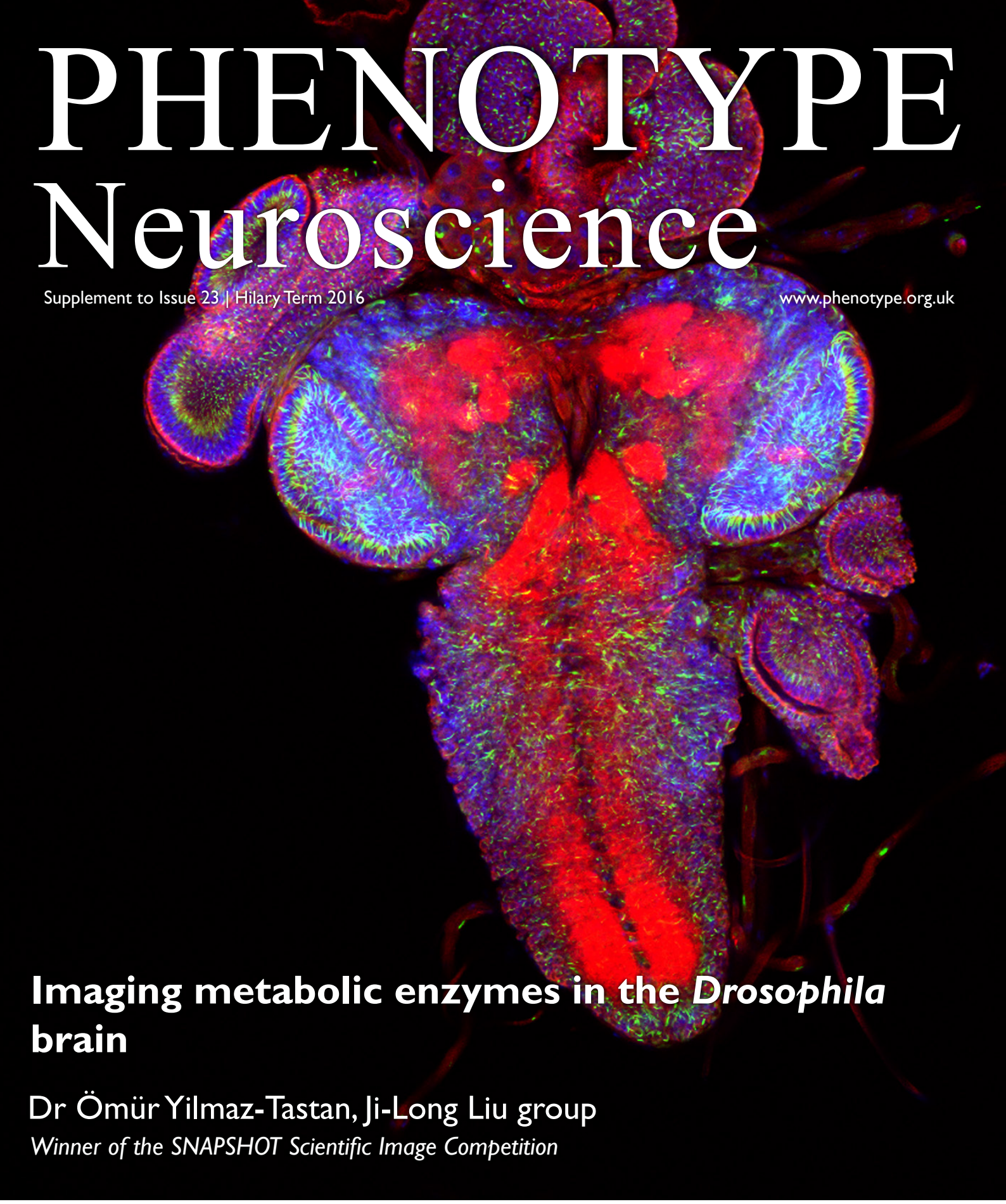
PHENOTYPE
Neuroscience

Supplement to Issue 23 | Hilary Term 2016

www.phenotype.org.uk



Imaging metabolic enzymes in the *Drosophila* brain

Dr Ömür Yilmaz-Tastan, Ji-Long Liu group

Winner of the SNAPSHOT Scientific Image Competition

Neuroscience

Encoding information in neural circuits

Unravelling the complexity of sleep

Psychology and music

OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

OUBS

CONTENTS

neuroscience

Cover Image: A *Drosophila* larval brain that overexpresses CTP synthase (green), showing Discs Large (red) which is important for cell polarity and proliferation, and nuclei (blue). CTP synthase marks a novel distinct filamentous structure (cytoophidium) that houses inactive metabolic enzymes ready for usage. Dr Omur Yilmaz-Tastan is a research assistant and laboratory supervisor in Dr Ji-Long Liu's lab at the department of Physiology, Anatomy and Genetics.

OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

MEDICALSCIENCES Skills Training

OXFORD UNIVERSITY PRESS

WILEY-BLACKWELL

EDITORIAL TEAM

OXFORD UNIVERSITY PRESS

SNAPSHOT

Research Image Competition

Win a £50 book voucher kindly provided by Oxford University Press!

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of the Trinity 2016 issue of *Phenotype*.

To enter, send images to rebecca.hancock@linacre.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

Write for Phenotype?

- The deadline for article submissions is Friday of 8th week, 11th March 2016
- We accept articles on any aspect of biological research, science impact & science education
- Articles can be either 650 or 1300 words

If interested, please get in touch: rebecca.hancock@linacre.ox.ac.uk

Work for Phenotype?

If you'd like to get involved in editing, production or management of Phenotype, please get in touch: rebecca.hancock@linacre.ox.ac.uk

Encoding information in neural circuits

by Inês Barreiros

Communication between neurons forms the neural basis of our capacity to perceive sensations. Skin peripheral afferent neurons contain mechano-gated ion channels that generate patterns of electrical transient impulses known as action potentials (APs). These are transmitted through the appropriate sensory pathways from neuron to neuron until they reach the integration unit responsible for their interpretation, the somatosensory cortex (1). How the brain translates those AP patterns into sensory information is a puzzling question in the neuroscience field. Which features of neuronal activity allow the cortex to distinguish whether we are touching a smoother or a rougher texture? This relationship between a stimulus and its activity pattern is known as the 'neural code'.

