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A Cross-Species Analysis of Prefrontal Cortex Homology Based on Anatomical Connectivity, Behavior, and Cell Types

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Abstract

The steepest rise in publications on prefrontal cortex (PFC) function over the past decade has been in mouse studies. If we adhere to cell layer organization criteria for what constitutes PFC, rodent researchers may be studying a different PFC to primate PFC. Indeed, this chapter reviews several unique aspects of primate brain: primate cortical evolution favored a clustering of cell types more than rodent; primate PFC is more specialized in the expression of interneurons compared to rodent; and where comparative transcriptomic studies of different cell types in PFC have been conducted, they reveal unique similarities only within primate species. In contrast to these differences between species, strong similarities are also reviewed: connectivity patterns across rodent and primate PFC, specifically agranular orbitofrontal cortex and anterior cingulate cortex, as well as common features of foraging with some innovations that may have contributed to PFC specializations in primate. The study of cell types should be better integrated in the study of PFC across species, and this integration should, in principle, be closely related to a characterization of the cells along a spatial and behavioral gradient that reflects phylogenetic refinement. Currently, few studies combine neural activity with molecularly defined cell types within a species, and even fewer take a comparative approach. Combining transcriptomically defined cell-type information with other characteristics, such as task-related signaling in PFC and their connectivity patterns across rodent and primate species, represents a major challenge to the field, but would be an impactful way forward.

Introduction

The zeitgeist of present-day neuroscience involves a fascination with the "central executive" which oversees and coordinates all behavior. Executive

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function is an umbrella term that includes the many different functions of the prefrontal cortex (PFC), including planning, self-ordered memory and monitoring, attentional set shifting (Fuster 1989; Luria 1966b; Robbins 1996), and cognitive control (Friedman and Robbins 2022), to name a few. The recent emphasis on mimicking executive functions is not surprising, given the rise in interest in using artificial intelligence and neural network architecture to support these functions (Tsuda et al. 2020). There is presently no shortage of research on PFC, probed with increasingly powerful tools and analyses in service of understanding the functions of this complex and heterogeneous region.

In a recent analysis of the prevalence and common misconceptions of what constitutes rodent frontal cortex, Laubach et al. (2018) found that while human PFC still held the lion's share of publications, followed by rats, then mice and monkeys (Figure 2.1a), the steepest rise in publication prevalence on PFC during the past couple of decades was actually in mouse (Figure 2.1b). Given this growing bias in model systems for PFC function, it begs the question: When one studies frontal cortex in rodents, how does this knowledge, *if at all,* translate to our understanding of PFC in primates? This is an established topic of consistent, heavy debate (Carlen 2017; Laubach et al. 2018). This debate arose many years ago as the Rose and Woolsey definition of PFC centered around anatomical connectivity to mediodorsal thalamus, with major input from MD thalamus into a clearly visible granule cell or "granular" Layer IV (L4) in PFC (Preuss 1995; Rose and Woolsey 1948). On the basis of this laminar or cell-layering (i.e., cytoarchitecture) criterion alone, rodents undisputedly lack a PFC. If we consider other criteria, such as connectivity, gene expression, electrophysiological properties, and behavior, we may make better comparisons across species.

I begin here by highlighting key limitations in the rodent model, especially those related to how findings from rodents may translate to human PFC, that have to do with gross anatomical differences in brain structure and shape. Unsurprisingly, primate brains have greater neuron numbers simply as a result of their folded-ness (i.e., deep sulci). This enhanced neuron number and

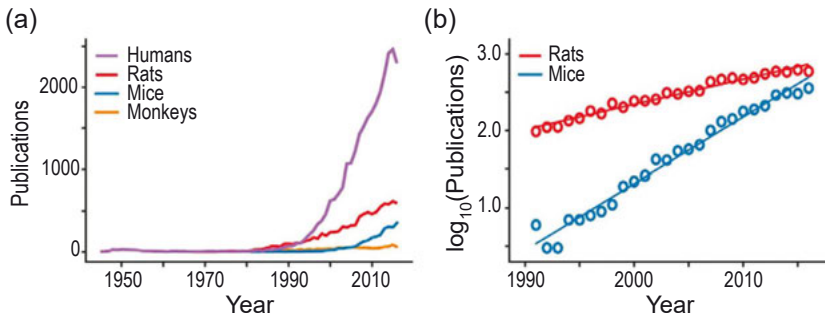

Figure 2.1 Publication trends (a) across species in (pre)frontal cortex and (b) number of published works per year. Reprinted with permission based on Laubach et al. (2018).

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cortical expansion is thought to be largely due to the proliferation of progenitor cells in the outer subventricular zone, OSVZ (Kriegstein et al. 2006; Lui et al. 2011). There are a few proposed mechanisms for this expansion, including radial glial cells acting as migratory guides for columnar distribution of neurons and intermediate progenitor cells contributing to increases in cell layers within each layer (i.e., lateral expansion of cortex, in primates). Indeed, humans have a "scaled up" primate brain (Herculano-Houzel 2009), meaning that the ratio of brain weight and neuron number is in accord with other primate brains of similar mass. Critically, however, rodent neocortex is nonfolded (i.e., lissencephalic, not gyrencephalic) so the ability to model human neocortical PFC developmental evolution is quite limited, perhaps especially using analyses of OSVZ expansion. A more complete integration of the cellular dynamics that subserves cortical development and evolution to include the molecular basis of the neural stem and progenitor cell diversity is generally lacking in the literature and should be explored across different primates, with full acknowledgment that it may have limited application to rodents. Another gross anatomical difference between rodent and primate PFC was highlighted by Vogt and Paxinos (2014) and Laubach et al. (2018), detailing that, comparatively, rodent brains lack curvature. They suggest that a primate specialization that contributed to this curvature is the expansion of the midcingulate cortex (MCC) wrapping around the genu of the corpus callosum, leading to the displacement of anterior cingulate cortex (ACC) more rostrally and ventrally. This primate expansion may have given rise to compression of tissue in the form of gyri in primates, but not rodents. Hence, this circles back to the difference between folded and nonfolded brains in primates and rodents, respectively.

