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Neural Oscillations: Phase Coding in the Absence of Rhythmicity

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In the brain, coding information in the phase of neural firing relative to some baseline oscillation offers numerous theoretical advantages. New research suggests this may occur even when the baseline frequency is highly irregular, as seen in bats and humans.

Neurons in the brain communicate by means of action potentials — brief membrane voltage 'spikes' that travel rapidly along the axon to stimulate the release of chemical neurotransmitters at the pre-synaptic bouton. Although the mechanisms of action potential generation and propagation are well established, there has been a long-standing debate in the neuroscience community over the manner in which trains of these spikes might represent external variables or internal states. Early studies suggested that neurons primarily encode information in their firing rate — the number of action potentials generated per unit time. However, theoretical and empirical evidence suggests that the brain can also encode information in the precise timing of neural activity [1]. Under one powerful and parametric form of temporal coding, the firing phase of a neuron with respect to an ongoing 'baseline' oscillation in the local field potential (LFP) is used to represent information. This baseline signal is often thought of as a 'clock', providing a common reference for firing phase across brain regions.

Phase coding of this nature has been well studied in place and grid cells of the rodent hippocampal formation: neurons that fire selectively when an animal visits specific regions of space to provide a 'cognitive map' of known locations. The rodent hippocampal LFP is dominated by ~8 Hz theta oscillations during self-motion and, importantly, the theta firing phase of both place and grid cells becomes progressively earlier as their spatial receptive fields are traversed [2,3]. Place and grid cell responses have also been identified in both bats and humans but, interestingly, in the apparent absence of a regular LFP oscillation, calling into question the existence of phase coding in these species [4–6]. Recent data from Eliav *et al.* [7] resolve this issue by demonstrating that a phase code for location is exhibited by place and grid cells in the bat hippocampal formation, relative to the LFP, despite the absence of any sustained rhythmicity in that signal.

In their new study, Eliav *et al.* [7] first examined hippocampal LFP power spectra and spike train autocorrelations from interneurons, grid and place cells in crawling and flying bats. Consistent with prior reports, they found no evidence for any regular oscillatory activity — that is, prominent peaks reflecting relatively high amplitude signals with an approximately constant frequency. The authors contrast this result with a publicly available rodent data set, where theta rhythmicity is clearly visible in the spike train of most hippocampal neurons during movement.

Nonetheless, by filtering in a wide (1–10 Hz) frequency band, Eliav *et al.* [7] went on to demonstrate that activity within a large proportion of the cells recorded in bats remains phase locked to the LFP, despite the frequency of that signal varying dynamically over a wide range. More importantly, they were able to show that a significant proportion of bat place and grid cells do exhibit phase precession — firing at progressively earlier phases of the irregular LFP signal as the spatial firing field is traversed. These results have two important implications for future studies of neural coding: first, they demonstrate that phase coding can be implemented in the mammalian brain in the absence of a regular baseline oscillation; and second, they show that the phase code for location exhibited by place and grid cells is preserved across species.

These findings also raise several further questions. First, is there any advantage in dynamically varying the baseline frequency over such a wide range? It is possible that, beyond the firing rate and phase of principal cells, changes in LFP frequency could reflect the encoding of additional behaviourally relevant information. Indeed, theta frequency in the rodent increases slightly but consistently with running speed [8], and Eliav *et al.* [7] show that the variable LFP frequency in bat hippocampus is also slightly but significantly higher during faster movement. Nonetheless, their results also demonstrate that cycle-by-cycle changes in LFP frequency are no different from a random shuffle, whereas some local structure would be expected if frequency were being used to represent some slowly varying quantity.

Alternatively, it is possible that changes in LFP frequency could modulate both synaptic plasticity within the hippocampus and the impact of hippocampal cell firing on downstream neurons. A specific phase offset between the firing of two cells corresponds to a much smaller temporal offset at high frequencies, such that the strength of connections between those cells are more likely to undergo activity-dependent synaptic plasticity, and the combined output of those cells is more likely to produce firing in a post-synaptic target. Hence, dynamic changes in LFP frequency could allow the robust phase code for location to be maintained while

modulating both the encoding of new memories and the influence of hippocampal activity on downstream regions.

Second, what is the functional role of hippocampal phase coding in spatial cognition and memory? It is well established that encoding information in the timing of spikes incurs a lower metabolic cost, facilitates rapid processing, and can be used to disambiguate stimuli that are presented simultaneously or generate similar firing rates [1]. In the rodent hippocampus, theta phase precession also produces rapid sequences of place or grid cell activity within each oscillatory cycle that correspond to the sequence of spatial firing fields being traversed on a behavioural timescale [9]. These 'theta sequences' encode movement direction, even when that diverges significantly from the direction the animal is facing (as represented by head direction cells) [10]. Moreover, theta sequences may be useful for simulating future paths, computing the distance and direction to a goal, and encoding behavioural trajectories in synaptic weights through a spike-timing dependent plasticity rule [11,12]. The latter hypothesis is supported by recent rodent data which indicate that, when place cell theta sequences are disrupted by passive movement of an animal through its environment, then those behavioural trajectories are no longer recapitulated as hippocampal replay events during subsequent sleep [13].

