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A novel technique for simultaneous bilateral brain infusions in a mouse model of neurodegenerative disease

Steven P. Bennett[a,b], Tim D. Boyd[a,b], Michelle Norden[a,b,c], Jaya Padmanabhan[a,b], Peter Neame[b], Inge Wefes[b,c], Huntington Potter[a,b,c,*]

[a] USF Byrd Alzheimer's Institute, Tampa, FL 33613, USA
[b] University of South Florida, Department of Molecular Medicine, Tampa, FL 33612, USA
[c] Eric Pfeiffer Suncoast Alzheimer's and Gerontology Center, USA

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ABSTRACT

A common problem faced by researchers using transgenic models to study disease is the phenotypic variability that exists within a group or colony of animals. Significant pathological analyses thus often require large numbers of mice to perform. Many lines of transgenic mice harboring the gene for human amyloid precursor protein (APP) with different mutations causing familial Alzheimer's disease have been developed over the past decade to study plaque deposition and other aspects of AD. However, variations in size, density, plaque number, and total amyloid load between animals of the same age and genotype have been identified by our lab and others. Therefore, to study the effects of compounds on amyloid pathology, it was imperative to develop a technique that would allow each brain hemisphere to receive different infusions. We have developed catheters that facilitate simultaneous bilateral infusion in mouse brains, thereby using the contralateral hemisphere of the same animal as an internal control while studying, for example, the effect of compounds on amyloid plaques, a pathological hallmark of the progression of Alzheimer's disease (AD). Several molecules have been identified within the plaques including the major component, the Aβ peptide, and two inflammation-related proteins, apolipoprotein E (apoE) and the serine protease inhibitor α-1-antichymotrypsin (ACT). In these experiments, ACT was infused unilaterally over a period of 28 days into the parenchyma and lateral ventricles of PS/APP mice and observed to associate with amyloid plaques, with minimal mortality. Utilizing the ACT/Aβ interaction, details of this procedure are discussed here in detail.

1. Introduction

Alzheimer's disease is the most common form of neurodegeneration, affecting over 5 million people nationwide. Approximately 1 person in 8 will be diagnosed with AD by the age of 65, and 1 in 2 by the age of 85 (National Institutes of Health U.S. Department of Health and Human Services). The disease is characterized by severe cognitive decline, leading to dementia and death within an average of 8 years from diagnosis. Pathologically, the disease outcome is massive neuronal loss, extracellular deposits of amyloid, and intracellular accumulation of neurofibrillary tangles (Selkoe, 1986). Several proteins have been identified within the plaques including Aβ, apolipoprotein E (apoE) (Wisniewski and Frangione, 1992) and α-1-antichymotrypsin (ACT) (Abraham et al., 1988). Aβ is the primary component of the plaques and is a 38–43 amino acid peptide proteolytically derived from the amyloid precursor protein (APP) (Hardy and Selkoe, 2002). Mutations in the APP gene that cause AD result in an increased production of pathogenic forms of Aβ (Tanzi et al., 1991). ApoE and ACT are part of a local inflammatory process in the brain. Aβ binds to ACT and ApoE, and these proteins serve to catalyze the conversion of Aβ into filaments *in vitro* (Ma et al., 1996; Wisniewski et al., 1994), and *in vivo* to form beta-pleated structured amyloid deposits (Bales et al., 1999; Nilsson et al., 2001).

ACT is a 68 kDa acute phase serum glycoprotein from the family of serine protease inhibitors (serpins). Systemically, it is released by hepatocyes and monocytes in response to inflammation and functions as a suicide inhibitor of cathepsin G released by neutrophils (Kalsheker, 1996). In the brain, ACT is known to be produced by and released from astrocytes in response to IL-1β stimulation by microglia following trauma, infection, or amyloidoses (Abraham, 2001; Das and Potter, 1995). ACT is highly upregulated in AD brain and is involved both in the formation of amyloid

* Corresponding author at: USF Byrd Alzheimer's Institute, 4001 E. Fletcher Ave., Tampa, FL 33613, USA. Tel.: +1 813 396 0660; fax: +1 813 866 1601.
E-mail address: hpotter@health.usf.edu (H. Potter).

