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Original Article

Awake intracerebroventricular delivery and safety assessment of oligonucleotides in a large animal model

Hector Ribeiro Benatti,[1] Rachel D. Prestigiacomo,[1] Toloo Taghian,[1,2] Rachael Miller,[3,4] Robert King,[2] Matthew J. Gounis,[2] Ugur Celik,[5] Stephanie Bertrand,[6] Susan Tuominen,[7] Lindsey Bierfeldt,[7] Elizabeth Parsley,[6] Jillian Gallagher,[1] Erin F. Hall,[1] Abigail W. McElroy,[1] Miguel Sena-Esteves,[1,8] Anastasia Khvorova,[4] Neil Aronin,[3,4] and Heather L. Gray-Edwards[1,2]

[1]Horae Gene Therapy Center, UMass Chan Medical School, 368 Plantation Street, Worcester, MA 01605, USA; [2]Department of Radiology, UMass Chan Medical School, 55 N Lake Ave, Worcester, MA 01655, USA; [3]Department of Endocrinology, UMass Chan Medical School, 368 Plantation Street, Worcester, MA 01605, USA; [4]RNA Therapeutic Institute, UMass Chan Medical School, 368 Plantation Street, Worcester, MA 01605, USA; [5]Center for Clinical Research, UMass Chan Medical School, 55 N Lake Ave, Worcester MA 01655, USA; [6]Cummings School of Veterinary Medicine, Tufts University, Grafton MA 01536, USA; [7]Department of Animal Medicine, UMass Chan Medical School, 368 Plantation Street, Worcester, MA 01605, USA; [8]Department of Neurology, UMass Chan Medical School, 368 Plantation Street, Worcester MA 01605, USA

Oligonucleotide therapeutics offer great promise in the treatment of previously untreatable neurodegenerative disorders; however, there are some challenges to overcome in pre-clinical studies. (1) They carry a well-established dose-related acute neurotoxicity at the time of administration. (2) Repeated administration into the cerebrospinal fluid may be required for long-term therapeutic effect. Modifying oligonucleotide formulation has been postulated to prevent acute toxicity, but a sensitive and quantitative way to track seizure activity in pre-clinical studies is lacking. The use of intracerebroventricular (i.c.v.) catheters offers a solution for repeated dosing; however, fixation techniques in large animal models are not standardized and are not reliable. Here we describe a novel surgical technique in a sheep model for i.c.v. delivery of neurotherapeutics based on the fixation of the i.c.v. catheter with a 3D-printed anchorage system composed of plastic and ceramic parts, compatible with magnetic resonance imaging, computed tomography, and electroencephalography (EEG). Our technique allowed tracking electrical brain activity in awake animals via EEG and video recording during and for the 24-h period after administration of a novel oligonucleotide in sheep. Its anchoring efficiency was demonstrated for at least 2 months and will be tested for up to a year in ongoing studies.

INTRODUCTION

Oligonucleotide therapeutics, including small interfering RNAs (-siRNAs) or antisense oligonucleotides (ASOs), offer promise in the treatment of several neurological conditions by knocking down expression of toxic proteins and enhancing expression of loss of function proteins (e.g., splicing modifications).[1] This class of therapeutics offers great promise in the treatment of previously untreatable disorders; however, they carry a well-established dose-related acute toxicity (generalized and myoclonic seizures) at the time of administration.[2,3] Neural functions include a range of regulated mechanisms of intracellular calcium homeostasis, neurotransmitter release, and electrical activity,[3] and the negative charge of an oligonucleotide into the central nervous system (CNS), for instance, can affect ion flux and lead to neuronal hyperexcitability and eventually seizures.[3,4]

One commonly used technique for the delivery of experimental drugs to the CNS is intracerebroventricular (i.c.v.) injection.[5–8] This allows direct access to the cerebrospinal fluid (CSF) in the cerebral lateral ventricles. Compared with other CSF delivery techniques into intrathecal spaces by cisterna magna or lumbar intrathecal space, i.c.v. injection offers the potential for better biodistribution to the cortex and deep brain areas simply because of its anatomical location.[6,7]

In this context, large animals are used to demonstrate safety and efficacy of genetic-based therapies for neurodegenerative disease, since rodents often do not recapitulate human neuroanatomy, immune responses, or phenotypes experienced by patients with neurodegenerative diseases.[9–11] Non-human primates have traditionally been the gold standard large animal model used in toxicology studies, but recent guidance from the FDA suggests use of alternative non-rodent species.[12–14] To that end, pigs and sheep have gained more visibility not only for toxicology studies,[5–7,15–17] but as models of translational neuroscience. The use of species with higher brain plasticity and

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Correspondence: Heather L. Gray-Edwards, UMass Chan Medical School, 368 Plantation Street, ASC6-2041, Worcester, MA 01605, USA.
E-mail: heather.grayedwards@umassmed.edu

