, and topographic pattern of cortical activation following each stimulus. Ethical approval was given by the NHS Health Research Authority (London – Surrey Borders) and the study conformed to the standards set by the Declaration of Helsinki.

There was a significant localised increase in average concentration of oxygenated haemoglobin (D[HbO]) following touch of the heel (maximum change of 0.35 μM at 15.8 s post-stimulus) and hand (0.33 μM at 9.2 s), from baseline (-500 – 0 s pre-stimulus; Bonferroni-correct t-tests; $p<.01$), at locations consistent with the S1 regions representing the foot and hand respectively. Conversely, there was a widespread D[HbO] increase following the heel lance, which topographically overlapped with both the heel and hand touch responses. Non-parametric permutation analysis in source-space revealed that heel lance and touch elicited a peak increase at the same latency and location (Euclidean distance between peaks = 1.65mm, $p=.156$; difference in peak latency = 2s, $p=.358$), however the response was significantly larger (maximum D[HbO]: 16.06 vs 5.43 μM, $p=.001$) and the overall area of activation more widespread following lance (D[HbO] overall area: 455.27 vs 259.51 mm^2, $p=.009$). Importantly, the two responses only partially overlapped (in S1 region representing the foot), and while the heel touch response extended towards the motor cortex, the heel lance response extended towards more ventral regions of S1.

The widespread S1 nociceptive topography discovered here implies that nociceptive processing in the infant S1 is not somatotopically organised, consistent with their poorly directed pain behaviour. Heel lance is one of many skin-breaking procedures commonly performed in neonatal hospital care and this study reveals the extent of cortical activation that follows just one of such noxious procedure. This contrasts with innocuous mechanical stimulation, such as touch, which activates a spatially restricted and somatotopically defined cortical area.

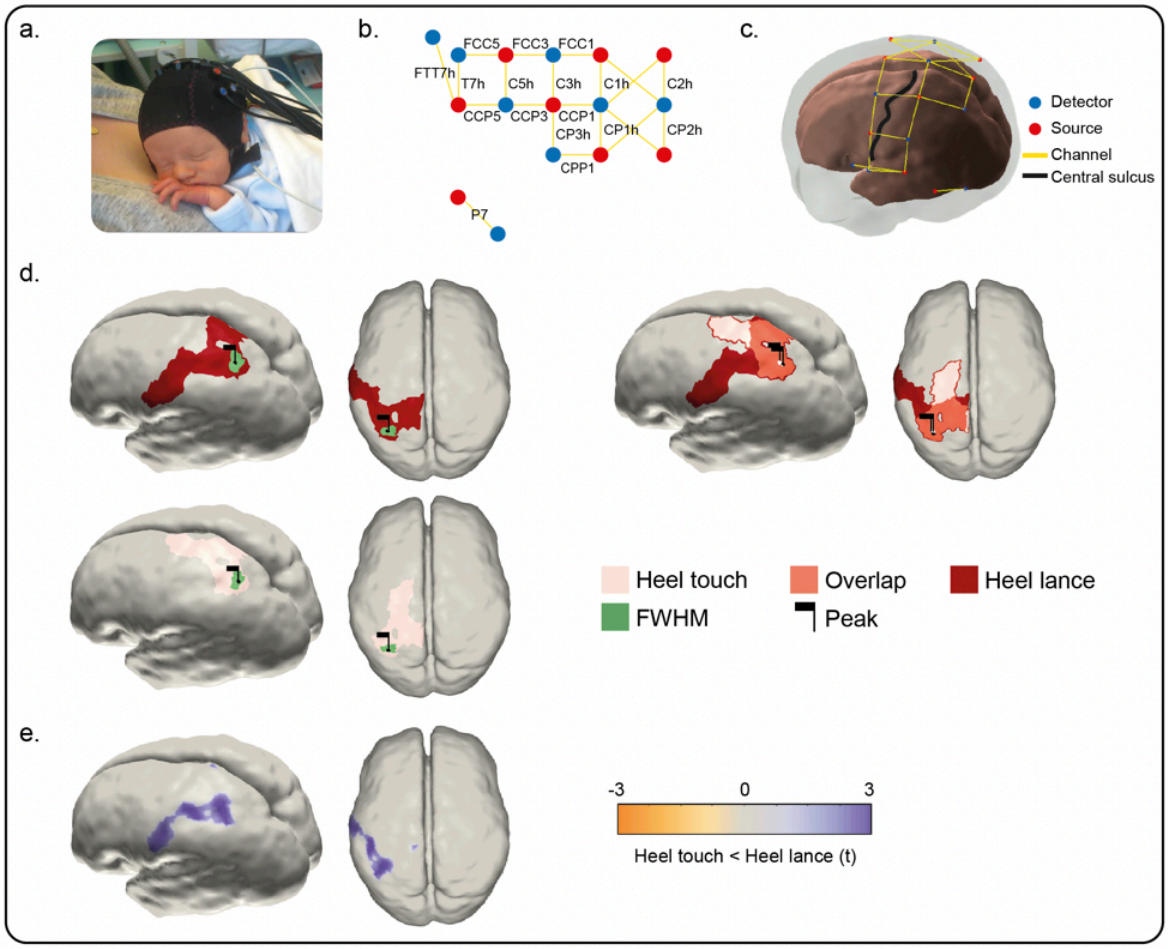

Figure 1. Multi-optode fNIRS setup and locations of peak and overall area of activation of the Δ[HbO] response to an innocuous (touch) and noxious (lance) mechanical stimulation of the heel. *(a) Typical fNIRS set up on a neonate of 35^{+2} weeks GA, 7 days PNA. (b) Channel locations according to the international 10-5 placement system. (c) Locations of the fNIRS sources, detectors and resulting measurement channels registered to a 39-week anatomical atlas. The central sulcus has been highlighted with a black line to mark the edge of the posterior gyrus (S1). (d) Overall area of significant changes in concentration of* oxygenated haemoglobin *(Δ[HbO]) following heel lance (red), heel touch (pink) and both (orange). Black flags demark the location of peak changes and green areas the extent of their full-width half-maximum (FWHM). (e) Comparison of response magnitude at each node. Results show the t-statistic at significantly different nodes within the areas of activation shown in (d).*

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Acknowledgements :- This work was funded by the Medical Research Council UK (MR/M006468/1, MR/L019248/1, and MR/S003207/1). RJC is funded by EPSRC Fellowship EP/N025946/1

C07

Spike propagation through the dorsal root ganglion, investigated using a modified working heart brainstem preparation, reveals a potential peripheral somatosensory gate.