Such substantial anatomical differences call into question if we are comparing similar brains across rodent and primate, especially since their nearest common ancestor occurred approximately 70 million years ago. In this vein, I recently reviewed evidence with Peter Rudebeck (Rudebeck and Izquierdo 2022) and concluded that along with comparative anatomy, there needs to be more thoughtful consideration of what different brains must do to obtain food in their natural environments, since different species evolved in unique foraging niches (Murray et al. 2011). In that review, we took an approach inspired by Cisek (2019): instead of highlighting functions that we think could have been subserved by different fronto-cortical systems (e.g., decision making, set shifting, flexible learning, working memory), we tried to follow the footsteps of evolution and assumed there was "phylogenetic refinement" of *clusters of functions* based on foraging niches. Below, I begin by reviewing commonalities across species having to do with neuroanatomical connectivity patterns and foraging behaviors that may have given rise to PFC specializations, and then consider more divergent results across species; information that may be most needed for a deeper understanding of comparative function across species *cell types* in PFC.

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Comparative Connectivity

The most anterior parts of macaque frontal cortex are either dysgranular or completely granular cortex, having a discernible granule cell L4. This includes orbital areas 13, 11, and 12 (ventrally), 10 and 9 medially, and areas 46 and 6 more laterally. More caudal areas of macaque orbital cortex (area 13) and areas 25, 32, and 24 more medially are agranular. Thus, similar to rats and mice, macaque caudal orbitofrontal cortex (OFC) and medial ACC are completely agranular. The medial wall of frontal cortex in rodent has historically been referred to as prelimbic (PL), infralimbic (IL), anterior cingulate (Cg1 and Cg2), but now more often referred to as areas 32, 25, and 24 with clear reference to their anterior-posterior (A-P) positioning. There is also the most ventrolateral portion of rodent frontal cortex that includes agranular insular, which I have suggested previously is not as well studied or understood as other subregions (Izquierdo 2017). Importantly, all these agranular subregions are shared by mammalian brains (Murray et al. 2011).

There are established "rules" about what could be considered PFC. First, as mentioned above, rodent frontal cortex is completely agranular, and the existence of a granule cell L4 has been for decades the primary definition of PFC in primate, with rats lacking any kind of homologue to prefrontal areas of primates (Preuss 1995). More importantly, moving away from this strict cytoarchitectonic criterion, we may better rely on other dimensions for species comparisons. Uylings et al. (2003) outlined five criteria for cross-species comparisons of PFC:

1. Cytoarchitectonic similarities
2. Connectivity patterns considering the density of those connections
3. Neurochemical distribution and receptor expression
4. Embryological development
5. Functional properties, including electrophysiological and behavioral similarities

Here, I emphasize criterion 2 (connectivity patterns) and criterion 5 (function), the latter with a focus on behavior. Electrophysiological comparisons are critically important as well, yet others have written on this topic (e.g., Seamans et al. 2008); for an updated comprehensive review, see Rich and Averbeck (this volume).

Laubach et al. (2018) provided a summary of the diversity of expert opinions in answer to the question: "What, if anything, is the rodent prefrontal cortex?" Their meta-analysis showed that there has been an overemphasis of the functions of the medial wall of frontal cortex compared to more lateral areas in rodents. Further, they reported that much of the diversity of expert opinions as to what constitutes rodent PFC may be partly due to the use of multiple and often inconsistent sets of anatomical nomenclature and acronyms to refer to the same subregions. Parcellation of subregions of rodent PFC may be conducted by using a similar framework as primate ACC: centered on gray

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matter location around the *genu* of the corpus collosum (i.e., along the A-P gradient). This proposal is substantiated by cross-species connectivity data, which I review next.

Several groups have suggested more attention should be given to the A-P axis as well as lateral over medial frontal cortex comparisons across species (Barreiros et al. 2021a, b; Izquierdo 2017; Rudebeck and Rich 2018; Wallis 2011). The most posterior and medial segments of nonhuman primate PFC are agranular and more similar in terms of connectivity to rodent (Heilbronner et al. 2016; Wise 2008). It is unclear, however, whether these map onto the most widely used anatomical atlases in rodents. For example, a recent review by van Heukelum et al. (2020) directly compared the structural and functional distinctiveness of cingulate cortex from both human (based mostly on diffusion tensor imaging) and rodent neuroanatomical tracing studies (Figure 2.2a, b). For this they used two different parcellations of cingulate cortex: a definition based on ACC and MCC along the A-P plane (Figure 2.2b) or the more widely used rat atlas Cg1/Cg2 designations that vary instead along the dorsal-ventral (D-V) plane (not shown). They found that the former, but not the latter, segmentation better reconciled functional results across species, referring to the A-P defined ACC as "homologous" and D-V Cg1/Cg2 segmentation as characteristically "nonhomologous." Connectivity of primate and rodent "homologous" ACC is strong with autonomic brainstem nuclei, amygdala, OFC, hippocampus, hypothalamus, and thalamus (van Heukelum et al. 2020), largely consistent with comparative studies (Floyd et al. 2001; Freedman et al. 2000). It should be noted, however, that there are ACC connections to autonomic regions in rat (e.g., nucleus of the solitary tract, magnocellular neurosecretory cell groups in the hypothalamus) that have not been observed or reported in macaques (Freedman et al. 2000).

In seminal work by Heilbronner et al. (2016), investigators used anatomical cases with anterograde tracers in rat and macaque ACC and OFC to study the extent to which cortico-striatal terminal inputs overlapped. They found that terminals into striatum overlapped extensively along with the medial wall and that area 25 in rats was most similar to area 25 in monkeys, similar to what van Heukelum et al. (2020) later reported in their metanalysis (Figure 2.2). Importantly, Heilbronner et al. (2016) also found similar patterns of connectivity across medial versus lateral OFC, with what they refer to as "homologous" segmentation along the striatum (Figure 2.3). Thus, using this striatal-based connectivity approach to study networks across species, Heilbronner et al. revealed largely conserved fronto-cortical inputs in rats and macaques. Since the sample in this study included various nonhuman primate species (*Macaca fascicularis, M. mulatta, M. nemestrina*) and rat strains (*Rattus Norvegicus*: Sprague-Dawley, Wistar, hooded strains), these findings are likely robust and generalizable in their conclusions of cross-species topography. Taken together, these studies (Heilbronner et al. 2016; van Heukelum et al. 2020) reveal the value of studying connections with striatum,

