Chapter 4
Developmental Sequences Predict Increased Connectivity in Brain Evolution: A Comparative Analysis of Developmental Timing, Gene Expression, Neuron Numbers, and Diffusion MR Tractography

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Abstract A conserved sequence in cell-type specification across mammals suggests that evolutionary changes in developmental timing may give rise to predictable changes in connectivity patterns across species. We here review the regularities in the timing of developmental events across species. We then use them to predict evolutionary changes in the number of cell types in order to identify evolutionary changes in the internal circuitry of the cerebellum as well as the gray and white matter of the isocortex across mammals. We survey what is known about the sequence and timing of cell-type specification in different brain regions and in

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various mammalian species. We find that lengthened developmental schedules predict a disproportionate increase in the number of locally projecting granule cells within the cerebellum and in the number of isocortical neurons projecting within or across cortical areas. Our main conclusion is that, as brains get bigger, neurons increasingly connect within their own major brain region.

Keywords Connections • Cortex • Diffusion MR • Evolution • Layers • Primate

4.1 Introduction

How connectivity patterns evolve has been an enduring question in the study of comparative neurobiology. Variation in brain size has been proposed to entail evolutionary changes in connectivity patterns (Deacon 1990; Striedter 2005). Yet, we still have few lines of empirical evidence to identify how connectivity patterns have evolved. Masterton and his colleagues compared the number of neurons that comprise different pathways in a large sample of mammalian species and showed that corticospinal neuron numbers largely covary with brain size (Nudo and Masterton 1990; Nudo et al. 1995). These data offer a glimpse as to how connectivity patterns evolve.

Connectivity patterns have been well characterized using anatomical tracer methods in a broad range of species (Kawamura 1973a, b, c; Kaas 1989; Striedter 2005; Schmahmann and Pandya 2009). Collectively, this large body of work has shown that projection patterns exhibit stereotypical patterns, which are relatively stable in mammalian evolution. For instance, cortical connectivity patterns exhibit a small-world network, wherein the majority of neurons project locally between cortical areas rather than over long distances (Sporns and Zwi 2004; Bullmore and Sporns 2012). This small-world pattern of connectivity is evident in primates such as macaque monkeys as well as in carnivores such as cats (Bassett and Bullmore 2006). As another example, neurons across the depth of the isocortex exhibit stereotypic patterns of projections. Upper layer neurons (layers II–IV) preferentially project within and across cortical regions, but many lower layer neurons (layers V–VI) project to subcortical structures (Gilbert and Kelly 1975; Barbas 1986; Nudo et al. 1995; Hof et al. 1995). That is, there is conservation in cortical neuron projection patterns in mammalian evolution.

The present review synthesizes findings from the field of evolutionary and developmental biology (evo-devo) to identify how evolutionary changes in developmental timing and conservation in the sequence of cell-type generation yields evolutionary changes in connectivity patterns. Given that different cortical layers consist of different cell types (Hof et al. 1995; DeFelipe et al. 2002; Belgard et al. 2011; Zeng et al. 2012) and neurons in upper (i.e., layers II–IV) versus lower layers (i.e., layers V–VI) exhibit stereotypical patterns of connectivity (Gilbert and Kelly 1975; Nudo et al. 1995; Barbas 1986; Rowell et al. 2010; García-Cabezas and Barbas 2014; Markov et al. 2014; Yamawaki et al. 2014), allometric variation in

cell-type numbers yield insights as to how connectivity patterns evolve. We specifically focus on the relative variation in the number of neuron subtypes such as isocortical upper layer (i.e., layers II–IV) and lower layer neurons (i.e., layers V–VI), as well as cerebellar granule and Purkinje neurons. Such an analysis shows that intra-regional projecting neurons within the isocortex and within the cerebellum become disproportionately more numerous as developmental schedules lengthen and brains expand.

4.1.1 Conservation in Developmental Sequences

The evo-devo approach has identified broadly conserved molecular developmental mechanisms and conserved cell-type specification across species (Finlay and Darlington 1995; Puelles and Rubenstein 2003; Puelles and Ferran 2012). Cell birth-dating studies performed in a broad range of mammals (e.g., rodents, primates, marsupials) show that the sequence of cell-type specification is highly conserved across mammals and that different brain regions vary in their duration of neurogenesis (Clancy et al. 2001; Workman et al. 2013). Some brain regions such as the isocortex and the cerebellum undergo neurogenesis for an extended period of time compared with other brain regions such as the thalamus and the medulla (Bayer and Altman 1991; Finlay and Darlington 1995; Rakic 2002; Workman et al. 2013). The protracted neurogenetic schedule of the developing isocortex and cerebellum is particularly evident in species with prolonged developmental schedules such as primates and marsupial mammals but is less evident in faster-developing species such as mice and rats because developmental events become more clearly separated in time in longer-developing species (e.g., primates; Workman et al. 2013).

