Avian brain chimeras

Contents [Hide]

A chimera is an organism made up of cells from two or more genetically distinct sources. While some chimeras (very rarely) arise naturally through the fertilization of an egg by more than one sperm cell, or from the very early fusion of fraternal twin embryos shortly after fertilization, the avian brain chimeras considered here are different because they are produced surgically by the substitution of presumptive nervous system cells between the early embryos of different bird species. The word “presumptive” is used because the transplanted cells have not yet definitively become nervous system cells at the time of transplantation. This is because the cell substitution takes place before major organ systems or blood vessels are formed in the embryo. The surgeries are guided by “fate maps” that identify which groups of embryonic cells give rise to particular parts of the developed brain. Avian brain chimeras are used in basic research examining the mechanisms responsible for the development of, and evolutionary changes in, neural circuits: complex groups of interconnected nerve cells located in many different parts of the brain that regulate particular perceptual, cognitive, and behavioral functions of organisms.

Underpinnings of the technique

This experimental system uses inborn species differences in neural circuit organization to identify and characterize interactions among cells in the developing brain that make important contributions to the development of neural circuit function.

Prenatal brains of different species are built differently

While the ability to predict an individual organism's personal characteristics from its genetic makeup is, and will likely remain, inaccurate, it is much easier to reliably predict particular species-level characteristics of the behavior of organisms. For example, human infants and the young of many vocally imitating birds (species that exhibit the ability for vocal imitation) will spontaneously produce (human) fluent speech or a small number of human speech phrases (vocally imitating birds) when interactively exposed to human speech from an early period in life. Other bird species such as chickens and quails (which do not exhibit vocal imitative abilities) will not; chickens and quails are also not able to learn to produce each other's vocalizations. However, chickens and quails, like dogs, may spontaneously learn (or can be taught) to respond differently to different human vocal commands. It is not that the hearing abilities or sound-producing abilities of chickens and quails are different in character from those of vocally imitating birds. Rather, there is some inborn difference in neural circuits within their brains that makes these species prone to do different things when presented with the same information. The avian brain chimera technique capitalizes on the existence of such circuit differences and uses them to discover the locations of cell populations that make a decisive contribution to circuit development. These are identified by finding groups of cells that, when transplanted from the donor to the host species, transfer donor behavioral characteristics to the host species that are normally not present (**Fig. 1*a***).

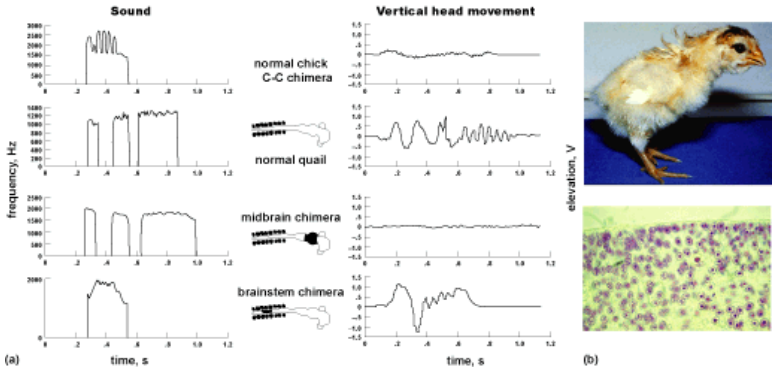

Fig. 1 (*a*) Quail-chick brain transplants change crowing behavior. (Left) A simplified representation of the form of the crow of juvenile chickens and quails induced by testosterone treatment. (Right) Head movement patterns measured with video object tracking. Midbrain transplants transfer the sound characteristics from quail to chick, while brainstem transplants transfer the head movement characteristics. “C-C chimera” represents animals that received control transplants of these same regions between two individual chicken embryos. (*b*) (Top) Quail-to-chick chimeric animal delivering a crowing vocalization. (Bottom) The quail-chick cell marker: chick cells are on the left part of the image; quail cells have large dark spots and are on the right side of the image.

Add to 'My Saved Images'

Inborn neural circuit developmental decisions

Every cell in an organism's body got there by splitting away from a preexisting cell (starting with the first cell formed by the union of the sperm and the egg) and, through a series of decisions guided by molecules both inside and outside of itself, gradually assuming its developed fate. One of the perennial questions that interests scientists about neural circuit development has been the extent to which these decisions are guided by genetic information (in the form of DNA sequences inside cells inherited from the sperm and egg). That is a question often phrased using the dichotomy of “nature” and “nurture.”