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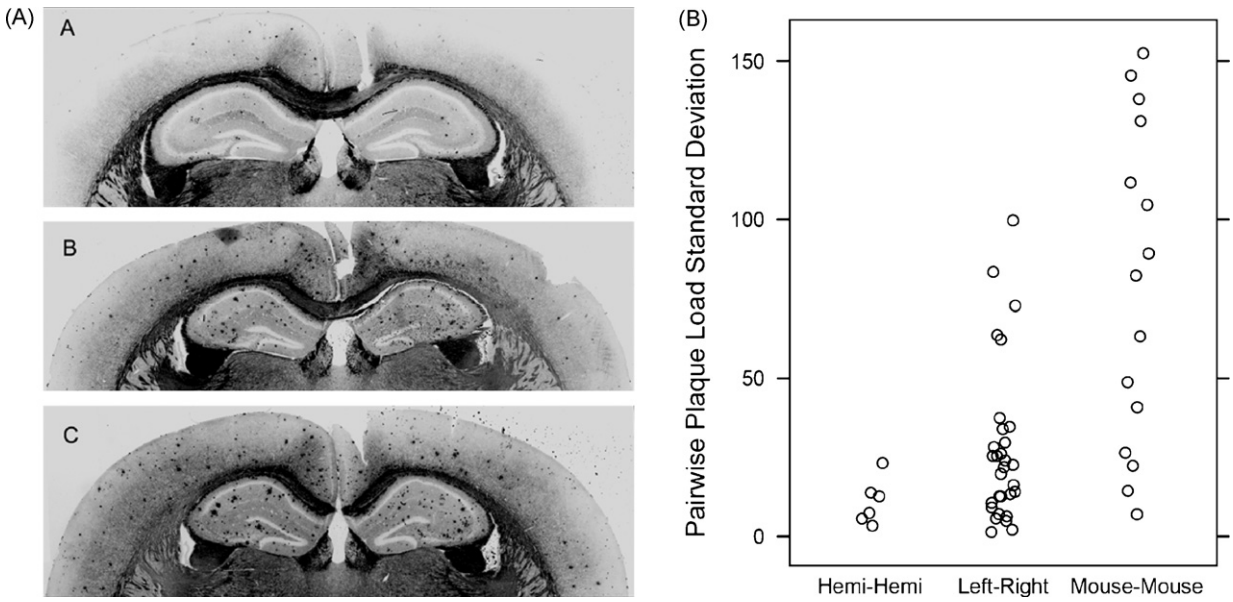

Fig. 1. (A) Thioflavin S staining on 25 μm coronal sections (montages) of 3 different PS/APP mice at 10 months of age showing plaque variability in the hippocampus and neighboring cortex. (B) Scatter-plot of plaque load data from 5 brain sections through 6 ten-month-old PS/APP mice comparing standard deviation (plaque variance) between total hemispheres of the same animal (28%), between sides of the same section (11%) and between animals (78%). All of the data from the left hemisphere are compared with the right for each mouse (Hemi–Hemi, 1 data point for each mouse); the left half of a single slice is compared with the right side (Left–Right, 1 data point for each slice); and a permuted comparison of each mouse with every other mouse (Mouse–Mouse, 1 data point for each pair of mice). Where data were aggregated (Hemi–Hemi and Mouse–Mouse), the mean of the aggregated counts was used to make the standard deviation comparable to the single slice data (standard deviation of two numbers).

plaques (Abraham et al., 1988) and in the phosphorylation of tau (Padmanabhan et al., 2006). Using a transgenic mouse carrying a mutated human presenilin gene (PS) and a mutated human APP gene (PS/APP), we observed the co-localization of ACT on existing amyloid plaques in adult PS/APP mice, utilizing a novel brain infusion technique.

