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Magnetic Implants in the Tongue for Assistive Technologies: Tests of Migration, Oromotor Function, and Tissue Response in Miniature Pigs

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Abstract

Objective—Uncertain biological consequences of titanium-magnet (Ti-mag) tongue implants constrain application of the Tongue Drive System (TDS), a brain-tongue-computer interface for individuals with severe physical impairment. Here we describe oromotor function and tongue tissue response following Ti-Mag implantation and explantation in the miniature pig, an animal model with a tongue similar in size to humans.

Design—A 1.8 × 6.2 mm Ti-mag tracer was implanted into the anterior tongue in five Yucatan minipigs. X-rays were taken immediately and >six days after implantation to evaluate tracer migration. In three minipigs, the tracer was explanted >16 days after implantation. Twenty-five days post-explantation, tongue tissue was harvested and processed for histological and immunohistochemical (IHC) markers of healing. In two minipigs tissue markers of healing were evaluated post-mortem following >12 days implantation. Drink cycle rate (DCR) was characterized to determine the impact of procedures on oromotor function.

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Ethical Approval

Animal studies approved by Emory University Institutional Animal Care and Use Committee

conflict of interests

There are no conflicts of interests

Results—Neither implantation (N=5) nor explantation (N=3) changed DCR. X-rays revealed minimal tracer migration (N=4, 0–4 mm). By histology and IHC a robust capsule was present two weeks post-implantation with limited fibrosis. Explantation produced localized fibrosis and limited muscle remodeling.

Conclusions—These findings suggest the safety of Ti-mag anterior tongue implants for assistive technologies in humans.

Keywords

tongue; Tongue Drive System; magnet; spinal cord injury

Introduction

Some 12,000 individuals in the United States suffer spinal cord injury (SCI) each year; of these, individuals with incomplete (39.5%) and complete (16.9%) tetraplegia have limited options for and high costs of assistive devices (Figures, 2011). Despite advances in neuroprostheses and brain-computer interfaces, individuals with severe SCI and other neurological diseases which lead to tetraplegia, such as amyotrophic lateral sclerosis and brainstem stroke, have limited options for control of their environments. Currently such control relies upon interaction via a voluntary motor system unaffected by the SCI, typically muscles of the neck, eyes, or face. However, these systems provide limited signal diversity and bandwidth for computer access, are difficult to decode from unintended activities, and cannot be used over extended periods because of inducing fatigue.

Volitional control of the tongue is spared, even in individuals with high-level SCI (i.e., C4 and above); for those individuals the tongue would appear to be the natural interface between cognition and computer because of its many degrees of freedom and facility for acquiring novel movements. Heretofore, the unique inherent abilities of the tongue as an interface has not been fully exploited due to limitations in translating voluntary tongue movements in a precise, reproducible, unobtrusive, practical, and safe manner. These limitations arise from the inaccessibility of the tongue inside the oral cavity and the requirement that interfaces do not impair preservative functions of the tongue in respiration, oral transport, and swallowing, as well as speech.

The Tongue Drive System (TDS) was specifically designed with these considerations in mind. A key innovation of the TDS is the translation of voluntary tongue movements to user-defined commands via real-time detection of the position of a small titanium-encased permanent-type magnet affixed to the anterior tongue in the form of a dumbbell-shaped tongue stud (Huo & Ghovanloo, 2010; Kim et al., 2012; Laumann et al., 2015). The TDS consists of an array of four 3-axial magnetic field sensors, mounted near the user's cheeks on a headset to track the position of a tiny magnetic tracer, the size of a lentil (3.18 mm length, 1.6 mm thickness). Any tongue movement results in changes in the magnetic field inside and around the user's mouth. The electronics on the headset wirelessly transmit the measured magnetic field variations from the sensor array to a nearby PC or smartphone, which runs a sensor signal processing (SSP) algorithm that removes the earth's magnetic field (EMF) components from the incoming signals, followed by a magnetic signal

classification method that can currently indicate 7 distinct positions of the magnetic tracer within the 3-D oral space in real-time. These tongue gestures are then translated to specific user-defined commands in real time, and used to access the PC/smartphone or control target devices, such as a wheelchair (Huo & Ghovanloo, 2010; Kim, et al., 2012).

