Improvement of mouse genome editing protocol and the application: investigation of the physiological roles of glycine receptor alpha 4 subunit.

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The glycine receptors (GlyRs) are ligand-gated chloride channels composed of alpha (α1-4) and β subunits. GlyR subunits play major roles in mammals CNS ranges from regulating simple sensory information to the modulation of higher-order brain function and are involved in several neurological diseases. The GlyR α4, unlike other GlyR subunits, received relatively little attention because the human ortholog is considered as a pseudogene due to a lack of transmembrane domain. Interestingly, GlyR α4 has been recently reported as a risk gene for several neurological diseases in humans including startle disease and an autism spectrum disorder. However, the physiological roles of GlyR α4 in mammal behavior and whether it is involved in neurological diseases are fully unknown.

In the dissertation, I first describe a developed protocol to generate the needed mice using the CRISPR/Cas9 system (Chapter 1). The protocol combines a modified method for cryopreserving 1-cell C57BL/6J embryos, which improved embryos' developmental rates, with optimized electroporation conditions. Using this protocol, I generated several lines of knockout mice and knock-in mice with high-efficient mutation rates (100%, 50%, respectively) and a low mosaic rate within 4 weeks. Secondly, I show the temporal and spatial expression profile of GlyR α4 in the brain and the comprehensive behavioral analysis of *Glra4* mutant mice to elucidate its role in mouse behavior (Chapter 2). GlyR α4 subunit is mainly enriched in the hindbrain and midbrain and its expression is gradually increased during brain development. The *Glra4* mutant mice showed a low percentage of entries into open arms of the elevated plus maze test compared to wild-type littermates and showed decreased amplitude and delayed onset of the startle response. Moreover, they exhibited increased sociality in the home cage during the active period.

Collectively, the work describes a modified method to expedite and enhance mouse transgenesis. Besides, the data shed light on the behavioral roles and spatiotemporal expression pattern of the GlyR α4 subunit and suggest that glycinergic signaling modulates social and startle behavior in the mouse.