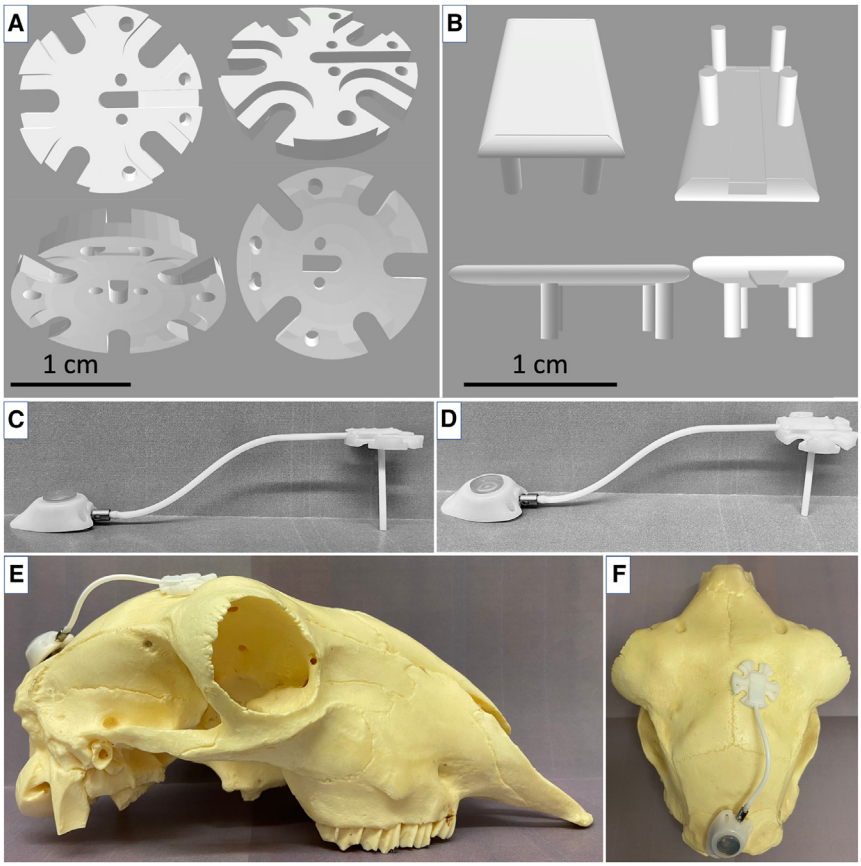


Figure 1. Prototypes and illustrative schematic models

(A) The top images represent the dorsal view of the disc prototype. The five openings on the sides are for fixing with ceramic screws in the animal's skull. The groove in the middle of the device is where the intracerebral catheter will be seated. The bottom images represent the ventral view of the prototype. It is curved to accommodate the shape of the skull. (B) The images represent different angles of the "fixation table" prototype. This fixation table prototype is fitted over the disc prototype over the groove to secure the catheter in place. (C) Lateral view of an assembly of the disc and table prototypes with the passage of the catheter. The other end of the catheter is connected to the Ommaya reservoir. (D) Top diagonal view of the same assembly described in (C). (E) Side view of a prototype use model in an ovine cranium. The prototype disc is fixed on the frontal bone of the skull of the ovine models, and the Ommaya reservoir is attached on the occipital bone. (F) Diagonal back view of the same assembly as the model described in (E).

gyrencephalic morphology better approximates these models to humans.[18]

However, for the development of pre-clinical studies of oligonucleotides administered directly into the CNS to produce reliable and accurate results, some barriers need to be overcome: (1) repeated administration into the CSF may be required for long-term therapeutic effect,[19] which is not possible for intracranial off the needle injections, and (2) the formulation of these compounds in cations has been postulated to prevent the acute toxicity, but a sensitive and quantitative way to track vacant or minor seizure activity in formulation development is lacking.

To overcome these challenges, implantable devices such as the Ommaya reservoir may offer a solution. Although Ommaya reservoirs have been used since the 1960s in patients,[20–23] the standard approaches used in neurosurgery do not work in large animals. Current anchorage systems include bone cement, cyanoacrylate adhesive, and sutures,[7,24,25] which although compatible with magnetic resonance imaging (MRI), computed tomography (CT), and electroencephalography (EEG), these methods are not standardized and depend on the surgeon's ability to create a stable anchorage.

In this study, we developed a method that allows multiple i.c.v. injections over a period of at least 60 days and the tracking of seizure activity in awake animals during and 24 h after the injection. The technique consists of using a 3D-printed anchoring system for plastic catheters fixed with ceramic screws. We demonstrate the effectiveness of this i.c.v. catheter anchorage prototype in sheep and its compatibility with MRI, CT, and EEG, which makes it ideal for translational laboratories of neurodegenerative diseases. Overall, this technique allows for reliable placement and stability of i.c.v. catheters to allow for long-term drug delivery in large animals and the ability to reliably track electrical brain activity during and 24 h following injection.

RESULTS

Accuracy

Ten animals had the disc prototype (Figure 1) fixed catheters implanted in the lateral ventricle. All sheep had normal sized ventricles (1–2 mm) according to presurgical MRI (data not shown). Both lateral ventricles were accessible for each animal. The choice of target was by surgeon's preference and the largest part of the ventricle, most commonly the caudothalamic notch.

Real-time X-ray and CT images demonstrated good placement of the catheters (Figure 2A). In one of the 10 animals, the catheter tip was deeper than expected, which was corrected by removing the fixation table prototype off the disc prototype and pulling the catheter out and replacing the fixation table. Subsequent imaging demonstrated ideal positioning (Figures 2B and 2C).

All animals had catheter position and viability confirmed via X-ray and/or CT, immediately after surgery, between 48 and 72 h before

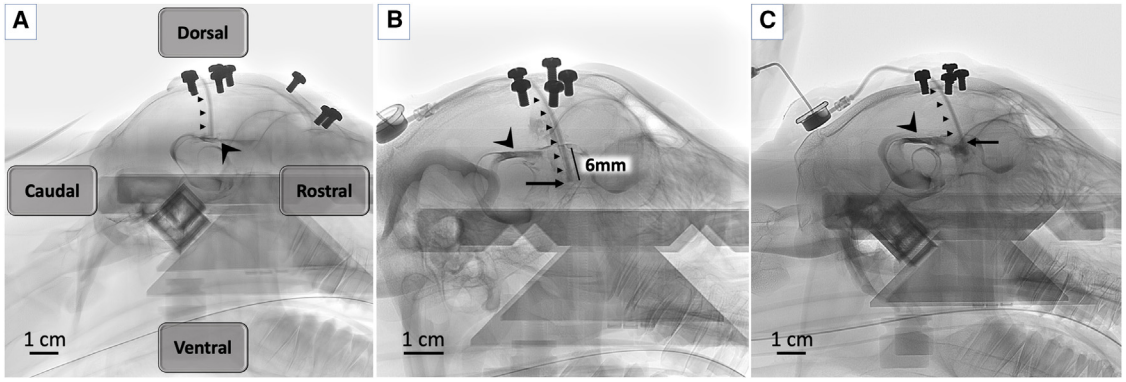


Figure 2. Lateral intraoperative X-ray images from intracerebroventricular (i.c.v.) catheter placement and reservoir replacement

(A) Confirmation of a good catheter placement and i.c.v. targeting. The arrow indicates the catheter. The big arrowhead indicates the ventricular system being filled by contrast. The small arrowheads indicate the catheter and the catheter tract. (B) Representation of a mistargeting. The ruler indicates 6-mm deviation of the catheter tip in relation to the i.c.v. The deeper position of the catheter tip was detected during surgery and corrected. The arrow indicates the catheter tip. The big arrowhead indicates the targeted lateral ventricular system being filled with contrast from reflux in the catheter track. The small arrowheads indicate the catheter and the catheter tract. (C) Correction of the catheter position in relation to the previous image. The arrow indicates the catheter tip. The big arrowhead indicates the ventricular system i.c.v. targeted. The small arrowheads indicate the catheter and the catheter tract.

test article administration, checking for positioning and reflux along the catheter tract. All catheters were well positioned, and we had no evidence of reflux.