The effectiveness of the TDS has been demonstrated in both SCI and healthy individuals in pilot studies with magnets attached by temporary adhesion to dorsal tongue epithelium or by tongue piercing (Huo & Ghovanloo, 2010; Kim, et al., 2012). However, these approaches are either impractical or undesired for long term use due to lingual and dental complications, the requirement of persistent hygienic maintenance and psychosocial resistance, particularly by older patients, to tongue piercing. An optimal solution is to implant a biocompatible titanium-encased magnetic tracer (Ti-Mag) in the tongue body obviating the need for maintenance (as with tongue piercing), while minimizing the risk of swallowing the magnetic tracer and damage to teeth and gums.

Features unique to tongue biology may impact the safety and reactivity of implantation of a free-standing device in the anterior tongue. Complex changes in tongue stress-strain patterns during oromotor behaviors (e.g., (Felton et al., 2008) as well as routine tonic and forceful tongue muscle activation in preservative behaviors (i.e., respiration, swallowing) may impact the formation of a sequestering implant capsule. Additionally, the rich capillarization of tongue muscles (Granberg, Lindell, Eriksson, Pedrosa-Domellof, & Stal, 2010) and presence of large ventral lingual arteries raises the possibility of embolization if a small device is not appropriately integrated into surrounding tissue.

Previously we demonstrated encapsulation and limited migration of a 0.5 mm stainless steel sphere injected into the anterior tongue of the rat (Mimche et al., 2016) suggesting the general safety of unanchored tongue implants. Here we test encapsulation, migration, and drink behavior of 1.8 × 6.2 mm cylindrical implantable Ti-mag that is injectable through a hypodermic needle into the anterior tongue of the mini-pig, a mammal with tongue similar in size to humans. Figure 1 shows a close up view of the Ti-mag implant used in this study. We also test the consequences of device explantation on mini-pig drink behavior, and tongue anatomy. Our preliminary study indicates limited impact of Ti-Mag implantation and explantation on tongue function.

Materials and Methods

Animal Subjects and Husbandry

Six adult male Yucatan mini-pigs (*Sus scrofa*, 18–25kg, Sinclair BioResources, Columbus, Missouri) were used in this study. All experiments were conducted in accordance with the Emory University Institutional Animal Care and Use Committee and the Eighth Edition of the Guide for the Care and Use of Laboratory Animals. Mini-pigs were housed singly to facilitate investigation of drink behavior by subject, fed daily, given access to water *ad libitum* via automatic dispenser system and weighed weekly. Mini-pigs were trained to drink dilute apple juice (AJ, 2/3 apple juice, 1/3 water) from a 32 ounce dog water bottle and conventional stainless-steel roller-ball nipple (i.e., with two roller balls to impede fluid flow,

Lixit, Napa, California), a behavior learned in 1–3 sessions. Subsequently, tests of drinking during consumption of ~400 cc AJ were conducted several times per week.

Experimental Procedures

Implantation and X-ray—Following at least seven days of acclimatization, mini-pigs (P1-P6) were anesthetized with ketamine (35mg/kg, IM), atropine (0.4 mg/kg, IM) and acepromazine (0.8 mg/kg, IM) or with xylazine (1 mg/kg, IM) and ketamine (35mg/kg, IM) and maintained on ~2% isoflurane. The tongue was washed with povidone-iodine antiseptic solution, the tongue tip was held by gauze or foerster clamp, and a sterile Ti-Mag tracer was injected into the superficial anterior tongue at or near midline by sterile injection assembly (syringe, 12-gauge hypodermic needle, metal plunger; Figure 1; Figure 2). Additionally, two sterile stainless steel pellets (0.9 mm) were injected anterior and posterior to the Ti-Mag for X-ray reference (sterile syringe/16-gauge injection assembly). Bleeding, when present, was minimal and resolved by pressure. While anesthetized, lateral and dorso-ventral X-rays of the oral cavity were taken (minXray, Illinois, model, HF100AP, 46 kVp, 0.712 mAs) to enable localization of implant relative to reference pellets and structures of the oral cavity. Meloxicam (0.4 mg/kg) was given IM.