Following the first experiments with activity recordings in muscle sensory neurons in 1926, it was generally assumed that afferent neurons encode sensory information using a rate coding strategy, in which information is carried by the average number of times that a neuron is activated (2). This was the first solid hypothesis for how information is encoded within brain circuits, and persisted as the most plausible one for decades. However, this coding strategy was later revealed to be insufficient to accurately codify stimuli involving temporal features or the cooperation of several sets of neurons. As a result, the concept of a coding strategy emerged in which the precise timing of APs carries important information, allowing for better discrimination between stimuli (Figure 1). This strategy, known as the temporal code, helps to distinguish between similar but distinct neural inputs by allowing the integration of a larger amount of information in the same activity pattern (3).

While the rate of APs seems to be essential to discriminate highly distinct features, precise timing is crucial to distinguish between similar stimuli or encode information about stimuli with a clear temporal structure, e.g. sound localization or stimuli frequency (4-6). Hence, to allow more accurate sensory encoding and perception, rate and temporal coding schemes are likely to complement each other (7). Additionally, activity of neuronal populations with distinct characteristics is likely to shift the processing of information towards one strategy or the other by being selectively activated by different kinds of stimuli.

In Dr Michael Kohl's group, in the Department of Physiology, Anatomy and Genetics at the University of Oxford, we aim to understand neural coding principles by simultaneously manipulating and recording the activity of specific neuronal populations, using a combination of electrophysiology and optogenetic tools. By expressing light-activated ion channels in cells that express specific gene promoters we are able to manipulate the activity of genetically defined neuronal populations. The activity of those neurons can be manipulated using pulses of light at specific wavelengths. Alterations at the single cell level are measured using patch-clamp recordings. Alternatively, we use genetically encoded calcium indicators to quantify activity changes at the network level. By measuring the properties of each neuronal population, and testing how their manipulation affects the brain's ability to discriminate between different stimulus pairs, we can gain important information concerning the principles underlying the proposed coding strategies.

