varied and sex hormone levels simultaneously monitored. Establishing this quantitative framework may enable expanding the classical model of sleep regulation to incorporate motivation (which we may call process M) as an additional factor. Second, having shown that dopaminergic VTA neurons are both engaged during and required for the modulation of sleep by motivation, this study will now enable the detailed circuit architecture underlying this process to be investigated. The fact that the dopaminergic projection from VTA to nucleus accumbens appears to be responsible for coupling motivational state to sleep regulation calls for more detailed investigation of the underlying neural elements. Electrophysiological recordings of nucleus-accumbens-projecting VTA neurons using optogenetic tagging (as in refs. 14,15) while monitoring both motivation and state transitions is likely to reveal how these neurons respond to both parameters at single-cell, single-action-potential resolution. Using viral tracing methods, the input–output relationships of these neurons can also be delineated. Together, these approaches will provide a much more detailed picture of how the relationship between motivation and sleep is implemented neurally. Finally, this study may have a broad, clinically translatable impact. As noted above, ADHD is a condition in which the relationship between motivation and sleep may be pathologically exaggerated. Could the dopaminergic actions of stimulants used to treat ADHD, such as amphetamine derivatives, be normalizing this exaggerated relationship? This, along with many other questions, can now be approached within the behavioral and circuit framework established by Eban-Rothschild *et al.*[6].

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A useful code for sequences

Nikolai Axmacher

A neural code for sequences needs to allow the recruitment of plasticity mechanisms that link successive items. New results suggest that this is achieved by coupling gamma band activity to specific phases of theta oscillations.

In their attempt to link cognitive functions to brain processes, cognitive neuroscientists inevitably face one of the greatest questions in neuroscience: what is the 'code of the brain'? In contrast to other codes, representations in the brain do not only need to provide reliable information about things in the world. They must also deliver this information in a way that allows the brain to usefully guide behavior. Thus, unraveling the code of the brain does not merely imply mapping individual object features onto spike rates in single cells or onto functional magnetic resonance imaging responses in specific brain areas. To unravel the code, it is not even sufficient to determine whether a certain item is encoded by the magnitude of cellular or network activity or by its specific timing (that is, whether a rate code or a phase code is employed). Instead, truly understanding the neural code involves finding out how brain representations of a given experience recruit those neural functions that are conducive to goal-directed behavior.

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An article by Heusser *et al.*[1] in this issue of *Nature Neuroscience* substantially advances our understanding of the neural code in this more profound sense. By testing predictions from an influential computer model[2,3], the researchers show for the first time that items within a sequence are represented such that the intrinsic properties of long-term potentiation can be recruited to link these items. Typically, events do not occur in a sufficiently rapid succession to fall into the temporal window of spike-timing-dependent plasticity, which lasts only a few tens or hundreds of milliseconds. Thus, there are likely to be mechanisms that bring the representations of these events closer together in time. In the model by Jensen, Lisman and Idiart[2,3], this is achieved by representing multiple items of a sequence via gamma-band activity (30–100 Hz) locked to consecutive phase ranges of theta (3–8 Hz) oscillations. While individual cycles of gamma activity are believed to represent individual items, it has been suggested that theta oscillations act as an internal 'pacemaker' of the brain.

Previous studies[4,5] have shown that gamma activity is modulated by the phase of theta oscillations in humans, a phenomenon known as cross-frequency coupling (CFC). Furthermore, in addition to the well-described phenomenon of phase precession of place cells[6], evidence from monkeys (for example, ref. 7) and humans (for example, refs. 8–10) has shown that specific contents are represented during distinct phases of neural oscillations and that phase coding is related to CFC[11]. However, no previous study has investigated whether consecutive items within a sequence are represented by locking of gamma cycles to consecutive phases of theta oscillations and whether this supports long-term memory for sequence information.

In the new study, participants studied lists of six consecutively presented trial-unique images. They were then asked which of two items from these lists occurred at an earlier list position, which required them to remember sequence information. During this task, the researchers measured their brain oscillations using magnetoencephalography. The authors report several findings that support the model of Jensen, Lisman and Idiart[2,3] (**Fig. 1a**). First, they tested sequence effects on the magnitude of CFC. When more and more gamma cycles occur during consecutive phase ranges of theta oscillations or when gamma activity also occurs during theta phases that are generally less preferred, a broader distribution of gamma activity across theta phases should result. Thus, the strength of CFC between gamma amplitudes and theta phases should be reduced. The authors indeed found such