Amyloid plaque load can vary significantly from animal to animal of the same APP transgenic genotype as well as from hemisphere to hemisphere of the same animal (Fig. 1). We analyzed the brains of 6 PS/APP mice at 10 months of age at 5 sections per brain front to back for variations in plaque load. Although variability does exist from hemisphere to hemisphere in this model per a given section (11%), we have demonstrated that the variability from animal to animal is almost 3 times that from that of total hemisphere to hemisphere comparison (78% from mean compared to 28%, respectively) (Fig. 1B). For this reason, we designed a bilateral delivery technique that would allow one of the two hemispheres to serve as an internal control. This technique allows researchers to use fewer mice than an experiment of this nature would typically require and with more accurate results. Thus ACT, for the purposes of our work, could be delivered to one hemisphere and the vehicle, artificial cerebrospinal fluid (aCSF), to the other. Current systems for drug/compound delivery to the mouse brain use pedestal cannulae which are commercially available, but tend to be bulky and irritating to the animal, restrictive to the researcher, and lead to high mortality rates. Furthermore, use of two independent pedestal-based cannulae infusions in the same mouse is not possible due to their large size. Therefore, we designed and constructed novel catheters that are implanted subcutaneously and contoured to the skull. They are minimally invasive and virtually eliminate iatrogenic mortality. Our data show that this method is reliable for studying the chronic delivery of ACT into PS/APP mice to study its effects on amyloid pathology. Moreover, because the catheters are custom made, they can easily be adapted to study the impact of virtually any compound in other mouse models of neurodegeneration or brain disease as well as on non-transgenic mice in order to study the effects of these compounds on normal physiology.

2. Materials and methods

2.1. Catheter construction

The catheters (patent pending PCT/US08/73974) used for this experiment were made by first taking a 1.25 cm length of 30 gauge stainless steel tubing (Small Parts Inc., Miramar, FL) and carefully inserting it into a 20 g needle (tip removed) to the appropriate depth under a dissecting microscope (Leica, Heerbruug, Switzerland), and bending it at 2.5 mm to approximately 90° being very careful not to crimp the tube. The remaining length of the tube was bent again at 5 mm to an angle of 120–160°, approximating the contour of each animal's skull. These metal cannulae were inserted into a 3 cm length of polyethylene (PE-10) tubing with an internal diameter of 0.28 mm and an outer diameter of 0.61 mm. One centimeter of the PE-10 was then itself inserted into a 4.5 cm length of sterile polyvinyl tubing (PV-50-I.D. 0.69 mm/O.D. 1.14 mm, Durect Corp., Cupertino, CA), held in place using a bead of Locktite 454 adhesive (Plastics One, Roanoke, VA), and cured overnight. The following day, sterile water was forced through each catheter assembly to ensure that the lines were not obstructed. The osmotic pumps were then filled with solution, attached to the catheters and primed and implanted as described below. The proven catheters were later custom manufactured by Braintree Scientific (Braintree, MA) with some minor changes: The adhesive was supplanted by heat sealing PE-10 to PE-50 tubing and, because the diameters of the PV-50 and new PE-50 were not exactly the same, a sheath of silicone (SIL 047) was placed over the PE-50 with an extra 2 mm overhang to attach it to the flow modulator of the pump, forming a tight, leak-proof seal (Fig. 2).