Catheter use and viability

The animals were allowed to recover from the catheter and reservoir implantation procedure for 2–3 days. Neurologic assessment of each animal was performed the day after surgery, before test article administration, for 2 days after test article administration, and monthly thereafter until termination, evaluating mentation and behavior and neurological exam including cranial nerve function, spinal reflexes, and proprioception testing.[26,27]

All i.c.v. catheters (10/10) were patent and able to be injected through the reservoir without resistance. This is indicative that the catheter tip is unobstructed and is in the ventricle. It was possible to aspirate CSF (approximately 100 μL) from the reservoir in only two out of 10 animals. This limitation is likely due to the small size of the sheep ventricle and abundance of choroid plexus.

Safety assessment during and after oligonucleotides administration

The channels used for EEG assessment were F3 and F4; C3, C4, and Cz; and O1 and O2 (Figures 3A and 3B). The trackIt device is kept close to the animal with an adaptation of a prolapse harness for sheep (Figure 3C).

We were reliably able to detect alpha, beta, gamma, delta, and theta waves for a 24-h period (Figure 4A). EEG abnormalities associated with oligonucleotide administration and prevention with formulation are detectable by EEG in a dose-dependent manner. The changes in waveform correlate with alterations in animal behavior phenotypes recorded by video.

Animals that received high doses of the test oligonucleotide (i.e., four times the baseline dose) showed EEG abnormalities, with sharp spikes and waves consistent with seizures (Figure 4B), followed by tonic-clonic movements (Figure 4C). After being reversed from sedation, the sheep showed inappropriate behavior in the subsequent 24 h (stargazing, apathy, low responsiveness to external stimuli). After the first 24 h, all animals subjected to high doses returned to normal behavior, expected for the species.

The sheep in the present study had catheters and reservoirs implanted to serve as models for the evaluation of efficacy and safety of a test article for the treatment of Huntington's disease. Electroencephalography during and 24 h after the infusion was briefly analyzed for the detection of changes in the wave pattern that were consistent with seizures so that the described method could be validated. Complete analyses are underway.

Catheter placement and viability after 60 days

The catheter system was evaluated at ∼2 months after surgery by MRI and CT to verify placement and functionality. MRI showed the tip was secured in the ventricle without evidence of migration. CT showed flow through the catheter, demonstrating complete viability and functionality (Figure 5).

Complications

1 day after the infusion, one of the animals was apathetic and had a fever. With suspected encephalitis, the animal was submitted to antibiotic therapy with ceftriaxone (25 mg/kg BID), a broad-spectrum antibiotic capable to cross blood-brain barrier[28] and fluid therapy (60 mL/kg/day subcutaneously) for 7 days and fully recovered. The same animal had dehiscence of the sutures at the implant site 10 days after the injection via the reservoir, exposing the prototypes, the catheter, and the reservoir. This animal had all implants removed and afterward fully recovered.

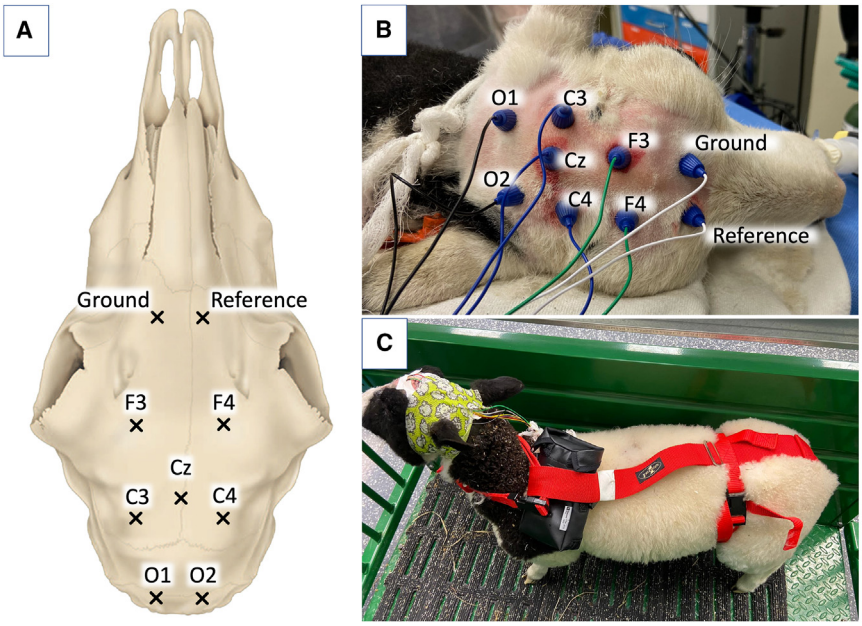


Figure 3. Electroencephalography electrodes channels

(A) Illustrative representation of a sheep's skull with the location of channels chosen for placement of electroencephalography (EEG) electrodes. (B) Demonstration of electrode placement (Ambu Subdermal Corkscrews – 47″) on a seated sheep for oligonucleotide infusion. (C) After reversal of sedation, still with the EEG electrodes attached to the scalp, the animal is kept with the trackIt device close to the body with an adaptation of a prolapse harness for sheep.

In another subject, 2 days after the implantation of the i.c.v. catheter and the Ommaya, it was noticed by palpation that the reservoir was loose in the subcutaneous space. The animal was sedated, and the stitches removed. It was observed that the catheter had ruptured at the connection with the reservoir. A new reservoir was reconnected to the loose end of the catheter, at the point where it had ruptured, and catheter patency and placement were confirmed by radiographs after administration of contrast. Anchoring was secure, and the catheter remained on target in the ventricle (Figure 6).