X-ray and Explantation—Seventeen or eighteen days following implantation, mini-pigs were anesthetized as above and lateral and dorso-ventral X-rays were taken to evaluate tracer migration and assist in explantation. These X-rays revealed the tracer implant to be present in only three mini-pigs (P1, P3, P4), indicating extrusion of the implant in the other mini-pigs (P2, P5, P6). In the three mini-pigs with implant, the tongue was washed with povidone-iodine antiseptic solution, the tongue tip held by gauze or a foerster clamp, and the anterior tongue protruded from the oral cavity by application of gentle tension at the tongue tip. Tracer location was identified by palpation or by dorso-ventral X-ray following insertion of a reference 25 gauge needle. A small incision was made through the dorsal epithelium dorsal to the tracer. Tongue tissue was gently spread with a forceps to expose the Ti-mag tracer, which was removed (Figure 2). The incision was closed with 4-0 polydioxanone (PDS) suture and animals were given meloxicam (0.4 mg/kg, IM).

Re-implantation and X-ray—X-rays revealed extrusion of the tracer in P2, P5 and P6, likely soon after implantation, possibly facilitated by action of the animal or much less likely the attraction between the magnetic tracer and the metal nipple on the automatic water system. Therefore we re-implanted new Ti-mag tracers in these three mini-pigs as described above with the addition of a single PDS 4-0 suture through the dorsal epithelium to close the needle hole created by injection (for schedule of experimental procedures see Table 1). To further minimize the possibility of tracer extrusion the automatic water dispenser nipple was removed from the run and water offered *ad libitum* in a rubber bowl.

X-ray—Seven days following re-implantation mini-pigs P2, P5, and P6 were anaesthetized as above and lateral and dorso-ventral X-rays taken to enable evaluation of tracer migration. These X-rays revealed extrusion of the tracer in one of the three re-implanted mini-pigs (P6) which was therefore not included in subsequent and histological analyses. Additionally, after prolonged healing to promote encapsulation, as part of a separate study P5 was

anaesthetized as above, intubated, maintained on isoflurane, and a head MRI taken to collect preliminary data on possible Ti-mag distortion of MRI imaging.

Euthanasia and Tissue Harvest—Mini-pigs were anesthetized with xylazine (1 mg/kg, IM) and ketamine (35mg/kg, IM) and euthanized by overdose of Euthasol (Virbac, Fort Worth, TX). Following euthanasia, the tongue bodies of explanted mini-pigs were immediately frozen in liquid nitrogen and stored at -78°C ; the tongue bodies of the two mini-pigs with implant *in situ* were placed into 10% buffered formalin for up to two weeks. Lacking persistent implantation, tissue from Pig 6 was not processed or analyzed.

Analysis of Drink Behavior

Collection of Drink Data—Drinking was studied ~3 times per week to evaluate impact of implantation and explanation procedures on oromotor behavior. In sessions of 2–6 minutes, mini-pigs drank AJ from the plastic dog water bottle with modified roller ball nipple affixed to the cage and drinking was assayed by simultaneous magnetic and audio recording of the movement of stainless steel metal balls in the nipple barrel, which were augmented with a small magnetic tracer, a pair of 3-axial magnetometers, and a microphone (for details of magnetic and audio recording setup see (Sargolzaei, Yang, Elahi, Sokoloff, & Ghovanloo, submitted)). All sessions were also recorded by video (30 FPS).

Measurement of Drink Cycle Duration—The two metal roller balls in metal tube that constitutes the body of the nipple operate as a normally-closed valve. Release of liquid requires application of tongue pressure at the nipple orifice to push the roller balls into the nipple tube (i.e., the state of roller “ball-up”); subsequently the roller balls descend to the resting position in the nipple orifice by gravity, blocking liquid release (i.e., the state of roller “ball down”). Movement of the roller balls was recorded in magnetic and audio modalities to determine drink cycle duration (DCD), defined as the time between two sequential “ball-down” states in milliseconds, and drink cycle rate (DCR), defined as the average number of ball-down transitions in a second in Hz. Briefly, a permanent-type magnetic tracer (4.8 mm in diameter and 1.6 mm in thickness, made of grade N52 NdFeB, KJ Magnetics, Pipersville, PA) was glued to the upper ball and a plastic cuff embedded with a pair of magnetic sensors (LSM303D, STMicroelectronics) was placed on the outside of the nipple tube with ~1 cm spacing to each detect the changes in the magnetic field as a result of magnetic tracer movements in 3 axes (for details see (Sargolzaei, et al., submitted)). The magnetic sensors sampled the changes in the magnetic field at a rate of 100 Hz and delivered the digitized samples to a PC through a microcontroller (LPC1768, NXP Semiconductors) and universal serial bus (USB). Additionally a unidirectional USB microphone (Mini Akiro microphone, Kinobo Inc.) was attached to the bottle and directed at the nipple to enable discrimination of sounds made by the metal balls during ball-up and ball-down movements. The acoustic signature of the ball movements in the metal tube was sampled at 44 kHz and delivered to the same PC via USB. A dual-mode sensor signal processing algorithm was used to discriminate between ball-up and ball-down states from the magnetic sensor raw data as well as the ball hitting the bottom of the nipple tube from the acoustic data. This information was then used to generate a time series indicative of “ball-up” (valve-open) and