Long before people knew about the role of DNA in inheritance, they were already making the distinction between inherited (internal) and environmental (external) sources of developmental information, by raising animals in controlled environments to see which aspects of their natural behavior would appear in spite of the fact that they lacked exposure to either parents or siblings (or to deliberately try to mold their behavior in particular ways). For example, in 1773, the British scientist Daines Barrington published the results of experiments on the developmental origin of bird songs based on these types of studies. Developmental biologists took this same line of experimentation to embryonic cells by the end of the ninteenth century, examining what happens when one of the first two cells that form the embryo is removed: Do you get half an animal or a whole animal? In the 1880s and 1890s Wilhelm Roux and Hans Driesch found that the answer to this question depended on the way the manipulation was performed. Killing one cell and leaving it attached to the other cell results in half an embryo, while separating the two cells results in a complete embryo of smaller size. The repercussions from this initial realization that embryonic cells make conditional decisions took a while to be realized. Many scientists believed such experiments simply demonstrated limited environmental modulation of mostly autonomous, preordained instructions (a modern version of this idea, the modulated blueprint, is shown in **Fig. 2**). During the past century, scientists have learned enough about the mechanisms of development to understand that the blueprint metaphor and the dichotomy of nature-versus-nurture that underlies it have been deeply misleading. Cell decisions are better visualized as “conversations” rather than blueprints because of the two-way nature of the processes that contribute to them (Fig. 2).

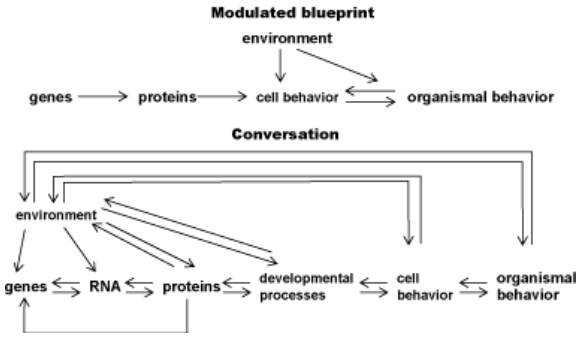

Fig. 2 Two different conceptions (modulated blueprint versus conversation) of the control of cellular decisions during brain development.

Add to 'My Saved Images'

The special challenges to understanding the development of neural circuits were first suggested in the 1920s by Hilde Mangold and Hans Spemann, who examined the emergence of the embryonic brain. They showed that the fate of future brain cells depended on fleeting communication between two preexisting populations of cells during a process called neurulation (differentiation of nerve tissue and formation of a cylindrical embryonic structure called the neural tube). As a result of this earlier communication, one of these cell populations goes on to produce brain cells, while the other population eventually dies and is no longer present at the time of birth. Many cells in the developing brain make fleeting contacts with other cells that they have no contact with later on. We do not know which of these interactions may be important for the function of neural circuits that a particular cell will contribute toward. The chimera technique enables investigators to find embryonic cells that contribute to the function of particular neural circuits; their interactions with other cells can then be followed to study the way in which these cells are involved in building particular neural circuits.

Use of marked cells to distinguish donor and host tissues

Initial work on avian brain chimeras has relied on a naturally existing difference between chicken and quail cells first discovered by the French developmental biologist Nicole Le Douarin, and used for fate-mapping studies in embryos (Fig. 1*b*). For reasons that are still not well understood, the geometry of the arrangement of DNA in the cell nucleus is different in chickens and quails, and any staining method that colors DNA can be used to reveal the species identity of cells. The recent development of nontoxic cell marking substances, and genetic techniques for creating embryos with marked cells, allows chimeras to be made between any species. Birds are preferable for this work because of easy experimental access at a critical time in development (at this same stage, mammalian embryos are

establishing connections to the mother's body that manipulations interfere with). Birds also have nervous systems of the same order of complexity as mammals, and exhibit many inborn species differences in both simple and complex behaviors.

Basics of brain switching

To create a brain chimera between two species, eggs are incubated for approximately 2 days to reach the state of embryonic development most conducive to transplants. At this stage, there are no blood vessels in the presumptive brain, and the basic regions of the future brain are laid out, but no cells have yet reached their developed state and are still fairly plastic (if moved they can turn into other kinds of cells than what they would have become if left in place). The eggs are removed from the incubator, turned on their side, and a 1-cm (0.4-in.) hole is cut in the shell to reveal the tiny white embryo on top of the yellow yolk (**Fig. 3**). A solution of sterilized dark food coloring is injected between the embryo and the yolk for visual contrast. An embryo of each species at the same state of development is selected; this can be gauged by the visual appearance of the embryos under a high-magnification surgical microscope. Tiny scalpels made of stainless steel wire are used to cut a small hole in protective membranes overlying the embryo, and the region of the presumptive brain (contained in the neural tube) is mechanically cut out of donor and host embryos using the microscalpels. The dissected fragment from the donor embryo is gently sucked up into a small glass tube filled with sterile fluid, and transferred into the host embryo, substituting for the same fragment removed from the host embryo. Because of the growth happening at this time, the fragment is completely reincorporated into the developing brain within a few hours, with no sign of any surgical intervention. At the end of the transplant, a piece of sterile wound-closure tape is used to close the hole in the egg, and the egg is returned to an incubator to undergo the rest of its development. Well-performed transplants yield a healthy chimeric animal with a morphologically normal brain. Since the brain now contains a population of foreign cells, the immune system of the host animal will reject these cells once cellular immunity is established at about 2 weeks after hatching, unless steps are taken to prevent such rejection from occurring.