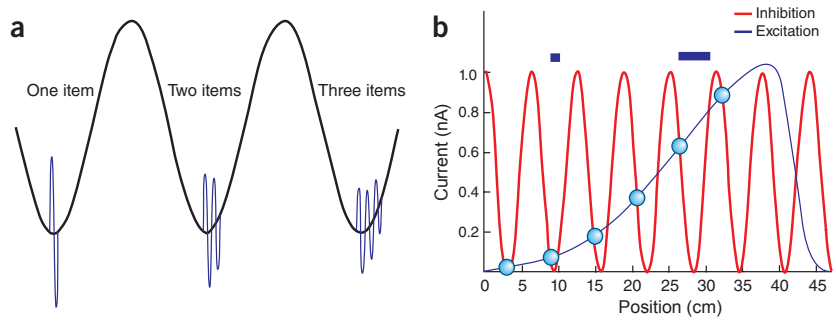

Figure 1 Phase coding of sequences. (**a**) Representation of sequences via cross-frequency coupling of gamma band activity (blue) to theta oscillations (black). Individual items are represented by single gamma cycles locked to specific phases of theta oscillations. During sequence encoding, the first items in the list are represented at the generally most preferred phase of theta (its trough), leading to pronounced cross-frequency coupling. Later in the list, items occur during less preferred theta phases as well, leading to a reduction of cross-frequency coupling strength. (**b**) Theta-inhibition model of phase precession. When rats approach the place field of a given place cell, the cell fires (circles) at progressively earlier theta phases. Longer excitable periods (blue rectangles) during specific phases of theta oscillations may result from a combination of theta-related inhibition (red line) and linearly increasing excitation (blue line) across consecutive theta cycles. However, this model also leads to a precession of excitable periods across theta cycles, whereas Heusser *et al.*[1] found that gamma cycles shift to later theta cycles. Panel **b** adapted from ref. 12, Nature Publishing Group.

a linear decrease of CFC strength across consecutive list positions. Source reconstruction analyses suggested a hippocampal origin of this effect, consistent with previous evidence for the role of the hippocampus in sequence encoding. However, the authors laudably caution against overinterpreting these latter results because hippocampal activity is notoriously difficult to measure noninvasively.

Second, they investigated whether the peak of gamma activity across the theta cycle shifted to later theta phases for later list positions. This analysis tests for phase coding even more directly because a reduction of CFC across list positions, by itself, could also result from non-systematically adding gamma cycles to variable theta phases. Indeed, the authors observed gamma activity at earlier theta phases for items early in the list than for items later in the list.

Third, while the authors did not test whether the linear reduction of CFC was behaviorally relevant, they did show that the shift in gamma activity across theta was. Only when participants were later able to remember the correct sequence of items did gamma power shift systematically to later theta phases across list positions. This important additional result underlines the functional relevance of the proposed phase-coding mechanism.

Interestingly, overall gamma power did not increase across list positions. This contrasts with the original proposal by Lisman and Idiart[2], according to which the processing of an increasing number of items is related to the accumulation of gamma cycles onto consecutive theta phase ranges. However, this original proposal was related to maintenance of multiple items in short-term memory, while the Heusser *et al.* paper investigates encoding of sequences into long-term memory. While both processes may rely on a similar coupling of gamma activity to theta phases and both recruit the hippocampus[5], they could differ with regard to important details, which might explain why some items are maintained for several seconds while others are encoded permanently. Multi-item short-term memory maintenance requires all items to remain immediately accessible, which could depend on their simultaneous representation by gamma cycles across multiple consecutive theta phase ranges. By contrast, more elaborate retrieval processes can be recruited during long-term memory of sequence information, and these items thus may not need to remain permanently represented via full gamma cycles.

The new results have several important implications. First, as discussed above, they suggest that the brain utilizes codes that adaptively recruit task-relevant processes such as spike-timing-dependent plasticity. Second, in a complement to previous results showing that enhancements of CFC support cognitive functioning[4,5,11], the new study demonstrates that reductions of CFC may also contribute: such reductions indicate that gamma activity occurs during more extended phase ranges of theta oscillations. This finding is consistent with the idea that oscillations in the theta[12] and alpha[13] frequency ranges are predominantly inhibitory, thereby restricting the occurrence of gamma activity to specific 'duty cycles'. Reduced inhibition implies more extended duty cycles and allows more information to be processed.

As with every discovery, questions for future research remain. First, do individual cycles of gamma activity indeed represent specific stimuli during sequence encoding? Multivariate pattern classification analyses have shown that decoding reliability of specific contents depends on the phase of low-frequency oscillations[9–11]. Within the framework of sequence encoding, one would predict that later list items can be decoded more reliably during later phases of theta oscillations.

Second, which mechanism controls the relationship of gamma activity to theta phases during sequence encoding? The authors showed that locking of gamma activity to later theta phases across list positions is not due to a shift in theta phase. However, how do gamma cycles at later list positions 'know' that they need to occur during more advanced theta phases? One possibility is that the results derive from a mechanism similar to one proposed for place cell precession (putatively, the cued recall of place sequences): an oscillating level of inhibition coupled to a linearly changing level of excitation[12] (**Fig. 1b**). Items early in the list are represented specifically at the theta phase that is generally most preferred, possibly because it reflects relatively low inhibition. As a result, CFC is high. By contrast, items at later list positions occur both during the most preferred and during less favorable theta phases, possibly because they recruit more excitatory activity (through an unknown mechanism). Notably, however, while place cell firing advances to earlier theta phases when rats approach their place field, in the current results, gamma shifts to later phases during sequence encoding. Future studies need to test whether similar mechanisms indeed account for the two phenomena and how their differences may be explained. But, despite these open questions, the results represent a significant advance in understanding how the brain utilizes an oscillatory network code in a manner appropriate for the induction of plasticity mechanisms that link items into a sequence.

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