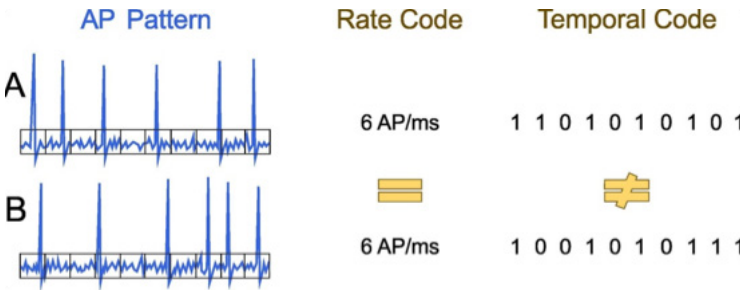


Figure 1: Rate vs temporal coding strategies. The AP patterns A and B represent the same or distinct information, according to the rate and the temporal coding strategies, respectively.

A better understanding of how the brain encodes information and the contribution of each neuronal population to this process will help us to solve one of the most fundamental neuroscience questions, allowing us to get closer to unravelling the mysteries of how the brain works.

References

1. Gerstner W, et al. (1997) Neural codes: firing rates and beyond. P Natl Acad Sci USA 94(24):12740–12741.
2. Adrian ED & Zotterman Y (1926) The impulses produced by sensory nerve-endings: Part II. The response of a Single End-Organ. J Physiol 61(2):151–171.
3. Miller JP (1994) Neural coding. Neurons cleverer than we thought? Curr Biol 4(9):818–820.
4. de Kock CP, et al. (2007) Layer- and cell-type-specific suprathreshold stimulus representation in rat primary somatosensory cortex. J Physiol 581(Pt 1):139–154.
5. Jadhav SP, et al. (2009) Sparse temporal coding of elementary tactile features during active whisker sensation. Nat Neurosci 12(6):792800.
6. O'Connor DH, et al. (2013) Neural coding during active somatosensation revealed using illusory touch. Nat Neurosci 16(7):958–965.
7. Kumar A, et al. (2010) Spiking activity propagation in neuronal networks: reconciling different perspectives on neural coding. Nat Rev Neurosci 11(9):615–627.

Inês Barreiros is a research assistant in Dr Michael Kohl's group in the Department of Physiology, Anatomy and Genetics.

Psychology and music

by Vani Rajendran

The earliest evidence for music-making dates back tens of thousands of years to a time when modern humans had only just emerged as a species. Since then music as a medium has evolved and diversified, accompanying the rise and fall of every great empire—packaging within it unique elements of human history. Music is indisputably linked with human thought, both in its creation and its consumption. Its enormous variety gives us a glimpse of life throughout different cultures and times.

Dr. Robert Saxton, Professor of Composition, and Fellow and Tutor in Music at Worcester College, Oxford, shares some insight into how human thought has shaped the evolution of Western music.

VR: Music history is characterised by its different periods. In your opinion, what factors influence transitions from one style to another?

RS: I don't think one can generalise about what creates transitions from one era to another, except to say that socio-cultural and political factors do enter the picture—for example the change from Latin to English in church music in 16th century England.

VR: So what do you think drives music nowadays?

RS: The fundamental shift in Western music was from music at the service of the Church to music as a commercial product. Although for centuries music was demanded by aristocrats and courts all over Europe, it was not a commercial product in the sense that it is now. This change came about largely due to the advent of the public concert and the rise of the industrial middle class. We appear now to have reached a situation whereby classical music has become part of the 'easy listening' culture and music. Rather than being an intellectual, social and expressive discourse, music has, in many respects, become a money-spinning product in the hands of the media and big business. The result is that, contrary to the situation in the late 18th century, the repertoire is what is demanded by those who sponsor concerts and orchestras, rather than profundity and progressive thought.

VR: In your opinion, what purpose does music serve to humans?

