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Review article

Molecular investigations of the brain of higher mammals using gyrencephalic carnivore ferrets

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ABSTRACT

The brains of mammals such as carnivores and primates contain developed structures not found in the brains of mice. Uncovering the physiological importance, developmental mechanisms and evolution of these structures using carnivores and primates would greatly contribute to our understanding of the human brain and its diseases. Although the anatomical and physiological properties of the brains of carnivores and primates have been intensively examined, molecular investigations are still limited. Recently, genetic techniques that can be applied to carnivores and primates have been explored, and molecules whose expression patterns correspond to these structures were reported. Furthermore, to investigate the functional importance of these molecules, rapid and efficient genetic manipulation methods were established by applying electroporation to gyrencephalic carnivore ferrets. In this article, I review recent advances in molecular investigations of the brains of carnivores and primates, mainly focusing on ferrets (*Mustela putorius furo*).

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1. Introduction

Recently, carnivores and primates have attracted more attention from neuroscience researchers than previously because molecular investigations of the brain using these animals are becoming feasible. The brains of carnivores and primates contain developed brain structures that mice do not seem to have. These structures include ocular dominance columns (ODCs) in the visual cortex, the magnocellular (M) and parvocellular (P) pathways in the visual system, and the gyrus and outer subventricular zone (OSVZ) of the cerebral cortex. Uncovering the physiological importance, developmental mechanisms and evolution of these structures using carnivores and primates would lead to our understanding of the human brain and its diseases, which are often difficult to investigate using mice. Although the anatomical and physiological properties have been intensively examined, molecular investigations of the formation, function, pathophysiology and evolution of these structures are still limited. This is not only because these structures are only poorly developed in mice, which are most commonly used for molecular investigations of the brain, but also because genetic methods that

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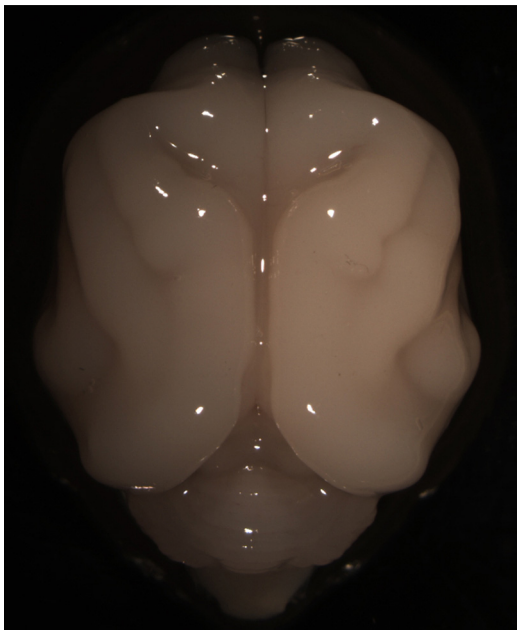

Fig. 1. The cerebral cortex of ferrets at P18. Note that cortical gyri are clearly visible.

can be applied to carnivores and primates were poorly available until recently.

To overcome these limitations, genetic techniques that can be applied to carnivores and primates have been explored. Pioneering research projects have reported successful application of virus vectors to make transgenic monkeys and marmosets (Chan et al., 2001; Lois et al., 2002; Sasaki et al., 2009). The injection of a lentiviral vector into marmoset embryos resulted in transgenic marmosets that expressed the transgene in several organs (Sasaki et al., 2009). Notably, germ-line transmission of the transgene was observed, and transgenic offspring developed normally (Sasaki et al., 2009). Although the creation of transgenic marmosets provides a new animal model for human disease (Okano et al., 2012), making transgenic animals takes time and effort, and requires special animal facilities. Because it was desirable to establish a rapid and simple genetic manipulation method for carnivores and primates, several groups reported post-natal electroporation protocols for ferrets to express transgenes into the retinal and superficial cortical neurons (Borrell, 2010; Huberman et al., 2005). To achieve transgenic expression in most layers of the cerebral cortex in ferrets, we successfully applied *in utero* electroporation to ferrets (*Mustela putorius furo*) as discussed below (Kawasaki et al., 2012, 2013). Combining the use of transgenic primates and the genetic manipulation of ferrets would greatly facilitate our understanding of the brain of higher mammals. In this article, I review recent advances in molecular investigations of the brains of higher mammals, mainly focusing on ferrets.

