hippocampal-dependent spatial navigation, suggesting that SWR events facilitate cognitive processes during wakefulness[16]. In their two-dimensional set-up, Pfeiffer and Foster show that the sequences during waking states reflect future plans rather than past experiences.

Functional connectivity within the hippocampal formation changes during distinct behavioural states. Whereas SWR events occur during sleep or quiet wakefulness, large-amplitude, low-frequency theta oscillations (4–12 Hz) characterize neuronal activity when an animal moves and during attentive wakefulness. Hippocampal firing during these theta states have been found to encode potential future options. For example, animals making decisions at a choice point on a T-shaped maze also show future-representing sequences, but these sequences occur during theta oscillations rather than SWR events[9,17]. A fascinating question is, what is the relationship between these two planning phenomena? Does one negate the need for the other?

It also remains unclear what triggers the hippocampal neural sequences associated with future-trajectory planning and how these sequences interact with other neural circuits. The hippocampus is only part of a complex neural network that involves several related brain structures. In humans, for example, planning processes entail an interaction of multiple structures, including prefrontal cortex[18,19]. What are these other structures doing during the planning events observed by Pfeiffer and Foster? In light of their remarkable results, researchers must now explore what processes generate these place-cell sequences, and how they are used in recalculating the journey home. ■

Brandy Schmidt *and* **A. David Redish** *are in the Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota 55455, USA.*
e-mails: schmidtb@umn.edu; redish@umn.edu

1. Pfeiffer, B. E. & Foster, D. J. *Nature* **497,** 74–79 (2013).
2. Tolman, E. C. *Psychol. Rev.* **55,** 189–208 (1948).
3. O'Keefe, J. & Nadel, L. *The Hippocampus as a Cognitive Map* (Clarendon, 1978).
4. Sutherland, G. R. & McNaughton, B. *Curr. Opin. Neurobiol.* **10,** 180–186 (2000).
5. Foster, D. J. & Wilson, M. A. *Nature* **440,** 680–683 (2006).
6. Davidson, T. J., Kloosterman, F. & Wilson, M. A. *Neuron* **63,** 497–507 (2009).
7. Gupta, A. S., van der Meer, M. A. A., Touretzky, D. S. & Redish, A. D. *Neuron* **65,** 695–705 (2010).
8. Diba, K. & Buzsáki, G. *Nature Neurosci.* **10,** 1241–1242 (2007).
9. Johnson, A. & Redish, A. D. *J. Neurosci.* **27,** 12176–12189 (2007).
10. Singer, A. C., Carr, M. F., Karlsson, M. P. & Frank, L. M. *Neuron* **77,** 1163–1173 (2013).
11. Johnson, A. Fenton, A. A., Kentros, C. & Redish, A. D. *Trends Cogn. Sci.* **13,** 55–64 (2009).
12. Tse, D. *et al. Science* **316,** 76–82 (2007).
13. Girardeau, G., Benchenane, K., Wiener, S. I., Buzsáki, G. & Zugaro, M. B. *Nature Neurosci.* **12,** 1222–1223 (2009).
14. Ego-Stengel, V. & Wilson, M. A. *Hippocampus* **20,** 1–10 (2010).
15. Wikenheiser, A. M. & Redish, A. D. *Hippocampus* **23,** 22–29 (2013).
16. Jadhav, S. P., Kemere, C., German, P. W. & Frank, L. M. *Science* **336,** 1454–1458 (2012).
17. Gupta, A. S., van der Meer, M. A., Touretzky, D. S. & Redish, A. D. *Nature Neurosci.* **15,** 1032–1039 (2012).
18. Spiers, H. G. & Maguire, E. A. *Neuroimage* **31,** 1826–1840 (2006).
19. Voss, J. L. *et al. Proc. Natl Acad. Sci. USA* **108,** E402–E409 (2011).

EARTH SCIENCE

Small differences in sameness

Fresh evidence shows that the iron isotopic composition of Earth's silicate component does not, as was previously thought, reflect the formation of the planet's core at high pressure nor losses of material to space.

ALEX N. HALLIDAY

Writing in *Earth and Planetary Science Letters*, Craddock *et al.*[1] provide strong evidence that iron-isotope differences between planetary samples reflect the origins of the samples themselves rather than isotopic fractionation during planet formation. Although this is a negative result, it says a lot about planet and core formation.