RS: Plato answered this in various ways but, in brief: music can aid those who have serious illness, it can enlighten as a spiritual experience, and it can incline us to certain modes of thought and behavior. Music is part of our essence, as we all dance and sing in one way or another, even if only internally. It can have a sociological function as 'group therapy' or 'group bonding' and, like sport, can bring people together (music and athletics were considered essential in Ancient Greece), and it brings comfort and solace.

Though the social context of Western music has undergone dramatic change over the last few centuries, we as humans have not changed much from a biological point of view. **Dr. Jan Schnupp**, Professor of Neuroscience and co-director of the Oxford Auditory Neuroscience Group, tells us more about the role our biology plays in the perception of sound and music.

VR: Our ancestors went to seemingly great lengths to manufacture instruments that would produce different pitches. What is the difference between any noise and a pitch?

JS: Noises have waveforms that are random and unpredictable. Sounds with pitch, in contrast, have a regular motif that is repeated. In nature, such regular sounds are usually created by physical oscillators: vibrating strings or membranes, insect wings or vocal cords. The pitch we hear tells us much about the sound source. High pitches are indicative of light oscillators under a lot of tension, while low pitches correspond to heavy oscillators at lower tension.

VR: So why is pitch more often the basis of music than noise?

JS: Both noises and pitches can be used to create interesting rhythms, but pitches in addition can carry melodies. The human ear and brain are remarkably good at discriminating different pitches. Even with little musical training, people can detect pitch changes by as little as 2%, so a musical pitch scale can give us a lot of different 'colours' to paint with. It is also possible that our brains have evolved to be particularly interested in pitch-based sounds, since most animal communication sounds have a periodic structure that lend them to pitch.

VR: Music is not dissimilar to language in the sense that both can be considered forms of communication. What are some similarities and differences in how music and language are processed?

JS: The happy/sad dimensions in music probably originate directly from the effect that emotional states have on the spoken voice. Thus, speech prosody and musical melody and rhythm are processed in much the same way and, as far as we know, by the same or overlapping brain areas. However, I think the music-language parallel is often overstated. Music is necessarily auditory; language can be written or signed in silence. Language has syntax and semantics that have no direct equivalent in music.

VR: Since music is clearly not our primary method of communication, what purpose do you think it serves to humans?

JS: Music is possibly the most important technology we have for manipulating our emotions, and its contribution to lifting our moods and facilitating human bonding cannot be underestimated.

Despite decades of cross-disciplinary research, no one truly understands why music came to exist, yet we can all attest to its pleasure. If one thing can be said with certainty, it is that music does not evolve in a vacuum; it evolves alongside us, simultaneously reflecting and influencing our thoughts in ways we are only beginning to understand.

Vani Rajendran is a DPhil student in Dr Jan Schnupp's group in the Department of Physiology, Anatomy and Genetics.

Unravelling the complexity of sleep

Sleep is a dynamic and highly intricate phenomenon that can be represented across different spatio-temporal scales, from long periods of behavioural quiescence occurring at a specific time of the 24h day, to localised millisecond changes in neuronal activity. Across different species, it is a highly conserved behaviour that is strictly regulated, suggesting it serves a critical biological role and confers an evolutionary advantage. Despite its apparent importance, the function of sleep remains an enigma, in part due to its remarkable complexity. Dissecting the physiological role of sleep remains an active area of research, particularly understanding the critical link between sleep and disease.

by Simon Fisher, Mathilde Guillaumin and Vladyslav V. Vyazovskiy

How is sleep regulated?

The dynamics of sleep and wake are primarily regulated by two processes. The first is governed by the internal circadian clock (or so-called Process C) and is mostly responsible for the timing and duration of sleep and wake across the 24h day. The second, homeostatic, process (also called Process S) tracks sleep-wake history and is reflected in a progressively increasing "sleep need" during waking and its dissipation during sleep. An important feature of the homeostatic Process S is that it has an 'intensity' dimension, such that the longer we stay awake, the deeper (and not merely longer) is our sleep. The conceptual framework of the two-process model (1) explains the interaction between these two processes and how they account for the timing, duration and intensity of sleep (Figure 1). However, it is important to keep in mind that sleep is not solely influenced by circadian and homeostatic processes. In fact, it varies greatly during our lifetime and can be modulated by external factors such as stress or the environment (such as ambient temperature and light, the latter having direct effects upon the circadian system). Understanding the mechanisms underlying sleep regulation will be an important step in helping us to answer the questions of why do we sleep and why is sleep so dramatically affected in disease.

