JOURNAL OF NEUROSURGERY:
Case Lessons

J Neurosurg Case Lessons 3(12): CASE21417, 2022
DOI: 10.3171/CASE21417

Xenotransplantation of porcine progenitor cells in an epileptic California sea lion (*Zalophus californianus*): illustrative case

*Claire A. Simeone, DVM,[1,2] John P. Andrews, MD,[3] Shawn P. Johnson, DVM, MPVM,[1] Mariana Casalia, PhD,[3] Ryan Kochanski, MD,[3] Edward F. Chang, MD,[3] Dianne Cameron,[2] Sophie Dennison, BVM&S,[4] Ben Inglis, PhD,[5] Gregory Scott, MA, DVM,[2] Kris Kruse-Elliott, DVM, PhD,[6] F. Fabian Okonski, MD,[7] Eric Calvo,[2] Kelly Goulet, RVT,[2] Dawn Robles,[2] Ashley Griffin-Stence,[2] Erin Kuiper,[2] Laura Krasovec,[8] Cara L. Field, DVM, PhD,[9] Vanessa F. Hoard, DVM,[10] and Scott C. Baraban, PhD[3]

[1]Sea Change Health, Kentfield, California; [2]Six Flags Discovery Kingdom, Vallejo, California; [3]Department of Neurological Surgery & Weill Institute for Neuroscience, University of California, San Francisco, San Francisco, California; [4]TeleVet Imaging Solutions, Oakton, Virginia; [5]Henry H. Wheeler, Jr. Brain Imaging Center, University of California, Berkeley, Berkeley, California; [6]Sage Veterinary Centers, Redwood City, California; [7]Department of Anesthesiology, Perioperative and Pain Medicine, Lucile Packard Children's Hospital at Stanford, Stanford School of Medicine, Stanford, California; [8]BrainLab, Westchester, Illinois; [9]The Marine Mammal Center, Sausalito, California; and [10]Pacific Marine Mammal Center, Laguna Beach, California

BACKGROUND Domoic acid (DA) is a naturally occurring neurotoxin harmful to marine animals and humans. California sea lions exposed to DA in prey during algal blooms along the Pacific coast exhibit significant neurological symptoms, including epilepsy with hippocampal atrophy.

OBSERVATIONS Here the authors describe a xenotransplantation procedure to deliver interneuron progenitor cells into the damaged hippocampus of an epileptic sea lion with suspected DA toxicosis. The sea lion has had no evidence of seizures after the procedure, and clinical measures of well-being, including weight and feeding habits, have stabilized.

LESSONS These preliminary results suggest xenotransplantation has improved the quality of life for this animal and holds tremendous therapeutic promise.

https://thejns.org/doi/abs/10.3171/CASE21417

KEYWORDS epilepsy; sea lion; MGE progenitor cells; interneuron progenitor cells; epilepsy xenograft; domoic acid toxicosis

Domoic acid (DA) is a neurotoxin produced by *Pseudo-nitzschia* algae.[1] DA toxicosis of California sea lions (*Zalophus californianus*) that consume the toxins is now widespread and a common cause of morbidity and mortality.[2] Unusually aggressive behaviors, vomiting, inappetence, marked lethargy, stranding in atypical locations, ataxia, seizures, coma, and increased mortality are observed in DA-intoxicated sea lions rescued for rehabilitation in California.[2–5] Many of these animals exhibit an acquired form of epilepsy (i.e., spontaneous recurrent seizures, hippocampal atrophy, mossy fiber sprouting, and interneuron cell loss) resembling human mesial temporal lobe epilepsy (TLE).[6] Similar to many human patients with TLE, treatments to control seizures in DA-intoxicated sea lions are urgently needed. Available options for wild animals only address acute toxicosis while in rehabilitation, and DA-exposed sea lions placed in long-term care can develop progressive disease despite administration of antiepileptic drugs (AEDs).[5]

A one-time cell transplant therapy, using GABAergic interneurons that efficiently integrate in local epileptic circuits, may offer an alternative. Studies of such transplants in mice suggest such GABAergic interneurons can function similarly to endogenous inhibitory neurons

ABBREVIATIONS AED = antiepileptic drug; CT = computed tomography; DA = domoic acid; GABA = γ-aminobutyric acid; MGE = medial ganglionic eminence; MRI = magnetic resonance imaging; QOL = quality of life; TLE = temporal lobe epilepsy.