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Peripheral nerves convey versatile information about the body's environment to the brain, which is interpreted as haptic, somatic and visceral experiences, including pain. Healthy nerves conduct action potentials from their peripheral endings to the spinal cord, where synaptic transmission first takes place. The 'Gate Control' theory assumes that the peripheral somatosensory signals are first integrated in the spinal cord and subsequently analysed in higher brain centres. Here we show that the dorsal root ganglia (DRG) are able to modulate nociceptive signals before they enter the CNS. Simultaneous whole nerve recordings were obtained from the C8 dorsal root (DR) and spinal nerve (SN) in the decerebrate working heart brainstem preparation of 18 day, Wistar rats of either sex (methods adapted from Paton. (1996)). These nerves innervate the median nerve supplying the forepaw. C8 ventral roots were transected. Using in-house custom written scripts to enable spike sorting, we were able to identify action potentials in the SN and match these to spikes propagated to the DR. Direct application of GABA (200 μM) into the DRG did not result in any changes in tonic activity in either aspect of the nerve (DR: 14.1 ± 1.2Hz Vs 14.2 ± 1.2Hz ($p= 0.95$), SN: 34.2 ± 2.5Hz Vs 35.1 ± 2.6Hz ($p= 0.8$); n= 19). Noxious stimuli (Randall Selitto) applied directly to the forepaw of the rat resulted in increased firing in both DR (12.7 ± 0.9Hz to 26.8 ± 1.3Hz, $p< 0.001$) and SN (31.4 ± 1.6Hz to 45.4 ± 2Hz, $p< 0.001$; n= 20). Randall stimulation during DRG application of GABA significantly reduced firing frequency in the DR (18.6 ± 1.1Hz, $p< 0.001$) but not in the SN (48.6 ± 2.3Hz, $p< 0.2$; n= 13), an effect we are interpreting as GABA-induced filtering of the conduction through the DRG. Spike sorting revealed that the extracellular action potentials traveling along C fibers were more likely to be filtered, in comparison to those in A-type fibers, suggesting that the DRG is able to selectively filter painful stimuli entering the spinal cord via GABAergic mechanisms. This notion was further confirmed in experiments with innocuous stimulation (cotton bud strokes, air puff) and proprioceptive stimulation. These stimuli also resulted in increased firing in both, SN and DR, however, direct application of GABA into the DRG during these circumstances was not able to reduce DR firing rate in the same manner seen with noxious stimulation. Taken together our findings indicate that peripheral somatosensory ganglia may represent a filter or even a 'gate' within the

somatosensory system and may serve as a novel therapeutic target for pain relief. As DRGs are not well protected by the blood-brain or blood-nerve barriers, this offers a unique opportunity to target the DRG with BBB-impermeable drugs thereby reducing central nervous system related side effects.

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C08

Perception of thermal comfort during skin cooling and heating

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Due to the static and dynamic activity of the skin temperature sensors, the cutaneous thermal afferent information is dependent on the rate and direction of the temperature change, which would suggest different perceptions of temperature and of thermal comfort during skin heating and cooling. This hypothesis was tested in the present study. Subjects (N = 12; 6 females and 6 males) donned a water-perfused suit (WPS) in which the temperature was varied in a saw-tooth manner in the range from 27 to 42 °C. The rate of change of temperature of the water perfusing the suit (TWPS) was 1.2 °C min−1 during both the heating and cooling phases. The trial was repeated thrice, with subjects reporting their perception of the temperature and thermal comfort at each 3 °C change in TWPS. In addition, subjects were instructed to report when they perceived TWPS uncomfortably cool and warm during cooling and heating, respectively. Subjects reproducibly identified the boundaries of their Thermal Comfort Zone (TCZ), defined as the lower (Tlow) and upper (Thigh) temperatures at which subjects reported slight thermal discomfort. During the heating phase, Tlow and Thigh were 30.0 ± 1.5 °C and 35.1 ± 2.9 °C, respectively. During the cooling phase, the boundary temperatures of Tlow and Thigh were 35.4 ± 1.9 °C and 38.7 ± 2.3 °C, respectively. The direction of the change in the cutaneous temperature stimulus affects the boundaries of the TCZ, such that they are higher durin cooling and lower during heating. These findings are explained on the basis of the neurophysiology of thermal perception. From an applied perspective, the most important observation of the present study was the strong correlation between the perception of thermal comfort and the behavioral regulation of thermal comfort. Although it is not surprising that the action of regulating thermal comfort is aligned with its perception, this link has not been proven for humans in previous studies. The results therefore provide a sound basis to consider ratings of thermal comfort as reflecting behavioral actions to achieve the sensation of thermal neutrality.

Acknowledgements :- The work was supported, in part, by the European Commission Horizon 2020 Research and Innovation action (Project number 668786: Heat Shield).

C09

Cortical microstate analysis reveals central nervous system modulation of responses to repeated noxious procedures in hospitalised human neonates.

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Pain habituation is an important physiological and psychological adaptation to recurrent or sustained noxious stimuli and is likely to preserve physical, emotional, and cognitive resources when the threat is unavoidable or if the stimulus is not life-threatening (De Paepe et al., 2019). This adaptive learning evolves over the lifespan as the brain develops in conjunction with exposure to a wide range of sensory experiences. However, at what stage in human development such habituation emerges is unknown.

Twenty-one human infants (10 preterm: median 34 completed postmenstrual weeks, 5 females; 11 term: median 39 completed postmenstrual weeks, 4 females) underwent two clinically-required blood tests (heel lances) in brief succession (median 7.5 minutes apart), on the same heel skin area due to an unsuccessful first attempt. We recorded brain electrical activity at the scalp (electroencephalography), flexion withdrawal reflex (electromyography), heart rate changes (electrocardiography) and facial expressions (video) time-locked to each lance procedure. Ethical approval was obtained from the NHS Research Ethics Committee, with informed written parental consent obtained prior to each study. Scalp-level cortical responses to the two stimuli were studied using an advanced global event-related field topography (microstate) analysis technique. This method identifies unique stimulus-related topographies, with their latency, extent of activation, and cycling behaviour post-stimulus, reflecting ongoing changes in the engaged network configuration of active cerebral sources (Michel & Murray, 2012). Our analysis determined a set of six and five microstates as optimal models for term and preterm infants respectively, given that they explained over 85% of the post-stimulus global scalp potential response.

Our results show that in both term and preterm groups, a common initial sequence of microstates (0-700/900 ms respectively) were engaged following both lances. Following a second lance there was no significant difference in the engagement of any of these microstates in this initial sequence, or accompanying physiological responses, in preterm infants. In contrast, there was a significant reduction in the engagement of the earliest microstate in term infants ($p=0.016$) following the second lance, which was accompanied by decreases in their flexion withdrawal reflex changes ($p<0.001$), heart rate changes ($p=0.022$) and facial expression scores ($p=0.002$). In the late part of the response, both age groups displayed distinct dominant microstates for first and second lance, suggesting that higher-level cortical processing is possible in preterm neonates, but is refined in developmentally mature term neonates.

Term neonates display habituation to repeated noxious stimulation as measured by cortical activation and by physiological responses and reflexive behaviours. The decreased short-latency cortical response suggests neonatal learning from the first noxious stimulation driving adaptions that reduce cortical processing of stimulus components common to repeated noxious events (Seymour & Mancini, 2020), thus potentially limiting energy wastage in favour of other goals. This was followed by distinct high-level processing of contextual factors unique to each stimulus. The failure of the preterm brain to habituate and thus adapt to repeated pain during a critical developmental period where high-level cortical processing networks are being established emphasises the vulnerability of preterm infants to repeated noxious stimulation.