Meteorites provide an invaluable archive of the circumstellar disk from which the terrestrial planets and asteroids formed. With the advent of accurate mass spectrometry and its application to meteorite samples, it was soon shown that this disk had relatively uniform isotopic compositions. For example, the uranium found on Earth has the same atomic mass as the uranium found in meteorites from the asteroid belt that lies between Mars and Jupiter, showing that the mix of isotopes from diverse primordial circumstellar-disk material was about the same. With the more recent development of a technique called multiple-collector inductively coupled plasma mass spectrometry, it has become possible to explore this 'sameness' to much higher precision and in many more elements. This has led to a search for small, mass-dependent isotopic differences that may have been imposed by effects that acted to separate (fractionate) the isotopes during planet formation. The resolution of such effects could help to confirm or refute theories about the dynamic processes that formed Earth and its metallic core[2–4].

This new area of isotope geochemistry has generated strong debate, because most isotopic differences reported so far have been small — less than about 100 parts per million per atomic mass unit (p.p.m. per AMU) — and have been obtained at the technical limits of what can be reliably resolved. Equally heated have been debates about whether systematic isotopic differences between samples can be scaled up to define planetary compositions at all. This is the focus of Craddock and colleagues' study.

Mass-dependent isotopic fractionation could in principle result from loss of planetary material to space through vaporization[2–5], or loss to a planet's core during core formation[6,7](Fig. 1). In both cases, there could be a slight difference in terms of the ease of incorporation of a lighter isotope in one phase relative to another. These two phases could be vapour and liquid in the case of material lost to space, or silicate and metallic liquids in the case of core formation. Both of these processes are relevant to Earth's formation by mass accretion, which probably occurred at the same time as core formation by means of a series of large, stochastic, gravity-driven collisions over tens of millions of years.

Chemistry-based arguments have accumulated that Earth, and/or the various protoplanets that it incorporated during accretion, may have lost material to space from their outer silicate portions through erosion during the impacts[3]. As Earth became bigger, the gravitational energy released by accretion would have generated temperatures at which silicates and metals should have been vaporized[2]. Major growth phases through collisions are termed giant impacts, and the last such collision between Earth and a smaller planet named Theia, often referred to as 'the giant impact', led to the formation of the Moon from condensation and accretion in the resultant disk of vapour and debris[2,4]. If some of the material was lost to space rather than re-accreted to Earth and the Moon, then elements that should have only partially entered the vapour phase at these temperatures and pressures, such as lithium, silicon and iron, might show resolvable isotopic differences.

The iron isotopic composition of lunar basalt rocks has been found[5] to be on average slightly enriched in the heavier iron isotopes (about 30 p.p.m. per AMU) compared with most terrestrial mantle-derived samples, mainly basalts. The average for data from Earth is in turn