INCLUDE WHEN CITING Published March 21, 2022; DOI: 10.3171/CASE21417.
SUBMITTED July 19, 2021. **ACCEPTED** December 6, 2021.

* C.A.S., J.P.A., and S.P.J. contributed equally to this work and share first authorship.

after transplant, with the potential to correct comorbid behavioral deficits.[7,8] GABAergic progenitor cells derived from embryonic medial ganglionic eminence (MGE) integrate into host circuits after transplantation, where they make functional inhibitory synapses,[8] efficiently suppress seizures, and correct comorbid behavioral deficits.[7,9] These observations are based on harvesting embryonic murine MGE progenitors and rodent acquired epilepsy models. Building on these studies, we recently established a protocol to harvest porcine MGE progenitors as a tissue source for xenotransplantation.[10] Progenitor cell xenotransplantation into marine mammals, specifically epileptic sea lions, has never been attempted, but, if successful, it could provide a route to disease-modifying therapies.

Illustrative Case

We report a pilot case of intrahippocampal porcine progenitor cell xenotransplantation in a California sea lion with refractory epilepsy and magnetic resonance imaging (MRI) characteristics of unilateral hippocampal atrophy.

Subject

The subject was an 8-year-old male California sea lion (*Z. californianus*) clinically diagnosed with epilepsy from earlier suspected DA toxicosis based on observed seizure-like activity, prolonged anorexia, radiological evidence of unilateral hippocampal atrophy, and absence of infectious disease agents (Fig. 1A).

Epilepsy

The observed course of epilepsy in the subject is as follows. The subject first stranded on the coast of San Luis Obispo County, California, in November 2017, and was admitted to a rehabilitation center for lethargy and disorientation but recovered rapidly and was released 2 weeks later. Over the next 2 months, the sea lion was