Although data from cell birth-dating studies are lacking in humans, an inspection of gene expression levels from the Allen Institute for Brain Science can provide some insights into changes in developmental maturation in humans (Hawrylycz et al. 2012; Miller et al. 2014). For instance, the *RBFOX3* gene (i.e., *NeuN*) differentially increases its expression over developmental time in humans in each brain region (Fig. 4.1). The observation that cerebellar *RBFOX3* expression increases well into the postnatal period compared with other brain regions, such as the thalamus, suggests that the prolonged period of neurogenesis timing that has been observed for nonhuman mammals is mirrored in the duration of changes in gene expression in developing humans. Both birth-dating studies of nonhuman primates (Rakic 2002) and variation in gene expression over developmental time in humans (Hawrylycz et al. 2012; Miller et al. 2014) yield similar results in illustrating the extended period of neurogenesis or maturation of some structures (Rakic and Sidman 1970; Rakic 2002).

Early in the development of the isocortex, neurons are generated within the ventricular zone. They undergo mitosis along the ventricular wall. As development progresses, proliferative cells exit the cell cycle and migrate outside of the proliferative zone to become neurons or glia (Bystron et al. 2008). As cells exit the

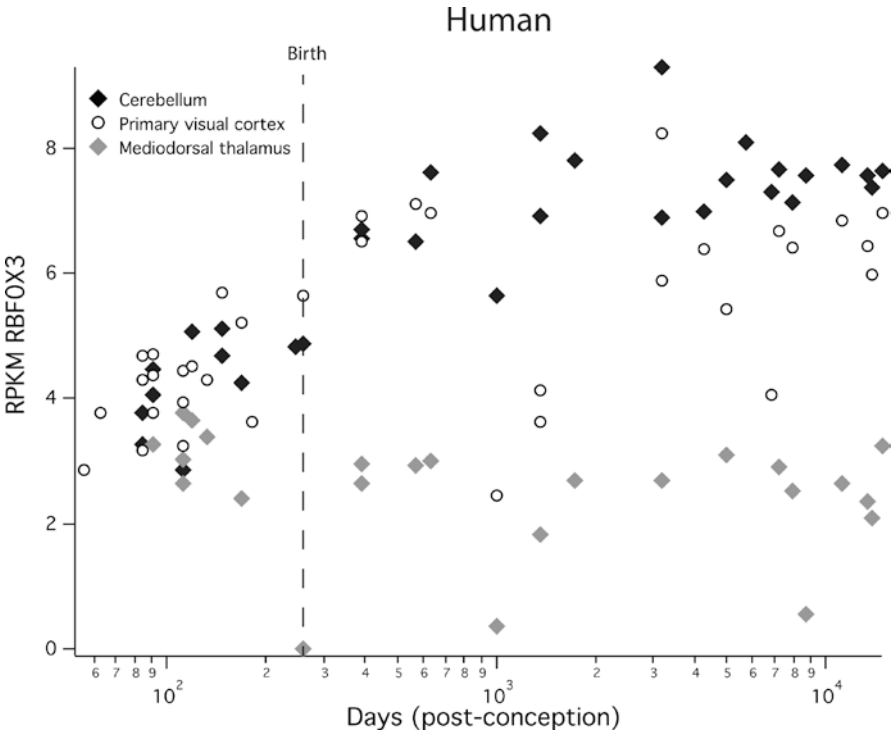

Fig. 4.1 Reads per kilobase of transcript per million (RPKM) of *RBFOX3* (start exon position, 77099243) in the thalamus, primary visual cortex, and cerebellar cortex over developmental time (age in days postconception) in humans. *RBFOX3* expression continues to increase in the isocortex and cerebellum over an extensive period of time, whereas RBFOX3 expression in the thalamus is relatively invariant. These data show that the maturation of the cerebellum extends for longer than the thalamus. These data are from the Allen Institute for Brain Science, brain atlas

proliferative zone, many neurons migrate along radial glia to the cortical plate (Rakic 2003). These radial glia can be seen with high-angular-resolution diffusion MR imaging tractography as shown in a human fetus at 17 gestational weeks (Takahashi et al. 2012; Fig. 4.2). These observations highlight the geometric structure of scaffolds that serve to give rise to the adult isocortex (Wilkinson et al. 1990; Wedeen et al. 2012). Diffusion MR tractography further shows that as neurogenesis wanes, scaffolds also regress and corticocortical tracts become evident (Takahashi et al. 2012).

The sequence of pyramidal cell-type specification is conserved in mammalian evolution. Neurons migrate to the cortical plate in an inside-out fashion such that infragranular layer neurons are born before granular layer neurons, which are in turn born before supragranular layer neurons (Sanderson and Weller 1990; Rakic 1974, 2002). The inside-out sequence of cell birth specification has been observed

