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Localized replacement of GABAA receptor γ2 subunits with bicistronic viral vectors

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Classical genetic methods used in neurobiology act slowly compared to the time scale at which neurons and neuronal circuits operate. However mutations affecting only the drug sensitivity of ion channels can be used for selective, reversible and fast manipulations. Here we applied lentivirus-mediated functional replacement of floxed, benzodiazepine insensitive point mutant GABAAR γ2 subunits (flox-γ2BIS) with wild type, benzodiazepine sensitive γ2 subunits (wt-γ2BS) to locally rescue the benzodiazepine sensitivity of GABAA receptors in targeted mouse CNS neurons. We compared three different strategies to co-express Cre-recombinase (Cre) and the wt-γ2BS subunit ensuring the reliable replacement of wt-γ2BS with the γ2BIS in all affected cells. Injecting a mixture of independent Cre and wt-γ2BS expressing lentiviruses in neocortex resulted in only partially overlapping expression of the Cre and the AU1-tagged wt-γ2BS as revealed by double immunofluorescent reactions. The bicistronic lentiviral construct containing IRES was expressed in very few cells and although co-expressed with Cre, the AU1-tagged wt-γ2BS showed cytoplasmic localization. More cortical cells expressed the bicistronic 2A peptide-containing constructs. Importantly, Cre was perfectly co-expressed with AU1-tagged wt-γ2BS targeted to plasma membrane of the infected cells. Our results demonstrate that the short 2A sequence based strategy is a reliable tool for achieving lentivirus-mediated GABAAR subunit replacement.