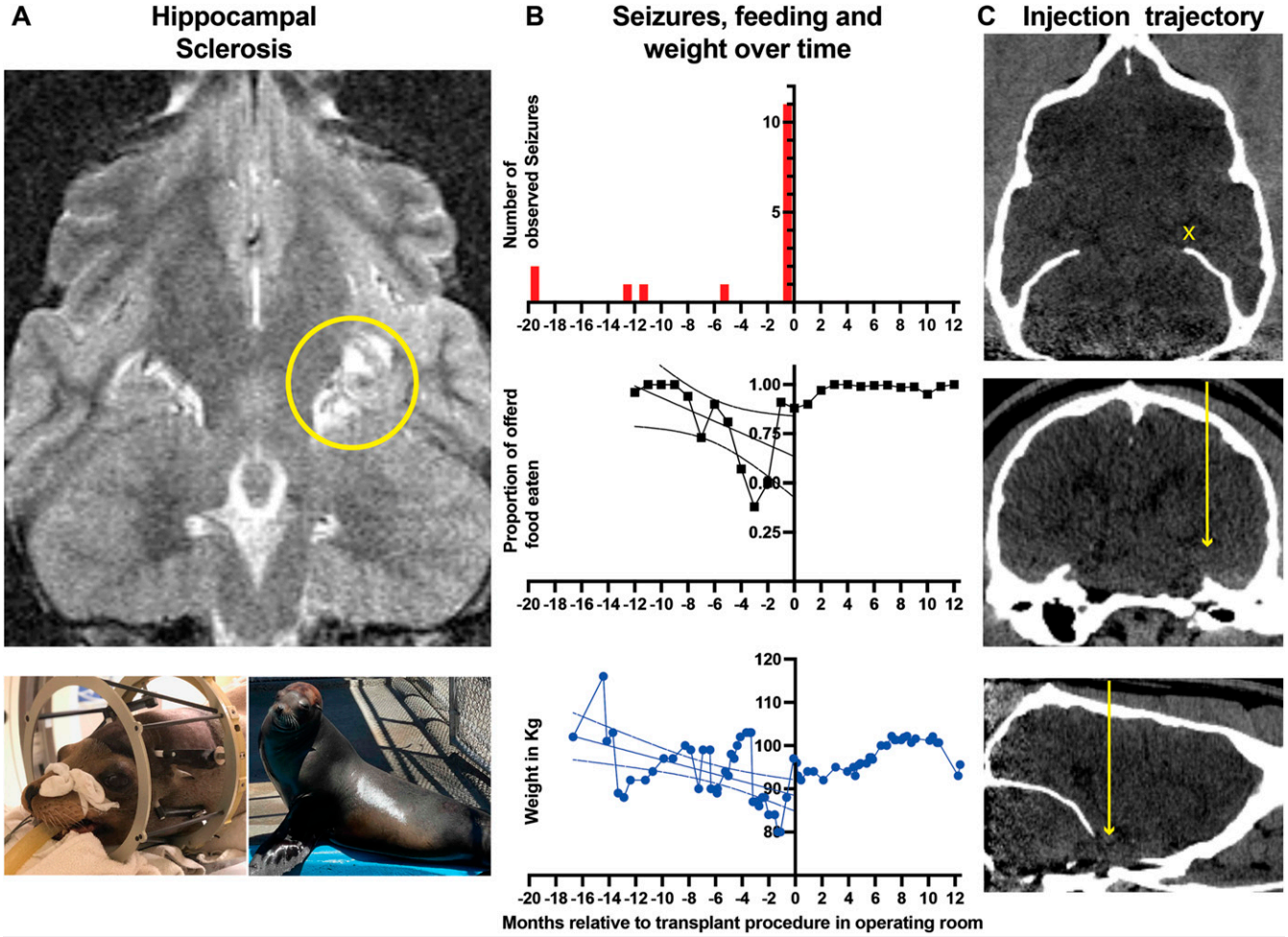

FIG. 1. A, *Top*: Transverse (axial) slice of a T2-weighted MRI scan of the sea lion subject. The *yellow circle* outlines the left hippocampus, showing relative atrophy compared with the contralateral hippocampus, as evidenced by increased T2-bright cerebrospinal fluid spaces of the temporal horn of the lateral ventricle. *Bottom*: The subject fitted with a stereotactic frame, intubated for a preprocedural CT scan (*left*), and the subject at leisure (*right*). **B:** Observed seizures (*top*), proportion of food eaten as a fraction of total food offered (*middle*), and average monthly weight in kilograms over time (in months), relative to date of the procedure (at month 0). Trend lines for variables before intervention are overlaid onto *middle* and *bottom panels* and are linear regressions with 95% confidence intervals of preintervention time points (i.e., not including points after month 0). R^2 of proportion of food eating = 0.32; R^2 of weight = 0.23. **C:** CT scan immediately before the procedure, showing transverse (*top*), dorsal (*middle*), and sagittal (*bottom*) slices. *Yellow X* delineates the hippocampus target (*top panel*); *yellow arrows* delineate injection needle trajectory.

rescued twice more. Veterinarians determined the sea lion was unlikely to survive in the wild due to habituation. He was deemed nonreleasable by National Oceanic and Atmospheric Administration Fisheries and transferred to a facility for permanent care. A convulsive seizure with impaired consciousness was observed in April 2018. A second seizure was observed in February 2019, and phenobarbital treatment was initiated as a first-line AED. Initially, an MRI scan obtained in January 2018 showed no structural abnormalities, but a second MRI study obtained in October 2018 showed evidence of unilateral hippocampal atrophy (Fig. 1A, top panel). Over the next 14 months, 3 additional convulsive seizures were observed (Fig. 1B, top panel) that were associated with periods of prolonged anorexia and reduced feeding (Fig. 1B, middle and bottom panels). Diazepam was added as a second AED in April 2020.

