A primate multilevel society exhibits distinct cortical molecular signatures

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Dear Editor,

Multilevel societies, characterized by nested layers of social organization, represent one of the most complex forms of social systems, and their adaptive benefits are only beginning to be uncovered (Grueter et al., 2017; Qi et al., 2023; Qi et al., 2014). The formation and stability of these societies are associated with advanced cognitive functions, including social interaction, decision-making, and emotional regulation. The neocortex, particularly the

prefrontal cortex (PFC), serves as the neural hub for these higher cognitive functions, empowering animals to integrate information, regulate emotions, and flexibly adapt to complex environments (Ma et al., 2022). The golden snub-nosed monkey (*Rhinopithecus roxellana*), a species that exemplifies a multilevel social system, holds the status of an endangered flagship species and is often hailed as China's "second national treasure", second only to the giant panda. Research on the brains of these monkeys has been hindered by limited sample availability, resulting in an incomplete understanding of how the uniqueness of their macroscale social organization correlates with alterations in molecular programming. Seizing a rare opportunity, we collected cortical samples from a male golden snub-nosed monkey. Using single-nucleus RNA sequencing (snRNA-seq), a recently developed technique that facilitates high-resolution transcriptomic classification of cortical cell types in both humans and model organisms (Ma et al., 2022; Tadross et al., 2025), we delineated the transcriptional profiles across various cell types within the dorsolateral regions of the prefrontal cortex (PFC). To detect evolutionary differences in cellular composition and gene expression patterns, we conducted comparative analyses with the cortical cellular architecture of marmosets (*Callithrix jacchus*)，a representative species of non-multilevel societies (Yamamoto et al., 2010). This approach aimed to identify molecular correlates associated with the formation and stability of multilevel social systems.

The study site was located in the Dapingyu (DPY) Area within the Qinling Guanyin Mountain National Nature Reserve in Shaanxi Province, China. The study group consisted of a free-ranging golden snub-nosed monkeys, the DPY-herd, which consisted of eight one-male units (OMUs) and one all-male unit (AMU), with a total of 114 individuals. From November 2022 to November 2024, we conducted behavioral observations of this troop. On November 21,

2024, at 10:39 AM, SZ, a former resident male, was seen falling from a tall tree, sustaining fatal spinal fractures presumably resulting from unit takeover conflict. Upon confirmation of his death, we collected tissue samples from the dorsolateral regions of the prefrontal cortex (PFC) from both cerebral hemispheres for subsequent analysis. We utilized droplet-based 10x Genomics snRNA-seq technology. Well-dispersed single nuclei were isolated through enzymatic digestion of the tissue, followed by the capture of their transcriptomes and the generation of cDNA libraries for sequencing (Figure 1A). After stringent quality control measures, we obtained 21,745 high-quality single-nucleus transcriptomes. Further analysis revealed four major cell clusters, each characterized by distinct gene expression profiles (Figure S1A–D). These clusters were classified into specific cell types based on canonical gene markers: glutamatergic excitatory neurons, GABAergic inhibitory neurons, glial cells, and non-neural cells (Figure S1E and F).

To date, multilevel societies have been primarily documented among Old World monkeys, with limited evidence of such complex social structures reported in New World monkey species (Grueter et al., 2017; Qi et al., 2023). Marmosets, a representative species of New World monkeys, typically live in extended family groups characterized by a small social units ranging from 5 to 17 members (Yamamoto et al., 2010), which starkly contrasts with the multilevel society structure of snub-nosed monkeys. To elucidate the molecular basis underlying the evolution of large and stable multilevel societies in species like the golden snub-nosed monkey, we compared single-nucleus RNA sequencing (snRNA-seq) data from golden snub-nosed monkeys with recently published brain transcriptomes of marmosets (Ma et al., 2022). Our goal was to identify molecular mechanisms potentially implicated in the formation and maintenance of multilevel societies. Using nonlinear dimensionality reduction (uniform manifold approximation and projection, UMAP), clustering analysis revealed global differences in gene

expression profiles within the dorsolateral prefrontal cortex (PFC) between the two species (Figure 1B). These clusters were further classified into distinct cell types based on canonical gene markers, such as *SLC17A7* for excitatory neurons (Figures 1C and 1D).