2.2. Transgenic mice

PS/APP (presenilin 1/amyloid precursor protein) mice and PS/APP/ACT mice were generated by crossing heterozygous PDGF-hAPP (V717F) mice with PDGF-hPS1 (M146L) on both Swiss Webster and C57BL/6 backgrounds. In some cases, PS/APP mice

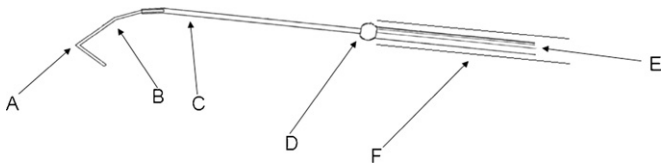

Fig. 2. Catheter assembly. (A) First bend of 30 gauge stainless steel tubing. (B) Second "contour" bend. (C) Polyethylene PE-10 tubing. (D) Heat seal. E- PE-50. (F) Silicone sleeve (SIL047).

were bred with mice harboring a gene for human ACT (hACT) under a GFAP promoter (Nilsson et al., 2001) were used to be compared to ACT-infused animals. Genotyping was performed using comparative real-time PCR (Bio-Rad iCycler, Hercules, CA). Pathogenically, these Alzheimer's mouse models are characterized by robust accumulation of amyloid plaques and the development of microgliosis between 6 and 10 months. The mice used in these studies ranged from 9 to 10 months of age.

2.3. Materials

Purified human α-1-antichymotrypsin was purchased lyophilized from Fitzgerald (Concord, MA) and was reconstituted in artificial CSF (Harvard Apparatus, Holliston, MA) to a concentration of 1 mg/mL. ACT was infused directly into the hippocampal parenchyma or lateral ventricles for 28 days using the novel catheters attached to Alzet osmotic minipumps (Alzet model 1004, Durect Corp. Cupertino, CA) with an average flow rate of 0.12 μL/h. The pumps and catheters were submerged in 0.9% sterile saline at 37 °C and primed for 48 h prior to implantation.

2.4. Intracranial infusions

All procedures involving experimentation on animals were performed in accordance with the guidelines set forth by the University of South Florida Animal Care and Use Committee. Animals (9-month-old PS/APP, 25–35 g) were anesthetized with 1–2% isoflurane, shaved and scrubbed with 10% betadine solution at the site of incision, and placed into a dual arm stereotaxic frame (Kopf Instruments, Tujunga, CA). A small (5 cm) incision was made, exposing the skull and neck, and double bladed scissors (10 cm curved Strabismus, Fine Science Tools, Foster City, CA) were used to form a subcutaneous pocket along the back of the animal into which 2 osmotic minipumps were inserted with catheters attached. One of the stereotaxic arms held an Ideal Micro-drill (Roboz Surgical Instrument Co., Gaithersburg, MD) holding a 0.32 mm diameter carbide drill bit. Using the drill bit on the stereotaxic arm to find the proper coordinates (from Bregma −2.2 to 2.5 mm anterior–posterior, ±2.2–2.5 mm medial–lateral), two holes were carefully drilled into the skull and a 30 gauge needle from a Hamilton syringe (Hamilton Co., Reno, Nevada) was attached to the second arm of the stereotaxic frame. The needle was inserted to the appropriate depth (2.2–2.5 mm corresponding to the posterior portion of the CA1/CA2 hippocampus) and allowed to sit for 5 min for the surrounding tissue to adjust. Alternatively, holes were drilled corresponding to the lateral ventricles at the following coordinates: from Bregma −0.2 mm anterior–posterior, ±1.0 mm medial–lateral, and to a depth of 2.2–2.5 mm. After removing the needle, the tips of the cannulae were held directly over the holes with forceps and then gently inserted straight into the preformed holes. Pulling back slightly on the catheters by the base of the osmotic pumps while inserting the cannulae provided stability which prevented them from moving and potentially damaging tissue while they were affixed. The cannulae were firmly affixed to the skull with Locktite 454 adhesive (Plastics One, Roanoke, VA) and secured down with a piece of nitrile, approximately 1 cm in diameter. After the adhesive cured, the scalp was closed with 6–7 silk sutures. For analgesia, the animal received an immediate subcutaneous dose of ketoprofen (10 mg/kg) and up to every 6 h, as needed, for a maximum of 48 h post-operatively. After the 28-day period, the animals were given an overdose (∼150 mg/kg) of sodium pentobarbital (i.p.) and were transcardially perfused with 0.9% saline. The brains were carefully removed and analyzed as outlined below. At this time, the integrity of the catheters was confirmed again by forcing water through them, and the pumps were also determined to be empty.