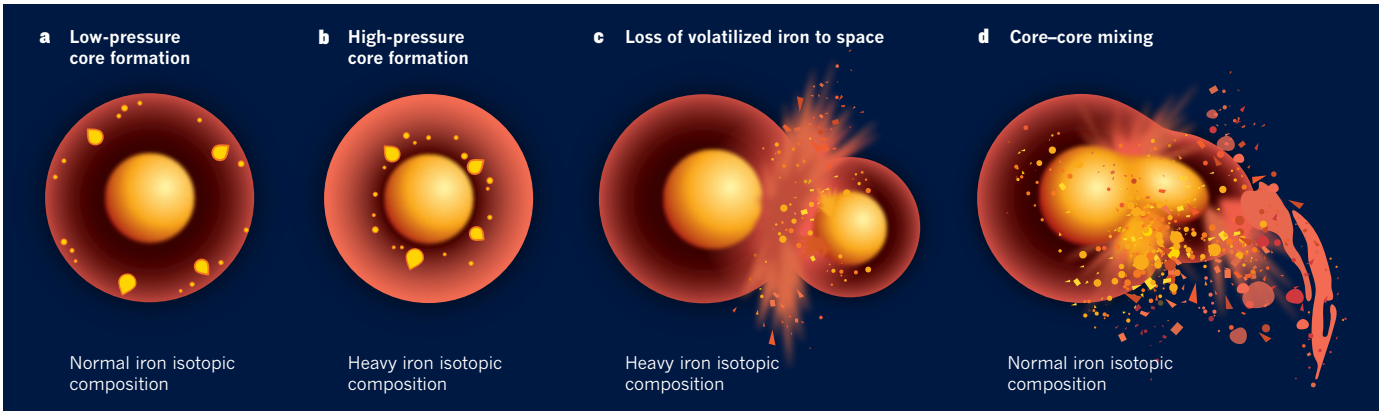

Figure 1 | Earth's formation and iron isotopic composition. Earth formed by the cumulative accretion of smaller planets and impactors. Melting from the accretion energy of these impactors would have led to segregation of dense metal (yellow) from the residual silicate of the planet (red to black, with black denoting a lower degree of melting), resulting in concomitant growth of its metallic core. The figure illustrates schematically how the iron isotopic composition of the silicate part could have been modified or left unchanged during this process, depending on the conditions of accretion and core formation. **a**, Formation of the core at low pressure is thought to leave the composition unchanged[6]. **b**, Conversely, this composition should become heavy if the core formed at high pressures[6,10]. **c**, If volatilized iron is lost to space during a collision between the proto-Earth and a small planet, then the iron isotopic composition of the residual silicate Earth should be heavy[5]. **d**, If the proto-Earth grows by repeatedly colliding with planets with low-pressure cores and the metal mixes directly with metal and silicate with silicate[13–15], the core will grow without a change in iron isotopic composition. Craddock and colleagues' results[1], coupled with earlier findings[6,8,10,11], provide evidence that **b** and **c** either resulted in no change in the iron isotopic composition, contrary to expectation, or were unimportant in the later history of Earth accretion and core formation.

slightly heavier (about 30 p.p.m. per AMU) than that for data on basalts from Mars and the asteroid Vesta. Further work confirmed that lunar basalts can indeed have a heavy iron isotopic composition, although this depends on the types of basalt analysed[8]. Furthermore, lunar basalts do not have a heavy isotopic composition for the light element lithium[9], which seems inconsistent with the idea that there were losses of lighter isotopes of iron during vaporization in the Moon-forming giant impact.

It has been argued instead that the Moon's apparent heavy iron isotopic composition might simply reflect that of the outer silicate part of Earth, which in turn was heavy because of the high pressure involved in core formation[6]. Recently, it was found that iron isotopes can become fractionated as a result of 'disproportionation' of ferrous iron into core-forming metal and oxidized ferric iron in the presence of perovskite minerals in the mantle[10]. Separation of this metal to the core is one mechanism that might explain why the silicate Earth is oxidized and why iron in terrestrial and lunar basalts is isotopically heavy, as it is for silicon[7].

More detailed studies of Earth[1,8,11], for which plentiful samples of the solid mantle are available, have raised the question of whether basalts are representative of planetary composition at all. In their favour, the mantle is compositionally heterogeneous, so individual fragments are not always representative. By contrast, basalts are derived by partial melting of large volumes of mantle and therefore provide a more effective method of averaging planetary heterogeneity. However, Craddock and colleagues' study clearly demonstrates that solid-mantle samples that have undergone melting have a lighter iron isotopic composition than basalts because of fractionation during melting. Furthermore, measurements[11,12] of chondrites, a group of primitive meteorites with a similar chemical composition to that of the Sun (if volatile elements are subtracted), show that the iron isotopic composition of the silicate Earth is like that of chondrites and so is no different from that of the Sun and the average Solar System.

These results imply that high-pressure iron-isotope fractionation, which has been demonstrated both theoretically[6] and experimentally[10], did not in fact substantially affect the silicate Earth's residual iron. The disproportionation of ferrous iron in the presence of perovskite may not have been the mechanism by which the iron in the silicate Earth became oxidized. Also, alternative models for core formation exist that do not involve segregation of metal at high pressures. For example, Earth's core and residual silicate may have grown in part in a more direct fashion by accreting smaller planetary objects that had their own low-pressure cores and by separate admixing of these objects' metal and silicate reservoirs through density differences[13]. There is supporting evidence for such core–core mixing, both theoretically[14] and in the silicate Earth's isotopic[13] and chemical[15] composition.

For some elements, such as silicon, the isotopic composition of the mantle is not fractionated greatly by melting and is heavy relative to both chondrites and samples from Mars and Vesta, with the most likely explanation being partitioning into the core[7]. The search is now on to determine which other elements have been isotopically fractionated by core formation and what this tells us about processes and conditions in the early Earth.

Craddock and colleagues' results also raise questions about the assumptions that have been made in simply comparing the iron isotopic compositions of basalts from Earth, the Moon, Mars and Vesta. It has been demonstrated over the past 10 years that the isotopic compositions of nearly all elements are the same in Earth and the Moon, leading to new models of lunar origins[4]. On this basis, the iron isotopic composition of the bulk Moon is probably also like that of chondrites, and the data for lunar basalts[5,8] may also reflect fractionation, but fractionation associated with melting on the Moon.

