Title: Intracerebral Injection Protocol
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Ai14 mice (Jackson Laboratory (JAX), STOCK# 7914) were used to assay the gene editing efficiency in the brain. All animal treatments and procedures were approved by the University of Wisconsin–Madison Animal Care and Use Committee. Mice were examined and determined to be in good health the day of injection. Mice were anaesthetized by intraperitoneal injection of a ketamine (120 mg/kg), xylazine (10 mg/kg), and acepromazine (2 mg/kg) cocktail. Stereotactic brain injections were performed using a Stoelting stereotaxic frame equipped with a Stoelting Quintessential Stereotax Injector (QSI). The right and/or left striatum was targeted at coordinates of AP +0.74 mm, ML ±1.74 mm, DV -3.37 mm using a 10 µl Hamilton syringe and 32-gauge 1 inch Hamilton small hub RN needle. The solutions delivered were 1.5 µl of NC-No Ligand or NC-CPP with RNP carrying guide targeting either Ai14 or a non-targeting guide at 20 µM RNP suspended in PBS or 1 µl of storage buffer. Solutions were intracerebrally delivered at a rate of 0.2 µl/minute. After the injection was completed, the needle remained in place for up to 5 minutes, then the surgical field was irrigated with sterile saline and the skin layers closed with surgical glue.