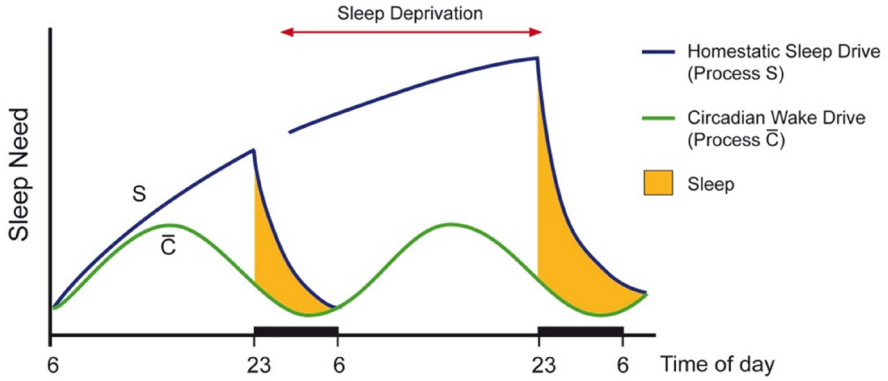


Figure 1: Time course of Process S and Process C (shown as an inverse function, C) during a typical 24h period and also following 24h of sleep deprivation. Note that Process S increases as an exponential saturating function during waking and decreases exponentially during subsequent sleep. Process C, on the other hand, represents the circadian drive for wakefulness during the day, which decreases at night. This induces a higher sleep propensity during the subjective night. The figure was adapted from (1), with permission from Springer, copyright (1982).

Historically, sleep was considered to represent a passive state during which the brain effectively goes 'offline' to permit its restoration and recovery. This viewpoint changed upon the discovery of rapid eye movement (REM) sleep (also called paradoxical sleep) in 1953 by Aserinsky and Kleitman (2) in which brain activity is more reminiscent of an active waking state, although notably muscle atonia (or relaxation) occurs through motor output inhibition. The two distinct states termed non-rapid eye movement (NREM) sleep and REM sleep alternate regularly across the sleep period. Usually we have four or five NREM-REM sleep cycles each night, while small rodents, such as mice, may have more than a hundred of such transitions every 24 hours. During NREM sleep, neurons in the neocortex fire quasi-synchronously in a characteristic slow pattern in which they switch every few hundred milliseconds between 'on' periods, when they fire as in an awake brain, and 'off' periods, when they cease firing altogether. The summation of excitatory and inhibitory currents generated by cortical and subcortical neurons can be recorded in the electroencephalogram (EEG) using electrodes placed on the surface of the skull or intracranially.

The master circadian clock is now known to reside in the suprachiasmatic nuclei (SCN) of the hypothalamus of the brain. However, the location or identity of brain mechanisms responsible for the homeostatic regulation of sleep remains unknown. Within NREM sleep, variations in the size or amplitude of EEG slow waves can be identified, which have been shown to reflect the preceding sleep-wake history: in both animals and humans, the longer the time spent awake, the higher the levels of EEG slow-wave activity (SWA; defined as spectral EEG power density < 4 Hz) during subsequent sleep (3). Thus, EEG slow waves reflect not only whether we are awake or asleep at any given moment, but also for how long we have previously been awake or asleep. Since we know a significant amount about the neuronal and network mechanisms underlying slow waves, this provides a unique opportunity to gain better insights into the mechanisms of sleep regulation in general. Various mathematical modelling approaches have been developed and utilised to investigate these mechanisms, among which the "two-process" model has been the most widely adopted. This model addresses both the circadian and homeostatic aspects of sleep regulation (1, 4) and can be used to predict the levels of SWA depending on the sleep-wake history of an individual. Presently, most elaborated versions of the model have been applied to human datasets. Our laboratory is currently adapting the model to data recorded from mice, whose spatio-temporal