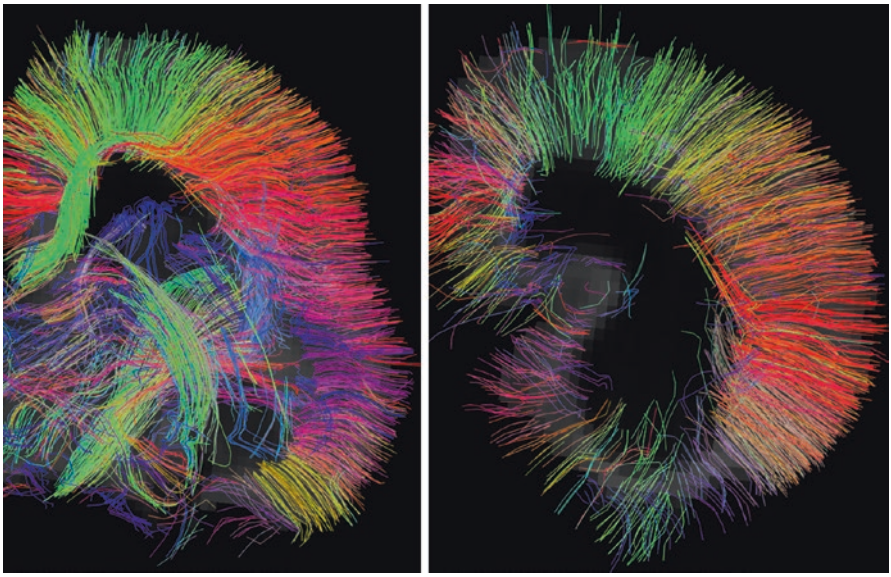

Fig. 4.2 High-angular-resolution MR tractography of a human at 17 gestational weeks. Coronal planes show pathways coursing radially from the proliferative zone along the ventricle to the outer surface of the cortical surface. The color-coding of tractography pathways is based on a standard RGB code, applied to the vector between the end points of each fiber (*red*, left-right; *green*, dorsal-ventral; *blue*, anterior-posterior). Images of these scans are from Takahashi et al. (2012)

in every mammalian isocortex studied so far (e.g., Fig. 4.3, primates, rodents, marsupial mammals; Sanderson and Weller 1990; Polleux et al. 1997; Marotte and Sheng 2000; Workman et al. 2013), but the sequence of neurogenesis that is characteristic of the mammalian isocortex is not observed in the pallium of reptiles and birds (Tsai et al. 1981; Goffinet et al. 1986; Striedter and Keefer 2000; Rowell and Ragsdale 2012). The most parsimonious interpretation of these data is that the inside-out sequence of cortical neurogenesis emerged early in mammalian evolution.

The cerebellum, likewise, exhibits a specific sequence in the birth order of cell types. Early in development, proliferative cells are located toward the ventricular surface and migrate radially outward throughout the developing cerebellum. As development progresses, an additional proliferative pool called the external granular layer forms, which consists of proliferative cells concentrated toward the cerebellar surface (Fujita et al. 1966; Ponti et al. 2006, 2008). As cells exit the external granular layer, these cells migrate inward to occupy the granular cell layer (Rakic and Sidman 1970). This outside-in pattern of neurogenesis observed in the developing cerebellum stands in contrast to the inside-out pattern of neurogenesis observed in the developing isocortex.

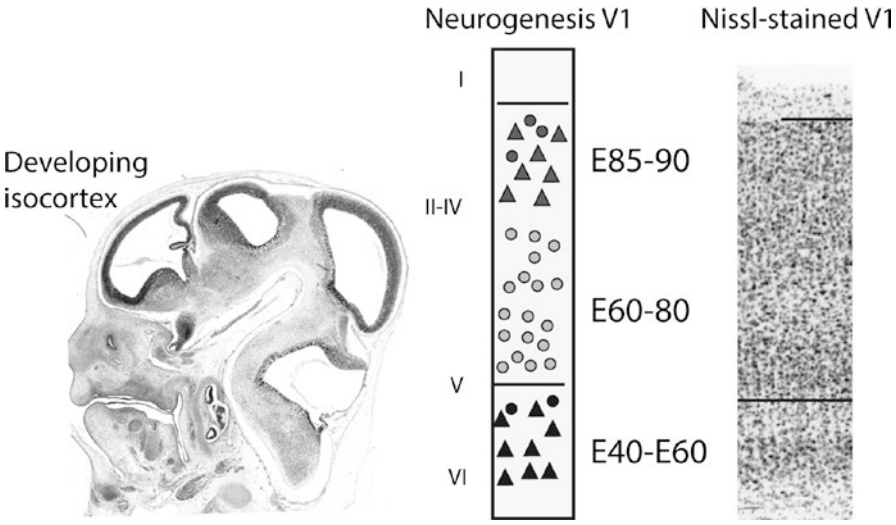

Fig. 4.3 Early in development, the developing isocortex consists of the ventricular zone. Neurons born found at various depths of the isocortex are born at different times. Early in development, the developing isocortex consists of the ventricular zone, which contains proliferative cells as shown here from a sagittal section of an embryonic tarsier. Representation of the birth order of lower and upper layer neurons in the macaque V1 cortex. Neurons found in lower layers are generated between embryonic day (E) 40 and 60. Neurons located in layer IV are generated between E60 and E80. Neurons located in upper layers are generated between E85 and E90. The tarsier (specimen ID# 1012) is part of the Hubrecht collection and was photographed at the Naturkunde Museum. Data on V1 neurogenesis timing are from Rakic (1974, 2002)