2.5. Preparation of brain extracts and Western blot analysis

The neocortex and hippocampus were each microdissected on ice, immediately snap frozen in powdered dry ice, and stored at −80 °C until use. The tissue samples were then dounce homogenized in cold RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) in the presence of a protease inhibitor cocktail (complete mini protease inhibitor tablet, Roche Diagnostics, Indianapolis, IN). Samples were subsequently spun at 20,000 × *g* for 30 min (Eppendorf 5417R, Westbury, NY) and the supernatants removed, aliquoted, and frozen at −80 °C. Equal amounts of protein from each sample were mixed with 2× sample buffer (Invitrogen, Carlsbad, CA), boiled for 5 min, and subjected to 10–20% Tris Tricine SDS gel electrophoresis (Invitrogen). Purified hACT and non-transgenic brain homogenates were used as controls. The electrophoresed proteins were wet transferred onto 0.2 μm nitrocellulose (Whatman, Dassel, Germany) and probed with a rabbit anti-mouse polyclonal antibody to ACT (1:1000, Dako, Glostrop, Denmark). The secondary antibody was horseradish peroxidase-conjugated donkey anti-rabbit IgG (Pierce, Rockford, IL). The immuno-labelled proteins were visualized with an enhanced chemiluminesence detection kit (SuperSignal pico, Pierce, Rockford, IL). After different exposures, autoradiographs were developed with a Konica/Minolta SRX101A (Tokyo, Japan), scanned using a Ricoh Aficio MP3010 (Tokyo, Japan) and quantified using Image J.

2.6. Histology and immunohistochemistry

Mouse brain tissues were fixed in 10% formaldehyde (formalin) for 72 h and then passed through a series of sucrose solutions (10–30%) over another 72 h. Brains were frozen to the peltier stage (Physitemp, Clifton, NJ) of a histoslide (Leica, Heerbruug, Switzerland) and sectioned coronally at 25 μm. After incubating the sections with blocking buffer (Tris-buffered saline with 10% normal goat serum, 0.1% Triton X-100, and 0.02% sodium azide) for 60 min, primary antibodies against ACT (1:500, Dako, Glostrop, Denmark) and Aβ (6E10, 1:1000, Covance, Princeton, NJ) were applied and incubated at 4 °C overnight. After thorough washing, the sections were incubated with secondary antibodies, Alexa 488 and 594 fluorophores (1:1000, 1:4000-Invitrogen) in the dark at ambient temperature for 2 h. This incubation was followed by a Hoechst (1:10,000, Sigma, St. Louis, MO) nuclear stain, and the sections were washed and sealed with Gel-Mount (Electron Microscopy Sciences, Hatfield, PA). The images were analyzed on a Zeiss Imager Z1 fluorescence microscope with a Zeiss Axiocam Mrm camera (Oberkochen, Germany) using Axiovision 4.7 software and quantified using Image J. For animal-to-animal, hemisphere-to-hemisphere plaque comparison, 5 sections from 6 mice (30 total) were stained using Thioflavin S to label the beta-pleated amyloid deposits from anterior to posterior hippocampus in each hemisphere of each mouse. Coronal sections of brain (25 μm) were mounted to slides and let to adhere overnight. The sections were then rinsed with deionized water and submerged in a 1% Thioflavin

S aqueous solution (Sigma–Aldrich, St. Louis, MO) for 5 min. The sections were differentiated in 70% ethanol for 5 min, rehydrated in 30% ethanol for 5 min, washed with deionized water coverslipped, imaged, and quantified as described previously.