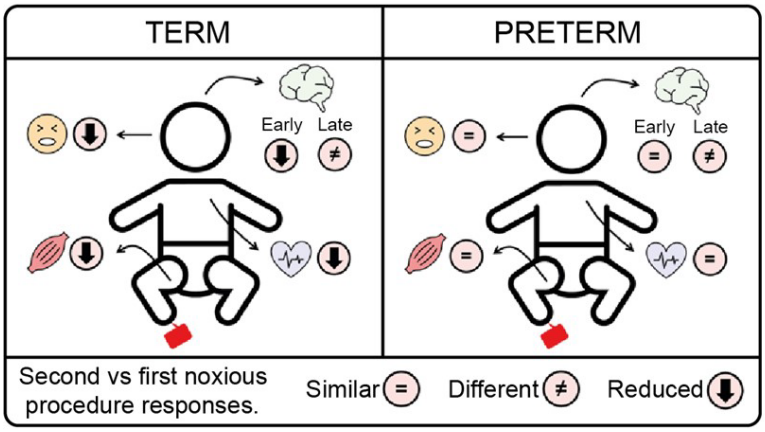

Figure 1: *Graphical abstract*

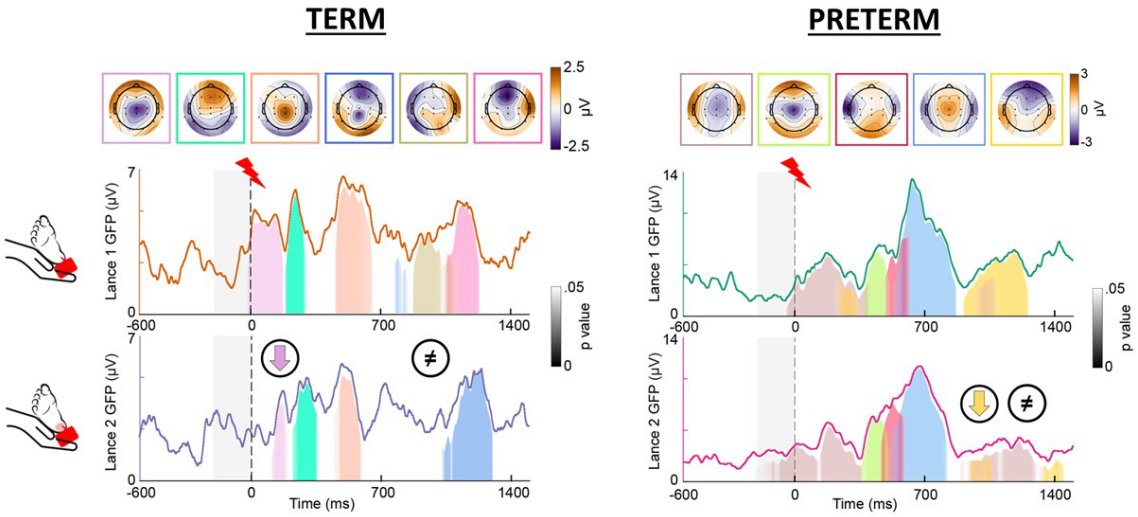

Figure 2: *Term (left) preterm (right) neonate global event-related field topography (microstate) analysis of electroencephalography data to repeated lances. Top panel: Microstates. Bottom panel: Microstate projection on first and second lance group average responses. GFP: global field power (spatial variation across the scalp at each time point). Downward arrows highlight microstate activity where the engagement was significantly (p<0.05) decreased following the second lance. ≠ highlights latencies where distinct microstates appeared across the two heel lances.*

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Reference 3 :- Seymour B & Mancini F (2020). Hierarchical models of pain: Inference, information-seeking, and adaptive control. *NeuroImage* **222**, 117212.

Acknowledgements :-

This work was supported by the Medical Research Council UK (MR/S003207/1) and the European Research Council (CoG 2015-682172NETS) within the Seventh European Union Framework Program. OB was supported by the Canadian Institutes of Health Research (FBD–170829). The research was performed at the University College London Hospitals (UCLH) Maternity and Neonatal Units and we thank the families of the infants that participated in this research.

C10

Two distinct functional modes for cochlear outer hair cells: implications for cochlear tuning at high acoustic frequencies

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Outer hair cells of the mammalian cochlea are part of an amplification system that enhances incoming sound. Pharmacological manipulations, a wide range of genetic mutations, the anatomical positioning of the cells and a fast voltage driven motility all provide evidence for involvement in cochlear tuning. It has long been proposed that OHCs can provide a force term that compensates the dynamics for any viscous damping of the basilar membrane. However, the (RC) low pass filtering of potential by the OHC membrane appears to limit the frequency at which potential driven 'electromotility' could contribute to cochlear mechanical tuning. Limiting OHC motile bandwidths have also been emphasized by recent in vivo optical coherence tomography (OCT) measurements and by in vitro patch clamp recording of isolated cells. To explain these data, considerations based on piezo electric descriptions of the OHCs (Iwasa, 2017, Rabbitt, 2020) suggest that the OHC membrane capacitance can be a source of power up to high acoustic frequencies.

The simplest theoretical models of the cochlear partition are those where, at each point, the OHCs provide feedback to the basilar (BM) and tectorial (TM) membranes, both of which can be resonant structures (Geisler, 1993). For a complete description, point models should be coupled into longitudinal cochlear modes to include wave propagation in the fluids; and ideally the models should be solved in the time domain to allow the incorporation of known non-linearities. The number of indeterminate parameters in such schemes can render these models prohibitively complex.

To clarify such proposals biophysically, I have constructed cochlear models that incorporate the inward current arising from the anion movement associated with deformation of the OHC motor protein prestin/SLC26A5 (Gale & Ashmore, 1994). This current is not limited by the RC low pass filter and can dominate the OHC current balance at high frequencies, extending the OHC operating bandwidth. Simple considerations indicate that the resulting OHC forces behave like positive feedback at low frequencies, but enhance any resonance of the BM (and of the TM) at high frequencies, producing a sharp resonance even though the transducer current is not primarily driving prestin/SLC26A5. Such modelling results may also explain qualitatively distinct auditory nerve frequency tuning curves observed at high and at low best frequencies. .

Reference 1 :- Gale JE, Ashmore JF (1994). Proc. Biol. Sci. 255, 243–249.

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Reference 4 :- Rabbitt, RD (2020). Proc. Natl. Acad. Sci. 117, 21880–21888.

C11

Expression of the glucagon like peptide-1 receptor in cerebrospinal fluid contacting neurons of the spinal cord: a potential role in cell proliferation and differentiation?