DISCUSSION

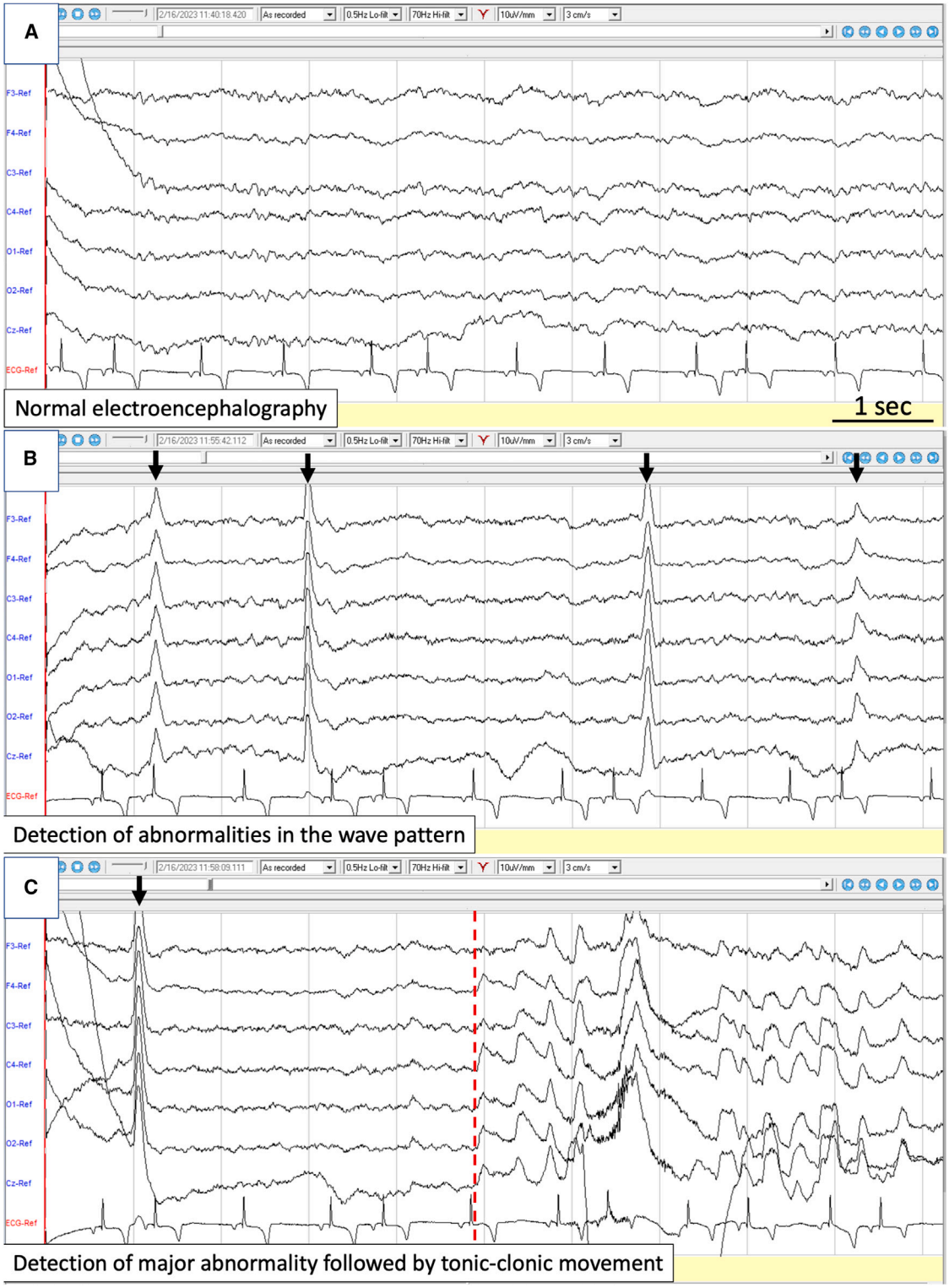
Among a wide range of uses for intracerebroventricular catheters with reservoirs, to our knowledge, this is the first report of a standardized, safe, MRI-, CT-, and EEG-compatible 3D printed sterilization-compatible fixation device for veterinary applications. This device can be modified to fit different cranial sizes and concavities to suit any species of large animal. Despite being most used for chronic and repeated intraventricular or intrathecal administrations,[29,30] intrathecal catheters have proved to be a great resource for the administration of experimental drugs in animal models but are prone to migration and mistargeting. Using the techniques and resources described in this paper, we outline a surgical procedure and device that ensures fixation in a way that is both MRI and electro-encephalography compatible and allows repeated dosage for experimental drugs for at least 60 days.

These are essential features in efficacy and safety studies for new test articles to be delivered to the CNS. Our results addressed and solved the necessity of multiple i.c.v. injections with minimally invasive procedures once the catheter is placed. Furthermore, neural functions include a range of regulated mechanisms of intracellular calcium homeostasis, neurotransmitter release, and electrical activity.[3] However, the negative charge of an oligonucleotide into the CNS, for instance, can affect ion flux and lead to neuronal hyperexcitability and eventually seizures.[3,4] For this reason, brain activity readouts are extremely important for the detection of potential subclinical seizures in safety studies. We were able to track brain electrical activity during and up to 24 h after oligonucleotide infusion, with video recording synchronized with the EEG reading.

The location of EEG electrodes on the human scalp is well established. The International System 10–20 of Electrode Placement is the most widely used method to describe the location of EEG electrodes on the scalp of patients.[31] However, interspecies differences between the shape and size of the skull of animals requires adaptation of the standard electrode placement system for recording surface EEG across species. Furthermore, the relatively small size of the skull in most animals compared to man also limits the total number of electrode positions that can be usefully recorded.[31] For that reason, we chose representative areas for the whole brain: frontal, central, and occipital; therefore, the channels chosen were F3 and F4; C3, C4, and Cz; and O1 and O2.

Regarding the analysis of the EEG, the animals in the present study serve as models for the evaluation of efficacy and safety of a test article for the treatment of Huntington's disease. Our aim was to describe and validate the method for the infusion of oligonucleotides into the i.c.v. in awake large animal models, only under sedation, and monitor brain activity in the subsequent 24 h. In this regard, our model proved to be successful, as it was possible to detect seizures during infusion and to monitor behavior by video, in synchrony with the EEG.

In terms of accuracy, the procedure is highly successful. Malposition of the catheter tip is described in up to 6% of Ommaya reservoir placements.[22] In the present study, the only catheter malposition was detected intraoperatively in one case and immediately resolved. Despite a small number of animals, the accuracy results of the present study (10/10) are comparable to the success of catheter and reservoir implants performed in humans.[32,33] The anchoring of the catheter via the disc prototype is reliable. In all implantations, once the fixation table was fixed in place, the catheter could be safely manipulated during the completion of the procedure and the suturing of the skin without moving from the ideal position.



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Interestingly, previous studies have reported backflow of contrast along the catheter tract for up to 4 days after implantation.[34] For this reason, a two-decade study conducted by Peyrl and colleagues restricted use of the catheter to after 5 days post implantation to allow for healing.[22] However, in this study, all animals had the ventricular catheter-reservoir system tested immediately after the end of surgery by iodinated contrast injection and fluoroscopy. There was no evidence of reflux in any of the animals. This leads us to hypothesize that the needle-guided catheter advancement method and its anchoring with disc prototype is efficient at preventing the creation of space for reflux.

Previous studies have reported infections of Ommaya reservoirs during chemotherapy administration in up to 19% of patients.[30,35] The first animal injected in the study developed encephalitis likely from contaminated injectate, as sterility was maintained during injection (e.g., sterilization of equipment, aseptic preparation of the injection site, and use of sterile gloves). The case was resolved with removal of the catheter and antibiotic therapy and fluid therapy. In addition, prophylactic therapies with ceftriaxone (15 mg/kg) can be instituted. Also, our model proved to be easy to correct intra-surgically and easy to repair and replace in case of catheter rupture. In the second case, the anchorage kept the catheter in place, although we suspect tension on the catheter from normal activity resulted in rupture.