“ball-down” (valve-closed) states, which was then turned into drink cycle rate, expressed in Hz (For further details see (Sargolzaei, et al., submitted)).

The raw magnetic sensor data is saved in a matrix with 6 columns, each of which corresponds to the data from a magnetic sensor axis. Each row of the matrix corresponds to one time sample (10 ms), which is recorded at 100 Hz. This (6×N) matrix is fed into a clustering algorithm based on unsupervised Gaussian Mixture Model (GMM) to determine whether each time sample, i.e. each row of the matrix, belongs to the “ball-up = 1” or “ball-down = 0” category. Once the entire matrix is classified, it turns into a time series, which indicates the transition times from one state to another. Since only two states have been considered here, the transition from one state to another is spontaneous. This time series is then filtered out by analyzing the periods of “ball-up” or “ball-down” that fall within the range of drinking cycle duration for the mini-pig (here set at 200–500 msec/drink cycle). DCD in each cycle are binned (5 msec width) and the modal drink cycle rate is determined for each trial as the inverse of DCD (expressed in Hz, DCR).

For the synchronously recorded 1-dimensional audio data, we used a supervised audio template matching process to validate the drink cycle rate generated from the magnetic data. In this case, an operator identifies and isolates a time window of a clear occurrence of the sharp sound of the roller balls dropping and hitting the bottom of the metal tube by listening to the audio and observing the waveform. The algorithm then uses that sample audio waveform as a template and slides it over the recorded audio waveform to indicate instances of above-threshold cross-correlation for generating the audio time-series, in which every time sample is zero except when the roller balls drop. This time series is then filtered out, similar to the magnetic data, by analyzing the periods of ball-drop-to-ball-drop events that fall within the range of drinking cycle duration for the mini-pig (200–500 msec/drink cycle). DCD in each cycle are binned (5 msec width) and the modal drink cycle rate is determined for each trial as the inverse of DCD (expressed in Hz, DCR).

Determination of Drink Behavior—Mini-pigs employed one of two drinking strategies which we call “licking” and “sucking”. In the licking strategy the tongue was protruded from the oral cavity, liquid obtained on the tongue dorsum, and the tongue retracted to deliver liquid into the oral cavity. In the sucking strategy the mouth formed a seal with the nipple and animals sucked while the tongue manipulated the nipple within the oral cavity. In both strategies, anterior movement of the tongue was required to move the nipple balls into the “ball-up” position and allow release of liquid.

In both drinking strategies, periods of nipple contact were often punctuated by periods during which the mini-pig disengaged from the nipple to completely swallow the liquid in their mouth. Therefore, to limit the study to cycles of drinking, we analyzed data for which the time from ball-down to subsequent ball-down (i.e., a drink cycle duration) was from 0.205 to 0.5 s (mini-pigs drink from an open container at ~2.25–2.5 Hz, see (Herring & Scapino, 1973), (Liu, Yamamura, Shcherbatyy, & Green, 2008) (Liu, Shcherbatyy, Kayalioglu, & Seifi, 2009); infant pigs suck from a bottle at ~ 4 Hz (Holman et al., 2013)). Impact of experimental procedures on drink dynamics was assessed by comparing modal drink cycle rate (DCR, expressed in hertz) measured by magnetic and by auditory modalities

from one to fourteen days before and from eight to fifteen days after successful implantation and explantation procedures.

Statistical Analyses—Repeated-measures analyses for drink cycle rate were performed with a means model via the SAS MIXED Procedure (version 9.4) providing separate estimates of the means by modality and procedure (pre-implantation, post-implantation, pre-explantation and post-explantation). A compound-symmetric variance-covariance form in repeated measurements was assumed for drink cycle rate and robust estimates of the standard errors of parameters were used to perform statistical tests and construct 95% confidence intervals (Diggle PJ, 1994). The model-based means are unbiased with unbalanced and missing data, so long as the missing data are non-informative (missing at random). Specific statistical tests were done within the framework of the mixed effects linear model. Statistical significance was defined as a 2-sided P value of less than .05.

