(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Merve Bilgic
Thesis Title	Common features of neural progenitor cells and cortical organization revealed by single cell transcriptome analyses of ferret cortical development		

(Thesis Summary)

The cerebral cortex underlies the cognitive abilities of mammals, including humans, and forms by sequential generation of a diversity of neuron and glia types from neural progenitor cells (NPCs). The diversity of neural stem cells is a fundamental characteristic of cerebral cortex development in gyrencephalic mammals, including primates and carnivora. Among these species, ferrets have emerged as a valuable model for mechanistic studies due to their cortical similarities to humans. However, the information on molecular and cellular features of their NPCs is limited. Thus, understanding the cellular diversity and transcriptomic profiles of RG subtypes in developing ferret brain is important.

In this study, the applicant conducted single-cell transcriptome analyses throughout various developmental stages in the ferret cortex to investigate the cellular diversity and temporal trajectory of NPC, revealing a conserved diversity and temporal trajectory of NPCs between the two species. These analyses identified remarkable conservation of RG cell types at the transcriptome and morphological level between ferrets and humans. In particular, the applicant examined the generation of truncated radial glia (tRG), a human-enriched RG subtype previously described in humans (Nowakowski et al, 2016). Furthermore, a combination of in silico and in vivo analyses identified that tRG differentiate into both ependymal and astrogenic cells. Comparative analyses with human datasets suggested a similar fate for tRG in humans. Furthermore, the timelapse imaging and slice culture experiments proposed that tRG emerge from asymmetric divisions of RG and that BMP signaling might play role in its maintenance.

Through transcriptomic comparison, this study indicated that tRG play a pivotal role in the formation of adult ventricles, which ultimately provides the architectural foundation for brain expansion. The findings in this study demonstrate that ferrets serve as a valuable model system for studying gyrencephalic brain features shared between ferrets and humans, which are otherwise challenging to explore in humans due to ethical and practical limitations. The comprehensive dataset generated in this study, containing ferret multiple developmental stages, provides a crucial resource for future investigations in cortical brain development.

(Form 2)

（Thesis Evaluation Summary）

The basic structure of the cerebral cortex is determined primarily by subtypes of neural progenitors and their dynamics. Even in the complex brain, represented by the formation of gyri, this is the case. However, several differences are known between simple brains such as rodents and the complex brains such as humans. It is not clear whether these unique characteristics of human brain formation at the progenitor cell-level are species-specific. The applicant provided a single-cell transcriptome dataset from the developing ferret cerebrum, allowing for the first time to compare them with human datasets. The applicant showed that the stem cell subtypes and their dynamics are highly homologous between ferrets and humans, both of which form folded brains. The applicant showed that truncated radial glia (tRG), a type of neural precursor that have been found during the late neurogenesis to early gliogenesis in humans and a few primates (not found in mouse), are also present during the neuro-glial transition stage in ferrets. Furthermore, the applicant's analysis suggested that tRG differentiate into cell types that contribute to cerebral expansion. The process of tRG formation was shown by time-lapse imaging of ferret cortical slices, an approach that would be ethically and technically difficult in humans.

This study provides a systematic single stem cell (neural progenitor cell) transcriptome dataset that is useful for comparative studies of mammalian cerebral development, shows that the ferret is an excellent model for complex brain research, and presents a comprehensive methodological model based on comparative analysis of single-cell transcriptomes.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of neuroscience, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On October 11th 2023, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

(Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd , yyyy