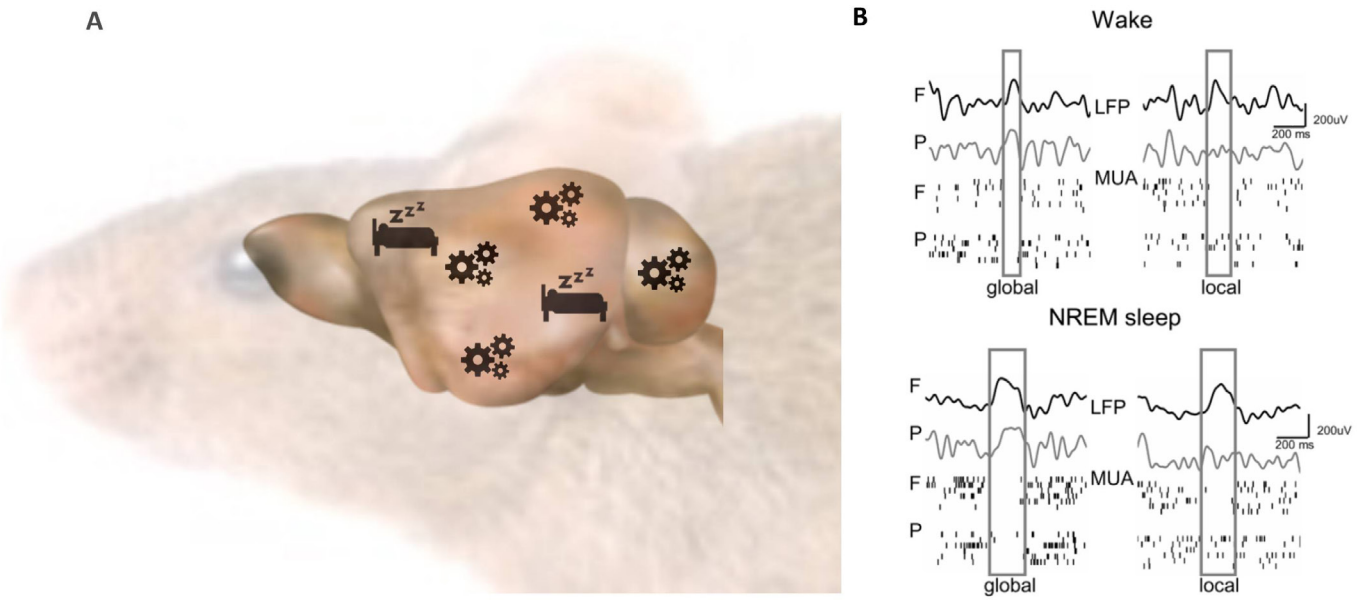


Figure 2: : Local versus global sleep. (A) Schematic representation of the ability of neurons to briefly go "offline" in some cortical areas of the brain while not in other areas; these periods of "local sleep", particularly evident during prolonged wakefulness, occur despite the animal appearing active and behaviourally awake. (B) Upper: wake local field potential (LFP) recorded in the frontal (F) and parietal (P) cortex, showing global or local frontal 2-6 Hz waves (boxes). Raster plots of the corresponding multiunit activity (MUA) are shown below. Lower: LFP recorded during non-rapid eye-movement (NREM) sleep depicting local or global frontal slow-waves (boxes). MUA raster plots are shown below. Panel B was adapted by permission from Macmillan Publishers Ltd: Nature (472(7344):443-447), copyright (2011). Available from http://www.nature.com/index.html

dynamics differ considerably from humans, with more fragmented polyphasic sleep episodes and state transitions exhibiting much faster time scales.