By adulthood, late-born granule cells are located within inner layers, whereas early-born Purkinje cells are located toward the outer cellular layer. Cell birth-dating studies in rodents and primates have shown that Purkinje cells are generated over a short interval, but granule cell production is generated for an extended period of time. For instance, Purkinje cell production occurs for roughly 5 days, but granule cell production extends over 140 days in rhesus macaques (Rakic 2002). In primates, as in other mammals, granule cell production extends into the postnatal period (Fig. 4.4; Bayer and Altman 1991; http://braindevelopmentmaps.org; Ponti et al. 2006, 2008). A similar situation is observed in nonmammalian species such as chickens or quail where Purkinje cells are generated over a short interval but granule cell production extends into the post-hatchling period (Gona 1976; Yurkewicz et al. 1981; Uray et al. 1987; Stamatakis et al. 2004). The most parsimonious interpretation of these data is that the sequence of cerebellar cell-type specification evolved early at least in sauropsids.

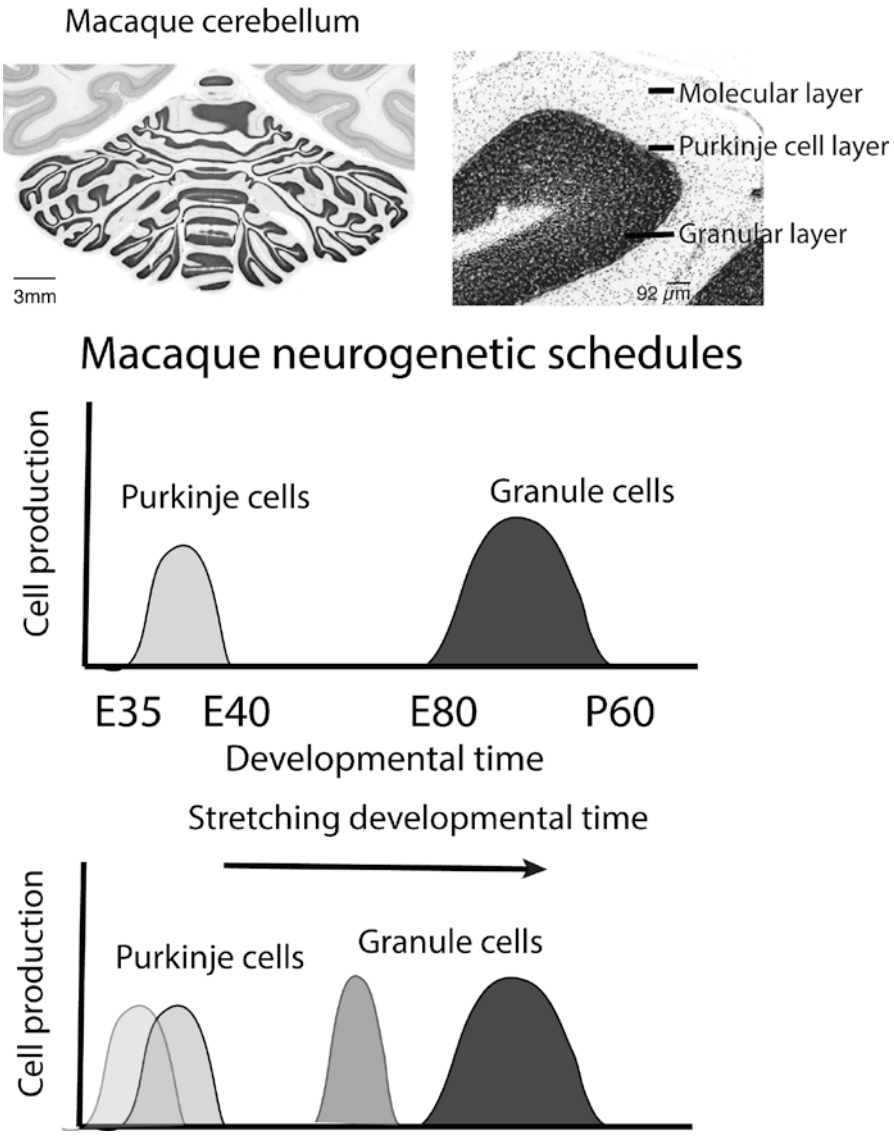

Fig. 4.4 A coronal section through the cerebellum of a macaque. Purkinje cells are born for a few days, but granule cell production extends into the postnatal period in macaques. Evolutionary changes in developmental duration entails that cells that are born late in development become disproportionately increased in numbers compared with neurons that are born early in development. In other words, the duration of granule cell production occurs for proportionately longer in longer-developing species. As a consequence, granule cell production disproportionately increases in bigger-brained species

4.1.2 Variation in Developmental Duration

The sequence in which neurons are born combined with variable lengths of developmental timing entail predictable changes in which cell types become amplified in bigger brains. A model proposed by Barbara Finlay and her collaborators states that as developmental schedules lengthen across species, neurons that are born late in development become disproportionately more numerous than neurons that are born early (Finlay and Darlington 1995; Cahalane et al. 2012, 2014). Consequently, this model predicts that as developmental schedules lengthen, the isocortex and cerebellum should become disproportionately enlarged relative to other regions because isocortical and cerebellar neurogenesis is protracted compared with that of other brain regions (Finlay and Darlington 1995; Reep et al. 2007; Workman et al. 2013). This model explains the allometric variation in the size of brain regions, the number of neurons that are contained within each brain region, as well as the allometric variation in the number of various cell types that comprise a given brain region (Fig. 4.5; Finlay and Darlington 1995).