The disc prototype anchorage proved to be highly effective, keeping the intraventricular catheter viable for use up to 60 days after implantation. All nine animals whose implants were maintained had catheter viability analyzed via MRI and/or CT via contrast injection (Omnipaque 350 mg/mL) through reservoir. All catheter systems remained completely patent after 2 months of the sheep maintaining normal activity without restriction. These data show the disc prototype maintains catheter fixation in the ideal position for long periods of time, enabling repeated injections. Future studies using repeated administration of compounds long term are planned using this technique.

To the best of our knowledge, our surgical procedure and disc prototype anchoring system for i.c.v. catheters is the first device that does not rely on bone cement, cyanoacrylate adhesive, and/or suture fixation in animals. Furthermore, to our knowledge, this is the first method of tracking electrical brain activity via electroencephalography and video for the 24-h period after administration of a novel test article in large animal models. Its anchoring efficiency has been demonstrated for at least 2 months and will be tested for up to a year in ongoing studies. Also, our technique has implications beyond oligonucleotide administration and may be of relevance for any research areas, (e.g., viral vectors, chemotherapeutics, small molecules, cell therapy). Due to its versatility between species, efficiency, compatibility with imaging and electrodiagnostic techniques, and applications for test articles of various natures, this model of i.c.v. catheter anchorage is ideal for use in translational laboratories for neurodegenerative diseases. We do not anticipate the necessity of replacing the reservoirs. Ideally, they should be patent and viable over the course of a year; however, we have not yet tested the model beyond 4 months.

MATERIALS AND METHODS

Study approval

All experimental studies involving animals were approved by the University of Massachusetts Chan Medical School IACUC (protocol A202100134) and performed according to the guidelines and regulations therein described.

Animals

Ten sheep (nine neutered rams and one ewe) were used in the study. The age at which the animals underwent surgery ranged from 8 to 16 weeks. There were no relevant differences in size and volume of the ventricles (data not shown).

Anchoring design and manufacturing

Prototypes and the final design of the model, nicknamed disc and fixation table, were designed using the online software Tinkercad (https://www.tinkercad.com), a computer-aided design software to export the model as Standard Triangle Language (STL) files. The STL files were sliced on the Preform software (Formlabs). The STL files were then printed using Form 2 stereolithography SLA Desktop 3D Printer (FormLabs, Somerville, MA, USA) with white resin (FormLabs; tensile strength of 65.0 MPa and a modulus of 2.8 GPa). The layer height was set to 100 microns, and the supports were autogenerated with 0.6-mm contact points. Once printed, the parts were washed with 91% isopropyl solution for 20 min and left for 1 day to let the remaining resin residues dry at room temperature. After support removal, sharp edges were sanded down using sandpaper (3M Pro Grade No-Slip Grip Sandpaper, 120-Grit). Before use, the prototypes were sterilized in a hydrogen peroxide (H_2O_2) chamber (Tuttnauer PlazMax P50).

Preoperative preparation

Sheep were pre-medicated with a combination of buprenorphine sustained release (0.27 mg/kg; administered subcutaneously 24 h before the procedure), acepromazine (0.05 mg/kg intramuscularly [i.m.]), and glycopyrrolate (0.004 mg/kg i.m.). Flunixin meglumine (1.1 mg/kg intravenously [i.v.]) was administered as an anti-inflammatory. Ceftiofur sodium (2 mg/kg) was administered as a prophylactic antibiotic. Sheep were anesthetized using ketamine (10 mg/kg) and midazolam (0.2 mg/kg) and intubated. Anesthesia was maintained by isoflurane gas (1%–3%).

Figure 4. Electroencephalography readouts

(A) Representation of a normal electroencephalography (EEG) reading. The scale bar represents 1 s of reading. (B) Detection of EEG abnormalities concomitant with oligonucleotide infusion. The black arrows indicate changes in the wave pattern. The peaks indicated by the arrows are consistent with seizure activity. (C) Detection of EEG abnormalities concomitant with oligonucleotide infusion. The black arrow indicates changes in the wave pattern. The dashed red line indicates the onset of tonic-clonic movements after the electrical peak indicated by the arrow, consistent with seizure activity.

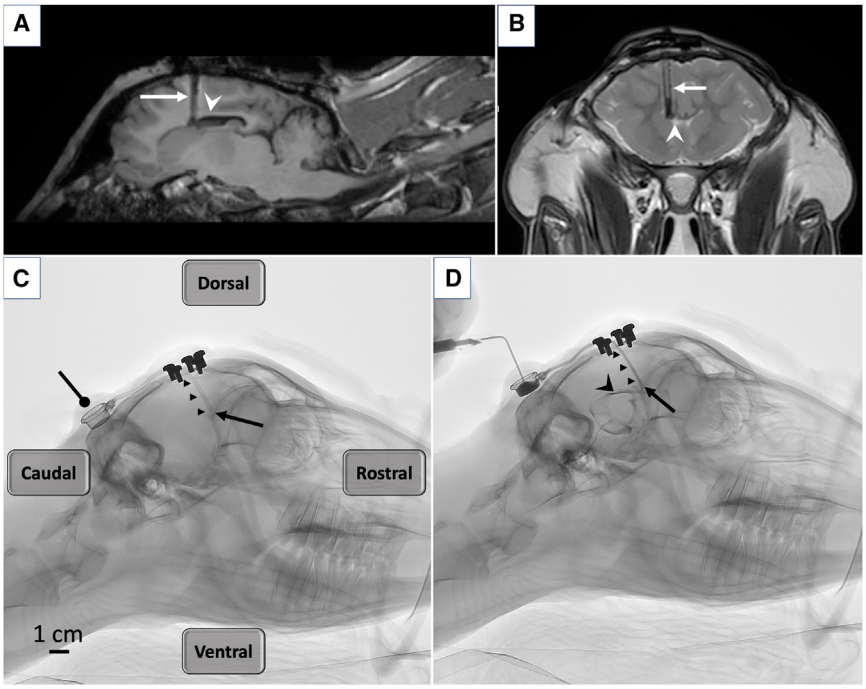

Figure 5. Verification of the viability and functionality of the catheter 60 days after implantation

(A) Sagittal view of magnetic resonance imaging indicating the location of the catheter. The white arrow indicates the catheter track. The white arrowhead indicates the lateral intracerebral ventricle. (B) Coronal view of magnetic resonance imaging indicating the location of the catheter. The white arrow indicates the catheter track. The white arrowhead indicates the left lateral intracerebral ventricle. (C) Lateral X-ray image of the animal's skull with pre-injection of iodinated contrast. The black arrow indicates the exact location of the catheter tip. The round-headed black arrow indicates the reservoir. The small arrowheads indicate the catheter and the catheter tract. (D) Lateral image taken immediately after administration of iodinated contrast. The black arrow indicates the exact location of the catheter tip. The big arrowhead indicates the ventricular system being filled by contrast. The small arrowheads indicate the catheter and the catheter tract.