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The ependymal layer of the spinal cord is widely considered a neurogenic niche. Within this layer exists many cell types, but two are of particular interest here; ependymal cells (ECs) and cerebrospinal fluid contacting neurons (CSFcNs). ECs may be latent spinal cord stem cells, becoming active after spinal cord injury. ECs are responsive to GABA[1], but the GABAergic source is unknown. CSFcNs have direct contact with the cerebrospinal fluid and are known to be GABAergic[2] . We have studied the properties of these unusual cells to understand their function and how they might interact with ECs. We show here that there is 100% colocalisation between polycystic kidney disease 2-like 1 (PKD2L1, a specific marker for CSFcNs) and vesicular GABA transporter (VGAT) in both juvenile (P18-P20, n=3) and adult (P30-P55, n=6) mice, indicating that this is a reliable marker for these CSFcNs.

Glucagon like peptide-1 (GLP-1) is a hormone that is secreted by the gut after food intake and is also produced by a subset of specialised neurons located in the brainstem. GLP-1R agonists exendin-4 and liraglutide neuroprotective and neurotrophic effects in models of Alzheimer's and Parkinson's diseases[3]. Using immunofluorescence labelling we found that most,

but not all, CSFcNs possess receptors for GLP-1 (90 ± 1.6%, n=4 adult mice). In calcium imaging experiments using acute spinal cord slices, obtained from VGATxGCaMP6f mice (n=7, P21-P33) terminally anaesthetised with sodium pentobarbital, CSFcNs respond to bath application of 1µM liraglutide through changes in their calcium activity levels, indicating that these receptors are functional.

We then asked if systemic administration of the GLP-1 agonist Exendin-4 could affect levels of cell proliferation in this neurogenic niche area, using the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU) as a marker of proliferating cells[1]. Injections of exendin-4 (0.5µM, i.p, every 24 hours for 4 days) *in-vivo* in healthy adult mice (n=5 control, n=5 test, 9 weeks old) resulted in a significantly lower number of EdU positive cells coexpressing an oligodendrocyte marker, (p=0.026, two sample t-test) and a significantly higher number of (p=0.05) neuronal marker expressing EdU positive cells compared to mice treated with vehicle. There was no overall difference (p=0.19) in the number of newly proliferated ependymal cells between groups, which is consistent with their limited proliferative nature in the physiologically normal state[4]. In simulated injury conditions using organotypic spinal cord slice cultures, application of 1µM liraglutide significantly increases the proliferation of cells within the ependymal layer after 10 days in culture (p=0.041).

To conclude, we have shown that the GLP-1 receptors on spinal cord CSFcNs are active, influencing the calcium activity of these cells. Furthermore, activation of GLP-1 receptors alters the number of proliferating spinal cells when given *in-vivo,* and results in an increased number of proliferating central canal cells in *ex-vivo* experiments. Further work will be done to explore the exact links between these cell types.

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Reference 2 :- Stoeckel, M.-E. *et al.* Cerebrospinal fluid-contacting neurons in the rat spinal cord, a gamma-aminobutyric acidergic system expressing the P2X2 subunit of purinergic receptors, PSA-NCAM, and GAP-43 immunoreactivities: light and electron microscopic study. *J. Comp. Neurol.* **457**, 159–174 (2003).

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Acknowledgements :- This research was funded by the EPSRC Centre for Doctoral Training in Tissue Engineering and Regenerative Medicine – Innovation in Medical and Biological Engineering, multidisciplinary collaboration of Faculties at the University of Leeds. Grant number EP/L014823/1.

C12

Inhibition of distension-induced afferent firing by Botulinum neurotoxin serotypes A and B in the mouse bladder

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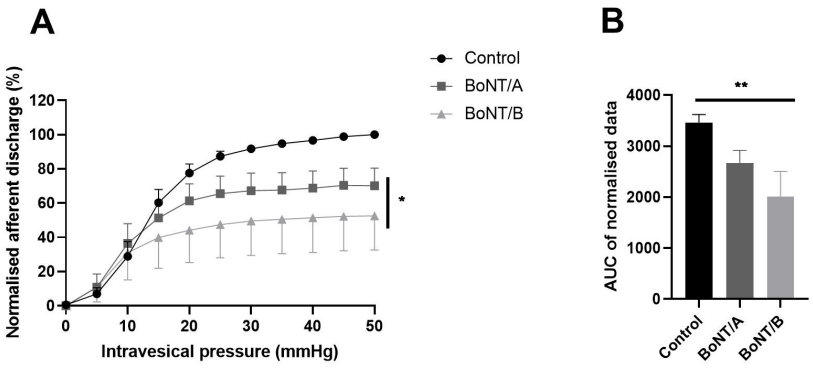
Background - Botulinum neurotoxin (BoNT) is a potent neurotoxin that silences cholinergic neurons through inhibition of vesicular release mechanisms. Intravesical injection of BoNT serotype A (BoNT/A) for patients with overactive bladder (OAB) unresponsive to anticholinergics was approved by the Federal Drug Administration (FDA) in 2013 (FDA, 2013). Despite its widespread use, the complete mechanism of action of BoNT/A is still unclear, as reduction of bladder sensation has been described in basic science and clinical literature. Most studies have investigated only BoNT/A. The aim of this study was to use an ex vivo bladder-nerve preparation to investigate the action of BoNT/A and BoNT/B on distension-induced afferent firing.

Methods – These studies were performed using adult C57BL/6J mice between 8 and 12 weeks old, sacrificed in accordance with Schedule 1 of the Animals (Scientific Procedures) Act UK 1986. An ex vivo bladder-nerve assay that allows the concomitant recording of afferent nerve firing and intravesical pressure was used to define the effect of BoNT/A and BoNT/B on bladder afferent mechanosensitivity. Nerve responses at 90 minutes post-BoNT treatment were normalized as a percentage of nerve activity of baseline distension prior to application of BoNT/A and BoNT/B at a concentration of 3 pM, or PBS in time control. All data are presented as mean +/- SEM, analysed using one-way and two-way ANOVA with Bonferroni post hoc test (Figure 1.)

Results – Time control preparations showed no difference in nerve responses, the 90-minute distensions retaining 94.48% (+/- 4.35%) of nerve activity of baseline distension ($p=0.3398$, $n = 11$). Figure 1: (A) BoNT/A significantly reduced bladder mechanosensation ($p <0.0001$; $n = 5$), responses decreasing by 29.8% (+/- 10.2%) compared to baseline. BoNT/B also significantly attenuated afferent firing ($p<0.0001$, $n = 6$), responses decreasing by 47.5 % (+/- 19.9%) compared to baseline. BoNT/B inhibited distension-induced afferent firing more potently than BoNT/A ($p <0.05$). (B) Afferent responses visualised as Area Under Curve (AUC) shows BoNTs significantly reduced afferent firing compared to control ($p=0.006$)

Interpretation of results – To our knowledge, this is the first study showing an effect of BoNT/B on bladder afferent nerve signaling. The results suggest that while both BoNT serotypes inhibit bladder mechanosensation, BoNT/B inhibits nerve firing more potently. Further studies are necessary to understand the underlying mechanism behind this effect.