Third, what generates the hippocampal phase code? Several potential mechanisms have been proposed. The oscillatory interference model posits velocity-controlled oscillators whose frequency varies with movement speed and direction such that their phase tracks displacement along specific preferred directions. Cells that receive input from multiple velocity-controlled oscillators with different preferred directions will then fire whenever those inputs are in phase [14,15]. If the LFP is assumed to reflect the summed activity of all velocity-controlled oscillator inputs, then a frequency difference between 'active' neurons and the baseline signal that is proportional to running speed follows naturally [14]. The absence of regular oscillatory activity in the bat hippocampal formation had previously been taken to causally disprove the oscillatory interference model [5]. However, phase and frequency are independent features of any oscillatory signal, such that the requisite phase relationships between velocity-controlled oscillator inputs can be maintained regardless of dynamic changes in the baseline frequency [16]. Indeed, the new results presented by Eliav *et al.* [7], where the relationship between location and firing phase is robustly maintained despite wide fluctuations in LFP frequency, are fully consistent with such a scheme.

Alternatively, phase coding could be generated by the interaction of periodic inhibition and a ramping depolarisation of individual cells [17]. In its most simple form, however, this model predicts that firing rate and phase would be correlated, which appears to be at odds with rodent empirical data [18]. Moreover, if wide fluctuations in LFP amplitude reflect variation in the level of inhibition, then the 'ramp' depolarization would need to precisely match that variation to produce a monotonic change in firing phase. It is interesting to note that both models predict an inverse relationship between LFP frequency and firing rate, as longer cycle durations provide a greater window for the temporal integration of afferent inputs and therefore a greater probability of firing. It is not clear from the data published by Eliav *et al.* [7] whether or not this is the case in the bat, and this should be tested in future work.

Finally, and perhaps most importantly, is irregular phase coding of the kind described by Eliav *et al.* [7] also observed in the human hippocampal formation? Like the bat, LFP power spectra and spike train autocorrelations recorded from the hippocampi of pre-surgical epilepsy patients rarely show regular oscillatory activity, although there is some evidence to suggest that phase locked neural activity is present and may be relevant for mnemonic function [19,20]. Inspired by Eliav *et al.* [7], future single unit recording studies can establish whether the computational advantages offered by phase coding are also harnessed by the human brain.

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Domestication: Colour and Flavour Joined by a Shared Transcription Factor

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The citrus family of fruiting trees is interwoven with Eastern and Western histories, and ritualised in at least four religions. A major gene has now been found and characterised that affects both visual appeal and flavour, revealing why citrus became so attractive to domestication.

The cultivation of citrus is likely to have begun over 3,000 years ago, originating in India, South China and South-East Asia. Key to its domestication must have been its visual appeal — imagine the shock of something so yellow or orange — and also its taste. In a paper by Butelli *et al.* [1] published in a recent issue of *Current Biology* we can suddenly understand how colour and taste are closely tied to each other, by job-sharing the same basic-helix-loop-helix (bHLH) transcription factor called *Noemi*.

Citrus is a group of flowering trees and shrubs that includes many well-known crops with high economic value, and is known for its value to the consumer for nutritional and medicinal benefits. Citrus has particularly high levels of ascorbic acid (vitamin C) and carotenoids (provitamin A) but is characterised by a distinct lack of anthocyanins: their presence would have made oranges and lemons red, but the fruit would have lost world-naming rights to at least three colours (orange, lemon, and lime)!

Anthocyanins are flavonoid compounds providing red, blue and pink hues, while carotenoids in citrus tend to be yellow and orange. Anthocyanins have both physiological and ecological roles: they are most prominent in young leaves where they protect developing tissues from light stress, and mature fruit and flowers where they attract both pollinators and seed dispersers. Furthermore, the biosynthesis of anthocyanins is linked to proanthocyanins (e.g. tannins) which have further roles, such as stress tolerance and strengthening the seed coat. However, several citrus varieties (or species, or accessions; the naming of this family is complicated by centuries of interspecific hybridization and apomixis) completely lack anthocyanins (in leaves and fruit) and proanthocyanins (in seeds). This trait is linked to fruit acidity; the loss of anthocyanin-linked colour correlates with a loss of fruit acid, with the pH of the fruit cells rising as much as 3 pH units, from pH 2.5 to 5.5! Butelli's paper [1] describes mutations in *Noemi*, a bHLH transcription factor that regulates both flavonoids and acidity, which have been selected for during the domestication of citrus. Selection may have been first on the appearance of purity (white flowers and bright yellow fruit) for religious rituals. In 'Yemen' and 'Greek' citron (the Etrog, used in Jewish Sukkot celebrations) and the ancient Chinese fingered Qingpi citron, leaves and flowers are anthocyanin-less and the fruit are no longer acidic. Even though these are rarely consumed —varieties carrying mutations in *Noemi* have crossed the continent, and so these mutations trace the spread and domestication of citrus over many centuries.

It is well known that MYB and bHLH transcription factors interact to control expression of the enzymes of the anthocyanin pathway (e.g., [2]). However, MYB transcription factors are a large family of genes (136 R2R3 MYBs in *Arabidopsis*) implicated in diverse roles, and bHLH transcription factors are equally numerous (139 in *Arabidopsis* [3]). Perhaps MYBs have received more attention simply because they are so much easier to clone; bHLHs typically have eight exons, while MYBs generally have three. It may also have to do with mutations in bHLH genes affecting multiple traits, making them more difficult to study.

In citrus it has been previously shown that an R2R3 MYB activator of anthocyanin biosynthesis called *Ruby* [4] is responsible for petal colour, young red