The area over the frontal bone was clipped and aseptically prepared using standard techniques. An incision was made over the frontal bone, and an MRI fiducial array was fixed to the skull. Animals then underwent 3T MRI (3T Phillips Ingenia) to generate 3D T1-weighted images for neuro-navigation (MPRAGE 0.5 mm isotropic voxels). Anatomical MRIs were acquired in a Phillips 3T scanner (Phillips Ingenia 3T; Philips Healthcare, Best, Netherlands) using an anterior coil (Philips Healthcare). The imaging protocol included 3D T1-weighted magnetization prepared-rapid gradient echo (MPRAGE) sequence (TR/TE 10/5 ms, flip angle [FA] = 8°, number of averages [NEX] = 8, matrix = 268 · 268, slice thickness = 0.75 mm, field-of-view [FOV] = 200 · 200 mm).

Lateral i.c.v. catheter and reservoir implantation through new anchoring system

After generating 3D T1-weighted images for neuro-navigation, the sheep were then placed in a radiolucent stereotaxic frame (Model 1530M, Khopf instruments) and registered to neuro-navigation equipment (Brainsight Vet, Rogue Research). The skin above the frontal and parietal bones were shaved and aseptically prepared using standard techniques.

Based on neuro-navigation MRI coordinates, a longitudinal incision was made, and the skull was exposed (Figure 7A). MRI-based neuro-navigation was used to determine the location of the

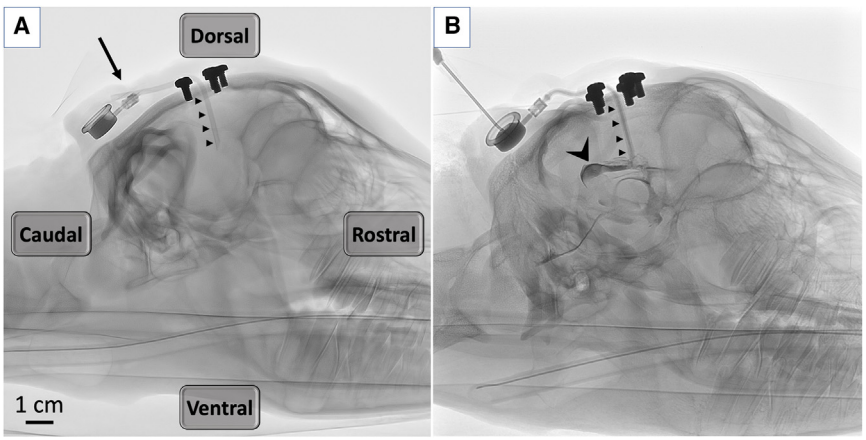

Figure 6. Lateral intraoperative X-ray images from intracerebroventricular catheter placement and reservoir replacement

(A) X-ray single shot showing the broken catheter at the junction with the reservoir. The black arrow indicates the rupture between catheter and reservoir. The small arrowheads indicate the catheter and the catheter tract. (B) X-ray single shot after reservoir replacement and contrast administration. The arrowhead indicates the ventricular system being filled by contrast. The small arrowheads indicate the catheter and the catheter tract.

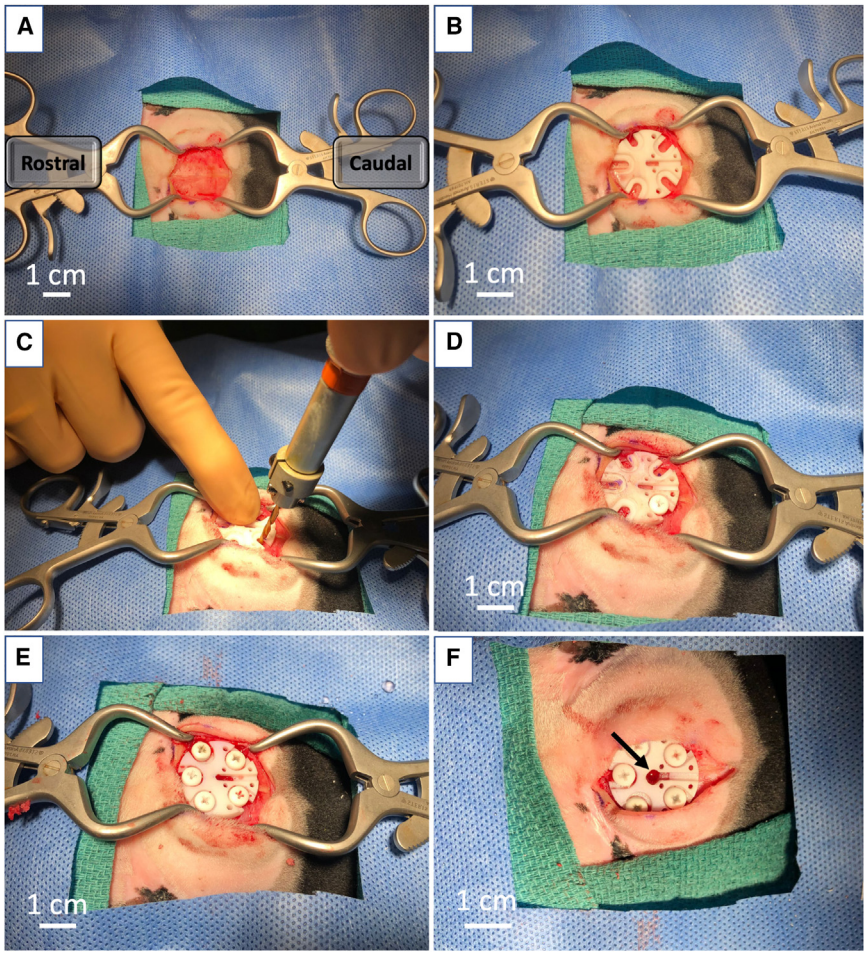

Figure 7. Placement and fixation of the prototype

(A) Longitudinal incision and exposure of the skull incision location is based on neuro-navigation MRI coordinates. (B) Positioning of the disc prototype over the exposed skull, with the center hole overlapping the craniotomy location determined by neuro-navigation. (C) The skull underneath the grooves of the prototype is manually drilled to create threaded holes for the placement of ceramic screws. (D) The holes are made sequentially in a star pattern to prevent unwanted movement of the disc prototype. For each screw attached, the center hole of the prototype is verified via neuro-navigation to ensure proper positioning for the craniotomy. Representation of the fixation of the first ceramic screw. (E) Representation of the fixation of the five ceramic screws. (F) After the prototype is properly fixed to the skull, the craniotomy is performed in the center of the prototype following the predetermined angle by neuro-navigation to access the intracerebral ventricle. The black arrow indicates the craniotomy.