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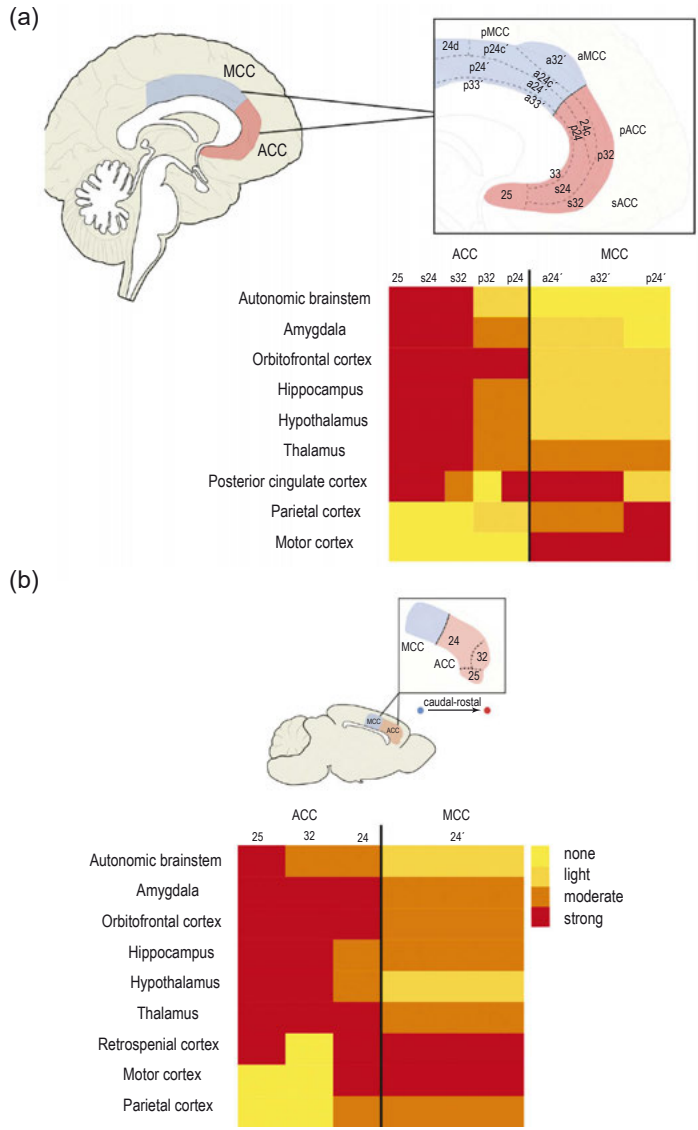

Figure 2.2 Similarity in primate and rodent anterior cingulate cortex (ACC) connectivity along the A-P axis. (a) ACC and mid-cingulate cortex (MCC) in humans. Mid-sagittal view of individual Brodmann's areas (top) and connectivity patterns (density) with other areas based mostly from diffusion tensor imaging (bottom). (b) The connectivity profile for the ACC/MCC nomenclature closely resembles the connectivity found in humans. See Brodmann's areas 25, 32 and 24 which are most anterior, different from a dorsal-ventral segmentation in Cg1 and Cg2 (not shown). Similarly strong connectivity can be found with amygdala, orbitofrontal cortex, hippocampus, hypothalamus, and thalamus. Modest-to-no appreciable connectivity with parietal and secondary motor cortex. Adapted with permission based on van Heukelum et al. (2020).

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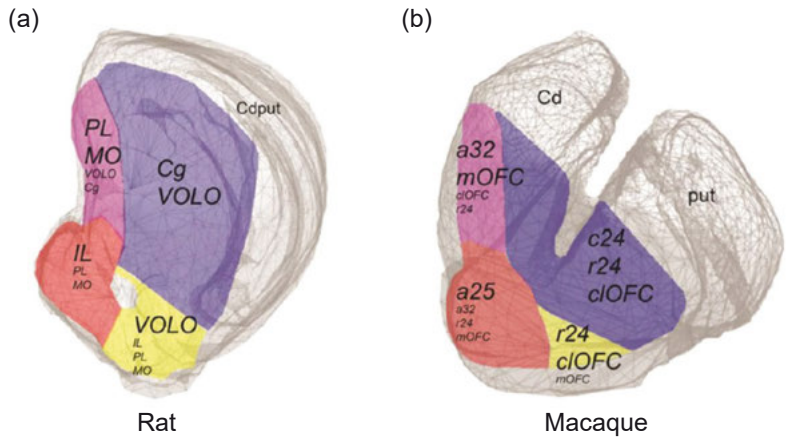

Figure 2.3 Similarity in parcellation of anterior cingulate cortex and orbitofrontal cortex inputs to striatum in rat and rhesus macaque. Striatal "segments" have unique combinations of fronto-cortical inputs and they are largely similar across rat (a) and nonhuman primate (b). Abbreviations: prelimbic (PL), infralimbic (IL), ventrolateral orbital (VOLO) cortex, medial orbital (MO) cortex, caudate (Cd), putamen (put), medial orbitofrontal (mOFC), cingulate (Cg), caudate-putamen (Cdput), caudolateral orbitofrontal (clOFC). Other divisions are also shown in Macaque: area 32 (a32), central area 24 (c24), rostral area 24 (r24), and area 25 (a25). Adapted with permission based on Heilbronner et al. 2016.

amygdala, other fronto-cortical regions, hippocampus, hypothalamus, and thalamus to assess rostral-caudal patterns of connectivity in PFC circuits, even if cytoarchitecture may not reveal as much similarity across species. Though recent anatomical tracing studies have been rigorously conducted within a single species (Barreiros et al. 2021a, b; Izquierdo 2017; Rudebeck and Rich 2018; Wallis 2011), it is a more powerful approach to study these patterns across species. Related to connectivity analyses, the Allen Brain Mouse Atlas (2011) provides a high-resolution, freely available anatomical reference along with a deep catalog of projection mapping experiments detailing axonal projections labeled by viral tracers. Similarly, with inclusion of the NIH Blueprint NHP atlas, there is a growing repository of selected gene analyses across the adult macaque brain that includes cellular marker genes with cortical area specificity as well as families of genes important to specific neural functions. Unfortunately, the Allen Brain Atlas lacks rat connectomics in their open-source atlas, which is by my estimation a missed opportunity in understanding the *functional* consequences of such comparative connectivity between rodents and nonhuman primates (NHP) because many classic, theory-driven behavioral experiments have been directed at understanding the substrates of these pathways in rats, not mice.