In early 2020, complete anorexia (1–3 days per month progressing to 6–17 days per month) was noted. Food consumption and monthly average weight showed a clear declining trend (Fig. 1B). Appetite stimulants (mirtazapine and capromorelin) were offered with minimal observed therapeutic effect. During this period, $\sim$25% body weight loss (from 103 to 79 kg) was observed. Progression of anorexia coincided with 11 more observed seizures over a 5-day period in September 2020, despite administration of therapeutic levels of phenobarbital.

With refractory seizures, increasing episodes of anorexia, and declining body weight, euthanasia was considered. As an alternative, a scientific-therapeutic collaboration led to a single-subject xenotransplantation trial modified to accommodate a large marine mammal. At 12 months after transplant, no seizures had been observed (Fig. 1B, top panel), appetite and weight had stabilized (Fig. 1B, middle and bottom panels), and behaviors appeared to be subjectively improved. The animal has continued postsurgery AED treatment (phenobarbital and diazepam) to reduce the risk of withdrawal seizures.[11,12]

Methods

Subject Tracking

When animals strand, they receive a plastic tag with a number on it. When they restrand, this number is checked with national registries. This was the method by which the subject of this report was identified and subsequently found to be nonreleasable and transferred to a facility for permanent care.

Xenotransplantation Procedure

The subject was anesthetized, intubated, and maintained on isoflurane and oxygen. With the subject under anesthesia, a computed tomography (CT) scan was performed to map the surgical trajectory to the left hippocampus, and the head was fixed in place for stereotactic implantation. Progenitor cells harvested from porcine embryos were prepared.[10] Freeze-thawed porcine embryonic MGE progenitor cells were prepared, and $>$90% viability was confirmed on-site, as described.[10] Procurement and preparation of porcine MGE progenitor cells is covered by a University of California, San Francisco Institutional Animal Care and Use Committee protocol (approval no. AN181254-02C). A custom-made 10-cm-long Hamilton needle (1.8 mm internal diameter; 2 outer sections, 5 cm length, 23-gauge, followed by 5 cm, 32-gauge blunt) was back-loaded with porcine progenitor cells. An entry point was chosen rostral and lateral to the occipital eminence, from which a trajectory orthogonal to the skull was used to enter the hippocampus. The sea lion was positioned in sternal recumbency and was prepped and draped in sterile fashion. A stab incision was made in the skin, and a twist drill was used to make a small burr hole for the injection needle. The MGE-loaded Hamilton needle was inserted slowly to a premeasured depth based on a preoperative CT scan. Progenitor cells injected at a rate of 5 nl/s in 3 locations along the trajectory: once at initial target depth, followed by withdrawal of $\sim$5 mm, repetition of the injection procedure, then withdrawing 5 mm and injecting once more. A total of 50,000 cells/site of MGE progenitors were injected along the single tract trajectory to the left hippocampus (Fig. 1C). After cell delivery, the injection needle was slowly withdrawn, and the skin was closed with an absorbable suture. The sea lion was maintained in a dry pen for 3 days postoperatively.

Pharmacological Treatments

The subject was maintained on cyclosporine for immunosuppression for 6 months, and peak and trough monitoring was employed. Because this drug has not been reported in sea lions, it is unknown what an immunosuppressive dose would be, but we aimed for serum levels consistent with dogs undergoing bone marrow transplant.[13] Immunosuppression consisted of cyclosporine (7 mg/kg once daily for 7 days, then 3 mg/kg twice daily for 2 months, then 2 mg/kg once daily) administered immediately before the procedure and for 6 months afterward to prevent MGE cell rejection. Cyclosporine serum levels were monitored (Clinical Pharmacology Laboratory, Auburn University) with 24-hour trough levels of 7 mg/kg once daily of 183–280 ng/ml. On the day of the procedure, an anti-inflammatory dose of dexamethasone (0.17 mg/kg once daily for 2 weeks) was started and then tapered to physiological levels over the course of 4 weeks. Prophylactic doses of antibiotics (ceftiofur 6.6 mg/kg) were administered during the perioperative period and for 1 week postoperatively. Long-acting buprenorphine slow release (0.2 mg/kg every 72 hours) was used for 10 days for analgesia. AED treatment consisted of phenobarbital (1.6 mg/kg once daily) and diazepam (0.05 mg/kg twice daily).