Analysis of Ti-Mag Migration

Lateral X-rays were compared between X-ray sessions to evaluate migration of the Ti-mag. Differences in head orientation and in anterior tongue posture between X-ray sessions precluded exact measurement of the Ti-mag implant relative to reference pellets and oral cavity structures. Therefore X-ray images taken immediately following implantation and more than six days after implantation (see Table 1) were overlapped, scaled to the width of the Ti-mag, and aligned by reference to radiopaque structures of the oral cavity and to the posterior reference pellet, which was less-affected by tongue position during X-ray. The merged image was imported into Neurolucida software (MBF Bioscience, Williston, VT), scaled to the 0.9 mm posterior pellet and the distance between the centers of the Ti-mag in the two images determined (average of two measurements). Failure of implantation in P6 and a disk-writing error during X-ray of P5 precluded evaluation of tracer migration in these two pigs.

Tissue Analysis

Evaluation of Implantation Site—Formalin-fixed tissue containing the implant (P2, P5) was rinsed in phosphate buffer and a scalpel-cut made perpendicular to the long axis of the Ti-mag such that the Ti-mag surface was entirely exposed at its midsection. Tissue on one side of the cut was gently removed leaving the Ti-mag embedded in the other side. The tissue samples *sans* implant was embedded in paraffin and sectioned at 6 microns. Sections were deparaffinized and rehydrated through a graded alcohol series and stained for standard hematoxylin and eosin (NovaUltra Hematoxylin and Eosin Stain Kit, IHC World), Picrosirius (NovaUltra Sirius Red Stain Kit, IHC World) and Trichrome Stain (Sigma-Aldrich-Trichrome Stain Masson Kit) and photographed (Olympus BH51, MicroFire digital microscope camera, Optronics, Goleta California).

Evaluation of Explantation Site—Tissue containing the explantation site was mounted on tongue depressors (Tissue-Tek® O.C.T. Compound, Sakura® Finetek) and cut at 8–10 µm in a cryostat (–21 degrees) in the transverse or sagittal plane. Cryosectioned tissue samples were stained for HE and for immunofluorescent (IHC) with antibodies (Abs) to embryonic myosin heavy chain (Developmental Hybridoma Studies Bank, Ab F1.625), to

dystrophin (Ab15277, Abcam) and to macrophage markers F4/80 (AbF4/80, Serotec, Bio-Rad) and CD68 (abSC7074, Santa Cruz Biological) and appropriate secondary antibodies (Alexa Fluor 488 anti-mouse and anti-Rat and Cy3 anti-rabbit). Slides were cover-slipped with vectashield (Vector Laboratories) and photographed (Olympus BH51, MicroFire digital microscope camera, Optronics, Goleta California; for details of reactions see (Slaughter, Li, & Sokoloff, 2005) and (Luo, Douglas, Burkholder, & Sokoloff, 2014).

Results

Animal Weight and Drink Measures

Animal weights increased throughout the experiment with minimal or no change following implantation or explantation (average gain at 41 days, 2.5Kg, range 1.8–3.4Kg).

Implantation and explantation procedures were well-tolerated, but extrusion of the magnet occurred in four of nine implantation procedures, likely immediately after implantation.

Drink cycle dynamics

Plots of modal drink cycle revealed minimal change by procedure (Figures 3 and 4). DCR was unchanged by explantation (Figure 4, Table 2) and by implantation when assessed in magnetic modality. Mean DCR was significantly different following implantation when assessed in the auditory modality, however, although statistically significant ($P = 0.0015$) the magnitude of the mean difference (3.58 minus 3.77 Hz = -0.19 (standard error = 0.041)) appears small.

Ti-mag Migration

Alignment of lateral X-ray images taken immediately after implantation and >six days post-implantation revealed no movement of the Ti-mag in P1 and P3 (i.e., complete overlap of the Ti-mag in X-ray sessions), and limited migration (<4.0 mm) in P2 and P4 (Figure 5). In these pigs differences in tongue posture during recording sessions may have contributed to the measurement of Ti-mag migration.