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Optogenetic and Chemogenetic Manipulations In Rodents and Primates

In addition to the many papers on rodent frontal cortex (Figure 2.1), in parallel there has been steady growth in NHP electrophysiological studies with high-channel count recordings (Berger et al. 2020; Mitz et al. 2017) and correspondingly sophisticated computational analyses to describe the neural correlates of high-order behavior and cognition. For an important discussion of the results of electrophysiological studies in PFC, see Rich and Averbeck (this volume). Importantly, viral-mediated technology to target the brain with cell-type specificity is increasingly more commonplace in NHP, including optogenetic manipulations. These perturbations work by precise light-gated excitation or inhibition of neural activity made possible by introduction of a viral vector in neurons to express light-sensitive channels, which are then responsive to different wavelengths of light (Deisseroth 2015). Optogenetic studies probing cortical circuits have demonstrated feasibility in NHPs (Diester et al. 2011) and have been especially useful to date in interrogating sensorimotor functions (El-Shamayleh and Horwitz 2019), though optogenetic efficacy in studying higher-order cognitive function and its potential application to psychiatry remains to be fully determined (Bliss-Moreau et al. 2022).

By comparison, chemogenetic techniques working through viral expression of mutant G-protein coupled receptors or designer receptors exclusively activated by designer drugs (DREADDs) (Armbruster et al. 2007) have been more widely applied to diverse behaviors in NHP than optogenetics. There is now evidence that success in using this technique depends on transduction level of the receptor and the ligand (i.e., actuator) used to activate these receptors in rodents, but perhaps most especially in NHPs (Eldridge et al. 2016; Grayson et al. 2016; Nagai et al. 2020; Roseboom et al. 2021; Upright and Baxter 2020; Upright et al. 2018). Conditional, pathway-specific DREADDs, often used in rodents, are also now being used in NHPs (Oguchi et al. 2021b; Oyama et al. 2022; Vancraeyenest et al. 2020; Wood et al. 2023). Yet despite these advances in tools in NHP experiments, due in part to the ease of working with the technology in rodent species (along with other critical factors such as lower cost of research, shorter lifespan of rodents, as well more limited access to training in working with NHPs), there have been steeper increases in the use of rodent models to study PFC function. Thus, at present there is not enough of a critical mass of papers for a thorough comparison of rodent and NHP studies using these techniques, but there is expected to be in the near future. In the interest of cross-species comparison with rodent, OFC and ACC connectivity with striatum, amygdala, and midbrain dopamine in macaque would be beneficial, as a great deal of pathway dissection has been conducted in rodent. On the other hand, in the interest of translation to the human primate, it may be best for macaque work to emphasize uniquely granular PFC region connectivity (e.g., to/from ventrolateral PFC, dorsolateral PFC).

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Foraging Innovations, Prediction, and Primate Specialization of PFC

Cue- and action-based learning in naturalistic environments requires a diverse set of neural processes. PFC functions that support flexible learning and decision making in such environments evolved in freely moving animals, yet these systems are frequently assessed in head-fixed animals. Head fixation enables precise cue presentation and the collection of data from hundreds and thousands of trials; thus, often better than tasks involving freely moving behavior at testing of computational models of neural responses and behavior. Conversely, learning paradigms in freely moving animals simulates more naturalistic foraging behavior with some amount of control, while animals have more options to engage in the required behaviors (or not), like in the real world. Along with recording and imaging thousands of neurons across long periods of time and multiple brain regions, the ecological validity of the behavior should be considered (Izquierdo 2021). Freely moving and head-fixed experiments may reveal the same underlying patterns of results, but there are also differences. For example, macaques are risk-seeking in head-fixed settings when tested in computerized gambling tasks, but risk averse while freely moving and foraging (Eisenreich et al. 2019). Given that pose estimation in freely moving rodents (Lauer et al. 2022; Mathis et al. 2020; Segalin et al. 2021) and NHPs (Bala et al. 2020) is an increasingly common and accessible method, this advance is predicted to better enable the incorporation of behavior as a correlate data stream to neural data than years before.

According to optimal foraging theory (Charnov 1976; Pyke 1984; Pyke et al. 1977), several factors contribute to an organism's enhanced fitness and profitable reward procurement. These include, but are not limited to, knowledge of a high-yielding food source (or "patch"), the nature of the food available in the current patch in comparison to others, when it is best to leave a patch, and the degree to which mobility is possible or an account of the travel time to different patches (Pyke 1984). Additional factors to the original theory include whether the animal has a central home or nest, the impact of uncertainty about the profitability of the reward environment (McNamara et al. 2013), and species "risk proneness" (Pyke 1984). Using the marginal value theorem, one can predict foraging behavior on the basis of energy-maximizing strategies across species (Charnov 1976) as well as time-minimizing strategies if there is greater risk of predation (Kie 1999). However, both rodent and primate species exhibit similar biases, leading to seemingly paradoxical or "suboptimal" behaviors in laboratory settings. For example, both species demonstrate myopic behavior when foraging, harvesting locally beyond what is predicted by optimal foraging theory, and exhibit a preference for immediate versus delayed rewards (Kane et al. 2019). Both rodents and primates also exhibit a paradoxical preference for information about the likelihood of obtaining reward, even if the information cannot change the outcome and when it comes at a cost (Bromberg-Martin

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and Hikosaka 2009; González et al. 2023; Jezzini et al. 2021; White et al. 2019). Mice, rats, and humans also have similar sensitivity to "sunk costs" (i.e., time dedicated to pursuing reward), resulting in a resistance to giving up on a reward once they have committed to pursuing it (Sweis et al. 2018). It is still unclear what these deviations from optimal behavior mean. The observed foraging "errors" or departures from optimal strategies observed across species could reflect cognitive or computational constraints or more covert learning processes that have yet to be fully understood (Harhen and Bornstein 2023).

