Non-fasted mice are used for local injections. All animals were anesthetized with ketamine/xylazine/acepromazine (K/X/A 120/10/2 mg/kg, 0.1 mL per 10 g intraperitoneal injection of 12/1/0.2 mg/ml K/X/A) . Approximately 5 minutes later, mice are given a subcutaneous injection of 5 mg/kg ketoprofen (0.05 mL/10 g of 1 mg/ml ketoprofen). The top of the head comprising the surgery area is shaved and the animal is positioned into the stereotax apparatus using the ear-bar and tooth-bar alignments. The mouse is placed on a water blanket connected to a temperature-controlled warm water circulator (Gaymar). Puralube ointment is applied to the eyes. The surgery area is cleaned with a chlorhexidine solution and saline. An incision is made down the middle of the skull using a Bard Parker #11 scalpel blade. To assist in the location of bregma, a small amount of 3% hydrogen peroxide is applied to the exposed skull surface.
We use a Hamilton Model 701 10 ul syringe with removable 1 inch, 32 gauge blunt needle. The injection syringe, which is held in place by the Stoelting Motorized Classic Quintessential Stereotaxic Injector (QSI; Cat. No. 53311) attached to the Stoelting stereotax unit, is lowered so that the needle is just above the skull surface to find bregma. The bregma coordinates (AP, DV, ML) are noted and the target injection coordinates are calculated relative to Bregma. We have been aiming for the striatum (+0.74mm AP, -3.37mm DV, and +/- 1.74mm ML) or the substantia nigra pars compacta (-2.92mm AP, -4.6mm DV, +/- 1.25mm ML). The needle is moved to each of the desired AP/ML injection sites and the skull marked with a sterile pencil. Using the surgical drill, a drill hole is made a the pencil marking for each of the injection sites. The syringe is moved to each of the drill hole locations and lowered to check the drill hole coordinate; if necessary, the drill hole is widened.
Prior to entering the brain, the syringe loaded with the RNP-NC solution is primed to ensure flow. The needle is then slowly lowered to the desired DV coordinate. 1.5 ul is injected at a rate of 0.2 ul/min using the programmable Stoelting QSI syringe pump. After the injection, wait 10 minutes and then slowly raise the syringe. This process is repeated for the other injection location(s). The skull incision is closed with VetBond skin glue and the animal is monitored until recovery from anesthesia. 1 mL of warm saline is administered subcutaneously during recovery. Mice are given a subcutaneous injection of 5 mg/kg ketoprofen (0.05 mL/10 g of 1 mg/ml ketoprofen) approximately 24h and 48h later. Mice are given access to soft, moistened food in addition to their normal chow during the 48 hours surgery recovery period. If necessary during post-surgical monitoring, antibiotic ointment is applied to the incision area. Tissues are typically collected 14 days after injection after transcardial perfusion with heparinized PBS.