Reference 1 :- FDA. HIGHLIGHTS OF PRESCRIBING INFORMATION These highlights do not include all the information needed to use BOTOX ® safely and effectively. See full prescribing information for BOTOX. BOTOX (onabotulinumtoxinA) for injection, for intramuscular [online]. Available from: www.fda.gov/medwatch. [accessed 24 June 2021].

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C13

Odour Adaption in the olfactory bulb

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Odour adaptation allows organisms to acclimate to their environment whilst remaining sensitive to changes in the odour landscape. The neural mechanisms underlying odour adaptation and whether this occurs in the olfactory bulb has not been thoroughly explored. Input to the olfactory bulb is provided by olfactory receptor neurons, which arrange themselves into functional units (glomeruli) based upon the receptor they express, and transmit sensory information to a pool of output neurons, the mitral/tufted cells. Olfactory receptor neurons receive feedback inhibition onto their axon terminals from periglomerular cells, while mitral/tufted cells are subject to feedback, feedforward and lateral inhibition via the dendrodendritic connections they form with periglomerular cells and deeply situated granule cells.

We used two-photon imaging to measure the glomerular activity of olfactory receptor neurons, mitral/tufted cells and periglomerular cells in anaesthetised mice, in response to 3s and 60s odour stimuli across concentrations spanning five orders of magnitude. We find that both the extent and the rate of adaptation varies widely across dorsal glomeruli (n=14, 125 glomeruli). Fast and slow adaptation was observed; slower adapting responses had time constants of seconds to over a minute (34.6% of observed responses), whereas fast adapting responses with time constants <2 s were present in a subset of glomeruli (14.4% of observed responses). Preliminary analysis shows that within the same glomerulus, the relative strength and identity of the odour influenced the rate of adaptation and the recovery from adaptation. Response kinetics in mitral/tufted cells were diverse and activity often persisted for >10 s after odour presentation (45.9% of observed responses from 48 glomeruli, n=5). In contrast, olfactory receptor neuron terminals and periglomerular responses were more homogenous and responses generally terminated at odour offset (with only 16.6% [73 glomeruli, n=7] and 8.3% [11 glomeruli, n=2] of responses exhibiting odour after-effects, respectively). We can provide a comprehensive view of odour adaptation across the different circuit components of the olfactory bulb; experiments exploring the role of inhibition are ongoing.

C14

Insulin inhibits GABAergic neurones in the dorsal vagal complex to control glucose metabolism

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C15

Deciphering olfactory bulb circuits modulated by metabolic signals

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Olfaction is important for the regulation of food intake and energy homeostasis and contributes to food choice and its consumption. Olfactory processing is influenced by the feeding status, satiety decreases and fasting increases olfactory acuity. Consistent with this, olfaction is modulated by changing levels of several metabolically regulated molecules such as insulin and glucose. Insulin is a key hormone for feeding, energy metabolism and cognition. The olfactory bulb has the highest density of insulin receptors in the brain. Previous studies have demonstrated the action of insulin on the mitral cells which are projection neurons of the olfactory bulb. Insulin increases the excitability of mitral cells by inhibiting a voltage-gated potassium channel (Fadool et al. 2004, 2011). However, it has not been fully characterized how insulin acts on the cells in the glomerular layer which are initial sites for odour information processing in the olfactory bulb. Here, we investigate how olfactory bulb circuits are regulated by metabolic signals and the role of these molecules on olfactory perception and feeding behaviour. Especially, we are investigating mechanisms underlying the role of insulin in the modulation of olfaction and identifying cellular targets of insulin in the mouse olfactory bulb. To explore these mechanisms, we are using variety of approaches including patch-clamp electrophysiology, 2-photon calcium imaging, immunohistochemistry and behavioural tests. We perform voltage clamp recordings from acute mouse olfactory bulb slices to test the effect of insulin on potassium currents in the glomerular layer. Our electrophysiology experiments demonstrate an effect of insulin on the cells in the glomerular layer; a subset show strong suppression of a voltage-gated potassium current. Following bath application of insulin (172 nM), current suppression was observed in 6 of 13 recorded cells from 11 mice. We use genetically encoded Ca indicators and 2-photon imaging to test action of insulin at the circuit level and our preliminary results from 3 mice suggest that insulin modulates olfactory-nerve evoked responses. We perform immunostaining to show the distribution of insulin receptors in the olfactory bulb and behavioural analyses to test olfactory sensitivity depending on the feeding state. Overall, this study will help to gain insight into the role of insulin in olfactory information processing by nutritional state and how this regulates olfactory-driven behaviour in mice.

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Reference 2 :- Fadool DA et al. (2011). PLoS ONE 6(9):e24921

C16

Mechanical and chemical sensing in cerebrospinal fluid contacting neurones of the mouse spinal cord

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Cerebrospinal Fluid-Contacting Neurons (CSFcNs) in the spinal cord are localised around the central canal, with an end bulb projecting into the cerebrospinal fluid (CSF). Based on their morphology and responses to various stimuli in non-mammalian vertebrates, they are hypothesised to play a mechanosensory and chemosensory role by detecting changes in CSF movement and composition. Within the CNS, PKD2L1 (Polycystin 2 Like 1, Transient Receptor Potential Cation Channel) is an ion channel subunit that is a cell-specific marker for CSFcNs and is a known to respond to both mechanical stimulation and changes in pH. The current study uses calcium imaging to examine effects of mechanical stimulation or changing pH on the level of activity of CSFcNs in spinal cord slices from mice in which the calcium sensor GCaMP6f had been expressed in CSFcNs.

Using two photon microscopy at room temperature (20-22 ^{o}C), the spontaneous Ca^{2+} spike rate in CSFcNs was 0.148 ±0.097 Hz (median ± IQR, n = 127 cells, N = 15 mice). The mean calcium spike rate was 3-fold higher when CSFcNs were mechanically stimulated through cell attached recordings of extracellular action potentials (EAPs; 0.43 ± 0.18 Hz, mean ± SD; n = 15, N = 10). Strikingly the different rates we measured with EAPs and Ca2+ imaging are almost identical to previous electrical recordings comparing spontaneous activity in wildtype (0.42 Hz) vs animals lacking the mechano-sensitive PKD2L1 channel (0.16 Hz) (Orts-Del'Immagine et al., 2016). Placement of an electrode against CSFcNs therefore elevates their spike rate and Ca2+ activity, likely due to the mechano-sensitive, large conductance and Ca2+ permeable PKD2L1 channels expressed in these cells.

The effects of pH on calcium signalling in CSFcNs in spinal cord slices were examined at room temperature (20-22 ^{o}C) using epifluorescence imaging (n = 24 cells). In initial analyses average spike amplitude decreased significantly ($p = 5.3 \times 10^{-7}$; Student's t-test) when the pH of bathing artificial cerebrospinal fluid was decreased from pH 7.34 (1.38 ± 0.05 DF/F; mean ± SEM) to pH 6.47 (1.07 ± 0.03 DF/F). However, at lower pH the sensitivity of GCaMP6f is reduced and when this was taken into account and calcium peaks normalised (Cho et al., 2017), the average spike amplitude significantly increased to 1.68 ± 0.05 DF/F; $p = 2.09 \times 10^{-6}$; Student's t-test). This is consistent with increases in firing rate reported in whole cell patch clamped CSFcNs in response to similar pH changes in lamprey spinal cord (Jalalvand et al, 2016).

