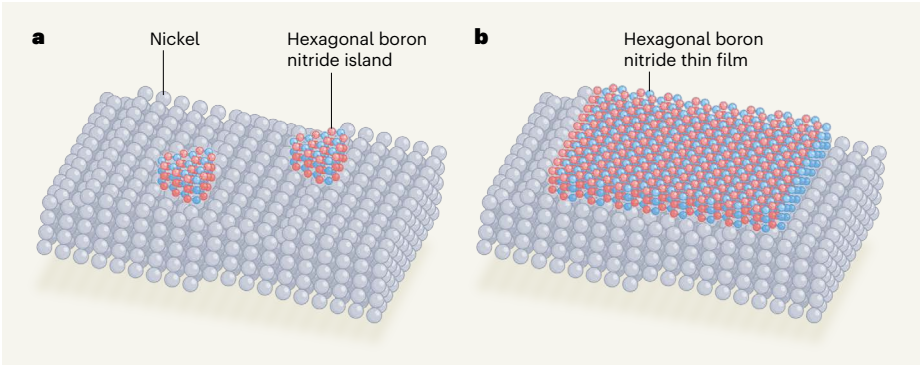

Figure 1 | Synthesis of a thin-film insulator with a uniform crystal structure. Ma *et al.*[3] devised a technique for growing thin films of the electrical insulator hexagonal boron nitride that had a uniform crystal orientation (a single crystal). The method enables growth of up to five single-atom-thick layers on a scale sufficiently large for use as a wafer – the substrate on which a microchip sits. **a**, In the authors' approach, islands of hexagonal boron nitride first nucleate at the stepped edges of a single-crystal substrate that exposes the (111) crystallographic plane of nickel. **b**, The nickel ensures that the islands have the same crystal orientation, and this leads to the growth of a single-crystal film when the separate islands coalesce.

thicknesses can lead to disparities in device performance, which ultimately limits the scale on which hexagonal boron nitride films can be used in electronics.

Clearly, a new technique for growing single-crystal multilayer hexagonal boron nitride films was required. Ma *et al.* have now delivered such an approach: a state-of-the-art growth technique for wafer-scale single-crystal hexagonal boron nitride films of up to five layers. The method uses a single-crystal sheet that exposes the (111) crystallographic plane of nickel – an atomic array with stepped edges.

Getting the temperature right is key to the success of the process. The team found that when the films are grown at 1,020 °C, a polycrystalline structure results. However, at higher temperatures of between 1,120 and 1,220 °C, the method gives rise to grains comprising three layers of hexagonal boron nitride with exactly the same crystal orientation (Fig. 1a). This, in turn, leads to the growth of a single-crystal film when the separate grains coalesce (Fig. 1b). During this initial growth stage, islands of trilayer hexagonal boron nitride nucleate at the stepped edges of the nickel surface, which ensures that the orientation of their crystal lattices are aligned. Over time, these islands grow larger and eventually merge without forming grain boundaries.

The authors showed that they could go beyond trilayer hexagonal boron nitride by varying the growth conditions, because the thickness is determined by surface-mediated growth, rather than by the precipitation mechanism. They succeeded in synthesizing samples containing five layers. However, controlling the thickness of multilayer samples was not straightforward. The binding energy of the sample to the nickel substrate decreases for one, two and three layers, but for samples comprising four or more layers, the binding energy does not change appreciably with each layer, making it difficult to control sample thickness above three layers.

Curiously, although the authors showed that it was possible to fabricate hexagonal boron nitride films comprising five layers, they could not synthesize samples containing four or six layers. The physics underlying this property remains unclear, as does the growth mechanism itself, and both will need to be elucidated before the technique can be used in soft electronics.

Finally, to be used as a substrate for transistors or as an insulating barrier between other 2D materials, hexagonal boron nitride films need to be around ten nanometres thick[11]. Whether or not Ma and colleagues' method can achieve such thicknesses is not known. However, a preliminary demonstration by the authors suggests that trilayer single-crystal films synthesized using this technique can increase the charge-carrier mobility in a molybdenum disulfide field-effect transistor.

Soo Ho Choi is in the Center for Integrated Nanostructure Physics and the Institute for Basic Science, Sungkyunkwan University, Suwon 16419, South Korea. **Soo Min Kim** is in the Department of Chemistry, Sookmyung Women's University, Seoul 14072, South Korea.
e-mail: soominkim@sookmyung.ac.kr

1. Haastrup, S. *et al. 2D Mater.* **5**, 042002 (2018).
2. Ciarrocchi, A., Tagarelli, F., Avsar, A. & Kis, A. *Nature Rev. Mater.* https://doi.org/10.1038/s41578-021-00408-7 (2022).
3. Ma, K. Y. *et al. Nature* **606**, 88–93 (2022).
4. Joo, M.-K. *et al. Nano Lett.* **16**, 6383–6389 (2016).
5. Vu, Q. A. *et al. 2D Mater.* **5**, 031001 (2018).
6. Lee, J. S. *et al. Science* **362**, 817–821 (2018).
7. Chen, T.-A. *et al. Nature* **579**, 219–223 (2020).
8. Wang, L. *et al. Nature* **570**, 91–95 (2019).
9. Shi, Y. *et al. Nano Lett.* **10**, 4134–4139 (2010).
10. Kim, S. M. *et al. Nature Commun.* **6**, 8662 (2015).
11. Vu, Q. A. *et al. Nature Commun.* **7**, 12725 (2016).