Discussion

This compassionate-use trial in a thrice-stranded, severely anorexic, presumed DA-exposed sea lion with multiple documented episodes of convulsive seizures attributed to a hippocampal lesion suggests porcine γ-aminobutyric acid (GABA) progenitors are promising candidates for xenotransplantation-based cell therapy. The rationale for attempting this lifesaving therapy is based on studies regarding origins and properties of inhibitory interneurons from embryonic MGE.[14] Control of seizure activity and rescue of comorbid behavioral deficits associated with acquired epilepsies was initially described in mouse models using murine embryonic MGE progenitor cell donors transplanted into a chemoconvulsant damaged hippocampus.[7,9] Murine MGE-derived cells migrate widely and differentiate to functional GABA-expressing interneurons capable of enhancing synaptic inhibition in these mice. Similar to AEDs that enhance GABA-mediated inhibition (i.e., benzodiazepines), therapeutic benefit of MGE transplant is most likely associated with addition of functional inhibitory interneurons into a hyperexcitable hippocampal network. To translate this strategy to larger mammals, we adapted a protocol using fetal pigs, a well-established source for xenotransplantation tissue.[15] A recent demonstration that porcine embryonic MGE progenitors migrate and differentiate into GABA-expressing interneurons in a manner similar to that described for murine embryonic MGE progenitors used for cell transplantation therapy[10] established this porcine cell source as a

viable candidate for treating larger animals. Because California sea lions with DA toxicosis represent a well-characterized example of acquired epilepsy in wildlife, and because prior studies described unilateral hippocampal atrophy with loss of inhibitory hippocampal interneurons in some of these animals,[6,16] it is not entirely surprising that successful MGE-based treatment of a sea lion mimics prior mouse studies.

Regarding limitations and potential confounds of the results presented here, there are several that cannot be definitively ruled out in the context of a case study. A structural lesion to the epileptic focus could in theory have an antiseizure effect. However, the likelihood of a lesion from the tract of a 32-gauge needle to result in seizure freedom seems extremely low. Indeed, the effect of differing needle sizes was previously tested in rodent models, with a finding that the present size had minimal disruption on mouse hippocampal anatomy,[11] and sham needle injections used to study the efficacy of MGE progenitor cell transplant in mouse models of temporal lobe epilepsy were never shown to be therapeutic.[12] Furthermore, for a parallel, stereoelectroencephalography[17] is routinely performed at epilepsy centers for human epilepsy, using multiple electrodes of comparable to greater diameter than the injection needle targeting possible epileptogenic foci, without a documented ablative effect on seizures.

Another change made at the time of the procedure was the immunosuppression protocol instituted to increase the likelihood that xenotransplanted porcine progenitor cells would engraft in the host brain. It again seems a highly unlikely explanation, given the low dosages of drugs used and the tapering down of these immunosuppressing agents over time, but we cannot absolutely dismiss the possibility that immunosuppressants could be contributing to the seizure-free status.

Observations

Although preprocedural monitoring of the subject's behavior and epilepsy was limited, and although this study describes a relatively short survival period, improvement in quality-of-life (QOL) measures and absence of observable convulsive seizures for $>$12 months offer a cautious enthusiasm. We followed immunosuppression protocols established for transplant of embryonic dopaminergic cells in patients with Parkinson disease. Because cell survival was noted 24 years after these procedures,[17] rejection risk may not be a limiting factor, and potential to generate pathogen-free pigs as tissue donors could further mitigate concerns. A limitation of the present procedure was the inability to obtain image verification during or after intrahippocampal delivery of MGE progenitors. Real-time MRI-guided xenotransplantation may be possible with coinjection of a contrast agent[18] to confirm anatomical localization and cell delivery at the injection site. Long-term monitoring of MGE-derived cells may be more difficult because these cells disperse and migrate in host brain, thus requiring single-cell resolution neuroimaging techniques. DA toxicosis is the most common cause of neurological abnormalities in stranded California sea lions,[19] and expected climate-driven increases in harmful algal blooms will continue to result in hundreds of sea lions (and other marine mammals) with DA toxicosis annually[1] that could potentially benefit from such therapy.