Tongue Histology

Histological analysis of the implant site was possible in two pigs, P2 at 13 days post-implantation and P5 at 54 days post-implantation (See Table 1 for schedule of procedures). In both pigs a fibrous capsule surrounded the Ti-mag with no giant cells and limited fibrosis (Figure 6). A thick capsule of fibroblasts was present at 13 days post-implant with a thinner capsule evident at 54 days post-implant. Tongue muscle adjacent to the capsule appeared normal and few fibers exhibited central nuclei indicative of degeneration/regeneration of muscle fibers. Migration of connective tissue into bore holes at the end of the Ti-mag casing indicated integration with the surround connective tissue (arrows in 6).

Histological analysis of the explantation site revealed fibrosis and fiber regeneration within a restricted area of the tongue body (Figure 7). Regenerating fibers did not always align with conventional tongue morphology. Macrophage markers F4/80 and CD68 were restricted to the explantation site (Figure 7).

Discussion

Summary

Implantation of a 1.8×6.2 mm Ti-mag tracer into the anterior tongue of the pig does not impact animal weight. Implant encapsulation and the absence of implant migration indicate a rapid integration of the implant into tongue tissue minimizing the risk of embolization into large ventral lingual arteries. Implantation and explantation procedures do not substantially alter drink behavior. These findings suggest the safety of implantation of small biomedical devices into the anterior tongue in humans.

Impact of Tongue Procedures on Drink Dynamics

By electromyography and sonomicrometry drink cycles of ~ 2.25 – 2.5 Hz were reported in adult mini-pigs drinking from open containers (Figure 19, (Herring & Scapino, 1973); Figure 6, (Liu, et al., 2008)). In our study, average drink cycle, recorded in magnetic and auditory modalities for each mini-pig, ranged from 3.21–3.88 Hz. Sucking cycles of ~ 4 Hz were reported in 3 week-old pigs during bottle drinking (Holman, et al., 2013) and the faster rate of drink cycles in the present study may in some measure reflect differences in DCR associated with open container versus bottle-drinking. In the present study we did not observe systematic differences in DCR in mini-pigs employing licking (P1, P2, P6) versus sucking (P3, P4, P5) strategies. Additionally, DCR was not changed following explantation and minimally changed following implantation. These findings suggest that neither the presence of the Ti-mag implant nor localized tongue damage associated with Ti-mag implantation and explantation interfere with normal drink dynamics. Previously, German et al. (German, Crompton, Levitch, & Thexton, 1992) reported that implantation of up to 6 metal rods of ~ 9 mm diameter $\times$ 1–3 mm length into the tongue body in mini-pigs and macaques did not interfere with normal feeding evaluated by cineradiography. In concert these data suggest limited impact of free-standing tongue implants on drinking and feeding in mammals generally.

Tracer Migration

The presence of large ventral lingual arteries raises the possibility of embolization if a free standing tongue implant is not integrated into surrounding tissue. Prior studies indicate that freestanding implants rapidly integrate with tongue tissue. In dogs, a polymer-coated magnet ($2.5 \times 15 \times 25$ mm³) implanted in the posterior tongue was encapsulated, did not migrate and did not cause systemic acute or chronic inflammation (Nelson, Boucher, & Stevens, 2005). Previously we demonstrated encapsulation and limited migration of a 0.5 mm stainless steel spherical pellet in the anterior rat tongue (Mimche, et al., 2016). In the present study, there was no or limited migration of the Ti-mag, suggesting rapid integration of the Ti-mag with surrounding tongue tissue, a conclusion supported by Ti-Mag encapsulation and tissue invasion of Ti-mag casing bore holes in P2 by 13 days (Figure 6). Ti-mag integration with tongue tissue was not accompanied by significant inflammation or fibrosis by HE, a finding supported by the presence of a thin, mature capsule at 54 days. We conclude that the persistent stress-strain experienced by tongue tissue during routine oromotor behaviors does not hinder tissue integration of a Ti-mag tracer.

In our study the Ti-mag was extruded in 4 out of 9 implant procedures, likely soon after implantation, possibly aided by interaction of the Ti-mag with the metal of the automatic water dispenser nipple and/or intentional scraping of the tongue on the teeth or cage. Although neither of these factors is an issue in human applications, routine closure of the needle track by suture or bonding medium (e.g. tissue adhesive) may be useful to minimize the possibility of Ti-mag extrusion.