The enigma of why we sleep

The reason we sleep remains largely unknown despite us spending approximately one third of our lives in this perplexing state, which we usually take for granted. It is often only when our sleep is disrupted that we become aware of its importance for physiological and mental well-being. It has been established that loss of sleep can result in performance deficits, cognitive slowing and reduced attention, and has been implicated in a number of major industrial environmental accidents including Chernobyl and the Exxon Valdez oil disaster. While the role of sleep as a whole is suggested to provide "recovery" from preceding waking, despite extensive research the field is yet to reach a consensus regarding the precise function(s) of sleep. Why is the function of sleep so elusive? One view is that sleep originated from a single vital function and gradually evolved to serve additional opportunistic roles providing adaptive advantages. It is well known that most, if not all, animals carefully studied to date sleep, even when facing the danger of being predated. Many hypotheses regarding the function of sleep have been proposed, including regulation of the immune system, remodelling of synaptic connections (neuronal plasticity), memory consolidation, energy homeostasis or the restoration of behavioural and cognitive performance degraded during prior waking (5). Another view is that sleep is a "default" state of the brain or the organism, or is a state of "adaptive inactivity". At any rate, it is becoming increasingly clear that to understand why we sleep, we first need to get a better understanding of what sleep is.

> "'Local sleep' occurs when some parts of the brain are asleep... while others remain awake"

A local component to sleep: implications for sleep/wake quality

In recent years it has become clearer that neither sleep nor waking is a continuous, homogenous or global "all or none" phenomenon, but rather both reflect pronounced changes in many behavioural and physiological variables. Complex differences can be found temporally within states and also in their global/local dynamics across the brain. For example, upon awakening cortical neuronal firing exhibits substantial variability, suggesting a fully awake brain state is not immediately re-established (6). Similarly, an intricate picture has been revealed at the beginning of NREM sleep episodes, where both time of day and preceding state history contribute to the characteristics and dynamics of EEG slow waves (7).

One concept our laboratory is interested in further understanding is the occurrence of 'local sleep' and its implications for behavioural performance and functioning. 'Local sleep' occurs when some parts of the brain are asleep or 'offline' while others remain awake (Figure 2A). According to this view, sleep may

emerge at the level of local neuronal networks as a direct consequence of fine synaptic modifications and regional changes in neuronal connectivity arising from specific preceding waking activities. In support of this concept, several studies have found local increases in EEG SWA after either passive stimulation or following active learning involving discrete brain areas. Furthermore, in awake rats after a period of prolonged wakefulness it was demonstrated that cortical neurons could briefly go 'offline' (as in sleep) in one cortical area but not in another. Intriguingly, these intrusions of 'local sleep' were associated with performance deficits, despite the animal appearing to be awake both behaviourally and from inspection of the EEG (8). Such periods of local sleep, defined based on local field potentials and multiunit activity (MUA) from different cortical regions, can occur in both waking and NREM sleep states (Figure 2B).

Local sleep is not necessarily maladaptive, but may have an important function, such as allowing some parts of the brain to "rest" while others continue to work. On the other hand, it may be disruptive for brain function. Stemming from this, one potential explanation for poor waking quality after sleep disruption is the unwanted intrusion of brief episodes of 'local sleep' during the day resulting in overall poor wake quality, as manifested in attention lapses and other deficits. For example, under certain situations (e.g., in sleep apnoea where frequent awakenings occur across the night), some areas of our brain may be unable to "fall asleep" and remain in a state of "local wakefulness", resulting in us experiencing sleep of poor quality even though behavioural observation or scalp-recorded EEG signals would suggest we have been asleep all night. Taking this concept further, we are interested in understanding the factors that determine sleep quality and the consequences of sleep loss on various aspects of behaviour and performance. We hypothesize that sleep quality, both in terms of how refreshed we feel upon awakening and how well we function during the day, depends precisely on the amount of local sleep and local wake that occurred during the night. We are currently testing this hypothesis using electrophysiology and behavioural tasks in mouse models.

Overall, our research aims to elucidate the mechanisms governing the spatio-temporal dynamics of brain activity during sleep to understand not only what sleep is but also why it is necessary. We employ a combination of EEG and neuronal activity recordings from individual cells in the mouse brain. We are hoping to provide a better understanding of the effects of sleep deprivation and disruption on subsequent sleep and performance. To this end, we utilise behavioural tasks to measure a range of cognitive functions, including learning and decision making. In addition, we are investigating the effects of ageing upon the properties of sleep homeostasis in general and in particular on the fine dynamics of slow wave oscillations. As part of the Oxford Sleep and Circadian Neuroscience Institute (SCNi), collectively our research is aimed at understanding the neural underpinnings of sleep, and in a wider context the relationships between sleep, circadian physiology and health, including the impact of sleep disruption upon psychiatric disorders and neurodegenerative disease. Such conditions are consistently associated with disrupted sleep patterns, and although most of them are both progressive and irreversible, the idea that the symptoms can be improved through the normalization of sleep abnormalities is now emerging (9).