craniotomy and trajectory to the lateral ventricle. The prototype was placed with the location for the craniotomy at its center, with the groove for the catheter pointing caudally. Pilot holes were hand-drilled using Rogue Research 2.36-mm drill to create threading compatible with the screws. The prototype was then fixed in place using 5-mm ceramic screws (Figures 7B–7E). Once stabilized, a 3-mm craniotomy was created in the center of the prototype using Hall Micro Free PRO8000SB High-Speed Drill and MicroAire, Med-Length Bur (ZB-125), 2.4 × 2.4 mm head, six flutes, 50.8-mm-long drill bit following the angle and trajectory determined by neuro-navigation (Figure 7F).

Using neuro-navigation MRI guidance, a spinal needle (20G x 6 inches) used as a stylet inside of a custom-made fenestrated flexible catheter (24-cm Custom Catheter, 2 Fenestrations 21-4590-24-C5 SAI Infusion Technologies) and lowered into the brain using the stereotaxic manipulator (Figure 8A). Backflow of CSF was used to confirm the location in the lateral ventricle. Placement was also verified by fluoroscopy and cone beam CT after injection of iodinated contrast (Omnipaque 350 mg/mL) (Figure 8B).

Once the placement was verified, the catheter was held in place using forceps, and the spinal needle was gently removed. Placement was once again verified by fluoroscopy after injection of iodinated contrast (Omnipaque 350 mg/mL) (Figure 8C). After placement was confirmed, the guide arm of the neuro-navigation equipment was removed, and the catheter was folded into the groove of the disc prototype and held in place by fitting the second part of the prototype, the fixation table, into the holes, thereby holding the catheter in place. The fixation table was then glued with surgical glue (3M Vetbond Tissue Adhesive 1469c), preventing catheter migration. Contrast was injected to confirm targeting (Figure 8D). The catheter was then attached to the reservoir (Port Elite, SAI Infusion Technologies) with 2–3 mm of slack in an S-shape to allow for small movements. Once the catheter was securely attached to the reservoir, a subcutaneous pocket was made under the skin using blunt-ended dissection. The reservoir was then placed subcutaneously, so that the surface where the injection would take place was completely covered by skin, avoiding the incision, and fixed in place with non-absorbable suture (ETHILON Nylon Suture 3-0) (Figure 8E). Placement was again verified by fluoroscopy as described above, after injecting Omnipaque 350 mg/mL through the reservoir using a Huber needle (Figure 8F).

The incision was closed using standard techniques. Animals were allowed to recover from anesthesia and returned to the pen when fully awake and sternal. After surgery, the animals received ceftiofur (30–50 mg/kg) for 3 days prophylactically.

Post-surgical pain management was based on the drugs mentioned in the presurgical procedures, buprenorphine sustained release (0.27 mg/kg subcutaneously) and flunixin meglumine (1.1 mg/kg i.v.). After the procedure, the animals were kept in smaller stables, accompanied by

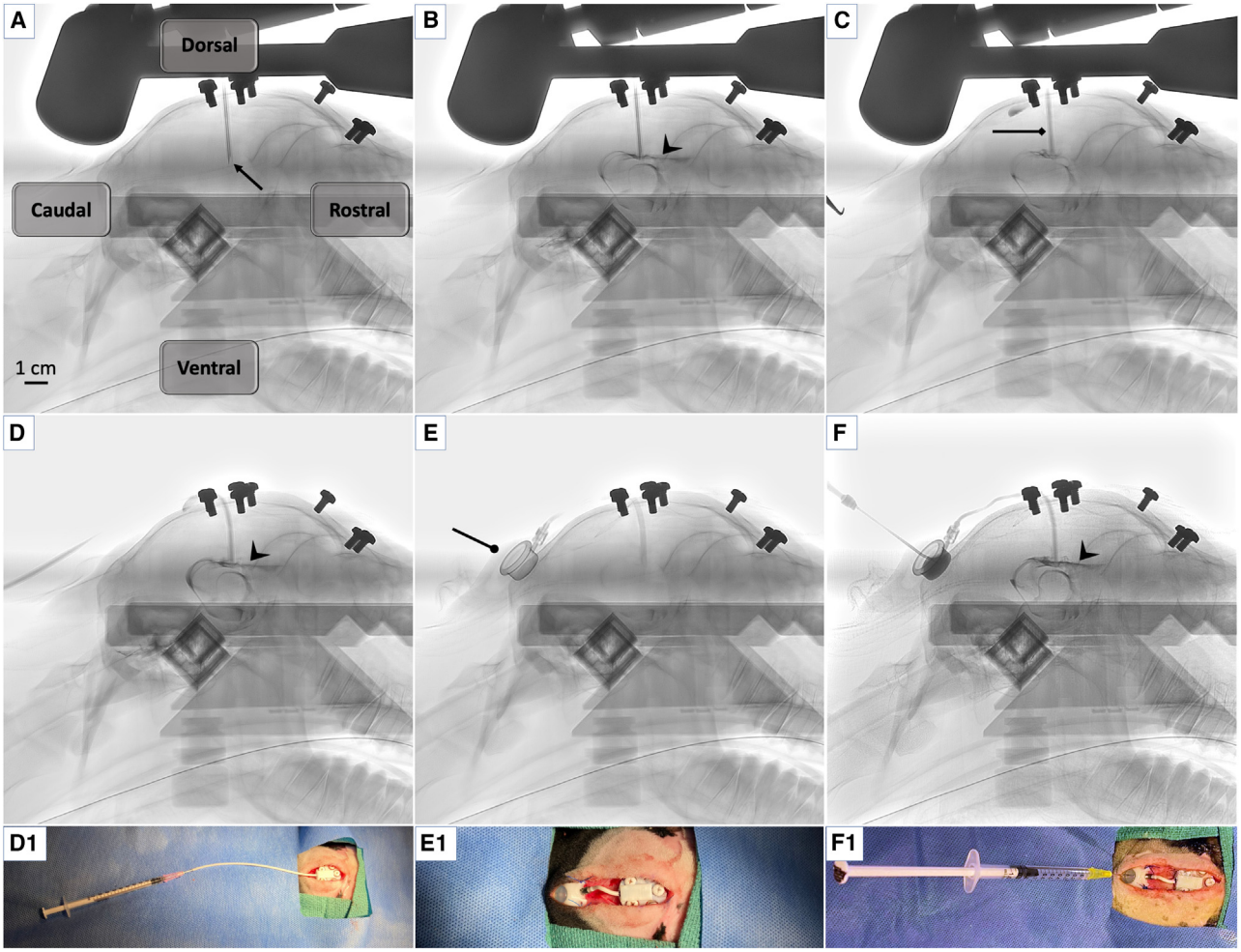

Figure 8. Intraoperative lateral X-ray images from intracerebroventricular catheter and reservoir placement

