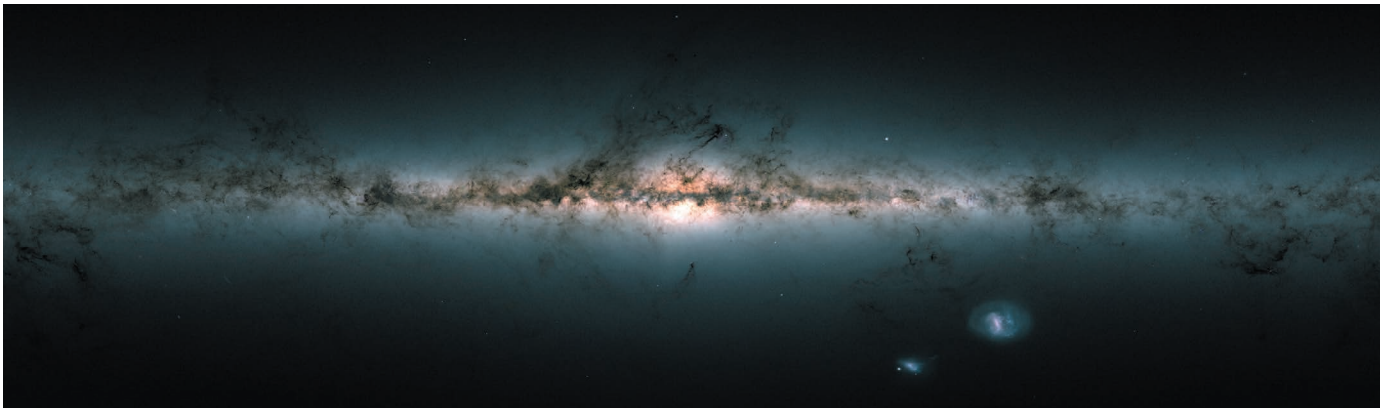

ESA/GAIA/DPAC

Figure 1 | View of the Milky Way based on data from the Gaia space observatory.

with large databases of the spectral and chemical properties of stars. Over the next decade, several international observatories will carry out massive surveys of the spectra of stars throughout the Milky Way. These surveys will provide new data to identify the characteristics of more stars from the satellite galaxy.

The Gaia mission will continue for another few years, sharpening our vision of the Milky Way. With Gaia's detection of even more stars that originated in the satellite galaxy, astronomers will be better able to determine the mass of this galaxy, and when the merger occurred. It might even be possible to learn about the star-formation history of the satellite galaxy before it collided with the Milky Way.

Helmi and colleagues named the satellite galaxy Gaia–Enceladus, in honour of the space observatory that provided the crucial data and after one of the Giants of Greek mythology. Enceladus was the offspring of Gaia (Earth) and Uranus (the sky). He was said to be buried under Mount Etna in Italy and responsible for earthquakes in the region. The authors suggest that this is an appropriate name because Gaia–Enceladus was a giant compared with other past and present satellite galaxies of the Milky Way. Furthermore, it shook our Galaxy, leading to the formation of the thick stellar disk. Regardless of the name, it is clear that the history of the merging event is written in the stars. ■

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BIOLOGICAL TECHNIQUES

Complementing the forebrain

A new technique, in which forebrain-precursor cells are ablated from early-stage mouse embryos and replaced with embryonic stem cells, promises to facilitate our ability to study the central nervous system. SEE LETTER P.126

JIMENA ANDERSEN & SERGIU P. PAŞCA

For the past few decades, it has been possible to directly manipulate an organism's genes early in development, to generate, for example, mice that harbour genetic modifications. Such transgenic mice have been powerful model systems in which to study human disease[1], but conventional approaches for generating these animals can be costly and time-consuming. On page 126, Chang *et al.*[2] describe an alternative approach to building complex mouse models with which to interrogate the function and diseases of the forebrain. The authors' approach could also be used to produce models focused on other regions of the central nervous system (CNS).