A critical function of PFC may be to incorporate new behaviors into the species' repertoire. As recently reviewed (Rudebeck and Izquierdo 2022), there may be several compelling explanations for PFC expansion and specialization, including foraging (Dunbar and Shultz 2017), and specifically "foraging innovations" that result in the organism's enhanced ability to procure reward such as sampling of new foods or adopting new strategies to obtain reward. Some of the most relevant factors in this regard include species' "time horizon" needed for successful foraging (i.e., not in the order of seconds or minutes, but rather days and weeks required to keep track of seasonal changes in resources), a rapidly accelerating metabolism that comes with a larger brain size (Pontzer et al. 2016), as well as species perceptual and physical capabilities (i.e., smaller body size and odor-guided navigation of rodents compared to larger body size and visually guided navigation of primates). Foraging innovations can be considered behaviors that enhance *prediction*, *evaluation*, and *action* (Figure 2.4). These behaviors involve assessment of stimuli, outcome, and possible actions that map nicely onto learning theory (Balleine and O'Doherty 2010; Holland 2008).

Murray et al. (2011) summarized the literature on the subregions of frontal cortex as performing either the "top-down biasing of behavioral control systems" or the "flexible alterations of foraging strategies." For example, ACC biases competition among multiple stimuli and actions, the medial wall (PL and IL, or areas 32 and 25) bias behavior toward goal-directed choices versus habits, respectively, and OFC biases behavior in favor of higher-valued rewards. What is absent from rodent frontal cortex that is uniquely present in primate (granular) PFC is what authors referred to as the ability to generate a representation of "valueless" reward. Specifically in human and NHPs, rewards can guide goal-directed behavior independent of their biological value, perhaps as a rich, visual representation of the food item made possible by the greatly expanded visual capacity in primate brains compared to rodents. In primates, these result from robust connectivity with vision association areas such as inferior temporal cortex and perirhinal cortex that relay the unique properties of reward and objects to granular PFC. As an empirical example of this "valueless" reward in primate brain, Murray and Rudebeck provide compelling evidence that macaque ventrolateral PFC mediates knowledge about the availability of reward, apart from its desirability (Murray and Rudebeck 2018; Rudebeck et al. 2017b).

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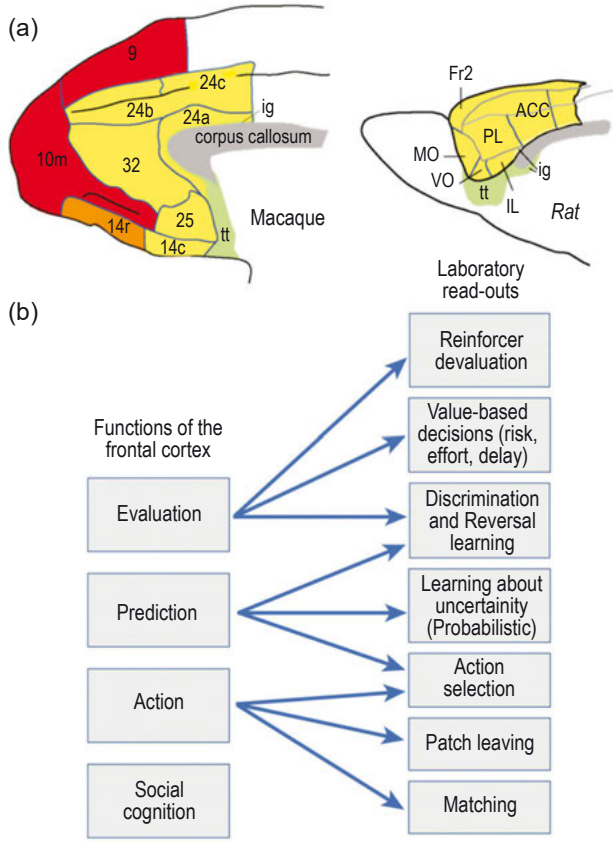

Figure 2.4 Foraging factors and PFC specialization in primates and rodents. If relying solely on cytoarchitectonic comparisons, rat frontal cortex is most similar to posterior and medial macaque PFC in that they are both agranular (yellow), as opposed to granular (red) or dysgranular (orange). There is allocortex in both species (green). These map onto ACC and OFC across species, though only a medial, not ventral, view is shown here (A). Foraging innovations were described in Rudebeck and Izquierdo (2022) as behaviors that enhanced Evaluation, Prediction, Action, and Social cognition (B). These functional categories (and the laboratory readouts of these categories) could be considered part of a "phylogenetic refinement" of brain and behavior. Social cognition is an important function of the frontal cortex, especially in primates, but outside the scope of the present review. Adapted with permission based on Rudebeck and Izquierdo (2022).

We extended the explanation in Rudebeck and Izquierdo (2022), highlighting that primates use highly developed visual capabilities to forage over larger ranges and longer time horizons, enhancing *prediction*: maintaining representations of both value and "valueless" reward would be essential for planning ahead for the right time to harvest different foods. Conversely, rats rely on olfactory capabilities to forage locally; thus foraging innovations in rodents

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may have arisen from adaptively enhancing the assessment of cues in their immediate environments, with short time horizons (i.e., favoring the *evaluation* function). We surmised that the *action* function may be most similar and overlapping across rodents and primates since it involves a convergence of pathways with a single outcome among many possible alternatives. Note that the laboratory readouts (Figure 2.4b) do not neatly map onto different subregions of either rodent frontal cortex or primate PFC, but the evidence suggests there is more shared support and less specialization of *evaluation* (i.e., reinforcer devaluation) than *prediction* (i.e., stimulus-based reversal and probabilistic learning). As an example, we recently found evidence in favor of less specialization in rat PFC during stimulus-based reversal learning. We conducted an experiment to compare local field potentials (LFPs) directly, specifically theta oscillations in OFC and ACC in rat frontal cortex during reversal learning (Ye et al. 2023b). We found strong support for OFC theta signaling of accuracy in reversal learning, unperturbed by chemogenetic inhibition of ACC (which expectedly did disrupt the ACC theta signal). Thus, we observed parallel, redundant signals of accuracy in both subregions of rat frontal cortex. Importantly, inhibition of ACC resulted in an impairment of early stimulus-based reversal learning, similar to those that follow OFC lesion or inhibition. This stands in contrast to a more specialized division of labor for stimuli (OFC) and actions (ACC) in primate PFC (Camille et al. 2011b; Rudebeck et al. 2008b). As another example, much like the finding of impaired confidence report in perceptual decisions following OFC muscimol inhibition (Lak et al. 2014), we find DREADDs inhibition of ACC also impairs confidence report in rats (Stolyarova et al. 2019). Collectively, these results suggest a lack of specialization of rat frontal cortex subregions for reversal learning and decision making under uncertainty, in behaviors requiring *prediction* (see also Jahn et al. 2014).