These data suggest that calcium imaging is an appropriate method to study mechanical stimuli in CSFcNs, but that caution is required when interpreting changes in calcium sensors in response to pH manipulations. CSFcNs appear to sense and respond to mechanical stimulation and changes to CSF

pH in the mammalian spinal cord. How this relates to their function in mammals remains to be determined.

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Reference 2 :- Jalalvand et al (2016). Current Biology, 26(10), 1346-1351

Reference 3 :- Orts-Del'Immagine et al., (2016). Neuropharmacology, 101, 549-65

Acknowledgements :- This work was supported by the following grants: SBF002\1033, MR/V003747/1 and WT104818MA

C17

Delineating inflammatory signalling mechanisms at the ER-PM junctions in sensory neurons

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Endoplasmic reticulum- plasma membrane (ER-PM) junctions are important aspects of the cellular structure characterised by close apposition between areas of the ER and PM (<30nm). These structures have many important functions including lipid transfer, protein transport, vesicle trafficking and also serving as multiplex signalling hubs. The latter property is indeed an extremely important aspect of signalling paradigms in excitable cells such as sensory neurons. In such cells, it has previously been shown that various signalling processes, such as G-protein coupled receptor mediated Anoctamin 1 (ANO1) channel activation or store-operated Ca^{2+} entry (SOCE), readily occur at ER-PM junctions and play an important role in inflammatory signalling. In order to understand junctional signalling, an important question needs to be addressed: which proteins are involved in formation and maintenance of these junctions in sensory neurons. Recently, we identified the junctophilin family of proteins as being important for ER-PM formation and SOCE as part of the inflammatory process in sensory neurons. Another set of proteins, that have been shown to play a role in ER-PM formation in various types of cells, are the Extended Synaptotagmin (Esyt) family of proteins. Interestingly, Esyt1 is activated by Ca^{2+} and is able to allow ER-PM proximity in a dynamic manner. Using immunohistochemistry we identified high expression levels of Esyt1 in rat dorsal root and trigeminal ganglion neurons (DRG, TG), with expression prevailing in mainly small-diameter neurons. Performed proximity ligation assay demonstrated close proximity between Esyt1, ANO1 or inositol trisphosphate receptors (IP_3Rs). In control conditions, there was baseline proximity detected between both Esyt1-ANO1 (1.62 ± 0.136 puncta per cell, n=65 neurons) and Esyt1-IP_3R (21.0 ± 2.68 puncta per cell, n=82 neurons). Interestingly, upon application of an inflammatory mediator, bradykinin, there was an increase in the incidence of proximity between Esyt1-ANO1 (11.2 ± 1.29 puncta per cell, $p<0.0001$, n=93 neurons) however, a significant decrease in the number of Esyt1-IP_3R proximity was also detected (7.41 ± 0.678 puncta per cell, $p<0.001$, n=75 neurons). Furthermore, live cell total internal reflection fluorescence (TIRF) imaging revealed that in DRG overexpressing an Esyt1-GFP protein, application of bradykinin was able to increase TIRF fluorescence (0.11 ± 0.02

$\Delta F/F_0$, n=6) suggesting that either there was movement of the ER to the PM upon stimulation or that Esyt1-GFP translocates closer to the PM within the existing ER-PM junctions. We also detected similar signals upon removal of extracellular Ca^{2+} (0.068 ± 0.0068 $\Delta F/F_0$, n=6), suggesting that Esyt1 translocation or ER-PM proximity can be effectively triggered by Ca^{2+} release from the ER. These data suggest that Esyt1 may be playing an active role in bradykinin-induced inflammatory signalling in sensory neurons and could be important for inflammatory pain generation.

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Ipsilateral responses in contralesional S1 may relate to recovery after peripheral nerve repair

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Transection of the major hand nerves leads to significant and long-lasting functional impairments. Animal models show functional changes in the contralesional hand area of primary sensory cortex (S1-deprived) in response to both stimulation of the repaired nerves of the injured hand as well to the (ipsilateral) uninjured nerves of the uninjured hand. Evidence for brain changes in human peripheral nerve repair patients is limited and their impact on functional recovery is unknown.

Objectives

We present preliminary results from an ongoing project using functional MRI to measure cortical responses to cutaneous stimulation of the hand in peripheral nerve repair patients and healthy controls. The results are preliminary since our target participant sample size has not been reached; current numbers include ten patients—three median, four ulnar, and three ulnar and median cases (time since repair, mean: 41 months; range: 10 – 82 months)—and 16 healthy controls.

Method

Vibrotactile stimulation was applied to the distal digit pads of each hand while inside the MRI scanner. S1 was defined functionally in each individual based on responses to stimulation of the digits of the contralateral hand combined with the estimated location of area 3b based on their

anatomical data. We then extract estimates of fMRI response levels within S1 to stimulation of the digits of the ipsilateral hand.

Patients and controls also completed novel tests of touch localisation on the volar surfaces of each hand; mean absolute and directional error were computed. Patients also completed a number of standardised clinical assessments, including the sensory Rosen test.

Results

We find significant hand-hemispheric differences in S1 ipsilateral responses levels in patients but not controls. A two-way mixed ANOVA identifies a significant interaction between group (patient, control) and hand (injured, uninjured) ($F(1, 24) = 4.767$, $p = .0.039$). Patient ipsilateral responses in S1-deprived (i.e., to the uninjured hand) are elevated compared to their ipsilateral response levels in the opposite hemisphere (i.e., to the injured hand), while no such hand-hemispheric differences are identified in healthy controls.

Inconsistent with our expectations, however, elevated ipsilateral response levels in patients do not exceed estimates of the range of normal variation as defined by our healthy controls. Greater ipsilateral responses in S1-deprived are nonetheless found to reliably correlate with higher sensory Rosen scores ($r = 0.82$, $p = .0035$) and better touch localisation ($r = -0.74$, $p = .0151$).

Conclusions

Our current results are difficult to interpret. There is a suggestion that ipsilateral response levels are elevated in the S1-deprived of patients, yet these levels do not exceed our estimates of normal variation in healthy controls. This makes it difficult to interpret these responses as possible markers of functional reorganisation following peripheral nerve repair. At the same time, the results identify a significant positive relationship between the strength of ipsilateral responses in S1-deprived and patient recovery. More data are needed to clarify these findings. Cautious interpretation is necessary, at present.

Compliance with ethical standards

The study has been approved by the ethic committees of Bangor University and Health and Care research Wales.