Tongue Tissue Response to Implantation

Factors specific to implant design, material and tissue determine the biological response to implantation of a metallic device (for review see (Anderson, Rodriguez, & Chang, 2008; Woodward & Salthouse, 1986)). Rhyanen et al (Ryhanen et al., 1998) reported a ~55 μm -thick capsule with a distinct inflammatory layer and occasional foreign body-giant cells following implantation of a 6 mm $\times$ 1.8 mm stainless-steel or titanium rod into paraspinal muscles in the rat. In contrast, McGeachie et al (McGeachie, Smith, Roberts, & Grounds, 1992) reported limited inflammation and a narrow capsule (10 microns thickness) 10 days following insertion of a 5 mm $\times$ 0.5 mm stainless-steel or titanium wire into the mouse tibialis anterior muscle. Similar to McGeachie et al., in P2 at ~2 weeks post-implantation we observed limited inflammation and no foreign body giant cells; however we noted a robust layer of connective tissue and fibroblasts. In contrast, in P5 at ~8 weeks a thin implant capsule was present. Previously, we noted a thin implant capsule ~four weeks following implantation of a stainless-steel sphere in the anterior tongue of the rat (Mimche, et al., 2016). Differences in time course of capsule maturation between studies may relate to a number of factors including the size, shape and surfacing of the implant and the species and muscle employed. Although limited in number, the present study suggests that persistent stress-strain experienced by tongue tissue is not an impediment to capsule maturation of a 1.8 $\times$ 6.2 mm anterior tongue implant.

Tongue Tissue Response to Explantation

Surgical removal of tongue body tissue in a mini-pig model of tongue reduction produced substantial tissue fibrosis and deficits in tongue movement (Perkins, Shcherbatyy, & Liu, 2008; Shcherbatyy, Perkins, & Liu, 2008; Ye, Abu, & Liu, 2010). In the same model, two parallel incisions of 2–3 mm depth along the length of the tongue body without tissue extirpation did not produce anatomical or functional change (Perkins, et al., 2008; Shcherbatyy, et al., 2008) (Ye, et al., 2010). In the present study the maintenance of drink measures following Ti-mag explantation indicates that observed localized tissue fibrosis and muscle fiber remodeling, including occasional divergence with normal fiber orientation, does not reach threshold for functional deficit by our measures.

Limitations of study

We recognize several limitations in our study. (1) The small sample size limits comparison with other studies, especially with regards to time course of implant encapsulation. (2) Due to differences in tongue posture and jaw position between X-ray sessions we can rule out the possibility of some tracer migration after implantation. However, several considerations indicate migration, if present, was minimal and likely occurred soon after implantation. Maximal measured migration was limited (from 0mm- 4mm) and Ti-magi tracer orientation

was unchanged in 3 of 4 mini-pigs. Additionally, in P2 there was tissue invasion of the Ti-mag casing bore holes at 13 days post-implantation indicating rapid integration with tongue tissue. (3) We measured DCR only and thus cannot address whether the presence of the Ti-mag or subsequent explantation impacted drink microstructure, for example transport time or tongue-nipple contact time.

Conclusions

A 1.8×6.2 mm cylindrical Ti-mag tracer implanted into the anterior tongue of mini-pig model does not migrate, does not substantially impact drink dynamics and is rapidly encapsulated with limited fibrosis. Magnetic tracer explantation produces local tissue damage and fibrosis, which are expected to be highly dependent on the explantation procedure, but does not disrupt drink dynamics. These findings suggest the safety of anterior magnetic tongue implants for use in assistive technologies, such as the Tongue Drive System, in humans with severe physical disabilities.

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Abbreviations

HE	Hematoxylin and Eosin
PS	Picrosirius Red
SCI	Spinal Cord Injury
TDS	Tongue Drive System
Ti-Mag	Titanium-encased Magnet
DCD	Drink cycle duration
DCR	Drink cycle rate

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Highlights

- Tongue Drive System (TDS) is an assistive technology with a magnetic tongue implant.
- TDS titanium-encased 1.8 mm × 6.2 mm implant does not migrate in the minipig tongue.
- Magnetic implantation/extirpation minimally disrupt drinking and tongue anatomy.
- Limited impact of implant suggests safety of this tongue-operated assistive device.

Figure 1. Titanium-encased magnet (Ti-mag) used in this study. The magnet (M) and un-assembled Ti-casing are shown. The assembled Ti-mag, with two bore holes, is shown in a 12 gauge needle. Calibration bar = 2 mm.