References

1. Borbély AA (1982) A two process model of sleep regulation. *Hum Neurobiol* 1(3):195-204.
2. Aserinsky E & Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 118(3062):273-274.
3. Achermann P & Borbély AA (2003) Mathematical models of sleep regulation. *Front Biosci* 8:s683-693.
4. Daan S, et al. (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. *Am J Physiol* 246(2 Pt 2):R161-183.
5. Krueger JM, *et al.* (2015) Sleep function: Toward elucidating an enigma. *Sleep Med Rev* 28:42-50.
6. Vyazovskiy VV, *et al.* (2014) The dynamics of cortical neuronal activity in the first minutes after spontaneous awakening in rats and mice. *Sleep* 37:1337–1347.
7. Cui N, *et al.* (2014) Long-term history and immediate preceding state affect EEG slow wave characteristics at NREM sleep onset in C57BL/6 mice. *Arch Ital Biol* 152(2-3):156-168.
8. Vyazovskiy VV, *et al.* (2011) Local sleep in awake rats. *Nature* 472(7344):443-447.
9. Wulff K, *et al.* (2010) Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nat Rev Neurosci* 11(8):589-599.

Vladyslav Vyazovskiy is Associate Professor of Neuroscience at the Department of Anatomy, Physiology and Genetics and is part of the Oxford Sleep and Circadian Neuroscience Institute (SCNi). Both Simon Fisher (postdoctoral researcher) and Mathilde Guillaumin (DPhil student) are members of Dr Vyazovskiy's lab.

absolute antibody

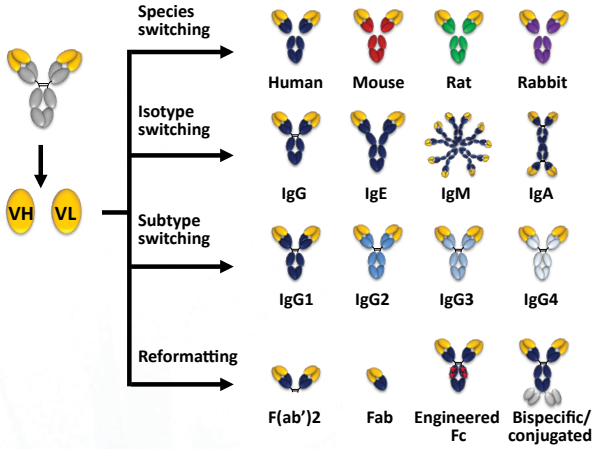
Catalogue

Classic Markers - F4/80 [Cl:A3-1(recombinant)], Ly6G [1A8], CD134 [OX86], CD200R [OX131], various anti-CD4 and CD8 clones

Research Tools - Anti-epitope tags, DNA-quadruplex, phosphotyrosine, FcR Block, isotype & loading controls
Research Grade Biosimilars - Campath, Cetuximab, Trastuzumab, Infliximab, Muromomab, Rituximab, Eculizumab and many more...

New Formats - Recombinant Fab fragments, dimerization-domain stabilized recombinant F(ab')2 (AbFab2™), Fc Silent™, goat **monoclonals**

Engineering



Sequencing

Sequence your hybridoma in 1-2 weeks.

Protect or patent antibody sequences.
Identification of aberrant/additional light chains or variable-domain framework mutations affecting clonality and expression levels.

Combine with our engineering and expression services to harness full potential of recombinant technology for your monoclonals.

Expression

Transient, serum-free, HEK293-based expression system.

6-8 week turnaround from sequencing of hybridoma to shipment of 1 mg - 2 g of recombinant antibody.

Ultra-pure (>98%), monomeric (>98 %) and low endotoxin < 0.5 (EU/mg).

www.absoluteantibody.com