4.1.3 Isocortical Development

Cell types within a given brain region can be distinguished by their birth order, their position, their gene expression, as well as their patterns of connectivity (Rakic 1974; Gilbert and Kelly 1975; Belgard et al. 2011; Zeng et al. 2012). A comparative analysis of isocortical neuron numbers in primates and rodents shows that upper layer neuron numbers become disproportionately amplified in larger brains relative to lower layer neurons (Fig. 4.6; Finlay et al. 1998; Clancy et al. 2001; Cahalane et al. 2014; Charvet et al. 2015, 2016, 2017a). The protracted production of upper layer neurons accounts for the differential increase in the number of upper layer neurons relative to lower layer neurons in bigger brains.

Because the laminar position of cell types in the isocortex can be distinguished by their specific patterns of connectivity, the disproportionate expansion of upper layer neurons yields specific consequences for connectivity patterns across taxa. As an example, we consider the allometric variation in the number of upper layer neurons and layer V corticospinal neurons (Nudo and Masterdon 1990; Charvet et al. 2015). Many somata of neurons that comprise the corticospinal tract are found within the frontal cortex (e.g., primary motor cortex; Nudo et al. 1995), and they form synapses with neurons within the spinal cord. Interestingly, the precise terminations of corticospinal tract neurons vary between species (Striedter 2005). To quantify corticospinal tract neurons, Nudo et al. (1995) applied horseradish peroxidase to the cervical spinal cord and quantified the labeled somata found in the isocortex. As is evident in Figs. 4.6 and 4.7, upper layer neuron numbers become disproportionately amplified relative to lower

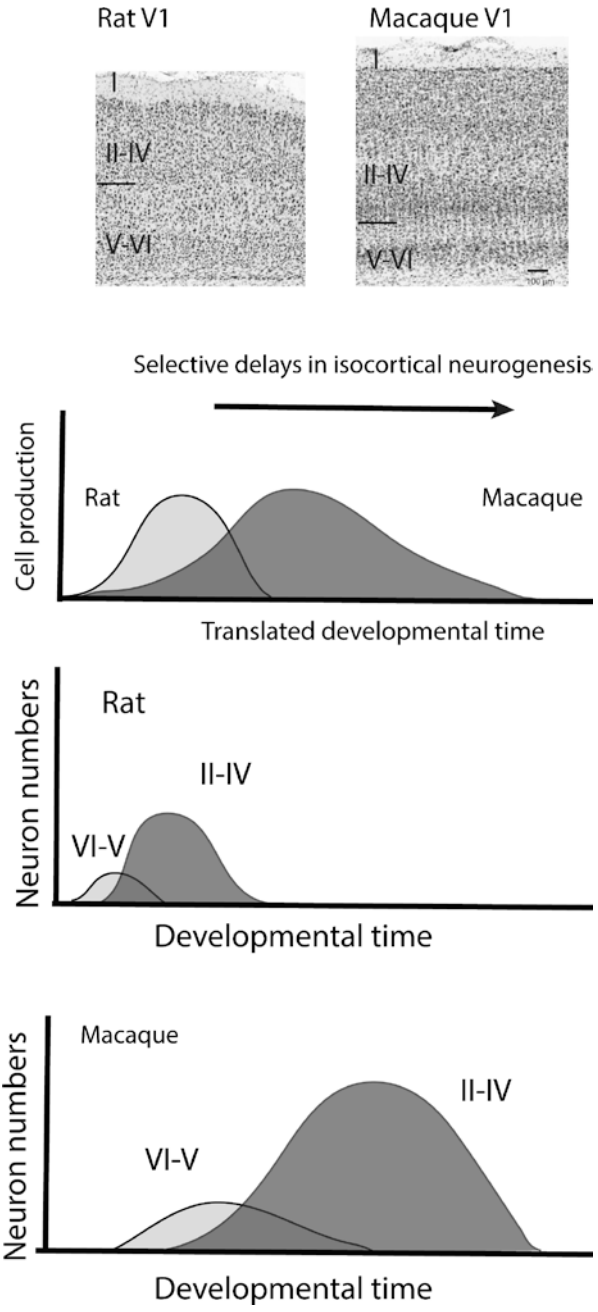

Fig. 4.5 Primates possess expanded upper layers compared with rats. Selective delays in isocortical neurogenesis in primates relative to rodents lead to a disproportionate expansion of cells that are born late in development. Because upper layer neurons are born after lower layer neurons, selective delays in isocortical neurogenesis entail that primates possess disproportionately more upper layer neurons compared with rodents. Images of Nissl-stained sections are screenshots from brainmaps.org