The authors declare no competing interests.

Neuroscience

Closing the window on memory linking

Andrea Terceros & Priya Rajasethupathy

An immune molecule has an unexpected role in memory formation – specifically, in limiting the window of time in which newly forming memories can be contextually linked. **See p.146**

Memories acquired close together in time often become linked, such that recalling one memory leads to recall of another. For instance, one of us (P.R.) recently visited the Metropolitan Museum of Art in New York City and remembered that the last time she'd been there, she had walked through Central Park just afterwards and spotted a rare and beautiful snowy owl. The museum and snowy owls had become forever linked in her memory. In 2009, two groups[1,2] observed that neurons in the brain can participate in multiple memory networks that are laid down closely in time, thus enabling memory linking. The activity of a transcription-factor protein called CREB promotes this neural co-allocation, but what limits it? On page 146, Shen *et al.*[3] make the unexpected discovery that the answer lies in an immune molecule that segregates memories by limiting the time available for linking to occur.

How does the brain know that two events are occurring closely in time? Addressing this question requires an understanding of how time is encoded in memory – a major unknown in memory research. Time can be encoded in an absolute or relative manner and encompasses varied scales. For example, some models suggest a moment-to-moment perception of time through a centralized internal clock[4]. Others suggest a more distributed representation, in which time is an emergent

property of the activity of neurons[5–7] and neural populations[8,9] that have an intrinsic capacity to encode relative time of varying durations. Still other models focus on longer-scale circadian time, which might segment behaviour and memories into regular 24-hour blocks[10].

But little is understood about the brain mechanisms that support each of these varied timescales, and even less about how they work together to create fluid memory representations. Shen *et al.* provide traction on this problem by studying how relative time, in the order of hours, might be coded into memory through the process of memory linking and delinking at the time of learning.

The authors began their study by establishing a behavioural assay for memory linking. The group placed mice in one context (context A), and then five hours later placed them in another (context B). Two days later, they placed the animals back into context B, where the mice received an electric shock. Finally, after two more days, the mice were placed back into context A. The animals exhibited a fear response (freezing on the spot) in context A, despite never having been shocked in that context, demonstrating memory linking. By contrast, when the initial placing of animals into contexts A and B was separated by 24 hours or more, memory linking did not occur (Fig. 1).

Shen *et al.* noticed changes in the expression of an immune receptor protein, CCR5, in neurons recently allocated to the memory network. CCR5 expression increased in the 12 hours after initial memory encoding, dropping back to baseline levels by 24 hours after encoding. This change parallels the time course of memory linking. Together, these observations suggest a possible role for CCR5 in closing the memory-linking window.

To test this role, Shen and colleagues manipulated the expression and activity of CCR5 in the hippocampus region of the animals' brains in the five hours between the exposures to context A and context B, when memory linking would presumably have occurred. The authors increased the activity of CCR5 in one of two ways: by optically activating a version of the protein that had been genetically engineered to respond to light; or by injecting animals with a CCL5 protein, which binds to and activates CCR5. Both manipulations impaired memory linking. By contrast, when the authors tested mice that had been genetically engineered to lack CCR5, they found that memories could be linked even when they were encoded up to seven days apart. These results provide key evidence for the role of CCR5 in controlling the duration of memory linking.

But how does CCR5 exert this role? To answer this question, Shen *et al.* recorded neural activity in the animals' hippocampi. They observed that many of the same neurons were active in contexts A and B when mice were placed in the two contexts five hours apart. The overlap between the neuronal populations activated in each context steadily declined as the separation between contexts was increased to one or two days. Mice lacking CCR5 exhibited greater overlap of the two neural ensembles if the events were separated by five hours, and the overlap was sustained even up to seven days' separation. These results support a model in which CCR5 reduces neural excitability and ensemble overlap, and thus memory linking.