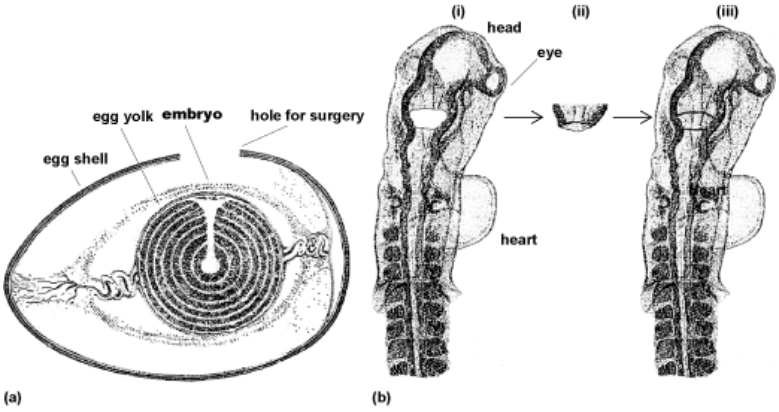

Fig. 3 (*a*) Diagram of a cross section through an incubated egg turned on its side, with a hole cut in the shell to give surgical access to the embryo. (*b*) Magnified view of an embryo at the time of surgery; the locations of the presumptive head, heart, and eye are indicated. (i) The region of the presumptive brain that is going to be transplanted is mechanically excised using a microscalpel from the donor and the host embryo, which are at the same stage of development and thus look very similar. The white area shows the excised region. (ii) The excised fragment from the donor is transferred to the host embryo. (iii) The donor fragment is placed in the incision inside the host embryo, substituting for the host fragment that was previously excised. Within several hours, it has healed seamlessly into place.

Add to 'My Saved Images'

These techniques have been used to successfully transplant species differences in a motor behavior (singing) between chickens and quails, and also to study neural circuits controlling inborn auditory perceptual differences between these two species. The technique has also been applied within species to study the etiology of epilepsy using transplants between normal and epileptic strains of chickens, and to study the development of male and female brain differences in quails. Current applications include studying brain differences implicated in vocal imitation behavior using transplants between birds that vocally imitate and those that do not, as well as studying brain mechanisms involved in migratory behavior, and the events that lead to brains first functioning as integrated systems before birth.

For background information See also: Aves; Brain; Cell lineage; Chimera; Developmental biology; Embryonic induction; Fate maps (embryology); Nervous system (vertebrate); Neurobiology; Neurulation; Transplantation biology; Vertebrate brain (evolution) in the McGraw-Hill Encyclopedia of Science & Technology

Evan Balaban

Bibliography

- E. Balaban, Brain switching: Studying evolutionary behavioral changes in the context of individual brain development, *Int. J. Dev. Biol.* , 49:117–124, 2005 **DOI:**10.1387/ijdb.041934eb
- E. Balaban, Cognitive developmental biology: History, process and fortune's wheel, *Cognition* , 101:298–332, 2006 **DOI:**10.1016/j.cognition.2006.04.006
- N. M. Le Douarin et al., Interspecific chimeras in avian embryos, *Methods Mol. Biol.* , 135:373–386, 2000 **DOI:**10.1385/1-59259-685-1:373
- K. Long, G. Kennedy, and E. Balaban, Transferring an inborn auditory perceptual preference with interspecies brain transplants, *Proc. Natl. Acad. Sci. USA* , 98:5862–5867, 2001 **DOI:**10.1073/pnas.091080098
- M. A. Teillet, C. Ziller, and N. M. Le Douarin, Quail-chick chimeras, *Methods Mol. Biol.* , 97:305–318, 1999 **DOI:**10.1007/978-1-60327-483-8_24

Additional Readings

- E. Balaban, Changes in multiple brain regions underlie species differences in a complex, congenital behavior, *Proc. Natl. Acad. Sci. USA* , 94:2001–2006, 1997 **DOI:**10.1073/pnas.94.5.2001
- M. Gahr, Male Japanese quails with female brains do not show male sexual behaviors, *Proc. Natl. Acad. Sci. USA* , 100:7959–7964, 2003 **DOI:**10.1073/pnas.1335934100
- M. A. Teillet, R. Naquet, and C. Batini, Transfer of an avian genetic reflex epilepsy by embryonic brain graft: A tissue autonomous process?, *Int. J. Dev. Biol.* , 49:237–241, 2005 **DOI:**10.1387/ijdb.041961mt