and gain of function simultaneously, investigating how *C9orf72* expression levels affect disease pathology in repeat expansion models and, additionally, how the presence of a repeat expanded allele alters the lifespan of *C9orf72*-null mice. The authors used two independent mouse models of *C9orf72* hexanucleotide repeat expansions. The first model involves somatic transgenesis, where 66 copies of the GGGCC repeat are packaged into an AAV9 viral capsid[10]. Early transduction in neonatal mice results in progressive motor and behavioral deficits accompanied by DPR pathology and neuronal loss, as compared to controls injected with only two packaged repeats.

The second mouse model involves a human bacterial artificial chromosome (BAC) transgenic model expressing 450 disease repeats, but not the entire coding sequence of the *C9ORF72* gene. This model has also been shown to demonstrate cognitive deficits as well as late onset motor phenotypes and DPR pathology[2]. Importantly, both models, while expressing the disease repeat, produce no functional full-length C9ORF72 protein from the transgene. Mating these non-C9ORF72-producing transgenes to loss-of-function *C9Orf72* mice provides a unique opportunity to study the effects of repeat expansions while assessing the contribution of reduced *C9orf72* expression on disease pathology. These experiments have not been possible with other *C9ORF72* BAC transgenic models, where the repeat containing BAC also encodes a full-length, functional C9ORF72 protein and, for one model, multiple copies of smaller, non-expanded repeats.

The results of these experiments were quite telling. The presence of the AAV66 repeat in the combination homozygous *C9orf72*-null mice exacerbated the premature death seen in the *C9orf72*-null models. In addition, the introduction of the AAV66 repeat to *C9orf72* heterozygous mice, where *C9orf72* expression was reduced by 50%, resulted in premature death where none had been previously reported. The authors also noted an increase in the accumulation of poly(GA) and poly(GR) in an age-dependent manner in the AAV66 mice in the presence of either a loss or reduction in endogenous *C9orf72* expression. The data point to a synergistic effect of damage from the repeat that is enhanced by lowered levels of endogenous C9orf72.

The authors go on to demonstrate similar finding in the *C9ORF72* BAC450 model, where the reduction or absence of endogenous mouse C9orf72 exacerbates cognitive defects as well as triggering the emergence of new motor-related deficits. Interestingly, the *C9ORF72* BAC450 transgene had no effect on reduced survival of the *C9orf72*-null mice as seen in those treated with the AAV66 transgene. Similarly to the AAV66 model, DPR pathology was intensified by the loss of C9ORF72 function in the BAC450 experiments. Reduced *C9orf72* expression also worsened hippocampal neuronal loss and glial activation. These results are summarized in Fig. 1. Attempts to reveal underlying mechanisms for these observations found loss of endogenous C9orf72 expression coincided with decreased levels of autophagy markers in the BAC450 model. Therefore, a working hypothesis is that *C9orf72* loss interferes with the autophagic response, which subsequently leads to toxicity through the accumulation of DPRs.

While there remains much to be learned about the endogenous function of the *C9ORF72* gene and mechanisms behind the pathophysiology of *C9ORF72* ALS/FTD, the publication by Zhu et al. has provided convincing evidence that the loss or lowering of C9orf72 levels in mice in the presence of a toxic repeat has synergistic negative effects and exacerbates some facets of the disease. These are important results when considering disease-modifying strategies that could potentially lower the *C9ORF72* levels in attempts to reduce the toxic load of the repeat or interfere with overall transcription of the gene. Fortunately, as the authors note, current ASO-based therapies target the repeat containing transcripts without lowering overall C9ORF72 levels. So whether your favored hypothesis in *C9ORF72*-driven ALS/FTD was a loss of function or a gain of function, as we may have suspected, they may both be correct. ❐

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Competing interests
The author declares no competing interests.

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EPISODIC MEMORY

Hippocampal firing rates count

Sun et al. discover that neuronal firing rates of hippocampal place cells code for periodically repeating events and that the rate code can flexibly transfer to new situations. These findings suggest that abstract neural representations of regularly occurring events may be foundational for performing complex cognitive tasks.