One of the more controversial perspectives has been that homology of PFC across species can be derived only if one finds the right level of PFC to make the comparison (Carlen 2017), with the recent strong suggestion that cellular-structural distinctions (cell types and morphology) are the most relevant dimensions of comparison of PFC across species (Le Merre et al. 2021). Though it will appear that I take a similar perspective here, I note that behavior and connectivity need to be incorporated as constraining factors (i.e., moderators) to these cell-type and morphological accounts.

Cell Types in PFC

Projection neurons make up 80% of all cortical neurons. Pyramidal neurons are the major class of these neurons, characterized by their triangular, pyramid-like, shape with both apical and basilar dendrites combining input from different cortical layers and sending this information to other brain regions. By comparison, intrinsic neurons, or interneurons, form synapses only within

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a particular brain region. Thus, the definitions of pyramidal and interneurons have mostly to do with their projections and not the source of their inputs (Masland 2004). Recent anatomical comparison of structural features of mouse and rhesus macaque Layer III (L3) pyramidal neurons in primary visual (V1) and frontal association areas shows that L3 neurons are broadly generalizable across these two areas in mouse, but not in monkey. In macaques, L3 lateral PFC neurons are much larger in size than V1 neurons and differ in their dendritic topology, but these neurons do not differ along these dimensions in mice (Luebke 2017). Thus, pyramidal neurons may not be the generalizable building blocks of cortical networks across species, at least if the classification is solely based on structural features.

A critical question is how transcriptome-defined cell types in PFC relate to their targets and functions across species. Yet single-cell transcriptomics (the collection of all the genetic readouts or expressed mRNA molecules in a single cell) and systems (behavioral) neuroscience have progressed largely as separate fields, rarely converging until recently (Lui et al. 2021). Single-cell RNA sequencing (scRNA-Seq) enables assessment of cortical or any type of cells clustered on the basis of morphological and physiological criteria (Yuste et al. 2020). Perhaps the first question to consider is what is meant by "cell type"? Yuste et al. (2020) suggest this definition should be based on data obtained from different methodological approaches, developmental stages, and species. According to these and other authors (Kepecs and Fishell 2014), cortical cell-type definition criteria could be based on (a) cell morphology, (b) connectivity motifs of interneurons with pyramidal cells, (c) molecular marker subtypes (i.e., parvalbumin, PV; somatostatin, SST; vasoactive intestinal peptide, VIP), and (d) intrinsic physiological properties of the neurons (e.g., fast-spiking, regular spiking non-pyramidal) (Figure 2.5).

Cortical inhibitory neurons can also be further classified as subtypes or subclasses via scRNA-Seq: *Pvalb, Sst, Lamp5, Vip, Sncg* (Bugeon et al. 2022). For example, Bugeon et al. (2022) report that modulation of responses to visual stimuli differ by subclass and activity can even be predicted by their transcriptional clustering. Ostensibly, this method could go beyond modulation of V1 activity by stimuli (Bugeon et al. 2022; Knoblich et al. 2019) and be extended or applied to behaviors more closely linked to PFC function, like reinforcer devaluation, set shifting, and reversal learning. Yuste et al. (2020:1464) also advise that transcriptomically similar cell types should in principle be related to the "proper levels of the anatomical structure"; in other words, a definition of the cells along a spatial gradient that corresponds with evolutionary distance between species. This overlaps considerably with a phylogenetic refinement mechanism proposed by Cisek (2019). Yet to align these datasets quantitatively in this way would require, as these authors describe, a "serious community effort," but would prove very worthwhile.

Cellular diversity afforded by interneurons may be a crucial evolutionary strategy to provide both stability and complexity (Kepecs and Fishell 2014)

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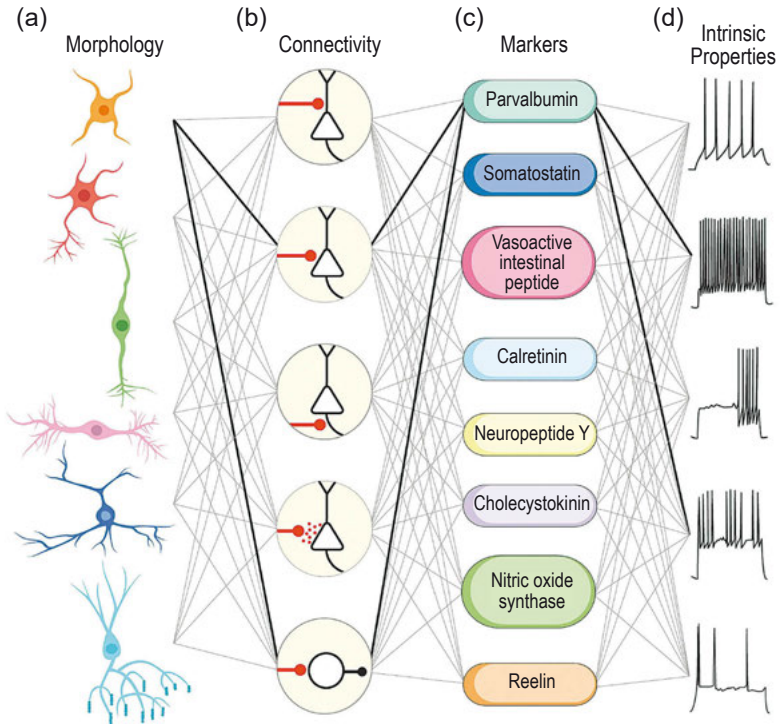

Figure 2.5 Classification of cell types that may be useful in guiding future cross-species transcriptomic studies. Cell types can be defined based on (a) morphology, (b) connectivity motifs, (c) molecular markers, and (d) intrinsic electrophysiological properties. Figure credit to Julia Kuhl, reprinted with permission based on Kepecs and Fishell (2014).