Lessons

This first-in-species case of interneuron xenograft in a California sea lion with refractory epilepsy associated with hippocampal sclerosis shows signs of therapeutic response in QOL measures and no further clinical seizures.

The multidisciplinary coordination of the technical procedure was the key to success. The participants roughly fell into 3 teams: (1) the veterinary surgical and anesthesia marine mammal specialists, (2) the cellular neuroscience team, and (3) the neurosurgical procedural team. An experienced veterinary surgical and anesthesia team was critical for a smooth induction, intubation, and CT scan, as well as for transferring directly from the CT scan to the surgical suite. The cellular neuroscience team prepped and aliquoted the porcine progenitor cells and designed and loaded a customized Hamilton needle for xenotransplantation intraoperatively. This division of responsibility by subspecialization allowed each team to operate within its field of expertise and allowed the procedure to proceed safely and efficiently.

From a technical standpoint, the cranial access from a neurosurgical perspective was similar to that used for a stab-incision insertion of an external ventricular drain—a stab incision and twist drill burr hole. Palpable cranial landmarks were identified on the scan and the animal to confirm anatomical alignment, namely the midline sagittal crest, from which to position the entry in the medial-lateral plane and the caudal aspect of the cranium for anterior-posterior alignment.

To confirm progenitor cell delivery into the targeted hippocampus, one method that could be useful in future versions of this procedure would be coinjection of a contrast agent that would allow the anatomical location of the injection to be monitored by imaging. Presuming there were no unanticipated effects of an iodine-based or CT-detectable contrast on the MGE progenitor cells, including a contrast agent in the cell suspension would be a simple addition to track cell injections.

The living marine mammal presents some difficulties in assessing cell viability in vivo. The injected cells will likely have a sharp decline in number after transplant, and the remaining cells with soma size of roughly 30–50 μm will likely migrate widely in the host brain. Visualizing these individual and widely dispersed neurons would require imaging resolution not currently possible in vivo. Detecting a secondary cell marker such as fluorescence from a cell line engineered to express luciferase would also be technically limited by the depth in tissue and calcified skull, limiting signal detection. Added to this, such a method would require genetic modification of cells, which would be suboptimal for a wildlife-focused study. Confirmation of cell integration was possible in rodent studies published over the past 2 decades using this same methodology combined with timed sacrifice and thin sectioning of brain tissue for detailed immunohistochemical analysis. Although useful, these approaches applied to experimental animals are not easily adapted to wildlife.

Further studies will be needed to provide evidence of efficacy and to optimize safety profile before translation to human trials. This seminal study, however, represents the first step toward validating the therapeutic effects of an MGE progenitor cell–based treatment of epilepsy in a large animal biologically distinct from rodent models in which this procedure was first developed. It has not escaped our notice that a cell-based therapy for refractory epilepsy in humans could provide a paradigm shift for epilepsy treatment, because it is mechanistically distinct from all approved clinical treatments for refractory seizures in humans.

Acknowledgments

Barbie Halaska, Abby McClain, Frances Gulland, and Padraig Duignan from the Marine Mammal Center are acknowledged.