3. Results

3.1. Animal recovery

Intracranial delivery of molecules, though invasive, is a common procedure to confirm results from *in vitro* experiments in an *in vivo* system and to test various compounds in transgenic animals via targeted delivery. As an additional advantage, the novel method described here bypasses concerns related to the blood–brain barrier permeability of the administered compound. ACT, a serine protease inhibitor that is known to contribute to the formation of amyloid pathology in Alzheimer's disease, was infused into the hippocampi or lateral ventricles of 9-month-old PS/APP mice for a period of 28 days. ACT was infused into one hemisphere and aCSF (vehicle) into the other hemisphere, thus allowing a comparative analysis to be made within the same animal. In all cases, the animals recovered very quickly from the procedure and resumed eating and grooming within minutes of awakening. All animals were monitored closely during the 4-week infusion. In only a few instances did animals scratch at the incisions, but in no case were they able to open the wound and remove or damage any of the implanted pumps and catheters. In most cases, the hair grew back within 2 weeks, and the animals became virtually indistinguishable from non-treated animals, with the exception of bulges on the haunches from the implanted osmotic pumps (Fig. 3A). The implants caused minimal discomfort to the animals, and procedural mortality was practically eliminated from these studies. When harvesting tissues following the 28-day infusion, the catheters were checked to ensure they were still connected to the pumps (Fig. 3B) and to the cannulae (Fig. 3C). The nitrile cap is indicated by the arrow in Fig. 3D. The entire assembly is shown attached to an Alzet osmotic minipump model 1004 (Durect Corp., Cupertino, CA) in Fig. 3E. Finally, when removing the brains only 2 small holes are visible (Fig. 3F) showing that there is no lateral movement of the cannulae during infusion. No serious damage or impairment was caused by the cannula, although tissue is displaced where inserted. Fig. 4 shows, on the left hemisphere, exactly where the cannula was placed and that surrounding tissue was intact following infusion. Fig. 5A and B show a region of the hippocampus anterior to the infusion site which also does not show signs of damage. To be noted, the gauge of the cannulae used in this experiment (30 g or 0.30 mm O.D.) is the same of those used in commercially available cannulae (Alzet, PlasticsOne), but without the irritation to the animal, inflammation, occasional motor impairment, and high mortality rates associated with them.

3.2. Bilateral brain infusion

Due to the increased variability in plaque load between animals, even within a largely inbred transgenic mouse line, it is often difficult to compare treated and untreated mice with a reasonably small number of animals. This phenomenon can be caused by phenotypic variability and/or differential expression of transgenes. Upon analysis of 6 untreated PS/APP mice at 10 months of age, we found that plaque load can vary significantly from animal to animal (78% mean standard deviation in pixel count). This fact makes it difficult to draw conclusions on the effects that different molecules might have on amyloid deposition or other features of the transgenic brain when comparing animals where hemispheres vary much less (28%) when compared. Fig. 1A and B demonstrates the variation in plaque load between hemispheres and within animals of this genotype. Therefore, in order to study promoters and inhibitors of AD pathology directly in the brain, it would be preferable (almost twice the efficiency) to compare the individually treated hemispheres of each animal rather than to compare the brains of littermates or matched cohorts from other litters. Western blots and immunocytochemical techniques demonstrated that our catheters functioned properly and that ACT was delivered successfully to the ventricles and parenchyma, respectively (Figs. 4–6). If ACT is infused into a PS/APP mouse that has plaques for it to interact with, it binds to the plaques and diffuses very little (Fig. 5C and D). However, if ACT is delivered into the hemispheres of non-transgenic mice it diffuses, but still remains in the intended hemisphere (Fig. 5A and B).