2. The ferret as a model animal for investigating the cerebral cortex

The ferret, like the weasel, badger and skunk, belongs to Mustelidae, which is a family of carnivorous mammals. They have an average length of about 50 cm and weight of about 1–2 kg. Ferrets have a long history as animal model subjects because they have developed brain structures that mice do not have. One of the most prominent features of the ferret brain is the formation of folds in the cerebral cortex (Fig. 1). Humans, monkeys and ferrets have gyrencephalic brains (*i.e.* brains with a folded cerebral cortex), while the brains of rodents are often lissencephalic (*i.e.* lacking cortical convolutions). Although the mechanisms underlying the formation of the cortical gyrus still remain unclear, it was proposed that the increased number of cortical neurons led to expansion and folding of the cerebral cortex during evolution. It is therefore important to uncover the mechanisms regulating the differentiation and proliferation of neural progenitors in the cerebral cortex during development (Borrell and Reillo, 2012; Dehay and Kennedy, 2007; Fietz and Huttner, 2011; Hevner and Haydar, 2012; Lui et al., 2011; Molnar and Clowry, 2012; Rakic, 2009).

Cortical neurons arise from radial glial cells (RG cells, also known as apical progenitors/apical RG cells/ventricular RG cells), the epithelial stem cells that line the cerebral ventricles and extend apical fibers and basal fibers (Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001). RG cells in the ventricular zone (VZ) undergo multiple rounds of asymmetric divisions and generate intermediate progenitor cells (IP cells/basal progenitors) that migrate into the subventricular zone (SVZ) and further proliferate to increase neuronal number (Haubensak et al., 2004; Noctor et al., 2004). Corticogenesis in carnivores and primates is distinguished by the appearance of the large SVZ that has an inner region (ISVZ) and an outer region (OSVZ), often split by a thin layer of fibers, called the inner fiber layer (IFL) (Smart et al., 2002; Zecevic et al., 2005). Recent studies identified a novel class of progenitor cells found in the OSVZ, termed OSVZ radial glial cells (oRG cells, also known as outer RG cells/basal RG cells/intermediate RG cells/translocating RG cells) (Fietz et al., 2010; Hansen et al., 2010; Reillo et al., 2011). Unlike RG cells in the VZ, oRG cells are unipolar, with a basal fiber that ascends toward the pia without an apical fiber that descends toward the ventricle. Because a major underlying cause of the expansion and gyrification of the cerebral cortex could be the increase in population size of neural progenitors in the OSVZ, a specialized germinal zone characteristic of higher mammals, it is important to investigate the mechanisms underlying the proliferation and differentiation of neural progenitors in the OSVZ. The ferret is a good option for investigating such mechanisms, given that genetic manipulation using ferrets has become feasible (Borrell, 2010; Kawasaki et al., 2012, 2013; Nonaka-Kinoshita et al., 2013; Pilz et al., 2013; Reillo et al., 2011). Recent studies reported genes expressed in the VZ and SVZ of mice and in various regions of the cerebral cortex in monkeys (Ayoub et al., 2011; Bernard et al., 2012). Manipulating these genes in ferrets would potentially uncover the mechanisms underlying the formation of the gyrus and the OSVZ in the cerebral cortex. In addition, because recent studies identified oRG-like progenitors in mice and marmosets, these animals also seem useful for examining the development of oRG cells (Garcia-Moreno et al., 2012; Kelava et al., 2011; Shitamukai et al., 2011; Wang et al., 2011). The identification of molecules whose expression patterns correspond to brain structures unique to carnivores and primates would facilitate our understanding of the development, function, pathophysiology and evolution of the brain. In addition, because a recent report uncovered four distinct morphologies of oRG cells in macaque (Betizeau et al., 2013), it would be intriguing to express GFP in oRG cells and examine the morphological diversity of oRG cells in ferrets.

Neuronal migration has also been examined using the cerebral cortex of ferrets (Anderson et al., 2002; Borrell et al., 2006; O'Rourke et al., 1992, 1995, 1997). Time-lapse confocal microscopy using cultured cortical slices showed that the majority of cells migrated along radial fibers, whereas a fraction of cells migrated orthogonal to the radial fibers (O'Rourke et al., 1992). After *in vivo* DiI focal injection, labeled cells migrated in all directions and over long distances (O'Rourke et al., 1997). These results suggest that cortical cells migrate not only radially but also non-radially, which may result in tangential dispersion.