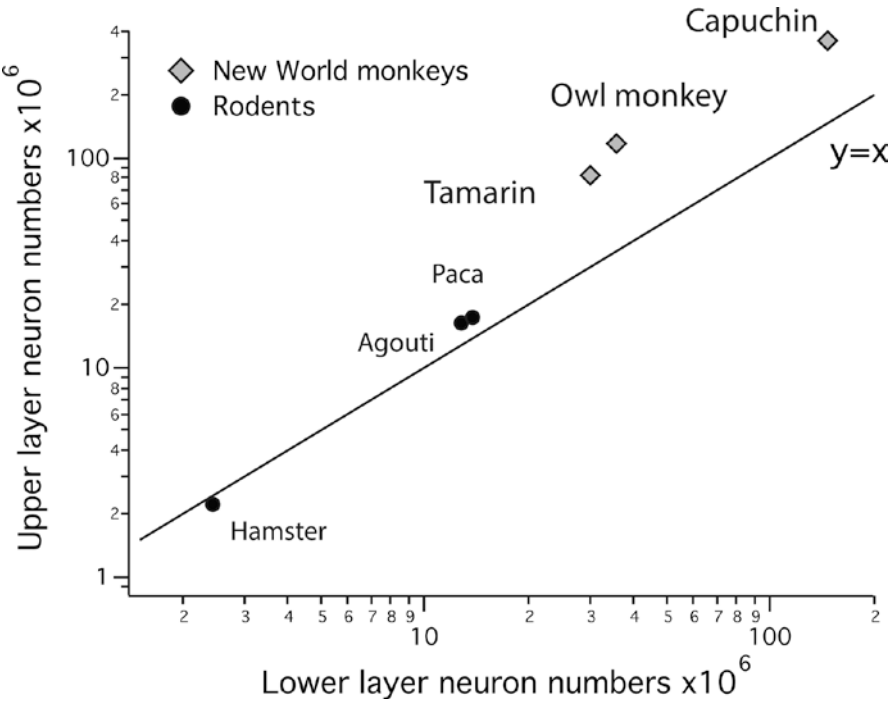

Fig. 4.6 Upper layer neuron numbers are plotted against lower layer neurons in primates and rodents. A linear regression is also plotted to highlight that upper layer neuron numbers expand disproportionately relative to lower layer neurons in primates and rodents

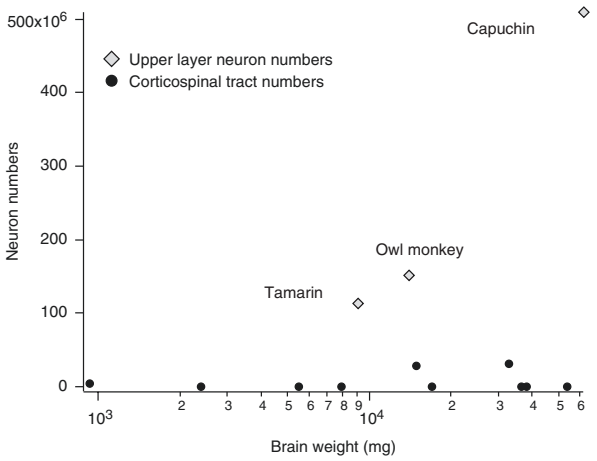

Fig. 4.7 Corticospinal tract neuron numbers (in a lower layer) and upper layer neuron numbers are plotted against brain weight in primate species. Upper layer neuron numbers expand disproportionately relative to corticospinal tract neuron numbers in bigger-brained primates. These findings show that cross-cortically projecting neurons increase with a positive allometry as brains expand. These data are from Nudo and Masterdon 1990 and Charvet et al. (2015)

layers and corticospinal tract neuron numbers as brains expand. The disproportionate expansion of isocortical neuronal numbers projecting within or to other cortical regions relative to isocortical neuron numbers projecting to subcortical structures may thus serve to increasingly modulate incoming and outgoing information in bigger brains.

4.1.4 Cerebellar Development

Similar to what is observed for the isocortex, cerebellar cell types can also be distinguished by their birth order, their position, as well as their patterns of connectivity. Although there are few quantitative studies examining the number of cerebellar cell types across species, a comparison between granule cells and Purkinje cell numbers in humans and rats show that the relative number of granule cells to Purkinje cell numbers is disproportionately increased in humans compared with rats. For instance, the ratio of granule cells to Purkinje cells in rats is less than 500, while in humans, the ratio of granule cells to Purkinje cells is approximately 3500 (Harvey and Napper 1988; Andersen et al. 1992). That is, granule cells are disproportionately more numerous in the bigger-brained humans than in the small-brained rat.

Within the cerebellum, incoming information from pre-cerebellar nuclei located within the pons projects onto granule cells. Granule cells, in turn, project to Purkinje cells (Voogd and Glickstein 1998). Purkinje cells also receive input from inferior olive neurons and project onto cerebellar nuclei as well as onto the neurons of the vestibular complex. Within this circuitry, granule cells become preferentially amplified, project locally, and increasingly synapse with Purkinje cells (Huang et al. 2014). The disproportionate increase in granule cell numbers in bigger-brained species entails increased modulation of incoming and outgoing information.