Notably, we observed an altered proportion of excitatory neurons in golden snub-nosed monkeys compared to marmosets (Figure 1E). Given this finding, we conducted an in-depth analysis of this characteristic manifestation within this neuronal population, aiming to uncover differences in gene expression profiles. Genes that met the criteria of $P < 0.05$ and a fold change ≥ 1.5 were designated as differentially expressed genes (DEGs). Specifically, within the excitatory neuronal populations, golden snub-nosed monkeys exhibited 2879 DEGs relative to marmosets, comprising 1728 upregulated and 1151 downregulated genes. The top twenty genes with the most significant upregulation or downregulation in excitatory neurons are presented in a heatmap (Figure 1F). Consistent with the highly social nature of snub-nosed monkeys, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed significant enrichment in the oxytocin signaling pathway. Additional significant enrichments were found in the dopaminergic and glutamatergic synapse pathways (Figure 1G).

To achieve higher resolution in cell type annotation, we further subdivided the four major clusters into 10 functional subtypes using layer- and subtype-specific canonical markers, consistently applied to both *Callithrix jacchus* and *Rhinopithecus roxellana*. Excitatory glutamatergic neurons were classified into four cortical layer-specific subtypes: L2/3 (marked by *CUX2*), L4 (*RORB*), L5 (*ETV1*), and L6 (*TBR1*). Inhibitory GABAergic neurons were categorized into four subtypes: SST+ (*LHX6*), PV+ (*PVALB*), VIP+ (*VIP*), and LAMP5+ (*LAMP5*). The remaining two clusters were retained as functionally distinct groups. All 10 subtypes were clearly separated in UMAP space and consistently annotated in both species (Figure S2A and B). Cross-species

comparison at the subtype level revealed notable differences in proportional cell type distribution. In excitatory neurons, *Rhinopithecus roxellana* showed a higher proportion of L6 neurons (0.85% vs. 0.49%) but a lower proportion of L4 neurons (27.1% vs. 32.5%) compared to *Callithrix jacchus*. Among inhibitory neurons, the PV+ subtype was nearly 4.5-fold more abundant in *R. roxellana* (4.9% vs. 1.1%), whereas the SST+ subtype was less represented (4.7% vs. 8.4%). Glial cells also showed interspecific differences in proportion (34.8% vs. 29.7%) (Figure S2C and D). Subtype-specific differential gene expression (DEG) analysis revealed distinct functional enrichments. In L5 excitatory neurons—enriched in *R. roxellana*—we identified 1803 upregulated genes, primarily associated with dopaminergic synapses (e.g., *SYT1*, *PLCB1*, *ARPP21*) and long-term potentiation (e.g., *DLGAP1*, *DLGAP2*) (Figure S2E and F). L2/3 and L4 excitatory neurons showed upregulation of genes involved in the oxytocin signaling pathway, while L6 neurons were enriched for genes related to phosphatidylinositol signaling and glutamatergic synapses (Figure S2G–I).

Within the inhibitory neuronal population, PV+ neurons (enriched in *R. roxellana*) exhibited 3101 DEGs, including upregulated *SYT1* and downregulated *PDE10A*. In contrast, SST+ and VIP+ neurons showed upregulation of genes associated with the oxytocin signaling pathway (e.g., *ERBB4*, *ADGRB3*, *ADGRL3*) (Figure S3A–D). These subtype-specific molecular profiles are consistent with a potential association between cellular specialization and divergence in social systems.