Figure 2. Photographs of the dorsal surface of the tongue during implantation and explantation. A. Tongue P1 immediately after implantation. Black arrows point to implantation tracts of Ti-mag (center arrow) and reference pellets. B. Tongue P4 immediately before explantation. C, D. Explantation of Ti-mag in P3. Calibration bars in A and B ~10 millimeters

Figure 3.

Drink cycle rate (Hz) determined by auditory (●) and magnetic (□) modalities for each pig (P1, P2, P3, P4, P5 and P6). Solid line indicates successful implantation, dashed line indicates explantation and dotted line indicates implantation with subsequent extrusion of Ti-magnetic tracer.

Figure 4.

Comparison of modal drink cycle rates (Hz) in trials before versus after implantation and in trials before versus after explantation. Modal DCR was significantly different following implantation when assessed in the auditory modality (Audio, $P = 0.0015$), however, the magnitude of the difference (3.58 minus 3.77 Hz) appears small.

Figure 5.
Overlap of lateral X-rays taken immediately following implantation and >6 days later
showing minimal migration in tongue body.

Figure 6.

A,B. Titanium-magnet (Ti-mag) implant in the tongue body of mini-pig 2 (P2) *in-situ*. C. Implant cavity following removal of Ti-mag (P2). D. Implant cavity in P2 with back-lighting; arrow indicates tissue “bridge” that filled hole in Ti-mag casing. E. Hematoxylin-eosin (HE) stained section (P5) showing implant cavity and tissue bridge (arrow). F,G. HE-stained section of implant capsule in P2. Note thick layer of fibroblasts at 12 days post-implantation. H,I. Masson’s trichrome stain (H) and HE (I) stained section of implant capsule in P5 showing tissue bridge (arrow). Note thin capsule at 54 days post-implantation. Calibration bars all 0.5 mm except G which is 100 microns

Figure 7.

A. Focal fibrosis (circle) following explantation (parasagittal section of the tongue in P1, Hematoxylin-Eosin). B. Higher power view of fibrosis in A. C. Localized expression of macrophage marker F4/80 at site of explantation (P4, anti-F4/80, FITC secondary). D. Demarcation of muscle fibers with anti-Dystrophin antibody in control side of tongue (P4, FITC secondary). E. Disorganized orientation of muscle fibers at site of explantation (P4, anti-Dystrophin antibody, FITC secondary). F. Focal area of regenerating muscle fibers following explantation (P3, anti-developmental myosin heavy chain, antibody F1.652, FITC secondary). Calibration bars 200 microns.

Schedule of Experimental Procedures (Days)

Table 1

	beginning of acclimatization and drink testing	implantation and X-ray	X-ray and explantation	re-implantation and X-ray	X- ray	sacrifice	duration of implantation (days)
Pig1	0	7	25			50	18
Pig2 [*]	0	8		35	42	48	13
Pig3	0	8	25			50	17
Pig4	0	7	25			50	18
Pig5 ^{*†}	0	8		35	42	79	54
Pig6 ^{*‡}	0	7		35	42	48	

^{*} Initial Ti-mag implant was extruded from tongue.

[†] Head MRI taken immediately prior to sacrifice.

[‡] Re-implanted Ti-mag extruded from tongue.

Table 2

Changes in Drink Cycle Rate (Hz) by Modality and Procedure

Modality	Procedure*	#Subjects (#Measurements)	Mean Drink Cycle Duration (Hz)	SE [†]	Lower 95% CI [‡]	Upper 95% CI [‡]	P Value [§]
Magnetic	PreI	5 (19)	3.60	0.08	3.43	3.78	0.66
Magnetic	PostI	5 (22)	3.57	0.14	3.24	3.89	
Magnetic	PreE	3 (13)	3.68	0.19	3.26	4.11	0.33
Magnetic	PostE	3 (10)	3.73	0.18	3.31	4.15	
Auditory	PreI	5 (17)	3.58	0.10	3.34	3.81	0.015
Auditory	PostI	5 (25)	3.77	0.14	3.44	4.10	
Auditory	PreE	3 (15)	3.72	0.12	3.43	4.00	0.52
Auditory	PostE	3 (18)	3.79	0.19	3.36	4.22	

* PreI = Preimplantation; PostE = Postexplantation

[†] SE = Standard Error of the Mean[‡] CI = Confidence Interval[§] P values apply to mean differences (PreI minus PostI).