4.1.5 Selective Changes in Neurogenesis Timing

In evolution, the conservation in the sequence of cell-type specification is superimposed on selective changes in the duration of neurogenesis timing (Workman et al. 2013). Heterochronies (i.e., developmental changes in the timing or rate of events) within some brain regions have been observed in a number of taxa such as primates, carnivores, as well as parrots and songbirds (Charvet et al. 2011; Workman et al. 2013). For instance, distantly related nocturnal species such as owl monkeys and cats exhibit selective delays in retinal neurogenesis relative to diurnal species, which led to a disproportionate amplification of late-born neurons relative to early-born neurons (Finlay 2008; Dyer et al. 2009; Workman et al. 2013). Because rods are born late in development, selective delays in retinal neurogenesis are associated with a disproportionate increase in the number of rods in nocturnal species compared with diurnal species. Evolutionary changes in developmental timing within the peripheral nervous system evolved in very distant lineages such as cats and owl monkeys.

As another example of heterochrony, parrots and songbirds selectively delay telencephalic neurogenesis relative to galliform birds. Evolutionary changes in devel-

opmental timing are concomitant with a number of changes in developmental processes. The selective delay in telencephalic neurogenesis in parrots is concomitant with delays in the decline in telencephalic cell cycle rates as well as an amplification of cells undergoing mitosis within the subventricular zone, which is an additional proliferative zone that lies superficial to the ventricular zone in development (Smart et al. 2002; Bystron et al. 2008; Charvet and Striedter 2008; Charvet et al. 2011; Martínez-Cerdeño et al. 2012; Dehay et al. 2015). Unlike ventricular zone cells, proliferative cells in the subventricular zone undergo mitosis at scattered locations throughout the subventricular zone (Smart 1972; Smart et al. 2002; Martínez-Cerdeño et al. 2012). The protracted neurogenetic schedules of parrots and songbirds may have fostered an increased duration of post-hatchling maturation in which juveniles may learn from conspecifics (Charvet and Striedter 2011).

Among mammals, birth-dating studies demonstrate that primates selectively delay isocortical neurogenesis relative to rodents (Workman et al. 2013). In adulthood, the selective delays in isocortical neurogenesis are concomitant with the expansion of the isocortex as well as increased isocortical neuron numbers in primates relative to many other taxa (Workman et al. 2013; Herculano-Houzel 2012). According to the model of "late equals large" cell populations that are born late in development become disproportionately more numerous relative to neurons that are born early in development (Figs. 4.4 and 4.5). Because upper layer neurons are generated late in the developing isocortex, upper layer neuron numbers should become disproportionately expanded in primates relative to other mammals such as rodents.

Although few studies have explicitly compared the number of cortical upper layer and lower layer neurons across mammalian species, data on upper and lower layer neuron numbers and densities have been gathered for primates, rodents, and manatees (Charvet et al. 2015, 2016; Reyes et al. 2015). Rodent brains are generally smaller and contain fewer neurons per brain mass compared with primates, which makes it difficult to specifically address whether primates exhibit disproportionately more upper layer neurons compared with rodents (Figs. 4.5 and 4.6; Herculano-Houzel 2012). A previous study compared upper layer neuron numbers in manatees relative to primates, because manatees have a large brain that is similar to that of some primates, which allows for a comparison between taxa of equivalent brain size and overall cortical neuron numbers. This study found that primates exhibit disproportionately more isocortical upper layer neurons compared with manatees (Charvet et al. 2015, 2016). It would be interesting to quantify upper and lower layer neuron numbers in a broader range of mammals to identify how upper layer neuron numbers in primates deviate from other taxa (Hofman 1985; Charvet et al. 2017a, b). The data so far supports the notion that selective delays in cortical neurogenesis in primates are concomitant with a disproportionate expansion of isocortical neurons and, in particular, upper layer neuron numbers.

4.2 Species Differences in Projection Patterns

The number of isocortical upper layer neurons (i.e., layers II–IV) projecting within or across cortical regions and lower layer neurons (i.e., layers V–VI) projecting to subcortical regions varies predictably with brain size. Yet, there are clear species