To further contextualize these findings, we integrated public snRNA-seq data from rhesus macaques (*Macaca mulatta*) (Ma et al., 2022), an Old World monkey with moderately complex social structures. Using the same annotation framework, we classified cells from both species into the 10 functional subtypes (Figure S4A). In contrast to the strong enrichment of the oxytocin signaling pathway in L2/3 and L4 excitatory neurons in the *R.*

roxellana–marmoset comparison, the *R. roxellana*–macaque comparison showed top enrichments in pathways such as prostate cancer (e.g., *DLG2*, *ANK2*, *PPP3CA*) (Figure S4B–D). The oxytocin signaling pathway showed markedly weaker statistical significance in the macaque comparison (L2/3: $P = 0.024$ vs. 3.43×10^{-11}; L4: $P = 0.041$ vs. 1.75×10^{-11}) (Figure S4E). Similarly, *R. roxellana* had only 531 upregulated genes in L5 excitatory neurons relative to macaques—less than one-third of the 1803 DEGs identified in the marmoset comparison. Key social behavior-related pathways such as the dopaminergic synapse also showed reduced significance ($P = 0.022$ vs. 3.38×10^{-10}) (Figure S4F and G). This graded pattern of transcriptomic divergence across two independent cross-species comparisons supports the interpretation that the observed cellular and transcriptional profiles correlate with the evolution of multilevel societies, rather than representing random species-specific traits.

Analysis of inhibitory neuron subtypes in the *R. roxellana*–macaque comparison revealed enrichments in pathways such as salivary secretion, aldosterone-regulated sodium reabsorption, human cytomegalovirus infection, and FoxO signaling (Figure S5A–D). These findings contrast with the prominent oxytocin and dopaminergic pathway enrichments observed in the *R. roxellana*–marmoset comparison. For instance, whereas VIP+ and PV+ neurons in the latter showed strong upregulation of genes related to oxytocin and dopaminergic signaling, respectively, the same pathways were significantly less enriched in the macaque comparison (VIP+: $P = 0.015$ vs. 2.64×10^{-10}; PV+: $P = 0.036$ vs. 1.85×10^{-11}) (Figure S5E and F).

This study provides the first single-nucleus transcriptomic profile of wild snub-nosed monkeys. Through cross-species comparison, we identified the most pronounced molecular differences in the dorsolateral PFC—specifically upregulation of the oxytocin and dopaminergic synapse pathways—between golden snub-nosed monkeys and marmosets, the latter representing a

phylogenetically distant species with a simpler, family-based social system. Notably, these pathway alterations were substantially less prominent in comparisons with rhesus macaques, which exhibit an intermediate degree of social complexity. This graded pattern of transcriptomic divergence helps mitigate phylogenetic confounding and supports a correlation between the observed molecular signatures and the evolution of multilevel societies. The upregulation of oxytocin and dopaminergic signaling pathways in golden snub-nosed monkeys relative to marmosets aligns with their complex social organization and is consistent with prior genomic evidence indicating enhanced receptor binding efficiency related to social bonding (Qi et al., 2023). The co-enrichment of both pathways further suggests a potential interplay between social motivation and reward processing (Charlet and Grinevich, 2017; Qi et al., 2023).

We also detected markedly reduced expression of *AUTS2*—a gene whose disruption is strongly linked to autism spectrum disorders (ASD) and associated social deficits in humans—in golden snub-nosed monkeys compared to marmosets. Given that *AUTS2* has undergone rapid evolutionary change in primates and is crucial for neurodevelopment, including neuronal migration and synaptic function (Oksenberg and Ahituv, 2013), its differential expression invites a compelling hypothesis. In humans, impaired *AUTS2* function is associated with reduced social aptitude, suggesting its normative expression is integral to typical social cognition. Conversely, the significant downregulation we observe in the highly social *R. roxellana*—which exhibits complex, multi-layered societies—compared to marmosets living in smaller, family-based units, suggests that a reduction in *AUTS2* expression might be permissive of the enhanced social integration and tolerance required for life in a large, stable multilevel society. We therefore cautiously propose that natural variation in *AUTS2* expression could be a contributing factor in the evolution of divergent social systems across

primates. This evolutionary hypothesis positions *AUTS2* as a high-priority candidate gene, warranting future functional studies to elucidate its specific role in shaping the neural underpinnings of primate sociality.

Compliance and ethics

The authors declare that they have no conflict of interest.