(A) Animal positioned in the stereotactic with the needle-catheter system inserted into the left lateral ventricle. The black arrow indicates the bevel of the needle. The black reflection at the top of the image is the guide arm of the neuro-navigation equipment. (B) Single shot subsequent to (A), after contrast injection. The arrowhead indicates the ventricular system being filled by contrast. (C) Single shot after removal of the needle from the catheter lumen and injection of contrast to confirm catheter location. The square-headed arrow indicates the catheter. (D) Confirmation of the location after removing the guide arm from the neuro-navigation equipment. The arrowhead indicates the ventricular system being filled by contrast. (D.1) Dorsal view of the surgical field for injection of iodinated contrast for verification of catheter placement. (E) Implantation of the subcutaneous reservoir connected to the catheter. The round-headed arrow indicates the reservoir. (E.1) Dorsal view of the surgical field after implantation and fixation of the Ommaya subcutaneous reservoir. (F) Confirmation of the location of the catheter before closing the incision, via injection of iodinate contrast through the reservoir. The arrowhead indicates the ventricular system being filled with contrast, indicative of good targeting. (F.1) Dorsal view of the surgical field for injection of contrast through the Ommaya reservoir for confirmation of catheter placement.

other sheep. These were monitored three times a day to check the incision and general condition of the animal. 72 h after administration of buprenorphine sustained release, the animal was accessed, and if it showed pain, a new dose of flunixin meglumine (1.1 mg/kg i.v. or i.m.) was administered.

siRNA

The oligonucleotides were manufactured as described previously.[6] The following modified sequences were used: V(mU)#(fU)#(mA) (fA) (mU) (fC) (mU) (fC) (mU) (fU) (mU) (fA) (mC)# (fU)#(mG) #(fA)#(mU)#(fA)#(mU)#(fA) for the antisense strand and Cy3-(fC) #(mA)#(fG) (mU) (fA) (mA) (fA) (mG) (fA) (mG) (fA) (mU) (fU) #(mA)#(fA)-DIO for the sense strand, where "#" denotes phosphorothioate linkage and "m" and "f" denote modification of the endogenous 2′OH group to 2′ O-methyl or 2′ fluoro, respectively, as described by Ferguson et al.[7] The oligonucleotide used in the present study has the function of non-allele-specific silencing of the translation of the Htt protein coding mRNA, as previously described.[6,7]

siRNA infusion and EEG analysis

For use of the catheter-reservoir system, sheep were sedated with dexmedetomidine (0.01–0.02 mg/kg). The sheep's head was shaved, from

the frontal bone region to the occipital bone. The entire area was aseptically prepared, and a subcutaneous injection of 200 μL of lidocaine 2% was performed in each channel site before placement of subdermal EEG electrodes (Ambu Subdermal Corkscrews – 47″, MedEnvoy Switzerland). The electrodes were then connected to the wireless Holter (trackIt EEG System F – T4A, Lifelines Neuro Company, Louisville, KY, USA), which sends the signals to the Lifelines Neuro matrix computer. The system allows video recording in synchrony with the tracking of brain electrical activity during and after the 24 h following the infusion.

After placing all the subdermal electrodes, a sterile field was placed over the injection site. With a Huber needle, the reservoir was flushed with 200–400 μL at a rate of 100 μL per min of sterile saline solution, to remove potential clogging at the catheter tip (e.g., by choroid plexus). To confirm the placement of the catheter, 100–200 μL of fluid was aspirated after 2 to 5 min for intraventricular system equilibration.

Once the placement of the catheter was confirmed, using a Huber needle, the oligonucleotide was injected into the lateral i.c.v. through the reservoir at a rate of 100 μL per min at 2 μM/ml, in the following doses: 25 mg/1 mL; 50 mg/2 mL; 75 mg/3 mL, and 100 mg/4 mL. After finishing the oligonucleotide infusion, the dead space of the reservoir and catheter was flushed with 400–500 μL of sterile saline at a rate of 100 μL per min.

The animal was reversed from sedation with atipamezole hydrochloride intramuscular (0.1–0.2 mg/kg) still attached to the EEG electrodes. The trackIt device is kept close to the animal with an adaptation of a prolapse harness for sheep (Premier 1, Washington, IA, USA, Figure 3C). After recovery, the sheep was placed in a pen with its dam to spend the next 24 h being monitored by video and having brain activity recorded (in synchrony with the video).

DATA AND CODE AVAILABILITY

All imaging and prototype files are available upon request. EEG data for quality assessment will be made available upon request. The remaining EEG data are a part of an ongoing study and will be made available upon completion of that study.

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AUTHOR CONTRIBUTIONS

H.R.B.: conceptualized and co-developed the surgical technique, conducted the surgeries, post-surgical care, oligonucleotide infusion, and EEG tracking and analysis, and was the primary author of the manuscript. R.D.P.: conducted the surgeries, post-surgical care, oligonucleotide infusion, and EEG tracking. T.T.: ran the MRI scanning and manuscript review and preparation. R.M.: co-conceptualized the surgical method and synthetized the oligonucleotide. R.K.: ran intra-surgical CT scanning and manuscript review and preparation. M.J.G.: ran intra- and post-surgical CT scanning. U.C.: provided the 3D-printed prototype. S.B.: was responsible for caring for and managing the sheep flock and provided post-surgical care. S.T.: assisted surgeries and post-surgical care. L.B.: assisted surgeries and post-surgical care. E.P.: conducted EEG tracking and analysis. J.G.: assisted surgeries and manuscript review and preparation. E.F.H.: assisted surgeries. A.W.M.: assisted surgeries. M.S.-E.: designed the prototypes. A.K.: conceptualized and synthetized the oligonucleotide. A.N.: provided resources and co-conceptualized the oligonucleotide. H.L.G.-E.: conceptualized and co-developed the surgical technique, conducted the surgeries, EEG tracking and analysis, and manuscript review and preparation.

DECLARATION OF INTERESTS

There are no conflicts to disclose.

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