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Spontaneous cortical activity in somatosensory networks gates their stimulus-driven recruitment in preterm human neonates

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Introduction

Developing sensory neuronal networks fire spontaneously in preparation to receiving external input (Molnár *et al.*, 2020). Preterm human EEG is characterised by high amplitude activity bursts with discrete voltage distributions across the scalp. Bursts can also be elicited with external stimuli such as touch (Whitehead *et al.*, 2016). While sensory evoked bursts may have voltage distributions similar to those that occur spontaneously, it is not clear whether they involve the same underlying cortical networks. To answer this question, we took advantage of network refractoriness, i.e. that spontaneous activation is followed by a period when the system cannot respond to further input (Fedirchuk *et al.*, 1999).

Methods

We recorded EEG in response to mechanical taps of hands and feet in 35 healthy infants of median 32 weeks corrected gestational age (CGA; range 28-35 weeks) and postnatal age (PNA) 7 days. (CGA = gestational age + PNA). There was a total of 101 stimulation trains (i.e. stimulated limbs) of 2-48 taps (mean 15). We then assessed how magnitude (power spectral density (PSD)) and distribution of endogenous activity preceding the stimulus affected the response to the tap. Ethical approval was obtained from the NHS Research Ethics Committee, and informed written parental consent was obtained prior to each study.

Results

Somatosensory stimulation evoked significant PSD changes in the delta, alpha-beta and gamma bands with peak changes of 6 dB at 1Hz (delta) and 13Hz (alpha-beta). PSD changes at lower frequencies were more widespread across the scalp than those at higher frequencies, which instead

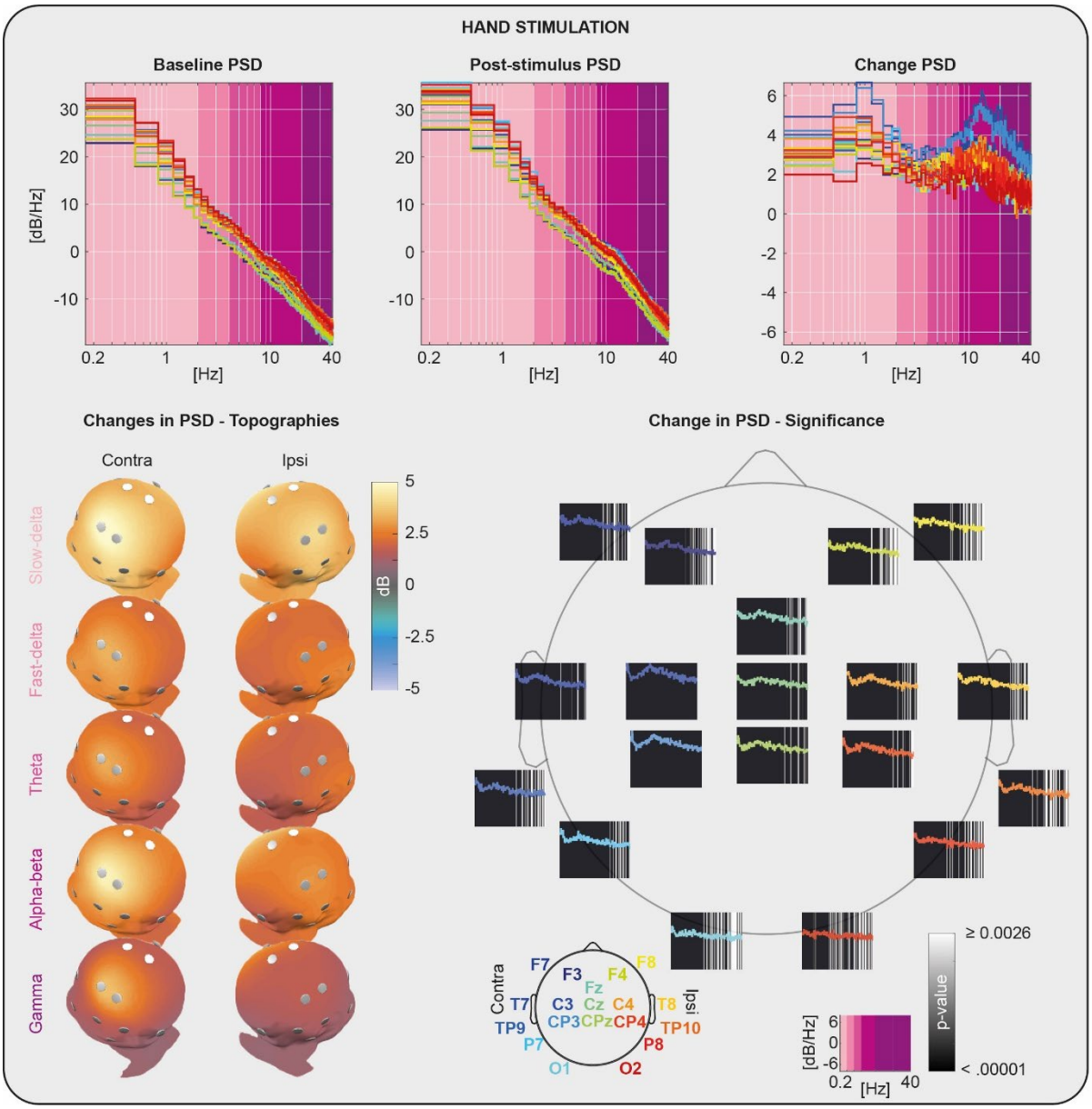
were localised at the somatotopic representation of the stimulated limb (i.e. central-contralateral for the hands and central-midline for the feet) (Fig. 1).

The PSD changes evoked in each band by tapping the limbs were inversely related to the PSD present before the stimulus (baseline) and to how similar its distribution was to that of the somatosensory evoked response (SIM) ($p < .0001$ after correcting for CGA; multiple linear model used: PSD change = b0 + b1 CGA + b2 baseline + b3 SIM + e; adjusted whole model r squared = 0.54 (delta), 0.50 (alpha-beta) and 0.27 (gamma)).

Conclusions

These results indicate that sensory stimulation elicits different oscillatory rhythms, which engage focal or extended networks. These same rhythms are present spontaneously and can make the brain refractory to an incoming external stimulus, which tries to engage the same network. These results are supportive that spontaneous activity in the preterm period represents the activation of maturing sensory cortical architectures in preparation to engagement with the external environment. This offers an etiological explanation for the characteristic discontinuous EEG pattern of preterm neonates.

Future work will examine the implications of these results for the *injured* preterm brain. Here, spontaneous firing is of higher magnitude than in healthy infants (Whitehead *et al.*, 2016), which correlates with longer subsequent periods of quiescent background, that increases the chance of a poor motor outcome (Koskela, Meek et al. submitted). It is possible that *excessive* refractoriness could perturb maturation of sensorimotor networks.