of neuronal firing patterns, especially relevant to PFC function that requires both. The diversity of interneurons in primate PFC may enable higher-dimensional neural representations important for behavior (Rigotti et al. 2013) and the dynamics of learning (Najafi et al. 2020). Programmed cell death of interneurons has been demonstrated to be a critical mechanism for adjusting the excitatory-inhibitory ratio, necessary for the assembly of neocortical circuits in mice (Wong et al. 2018). Though more cross-species studies are needed, several groups have conducted the important work of comparing cell types and gene expression patterns across rodents and primates (Hodge et al. 2019; Krienen et al. 2020). Different from the conclusion based on dendritic topology across pyramidal cells by Luebke (2017), Hodge et al. (2019) used scRNA-Seq in mice and a comparable single nucleus RNA-Seq (snRNA-Seq) method in humans and found largely conserved cortical cellular architecture across species and found similar functional gene families that discriminate inhibitory neuron types in both humans and mice, and homologous clusters of excitatory neuron projection targets. Where there was clear divergence across species

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was in the expression of genes associated with connectivity and signaling in homologous cell types. For example, gene families with the most divergent expression patterns included neurotransmitter receptors (especially serotonin), ion channels, and cell adhesion molecules. Hodge et al. suggest these differences likely impact *microcircuit* function, and even offer the possibility that this divergence could be one of the causes for the failure of preclinical studies in mice to translate to effective therapeutics in humans. Notably, an important limitation of this study as it relates to PFC is that the mouse tissue samples were obtained from V1 and a premotor area, anterior lateral motor cortex, not PFC per se. Nevertheless, these results highlight the need for more human and NHP studies to understand human brain disease as well as more investigation of local or microcircuit function.

Although the origins of interneurons may be conserved across species, the extent of homology of interneuron or interneuron subtypes within and across rodent and primates was poorly understood until recently. To study this, Krienen et al. (2020) conducted scRNA-Seq to profile expression of interneurons across brain regions, including neocortex and specifically PFC, across three primates (human, macaque, and marmoset) and rodent (mouse). They found that the same genes (*Sst, Pvalb, Vip, Lamp5*) were expressed in nonoverlapping neocortical interneurons across species and that their origins are similar: interneurons arise from medial and caudal ganglionic eminences (MGE and CGE) with MGE giving rise to *Sst* and *Pvalb* interneuron types, and CGE giving rise to *Vip* and *Lamp5* types. Interestingly, mouse frontal cortex contained these interneurons in proportions similar to those found in V1, but primates have significantly higher proportions of interneurons in PFC relative to V1. Additionally, there is evidence of "homologous interneuron types" readily identified by their RNA-expression patterns across species, with only a small fraction of "marker" genes being shared in another species. These marker genes vary less among primates and also show spatial expression gradients in primates more than rodents. Altogether, this suggests there is more specialization in the expression, but not the origin, of primate PFC interneurons compared to rodents.

Another cross-species transcriptomic analysis of the two cortical subtypes, glutamatergic (Glu) projection neurons and GABAergic interneurons, yields similar conclusions. This analysis included human, chimpanzee, and rhesus macaque (Kozlenkov et al. 2020) and revealed a pattern of cell-type evolution of gene regulatory elements (GREs), such as promoters and enhancers that drive and stabilize mRNA transcription. Using a combination of methods to isolate Glu and GABA nuclei in rhesus macaques, chimps, and humans, Kozlenkov et al. found several GREs in support of similar "concordant" evolutionary gene expression changes. Importantly, they found that GREs undergo subtype-specific changes more than GREs that are shared by different cell types. Similar results have been obtained by Khrameeva et al. (2020), showing that astrocyte and oligodendrocyte progenitor cells exhibit more differences

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than neurons across macaques, bonobos, chimpanzees, and humans and that the unique expression differences found in the human brain fall along neocortical and subcortical networks, similar to those revealed by neuroimaging studies. Though large-scale cell transcriptomic analyses have been conducted in different tissues in macaques (Han et al. 2022), there is no such transcriptomic atlas for macaque central nervous system or specific subregions of PFC. There is, however, a transcriptomic atlas of marmoset central nervous system (Lin et al. 2022) which could provide a useful resource to compare the evolution of PFC in New and Old World monkeys. Taken together, these findings suggest that primate cortical evolution favored a clustering of cell types.

Few studies have directly compared rodent and NHP cell-type function, either behaviorally or electrophysiologically. In a rare example, Povysheva et al. (2008) compared anatomical and physiological characteristics of PV-positive basket interneurons (multipolar GABAergic interneurons) in PFC of macaques and rats. Whereas there were several similarities (such as soma size, dendritic length, axonal horizontal, and vertical arbor span), macaque PV basket cells were found to be generally more excitable yet the frequency of the miniature excitatory postsynaptic potentials was higher in rats than macaques. Povysheva et al. deduced that these structural differences translate to differences in electrophysiological properties of the cortical networks, and ultimately may contribute to species differences in PFC function. This is reminiscent of the idea suggested earlier that there is species divergence in local, or microcircuit, function in PFC networks.

Laminar and Functional Patterns Among Cell Types

While rodent frontal cortex Layer I (L1) contains pyramidal neurons and GABAergic interneurons, Layer II and III (L2/3) contain cortical-projecting cortical cells or intratelencephalic neurons. L5 is the major output layer that contains both cortical-projecting and pyramidal tract cells targeting subcortical regions, and finally Layer VI (L6) mainly constitutes cortico-thalamic relay cells (Anastasiades and Carter 2021). Optogenetic inhibition of L2/3 pyramidal neurons in mouse medial frontal cortex results in intact behavioral flexibility as measured by probabilistic reversal learning. Conversely, selective silencing of deep layer pyramidal cortico-striatal and cortico-thalamic neurons (L5/6) does impair performance on this task (Nakayama et al. 2018). Interestingly, inhibition of interneuron-mediated "local" pyramidal neurons in mouse medial frontal cortex (in VGAT-ChR2 mice) produces enhanced premature responding and choice bias but intact reversal learning, suggesting dissociable roles of cell types on behavior that depend on laminar location. In NHPs, projections from agranular cortices (e.g., caudal orbitofrontal cortex) terminate mostly in upper layers of granular cortices (e.g., lateral PFC), and projections from granular cortices terminate mostly in the deep layers of

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agranular cortices (Rempel-Clower and Barbas 2000). As described above in rodent, laminar organization in NHP PFC—whether the cells influence local or long-range projections—may similarly be tightly associated with their putative roles in behavior, but this has yet to be fully elucidated.