Li Yuan and Stefan Leutgeb

For almost any task that requires cognitive control, we need to keep track of a sequence of events, sometimes in circumstances when each element in the sequence is exceedingly similar. This is not only the case in mundane circumstances but also in one of the most exciting settings, such as the Olympic Games (Fig. 1). In the 1,500-m run, how do elite athletes' brains distinguish between each of the four laps to correctly time their final sprint? Keeping track of the lap count probably does not require much effort because they have trained extensively to automatically update their mental image as they run along.

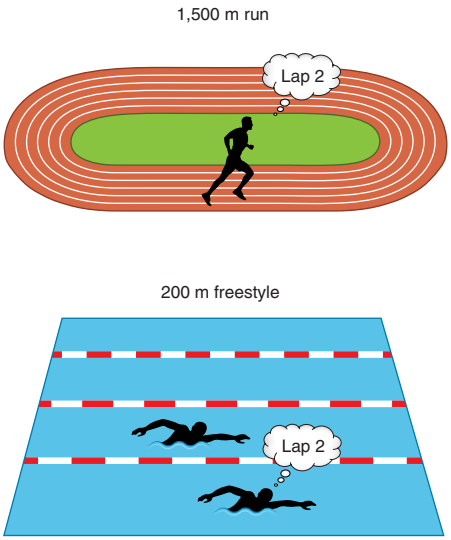

Fig. 1 | Firing rates of hippocampal cells keep track of lap count. Top: when applying the current findings to the human brain, neuronal activity patterns in an athlete's brain differ between each of the four laps of a 1,500-m run in a standard sports stadium. Bottom: when a track athlete cross-trains by swimming four laps of 50 m each, lap-specific neuronal firing patterns from the track transfer to the pool, which implies that the hippocampal neural code generalizes to represent regularly occurring events.

Nonetheless, lap information is vital because runners need to update their race strategy based on the distance to the finish line.

Despite the importance for cognitive function, neuronal activity patterns that correspond to abstract entities, such as early and late events in an episode, can be challenging for the brain to compute. These entities may not be associated with any particular set of unique sensory information, and reliance on the external world could even interfere with the representations of lap number. For example, views from within the stadium at the Olympic Games repeat during each lap, whereas the first lap in the Olympic stadium differs from the first lap in a stadium where qualifying events are held.

Among the many brain areas that could provide a signal about timing, the hippocampus is unique in that neuronal firing patterns here not only gradually change over time[1,2], but also resolve ambiguities that result from overlapping sensory features between events[3,4]. However, the neural code for periodically repeating events, such as laps that differ from each other only by when they occur, has been elusive. To address this question, Sun et al.[5] recorded large populations of hippocampal CA1 cells with a calcium imaging technique in an elegant behavioral design. While mice ran laps on a rectangular track, they were rewarded on only every fourth lap. As has been established in the Nobel Prize-winning discovery of 'place cells' by John O'Keefe[6], the default firing pattern for most hippocampal neurons is to be selectively active at a particular location ('the place field') along the track.

Place fields of various hippocampal cells were of course observed along the entire length of the track in the present study[5], but the intermittent reward on only every fourth lap resulted in the emergence of an additional intriguing phenomenon. When laps were counted with reference to reward delivery, the firing rate within the place field was consistently higher on a particular lap compared to other laps on the maze. For example, there was a population of cells that was most active on every lap 1 after reward delivery and another that was most active on every lap 2, and so on. The lap selectivity was already evident on the first day of testing but the abundance of lap-selective cells increased with further training. Similar reproducible rate changes within the place field had previously been found when sensory cues were varied within an environment or in response to particular running trajectories or food rewards[7–9], but none of the previous studies had shown that rate-coding for repetitive events can also occur when there are no immediate or immediately preceding sensory cues in the environment that distinguish between the events.

Furthermore, hippocampal coding has previously been shown to change over time in identical environments, such that any two identical events at different times have a different bar code of activity patterns, which could be used to uniquely identify an event[1,2,10]. Such gradually changing firing patterns typically occur over a longer time scale of minutes to hours, and repetitive patterns were not observed, not even for corresponding time points within the circadian clock[2]. The new results by Sun et al.[5] are thus unique in that they show not only distinct but also periodically repeating patterns for events that can only be distinguished by when in a sequence they occur.