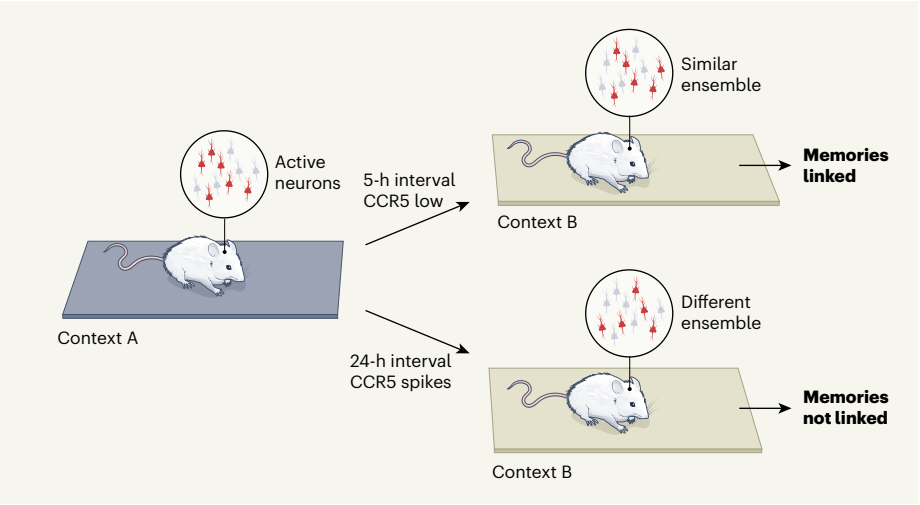

Figure 1 | The immune receptor protein CCR5 regulates memory linking. Shen *et al.*[3] designed a memory-linking task in mice to study the window of time in which memories of two environments (contexts A and B) can become linked. First, they placed a mouse in context A, and analysed the most active neurons (red) in the brain's hippocampus — these cells were recruited to memory networks that enabled the animal to recall context A. Either 5 or at least 24 hours later, the authors placed the animal in context B. In the five-hour window, the activity of CCR5 (which reduces neuronal activity) remained low, meaning a similar ensemble of excitable neurons was recruited to memory networks in context B. Memories of contexts A and B were therefore linked. By contrast, in a 24-hour gap, CCR5 activity increased (thereby reducing the activity of context-A neurons) before returning to baseline levels. This CCR5 spike meant that a different neuronal ensemble was recruited to the memory network for context B, and the memories were not linked.

The authors made the interesting observation that middle-aged mice have a higher expression of CCR5 than do their younger counterparts. They hypothesized that suppressing CCR5 could reduce the age-related deficits in memory linking that occur in these middle-aged animals. To test this, they infused a pharmacological inhibitor of CCR5, maraviroc, into the hippocampi of middle-aged mice. This indeed improved the animals' ability to link memories formed five hours apart. Because memory deficits in ageing and in Alzheimer's disease might be dominated by deficits in retrieval (and thus memory linking) rather than memory storage[11], which involves separate brain circuits[12], these results could have clinical implications.

The discovery of an unexpected role for CCR5 advances our basic understanding of the brain mechanisms that shape memory linking. More generally, it underscores an increasing awareness of the role of immune molecules in cognitive functions, and hints at exciting therapeutic possibilities.

To return to the question of time in memory, it will be important to determine how memory linking at the time of learning informs the relative time-stamping of these memories during long-term storage and retrieval. It will also be interesting to determine whether other regulators of neural excitability, such as ion-channel proteins and neuromodulatory molecules that act across diverse timescales, collectively support CREB and CCR5 in sculpting a flexible, context-dependent linking duration. More broadly, the exploration of brain activity during similar behaviours but on more-granular or -continuous timescales will lead to a better understanding of how memories are organized into sequences, episodes and, ultimately, a chronology.

Andrea Terceros and **Priya Rajasethupathy** are in the Laboratory of Neural Dynamics and Cognition, The Rockefeller University, New York, New York 10065, USA.
e-mail: priya@rockefeller.edu

1. Han, J.-H. *et al. Science* **323**, 1492–1496 (2009).
2. Zhou, Y. *et al. Nature Neurosci.* **12**, 1438–1443 (2009).
3. Shen, Y. *et al. Nature* **606**, 146–152 (2022).
4. Treisman, M. *Psychol. Monogr.* **77**, 1–31 (1963).
5. Robinson, N. T. M. *et al. Neuron* **94**, 677–688 (2017).
6. Tsao, A. *et al. Nature* **561**, 57–62 (2018).
7. Heys, J. G. & Dombeck, D. A. *Nature Neurosci.* **21**, 1574–1582 (2018).
8. Rubin, A., Geva, N., Sheintuch, L. & Ziv, Y. *eLife* **4**, e12247 (2015).
9. Cai, D. J. *et al. Nature* **534**, 115–118 (2016).
10. Hastings, M. H., Maywood, E. S. & Brancaccio, M. *Nature Rev. Neurosci.* **19**, 453–469 (2018).
11. Roy, D. S. *et al. Nature* **531**, 508–512 (2016).
12. Yadav, N. *et al. Nature* (in the press).

The authors declare no competing interests.
This article was published online on 25 May 2022.