Gao et al. (2022, 2023) have conducted tour de force studies on the spatial gradients of cell types in mouse frontal cortex. To determine whether single neurons project to specific targets, they reconstructed the projection patterns of genetically identified cell types, generating a "single-neuron projectome" in mouse. They found that the same transcriptome subtype corresponds to multiple projectome subtypes in different fronto-cortical regions (Gao et al. 2022) and identified morphological scaling of soma-dendrite combinations across lamina and subregions of frontal cortex. Combinations of dendrite-axon organization corresponded to cytoarchitecture and revealed a columnar organization of projection neuron subtypes in mouse frontal cortex (Gao et al. 2023). These are important studies; however, it will be important to integrate a comparative approach in the future since it is unclear if rat, macaque, and human frontal cortex follow similar principles of organization as mouse.

Few studies have combined electrophysiological recordings or calcium imaging data—either single cell, population, or LFPs—with molecularly defined cell-class information. Combining transcriptomically defined cell-type information with other characteristics, such as task-related signals in PFC as well as their connectivity patterns, represents a major challenge in the field. Using miniscope Ca^{2+} imaging in mice, Pinto and Dan (2015) found that pyramidal neurons exhibited much more functional heterogeneity in terms of task-related signaling on a go/no-go task than interneurons, and pyramidal neuronal responses varied across lamina. Interestingly, even though interneurons of the same subtype (SST+, PV+, VIP+) were more similar to each other, each subtype signaled different task-related events.

Returning to one of the classifications or criteria of cell types, the *connectivity motifs*, this classification may be especially informative as it relates most directly to integrated systems and microcircuit function. Using scRNA-Seq, Lui et al. (2021) studied the laminar distribution of cells expressing cluster-specific marker genes across both ventromedial and dorsomedial frontal cortex in mouse and found largely similar ratios for those marker genes. Of all the cell types they studied, the most specific marker genes for L5 were *Npr3* and *Tshz2*. Liu et al. discovered a great deal of redundancy in the projection targets of those neurons from multiple cell types. Not surprisingly, there was a complex collateralization pattern of various cell types in mouse frontal cortex to several target regions important in reward and cognition, such as amygdala and nucleus accumbens, which they referred to as "a many-to-one and one-to-many" mapping of cell type and projection targets. Specifically, they found that different cell classes signaled diverse aspects of task encoding as measured by calcium imaging, indicating that each transcriptomic type makes different contributions to behavior. In fact, connectivity patterns can be highly

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heterogeneous even within narrowly genetically defined cell clusters. It will be very useful to approach such an investigation in a comparative way in the future, to apply this technique to rat and macaque circuit dissection. Another powerful technique is multiplexed analysis of projections by sequencing, or MAPSeq (Kebschull et al. 2016). This high throughput method maps the projections of (thousands to millions of) single neurons by labeling sets of neurons with random "barcode" RNA that can then be extracted and sequenced from the putative projection zone or area. To my knowledge, only one group has used this approach in macaques (Zeisler et al. 2023), thus suggesting that this is a nascent approach.

Aside from these transcriptomic methods, other methods to study pathways are still commonly used, such as fMRI (Schaeffer et al. 2020) and mesoscopic mapping of pathways using tissue clearing methods (Xu et al. 2021). More traditional tract-tracing approaches fill neurons with proteins, often virally, so that their connectivity can be revealed using microscopy after the experiment. These methods often include retrograde Cre and Cre-dependent DREADDs. However, many limitations exist with these techniques, including lack of uniformity of expression, collateralization, and unpredictable transsynaptic viral expression. Transcriptomic methods to identify pathways identified by cell type across species offer a powerful way forward.

Stability and "Combinatorial Complexity" in PFC

Together, pyramidal and interneuron activity in PFC provide stability and "combinatorial complexity" (Kepecs and Fishell 2014), both critical for adaptive behavior in rodent and primate species. A purely excitatory network consisting of only pyramidal neurons would be unstable. Interneurons not only provide balance; they normalize local excitatory circuits and can provide feedforward inhibition, as a sort of "gain control," allowing for more temporal precision in neural activity. Superficially, this overlaps with the idea that mixed selectivity in PFC is important in generating high-dimensional representations for adaptive behavior (Fusi et al. 2016) that can be refined by learning and experience, shaped by excitatory and inhibitory (sub)networks (Najafi et al. 2020). As an example of this, our group performed bidirectional chemogenetic activation (hM4Di and hM3Dq-mediated) studies of pyramidal neurons in ACC on behavior, targeted with DREADDs on a calcium/calmodulin-dependent protein kinase IIα (CaMKII) promoter (Hart et al. 2020). Surprisingly, we found that *either* increases or decreases in ACC population activity produced impairments on effort-based choice in rats. In fact, a heterogeneous population would be more susceptible to perturbation by bulk inhibition or excitation, as demonstrated by the results of our DREADD manipulations. More interestingly, 1P calcium imaging (with GCaMP also driven by a CaMKII promoter) in freely behaving rats revealed that population activity was most predictive of choice,

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not individual cells. It may be an excitatory/inhibitory ratio in frontal cortex that computes (in our case here) relative cost-benefit, sending appropriate outputs to downstream targets that are more or less influential based on their laminar location. It could also be that not targeting specific cell types may serve to introduce noise and decrease signal-to-noise ratio in value-based choices. A caveat is that recent studies have determined that CaMKII and synapsin promoters exhibit more similar cell-type preferences than previously thought (Radhiyanti et al. 2021; Veres et al. 2023; Watakabe et al. 2015), transducing both excitatory and inhibitory neurons. In the future, it will thus be important to target interneuron function selectively in these cognitive processes, for direct comparison with pyramidal neuron involvement.

Conclusions

In this chapter, I have reviewed connectivity patterns across rodent and primate PFC and highlighted ways in which foraging behaviors may have given rise to PFC specializations. I also provided evidence in support of increasing efforts to study PFC cell types across species, with an appreciation for laminar and behavioral gradients that have undergone "phylogenetic refinement." Few studies combine neural activity with molecularly defined cell types within a species, and even fewer take a comparative approach. Across rodent and primate species, connectivity motifs likely provide the stability and complexity needed for the myriad executive functions of PFC. The field needs more studies that combine transcriptomically defined cell-type information with connectivity patterns and behavior-related signals in PFC across species. Collectively, this requires an integrative approach that incorporates the study of genes, neurophysiology, and behavior in both rodents and primates. These studies could be aimed at studying the evaluation function of PFC (i.e., value, value-based decision making), as there is substantial cross-species concordance of findings in this domain.

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