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Supplemental information

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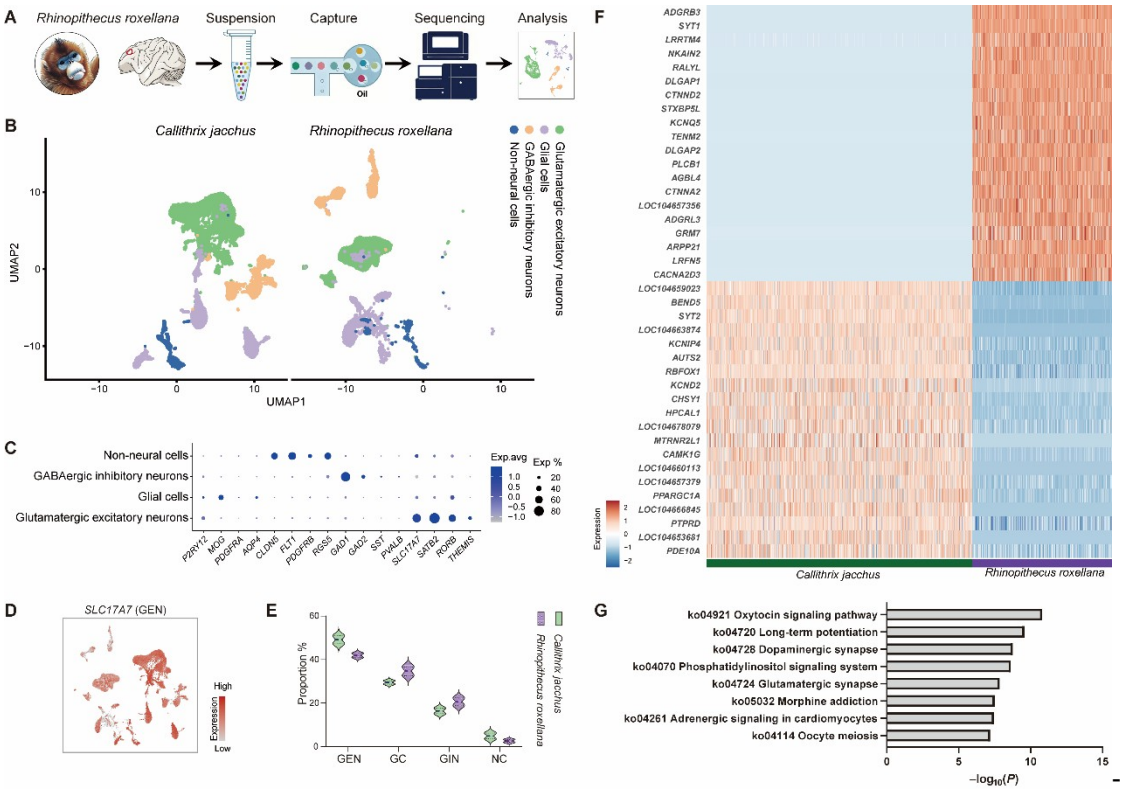

Figure 1. snRNA-seq unveils distinct gene expression profiles in neural populations of golden snub-nosed monkeys (*Rhinopithecus roxellana*) compared to marmosets (*Callithrix jacchus*).

A, Schematic overview of the snRNA-seq experimental design, with emphasis on the dorsolateral regions of prefrontal cortex (PFC) (indicated by red highlighting) subjected to analysis. B, UMAP visualization of gene expression profiles in the dorsolateral regions of PFC of golden snub-nosed monkeys and marmosets. C, Gene expression signatures of distinctive genes within four cellular subtypes. D, A representative feature plot illustrating the expression distribution of the excitatory neuronal marker gene *SLC17A7*. E, Violin plots illustrating the proportional distribution of distinct cellular populations between golden snub-nosed monkeys and marmosets. GEN, glutamatergic excitatory neurons; GC, glial cells; GIN, GABAergic inhibitory neurons; NC, non-neural cells. F, A heatmap illustrating the fold changes of the top 20 differentially expressed genes within the excitatory neuron populations of golden snub-nosed monkeys and marmosets. G, Among the upregulated differentially expressed genes in golden snub-nosed monkeys compared to marmosets, a bar chart revealed that the oxytocin signaling pathway was the most significantly enriched pathway. Characteristic terms are displayed in rows, with the corresponding $-\log_{10}(P)$ shown in columns.