In conventional transgenic approaches, genes are modified in mouse embryonic stem (ES) cells, which are pluripotent — they can give rise to all cell types of the animal's body. The genetically engineered cells are then injected into a mouse embryo at an early stage of development called the blastocyst stage. The result is a chimaeric mouse, in which some cells are genetically modified and some are not. If the animal's eggs or sperm contain the genetic modification, it can be bred to produce offspring in which all the cells are modified (Fig. 1a). This approach, although recently accelerated by gene-editing technologies such as CRISPR–Cas9, remains expensive and labour-intensive. Moreover, unless the genetic modification is engineered to be expressed in only certain cell types, this approach is limited to genes that are not essential for early embryonic development.

More than two decades ago, the laboratory that performed the current study devised a technique called blastocyst complementation, to circumvent these limitations in the immune system[3]. The approach is based on the fact that, if the development of a particular organ in the host is disabled, a vacant niche is created that can be filled by tissue derived from newly introduced ES cells. In that paper, the authors used RAG2-deficient mice, which do not have mature immune cells called T and B cells. They showed that embryos of this strain could give rise to mice that generated T and B cells normally if blastocysts were injected with wild-type mouse ES cells. Moreover, these immune cells were exclusively of donor origin.

Blastocyst complementation has since been used to generate the lens of the eye[4], the kidney[5] and the heart[6]. The technique has also been used to generate pancreases made mainly of cells from another species, by injecting mouse cells into pancreas-disabled rat blastocysts and vice versa[7]. This type of animal, called an interspecies chimaera, offers great potential both for understanding fundamental principles

in biology and for regenerative medicine, in which the ultimate goal is to grow organs that carry the genome of a specific individual.

Chang *et al.* developed an approach that they call neural blastocyst complementation (NBC), which involved disrupting the developing mouse forebrain — the region that will generate the brain's hippocampus, cerebral cortex and olfactory bulbs, among other structures (Fig. 1b). They crossed existing transgenic mouse strains to produce embryos in which diphtheria toxin subunit A was expressed specifically in forebrain progenitor cells. This led to the death of these cells, and therefore to mice that lacked forebrain structures. But wild-type ES cells injected into blastocysts of this strain could repopulate the forebrain niche. The authors found that the forebrain structures in the resulting animals were reconstituted. These mice were indistinguishable from controls in a set of behavioural assays.

The researchers next showed that this approach could be coupled with CRISPR–Cas9 editing of donor ES cells as a way of interrogating the function of genes of interest in the forebrain. As a proof of principle, they focused on the gene *doublecortin* (*Dcx*), which encodes a protein involved in neuronal migration and causes malformations of the cerebral cortex when mutated in patients. Chimaeras produced using *Dcx*-deficient donor ES cells exhibited hippocampal defects that mimicked those observed[8] in transgenic mice lacking *Dcx*.

Chang and colleagues' NBC approach could facilitate the study of brain development and disease in several ways. First, generating transgenic animals in which both copies of a gene are mutated requires multiple breeding steps: even if both copies of the gene are mutated in the injected ES cells, the chimaeric mice must be crossed to wild-type partners to produce offspring with one normal copy, and an extra step is needed to produce animals in which both gene copies are mutated. The need for these breeding steps is eliminated with NBC. Similarly, numerous modifications can be introduced into donor ES cells at the same time using CRISPR–Cas9, rather than being brought together through complex breeding strategies. This could accelerate studies into disorders involving more than one gene, such as autism spectrum disorders, and improve our understanding of gene–gene interactions during development.

Second, although Chang *et al.* chose to ablate forebrain progenitors, the same principle can be applied to other regions or cell types in the CNS. Moreover, alternative approaches for targeted genetic ablation could also be used, such as the removal of genes essential for development of a specific brain region, or the forced expression of proteins that induce programmed cell death. However, because most of the proteins that regulate development have roles in various cell types and at a range of