References

1. Scholin CA, Gulland F, Doucette GJ, et al. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature.* 2000;403(6765):80–84.
2. Gulland FM, Haulena M, Fauquier D, et al. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *Vet Rec.* 2002;150(15):475–480.
3. Goldstein T, Mazet JA, Zabka TS, et al. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proc Biol Sci.* 2008;275(1632):267–276.
4. Ramsdell JS, Gulland FM. Domoic acid epileptic disease. *Mar Drugs.* 2014;12(3):1185–1207.
5. Simeone C, Fauquier D, Skidmore J, et al. Clinical signs and mortality of non-released stranded California sea lions housed in display facilities: the suspected role of prior exposure to algal toxins. *Vet Rec.* 2019;185(10):304.
6. Buckmaster PS, Wen X, Toyoda I, Gulland FM, Van Bonn W. Hippocampal neuropathology of domoic acid-induced epilepsy in California sea lions (*Zalophus californianus*). *J Comp Neurol.* 2014; 522(7):1691–1706.
7. Casalia ML, Howard MA, Baraban SC. Persistent seizure control in epileptic mice transplanted with gamma-aminobutyric acid progenitors. *Ann Neurol.* 2017;82(4):530–542.
8. Hsieh JY, Baraban SC. Medial ganglionic eminence progenitors transplanted into hippocampus integrate in a functional and subtype-appropriate manner. *eNeuro.* 2017;4(2):e0359-16.2017.
9. Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC. GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. *Nat Neurosci.* 2013;16(6):692–697.
10. Casalia ML, Li T, Ramsay H, Ross PJ, Paredes MF, Baraban SC. Interneuron origins in the embryonic porcine medial ganglionic eminence. *J Neurosci.* 2021;41(14):3105–3119.
11. Schmidt D, Baumgartner C, Löscher W. Seizure recurrence after planned discontinuation of antiepileptic drugs in seizure-free patients after epilepsy surgery: a review of current clinical experience. *Epilepsia.* 2004;45(2):179–186.
12. Yardi R, Irwin A, Kayyali H, et al. Reducing versus stopping antiepileptic medications after temporal lobe surgery. *Ann Clin Transl Neurol.* 2014;1(2):115–123.
13. Graves SS, Storer BE, Butts TM, Storb R. Comparing high and low total body irradiation dose rates for minimum-intensity conditioning of dogs for dog leukocyte antigen-identical bone marrow grafts. *Biol Blood Marrow Transplant.* 2013;19(11):1650–1654.
14. Anderson SA, Marín O, Horn C, Jennings K, Rubenstein JL. Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development.* 2001;128(3):353–363.
15. Fink JS, Schumacher JM, Ellias SL, et al. Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. *Cell Transplant.* 2000;9(2):273–278.
16. Cook PF, Hoard VA, Dolui S, et al. An MRI protocol for anatomical and functional evaluation of the California sea lion brain. *J Neurosci Methods.* 2021;353:109097.
17. Stoker TB, Blair NF, Barker RA. Neural grafting for Parkinson's disease: challenges and prospects. *Neural Regen Res.* 2017;12(3): 389–392.
18. Nicholls FJ, Rotz MW, Ghuman H, MacRenaris KW, Meade TJ, Modo M. DNA-gadolinium-gold nanoparticles for in vivo T1 MR imaging of transplanted human neural stem cells. *Biomaterials.* 2016;77:291–306.
19. Simeone CA, Gulland FM, Norris T, Rowles TK. A systematic review of changes in marine mammal health in North America, 1972-2012: the need for a novel integrated approach. *PLoS One.* 2015;10(11):e0142105.

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Baraban, Simeone, Andrews, Johnson, Kochanski, Hoard. Acquisition of data: Simeone, Andrews, Johnson, Casalia, Kochanski, Chang, Inglis, Scott, Kruse-Elliott, Okonski, Griffin-Stence, Field, Hoard. Analysis and interpretation of data: Baraban, Simeone, Andrews, Johnson, Dennison, Inglis, Scott. Drafting the article: Baraban, Simeone, Andrews, Johnson. Critically revising the article: Baraban, Simeone, Andrews, Johnson, Casalia, Dennison, Scott, Field, Hoard. Reviewed submitted version of manuscript: Baraban, Simeone, Andrews, Johnson, Chang, Dennison, Scott, Kruse-Elliott, Okonski, Griffin-Stence, Krasovec. Statistical analysis: Andrews. Administrative/technical/material support: Simeone, Chang, Inglis, Kruse-Elliott, Calvo, Kuiper, Krasovec, Field. Study supervision: Baraban, Simeone. Animal caregiver: Cameron. Veterinary technician: Goulet. Animal observation and training: Griffin-Stence.

Supplemental Information

Preprint Server

An earlier version of this article can be found on a preprint server.
Preprint server name: https://www.biorxiv.org.
Preprint DOI: https://doi.org/10.1101/2021.07.30.454497.

Correspondence

Scott C. Baraban: University of California, San Francisco, San Francisco, CA. scott.baraban@ucsf.edu.