Recently, it has been argued that zinc isotopes in lunar samples were fractionated during the giant impact[16]. The new data from Craddock *et al.* greatly strengthen the argument that mass-dependent isotopic fractionation of elements less volatile than zinc, such as iron, magnesium and lithium, did not occur as a result of losses to space. If material was lost to space during accretion[3], it happened without isotopic fractionation of these elements, possibly because accretion was not as energetic as has been thought. Intriguingly, some of the latest simulations of the Moon-forming giant impact provide some support for this latter view[4]. ■

Alex N. Halliday *is in the Department of Earth Sciences, University of Oxford, Oxford OX1 3AN, UK.*
e-mail: alexh@earth.ox.ac.uk

1. Craddock, P. R., Warren, J. M. & Dauphas, N. *Earth Planet. Sci. Lett.* **365,** 63–76 (2013).
2. Pahlevan, K. & Stevenson, D. J. *Earth Planet. Sci. Lett.* **262,** 438–449 (2007).
3. O'Neill, H. St. C. & Palme, H. *Phil. Trans. R. Soc. Lond. A* **366,** 4205–4238 (2008).
4. Ćuk, M. & Stewart, S. T. *Science* **338,** 1047–1052 (2012).
5. Poitrasson, F., Halliday, A. N., Lee, D.-C., Levasseur, S. & Teutsch, N. *Earth Planet. Sci. Lett.* **223,** 253–266 (2004).
6. Polyakov, V. B. *Science* **323,** 912–914 (2009).
7. Armytage, R. M. G., Georg, R. B., Savage, P. S., Williams, H. M. & Halliday, A. N. *Geochim.*

Cosmochim. Acta **75,** 3662–3676 (2011).
8. Weyer, S. *et al. Earth Planet. Sci. Lett.* **240,** 251–264 (2005).
9. Magna, T., Wiechert, U. & Halliday, A. N. *Earth Planet. Sci. Lett.* **243,** 336–353 (2006).
10. Williams, H. M., Wood, B. J., Wade, J., Frost, D. J. & Tuff, J. *Earth Planet. Sci. Lett.* **321–322,** 54–63 (2012).
11. Schoenberg, R. & von Blanckenburg, F. *Earth Planet. Sci. Lett.* **252,** 342–359 (2006).
12. Craddock, P. R. & Dauphas, N. *Geostand. Geoanal. Res.* **35,** 101–123 (2011).
13. Halliday, A. N. *Nature* **427,** 505–509 (2004).
14. Dahl, T. W. & Stevenson, D. J. *Earth Planet. Sci. Lett.* **295,** 177–186 (2010).
15. Rubie, D. C. *et al. Earth Planet. Sci. Lett.* **301,** 31–42 (2011).
16. Paniello, R. C., Day, J. M. D. & Moynier, F. *Nature* **490,** 376–379 (2012).

STRUCTURAL BIOLOGY

Active arrestin proteins crystallized

Arrestin proteins regulate cellular signalling cascades initiated by ubiquitous G-protein–coupled receptors. Crystal structures reveal that two arrestins undergo similar structural changes on activation. SEE LETTERS P.137 & P.142

VALENTIN BORSHCHEVSKIY & GEORG BÜLDT

Physical abilities, cognitive skills and many other activities of humans and other organisms are generated by the coordinated actions of millions of cells. Each cell contributes to these activities by interacting with molecules outside itself. Many of these interactions are mediated through G-protein-coupled receptors (GPCRs), which in turn activate the eponymous intracellular G proteins, and so act as the starting points for numerous cellular signalling pathways. Arrestin proteins regulate the activity of these pathways by interacting with GPCRs, thus preventing G-protein-induced signalling and/or inducing additional signalling through G-protein-independent pathways. Two papers[1,2] in this issue report the first X-ray crystal structures of arrestins in their active states*.

GPCRs are members of the largest family of membrane proteins. There are more than 800 different GPCRs, most of which are activated by small molecules (agonists). One exception is rhodopsin, a well-characterized GPCR found in the light-responsive cells of the retina. Rhodopsin is activated by photons, which change the isomerization state of retinal, its covalently attached cofactor. But all GPCRs have similar interaction partners downstream: after activation of a GPCR, signalling begins when a G protein binds to the receptor, and is terminated by the binding of an arrestin molecule, which is triggered by the phosphorylation of several amino acids at the carboxy-terminal end of the receptor.