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C20

Severe hypoxia reduces the randomness and variability of carotid body (CB) sensory patterning

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Background: The carotid body (CB) detects and translates blood-borne stimuli into patterns of neural discharge to initiate corrective chemoreflexes. Elevated CB activity has been implicated in the pathophysiology of cardiorespiratory diseases including hypertension and cardiac arrhythmia (1). Hypoxia increases CB activity and the steady-state magnitude of its sensory output in an intensity-dependent manner. However, little is known about the patterning of CB sensory discharge. It is hypothesised that a shift from a random action potential pattern to a more regular pattern determines the physiological and pathological responses elicited by different intensities of hypoxia.

Aims: This study sought to analyse differences in the randomness and variability of action potential (AP) firing patterns recorded from CB chemoafferent fibres during graded hypoxic stimulation.

Methods: Ex vivo extracellular nerve recordings were made from Wistar rat CBs (male, aged 5-20 weeks, n=19), removed under terminal anaesthesia (2.5-4% isoflurane in O2 at 1.5L.min-1). Single-fibre activity (n=21) was distinguished using Spike2 (version 9.05) software. Data was grouped into five hypoxic intensities defined by their mean AP frequency range; 0-2Hz (baseline), 2-10Hz, 10-20Hz, 20-30Hz, over 30Hz. Instantaneous frequency (IF) and Poincaré plot analysis, which characterised inter-spike interval (ISI) distribution, were used as quantitative measures of AP patterning. Values are expressed as mean ± S.E.M, compared using one-way ANOVA followed by Dunnett's post-hoc analysis.

Results: Compared to baseline, the IF distribution of chemoafferent firing during severe hypoxia (over 30Hz) was not random (IF SD:Mean ratio; 2.4±0.127 vs 0.535±0.034, $p<0.001$). Standard measures of dispersion obtained from frequency-normalised Poincaré plots, SD1 and SD2, were both significantly reduced in severe hypoxia (Figure 1 A-D) leading to a lower overall variability between successive ISIs (Area of ellipse; baseline: 5.52±0.532AU vs over 30Hz:1.401±0.092AU, $p<0.001$) (Figure 1A & E). No changes between baseline and mild hypoxia (2-10Hz) were observed in the randomness or variability in AP firing, indicating that a minimum intensity is required to modify CB sensory patterning. 2-10Hz, but not over 30Hz hypoxia significantly increased the maximum IF

compared to baseline (baseline:109.9±14.4Hz vs 2-10Hz:160.0±8.55Hz, p<0.01), thus suggesting that some features of CB sensory discharge are not correlated with stimulus intensity.

Conclusion: This data shows that higher intensities of hypoxia modulate CB sensory patterning, most notably by becoming less random and more regular. Investigation of their functional significance and the impact of disease states may establish CB discharge patterning as an alternative therapeutic target. Future studies may also evaluate CB chemoafferent responses to other stimuli (hypercapnia, acidosis, mitochondrial inhibition) which may generate different chemoafferent patterns compared to hypoxia.

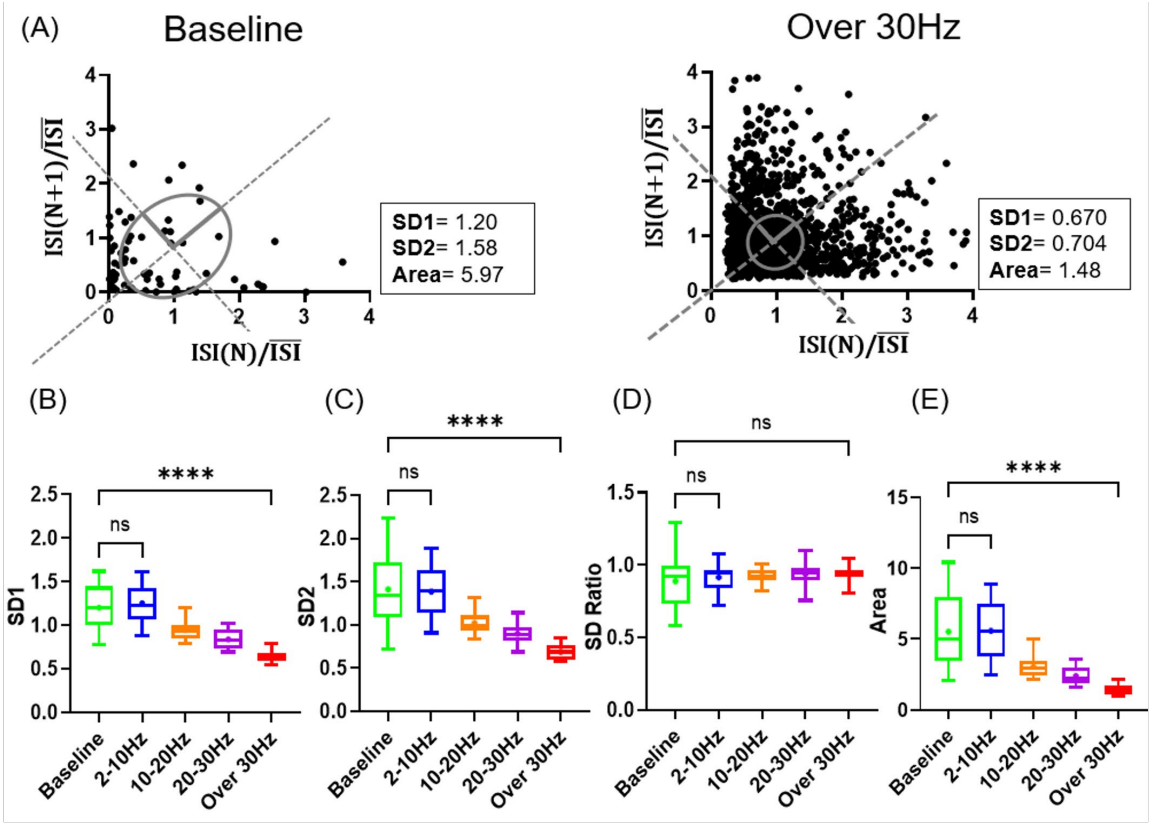

Figure 1. Increasing intensity of hypoxic stimulation reduces the frequency-normalised spike-spike variability of single fibre carotid body chemoafferents.

A) Example Poincaré plot analysis at Baseline and the peak hypoxic response (Over 30Hz). Calculated SD1,SD2 and Area values were used to construct an ellipse fitted along the line of identity where 'ISI(N)/ $\overline{ISI}$ = ISI(N+1)/$\overline{ISI}$'. The centre of the ellipse represents the mean of the dataset. B), C), D), E) represent grouped data from *n=21* chemoafferent fibres, derived from *n=19* CB preparations. Individual box and whisker plots represent spread of data within a selected AP frequency range with 25th percentile, median and 75th percentile forming the box, and whiskers extended out to indicate minimum and maximum values. + represents the mean spread of grouped data values. B) Mean SD1 C) Mean SD2 D) Mean SD Ratio E) Mean area of fitted ellipse, representing overall variability. 'ns' denotes no statistical significance whilst **** denotes a significance level of p<0.0001, for AP frequency ranges of 2-10Hz and Over 30Hz, compared with Baseline group; one-way repeated measures ANOVA with Dunnett's post-hoc analysis.

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