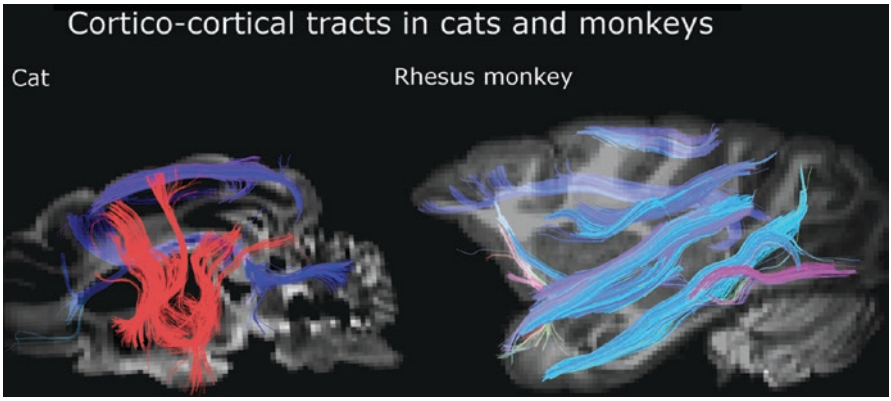

Fig. 4.8 Corticocortical tracts in a cat and a macaque were reconstructed from diffusion spectrum MR scans and in accordance with previous descriptions of tract-tracing studies in cats and macaques. Many tracts course across the anterior to posterior direction in the macaque, but a number of tracts course across the dorsal to ventral direction in cats. Except for the dorsal-ventral pathways in the cat, the color-coding of tractography pathways is based on a standard RGB code, applied to the vector between the end points of each fiber (*red*, left-right; *green*, dorsal-ventral; *blue*, anterior-posterior). The pathways coursing across the dorsal-ventral direction in the cat are shown in a single color (*smoky red*). These scans were published previously (Takahashi et al. 2011, 2012, Charvet et al. 2017a)

differences in the terminations of corticocortical projection patterns. Figure 4.8 shows corticocortical tracts reconstructed with the use of diffusion spectrum imaging (Takahashi et al. 2010, 2011; Wedeen et al. 2012). We referred to tract-tracing or lesion studies that identified corticocortical tracts in cats and macaques to ensure the accuracy of the diffusion MR tractography of these brains (Schmahmann and Pandya 2009; Diamond et al. 1968; Kawamura and Otani 1970; Kawamura 1973a, b, c; Paula-Barbosa et al. 1975; Poldrack and Farah 2015). As is evident in Fig. 4.8, trajectory patterns of corticocortical tracts clearly differ between the two species with macaques exhibiting many tracts aligned along the anterior-posterior axis, whereas cats exhibit a number of tracts coursing in the dorsal-ventral direction. Although cell birth order and position can predict the number of specific cell types projecting within or outside their major subdivisions, the trajectories and therefore terminal locations of corticocortical tracts clearly vary between species.

4.3 Developmental Sources of Change in the Brain and Behavior

Our analysis supports the notion that the number corticocortically projecting neurons in the isocortex and granule cells projecting to Purkinje cells devoted to modulating incoming information becomes disproportionately increased in bigger brains. Evolutionary changes in brain size are concomitant with the amplification of isocortical and cerebellar cell types that are born late in development such as upper layer neurons and granule cells. These observations suggest that allometric variation in

cell-type numbers have emerged from wholesale changes in developmental timing rather than selected changes in the developmental mechanisms generating a specific cell type (Finlay and Darlington 1995). The increase in relative numbers of intra-regionally connecting neurons observed in the isocortex and locally projecting granule cells in the cerebellum would provide a powerful matrix to modulate incoming sensory information to mediate various behaviors in bigger-brained species.

Evolutionary changes in behavior might emerge through evolutionary changes in developmental timing, increased neuron numbers, or through a combination of all of these variables. We argue that, among these variables, behavioral changes are likely to have emerged from changes in developmental timing. Developmental schedules are associated to some degree with the duration of postnatal development. An extended duration of developmental timing may promote an extended period of postnatal maturation, a prolonged period of parental care, which may foster learning from conspecifics (Charvet and Finlay 2012). The covariation in developmental timing, brain expansion, and allometric variation in neuron numbers projecting locally within the cerebellum and those isocortical neurons projecting either within or across the cortical areas can together foster increased learning from conspecifics. The brain is intrinsically plastic, and environmental exposure can further sculpt what is learned. The flexible nature of the brain, coupled with variable lengths of developmental duration, may channel what information is modulated in bigger-brained species.

Within this extended developmental schedule and prolonged period from which to learn from conspecifics, what is learned is contingent on what is rewarding (Young and Wang 2004). The mesolimbic system is characterized by dopaminergic projections from the ventral tegmental area to the nucleus accumbens, and this circuit has been implicated in mediating a range of naturally rewarding behaviors such as pair bonding and bird song (Goodson et al. 2009; O'Connell and Hofmann 2011). Evolutionary changes within this circuitry may arise through changes in receptors that serve to modulate reward-related behaviors. For instance, intra- and interspecific variation in the dopamine D4 receptor (DRD4) have been noted and may be associated with a number of changes in behaviors related to reward (Ebstein et al. 1996; Ding et al. 2002; Wang et al. 2004; Vallender 2011, 2012; Yamamoto et al. 2013). These observations are increasingly at odds with the notion that evolutionary changes in brain region size account for changes in select behaviors (Healy and Rowe 2007). Rather, evolutionary changes in reward circuitries and developmental timing may be powerful substrates through which evolutionary changes in behaviors emerge.

4.4 Summary

We have surveyed which cell types become preferentially amplified in bigger brains. We have remained relatively agnostic as to the precise targets of these cell types. Our overview of evolutionary changes in the number of isocortical and cerebellar

neuronal populations shows that isocortical neurons increasingly project either within or across cortical areas and that cerebellar neurons increasingly project locally. In other words, neurons projecting within their major brain subdivision become disproportionately amplified in bigger brains.

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