After discovering the lap selectivity of hippocampal cells, the authors ask whether the activity patterns could be lap-specific for a number of more trivial reasons. For example, cells could respond to a particular distance traveled or at a particular time after reward delivery. If this were the case, doubling the length of the track should systematically delay the firing of these cells. However, the cells' activity patterns remained specific for their preferred lap number and, for the most part, also specific to their firing location on the short track. What if the number of laps between reward delivery was increased from 4 to 5? This resulted in the interesting outcome that lap 1 and lap 2 cells remained specific for their preferred lap while former lap 3 and lap 4 cells now switched to laps 4 and 5, respectively, as if proximity to reward were most relevant to the firing pattern of these cells. These results already give a hint that the lap-specific information generalizes in interesting ways.

Could this information thus also transfer to other events that are repeated? Perhaps not for counting the number of trips to the Olympics because those would, at least for most of us, be too infrequent and not have the embedded structure of repeating within a larger context. However, as the authors point out, this type of hippocampal coding may generalize to keeping track of how far into a multicourse meal we are across different restaurants. Or, in the hypothetical case of an Olympic runner of the 1,500-m race who cross-trained by swimming a 200-m freestyle race, we would expect hippocampal cells that are most active during the second lap on the track to also be most active during the second leg in the pool (Fig. 1).

It is feasible to test a close analog of the latter possibility, but rather than forcing mice to swim, the authors transferred mice that had been rewarded on every fourth lap on the rectangular maze to a circular maze where they were also rewarded on every fourth lap. As expected from previous studies that showed substantially different hippocampal activity patterns across mazes[11], the spatial firing patterns of cells did not correspond across these maze designs. Despite the completely reorganized spatial firing patterns, cells generally retained their lap selectivity. For example, cells that were most active in lap 3 on the rectangular maze would immediately be most active in lap 3 on the circular maze, even though the place fields across track shapes were not at corresponding positions along the track. This is a striking example of how the hippocampal rate code identifies the position in a sequence of events across environments even when the associated spatial coding completely reorganizes.

This raises the question whether the opposite scenario can also be observed, i.e., whether the hippocampal spatial code can be retained while lap coding reorganizes. Because this question cannot be readily addressed with behavioral manipulations, the authors took advantage of the previous finding that hippocampal place fields persist when inputs from the medial

entorhinal cortex are inactivated[12], and they reproduced the previous finding. However, the firing rates within the retained place fields were no longer favoring the same lap as before the entorhinal inactivation. It is possible that lap selectivity is either precluded without entorhinal inputs or, as suggested in examples in their Fig. 6 (ref. [5]), reorganized so that a former lap 2-selective cell would, for example, now have become lap 3-selective. Reorganization as opposed to reduction of lap-selective activity could occur if inactivation is only partial, such that reduced inputs from a subset of layer III cells allow for inputs from a different subset to now dominate. Irrespective of the precise nature of the influence of entorhinal cortex on lap selectivity, the results convincingly show that entorhinal inputs to the hippocampus have a pronounced effect on coding for lap count as opposed to spatial coding.

The finding of a contribution of entorhinal cortex to specifically keeping track of a sequence of events raises the question of whether abstract representations of regularly repeating events may have any resemblance to the periodic representation of space that is provided by grid cells in the medial entorhinal cortex[13]. It will thus be interesting to determine whether firing patterns that are organized with reference to prominent event boundaries also exist in medial entorhinal cortex and, if found there, whether these entorhinal firing patterns show periodicity that relates in interesting ways to the spatial periodicity of grid cells in the open field. For example, grid cells could be influenced by not only the location of reward delivery[14,15], but also by more abstract event boundaries, such that they remain active during particular segments of an episode irrespective of the exact time and distance that is covered within a segment.

Even without these follow-up studies, the new results raise the intriguing possibility that the interconnected entorhinal and hippocampal regions have the capability of generating firing patterns for the position within a sequence without being tightly bound to time or distance. Future work will need to determine how such sophisticated coding for segments within a larger episode is used for cognitive functions, which will be an even more challenging question than the discovery of these patterns. When watching the Olympics, we will thus not only admire the athletic accomplishments but also have a better appreciation of how athletes' brains effortlessly generate abstract neural codes that are the foundation of solving complex cognitive problems. ❐

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Competing interests

The authors declare no competing interests.