If we understand the specific interactions between biomolecules in space and time, we can picture the molecular events that underpin macroscopic biological processes. Scientists have therefore invented numerous chemical and physical methods to study these interactions, with X-ray crystallography generally having the predominant role. Rhodopsin was the first GPCR to be crystallized and to have its structure solved to high resolution[3], and the interactions of this receptor with other molecules in the visual system have been intensively studied. To establish how GPCRs interact with arrestins on the atomic scale, the method of choice would be to crystallize the two proteins together for X-ray analysis. But in most cases this is difficult. The two papers published today report impressive procedures for obtaining crystal structures of activated arrestins for which co-crystallization had failed.

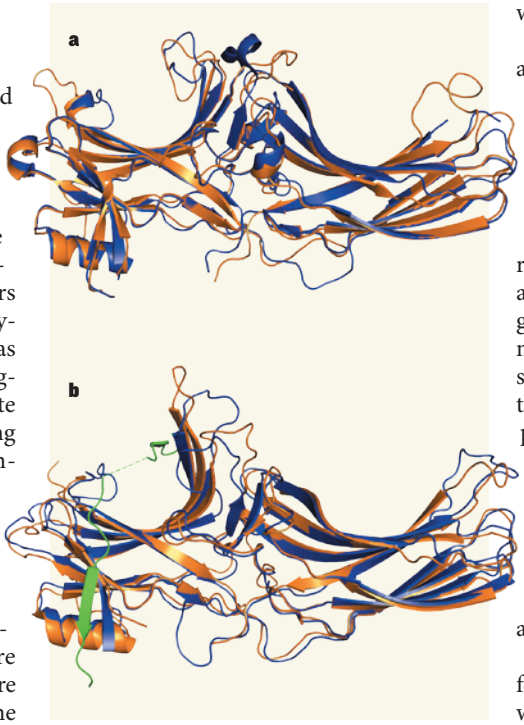

Figure 1 | Comparison of inactive and active arrestins. a, The structures of visual arrestin[5] (orange) and β-arrestin-1 (ref. 8; blue) in their inactive states are shown superimposed on each other. The N-terminal domain is on the left, and the C-terminal domain is on the right. **b**, Here, the active-state structure of visual p44 (a naturally occurring 'splice variant' of visual arrestin) reported by Kim *et al.*[1] (orange) is superimposed on the active structure of β-arrestin-1 described by Shukla and colleagues[2] (blue). The structure in green is a phosphorylated peptide that corresponds to 29 amino-acid residues of the C-terminal end of the V2 vasopressin receptor, used by Shukla *et al.* to activate β-arrestin-1. The broken line in the peptide structure denotes some amino acids that could not be traced in the X-ray crystal study. The similarity of the pairs of structures in **a** and **b** suggests that the arrestins in **b** are fully activated. The superpositions were aligned and refined with respect to the whole structures.

Kim *et al.*[1] (page 142) describe a method for activating p44, a naturally occurring variant of visual arrestin-1 in which 35 amino-acid residues at the C terminus have been replaced by a single alanine residue. In contrast to full-length arrestin-1 (refs 4,5), which binds only to light-activated, phosphorylated rhodopsin, p44 binds to rhodopsin with high affinity regardless of whether the receptor is activated and/or phosphorylated. The same research group had previously shown[6] that opsin (retinal-free rhodopsin) behaves much like rhodopsin in the active meta II state — the intermediate that allows the enzyme rhodopsin kinase to phosphorylate the C-terminal domain of rhodopsin so that full-length arrestin-1 can bind and deactivate the receptor. In the current study, Kim and colleagues attempted to crystallize p44 in the presence of opsin. The co-crystallization failed, but the authors did obtain crystals of p44 alone, which they analysed by X-ray crystallography.

The resulting structure revealed major conformational changes in p44 when compared with unactivated full-length arrestin-1. The salient differences are a 21° twist between the amino-terminal and C-terminal domains in p44, and local changes of loop conformations and interacting hydrogen-bonding networks. The researchers attribute these conformational changes to the active form of p44, assuming that the opsin present during crystallization caused p44 to adopt this form. Whether this structure represents fully activated p44 is open to question — the protein is highly flexible, which means that crystal-packing effects might have induced the large conformational changes observed. A previously reported structure[7] of p44 crystallized in the absence of opsin revealed much smaller conformational changes than in Kim and co-workers' study.

In the second arrestin paper, Shukla *et al.*[2] (page 137) describe the crystal structure of

*This article and the papers under discussion[1,2] were published online on 21 April 2013.