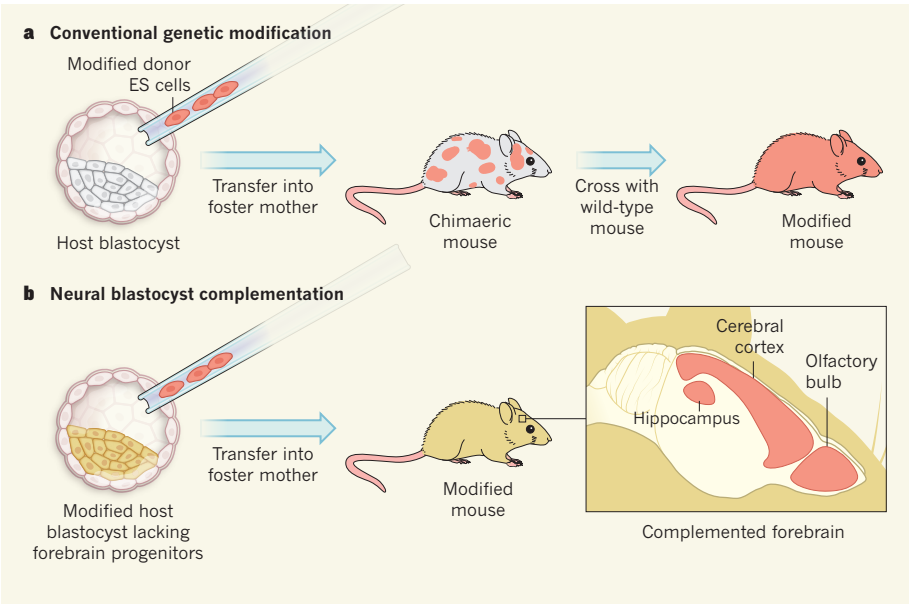

Figure 1 | Two ways to generate genetically modified mice. **a**, Genetically modified mice are usually generated by engineering mouse embryonic stem (ES) cells to harbour a desired mutation, and injecting the modified cells into a host embryo at the blastocyst stage. The embryo is then implanted into a foster mother. The donor and host ES cells mingle, giving rise to chimaeric mice consisting of both cell lineages (indicated by colouring). By breeding with wild-type mice, chimaeric animals can pass the mutation down through their eggs or sperm, producing offspring in which all cells are genetically modified. **b**, Chang *et al.*[2] describe an alternative approach called neural blastocyst complementation to produce mice whose forebrain cells all carry a desired genetic modification. The authors generated host embryos in which diphtheria toxin subunit A was expressed only in forebrain progenitor cells — these cells therefore died, leaving a vacant niche. The group injected the host blastocysts with donor ES cells that harboured a genetic modification of interest, and implanted them into foster mothers. The donor cells filled the vacant niche, repopulating the forebrain (but not other brain regions) of the resulting mice.

embryonic stages, caution is warranted when designing targeted ablation strategies, to avoid undesired side effects.

Third, this study raises the possibility of generating interspecies chimaeras of the CNS. In particular, the generation of human–animal chimaeras, in which human cells are integrated into an animal's neural circuits, would allow some human-specific brain features to be studied in a more physiological environment than can be provided by current *in vitro* systems[9]. However, the ethical implications of this work need to be closely considered[10]. Indeed, organizations such as the International Society for Stem Cell Research recommend restrictions on experiments that incorporate human cells into animals early in development, together with specialized oversight and review of the research[11].

There are technical difficulties, too. Although human cells such as neural-precursor cells have successfully been transplanted into mouse embryos to generate chimaeric tissues[12], it has proved harder to efficiently generate whole-organism chimaeras from blastocyst injections. This barrier could be overcome by strategies that confer a selective advantage on or enhance the survival of donor human cells, or by the development of techniques to better monitor the state of human pluripotent stem cells. For instance, human pluripotent stem cells that are in a ground state known as naive can be engrafted into pig and cattle blastocysts, but show little chimaera-forming abilities; by contrast, pluripotent stem cells in a different cell state known as intermediate can be engrafted and generate differentiated progeny[13].

Nonetheless, the model developed by Chang *et al.* provides a powerful tool for neuroscientists to study mammalian brain development and evolution. It will no doubt expand our ability to investigate the mechanisms of neuropsychiatric disorders. ■

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