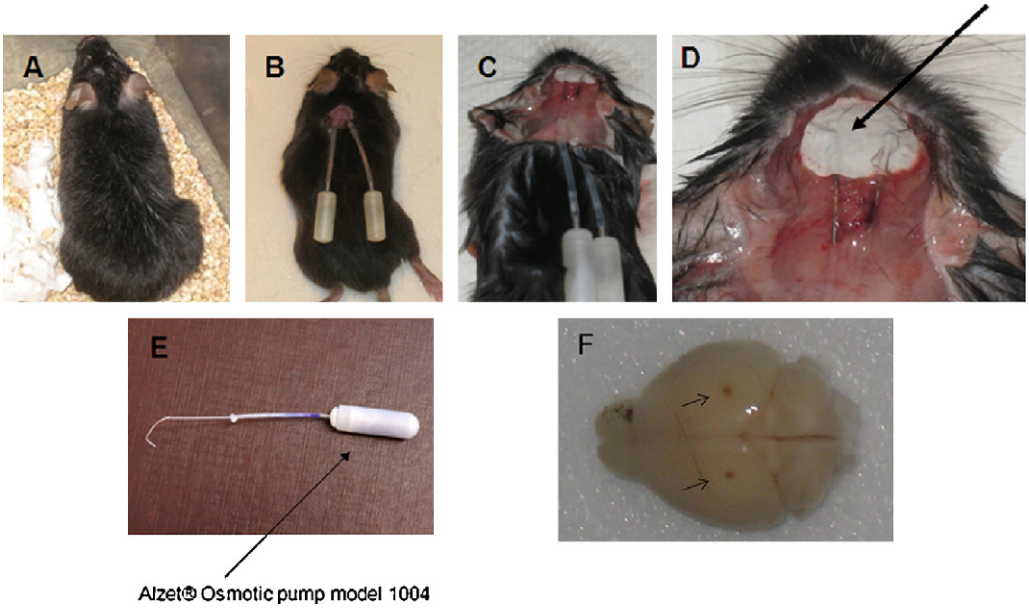

Fig. 3. A 10-month-old PS/APP mouse with 2 osmotic pumps following 28 days of infusion while still active (A) and after transcardial perfusion showing the Alzet pumps (B) and cannulae (C) still securely attached. The arrow in (D) shows the nitrile cap used to affix the catheters to the skull and (E) shows the entire assembly attached to an Alzet 1004 osmotic minipump. When the brain is removed, only small holes are visible where the cannulae were inserted (F).

3.3. Interaction of ACT and amyloid

It is well established that ACT is localized, together with the Aβ peptide, in the amyloid plaques of Alzheimer's disease (Abraham et al., 1988). However, the mechanism and effect of this interaction are still under investigation. Previous *in vitro* experiments indicated that ACT can bind to Aβ and become integrated into amyloid filaments as they are formed, and the same mechanism was presumed to occur *in vivo* (Mucke et al., 2000). Using the application method described here, we successfully delivered ACT into individual brain hemispheres of adult PS/APP mice and found that ACT binds to the perimeter of existing plaques in a similar manner as found for ACT association with amyloid plaques in PS/APP/ACT transgenic mice (Nilsson et al., 2001) (Fig. 7). While the application of exogeneous ACT did not lead to increased amyloid load during the time frame of these experiments, it should be noted that ACT was infused for only 28 days. A longer exposure could result in an increased plaque load and is currently being investigated. At the same time, while ACT is known to enhance plaque formation, the perimetrical binding of ACT to pre-existing Aβ plaques, as shown here, might indicate that ACT involvement in AD pathogenesis not only occurs concurrent with plaque formation but also afterwards. This conclusion is consistent with the fact that ACT is highly upregulated in adult AD brains (Abraham et al., 1988) and also induces the phosphorylation of tau (Padmanabhan et al., 2006).

Although there is evidence that ACT's involvement in AD is not restricted to its participation in plaque formation, the purpose of using ACT in this model was to demonstrate the utility of our infusion system. The catheters and cannulae described here served as an outstanding tool for targeted delivery of ACT into individual brain

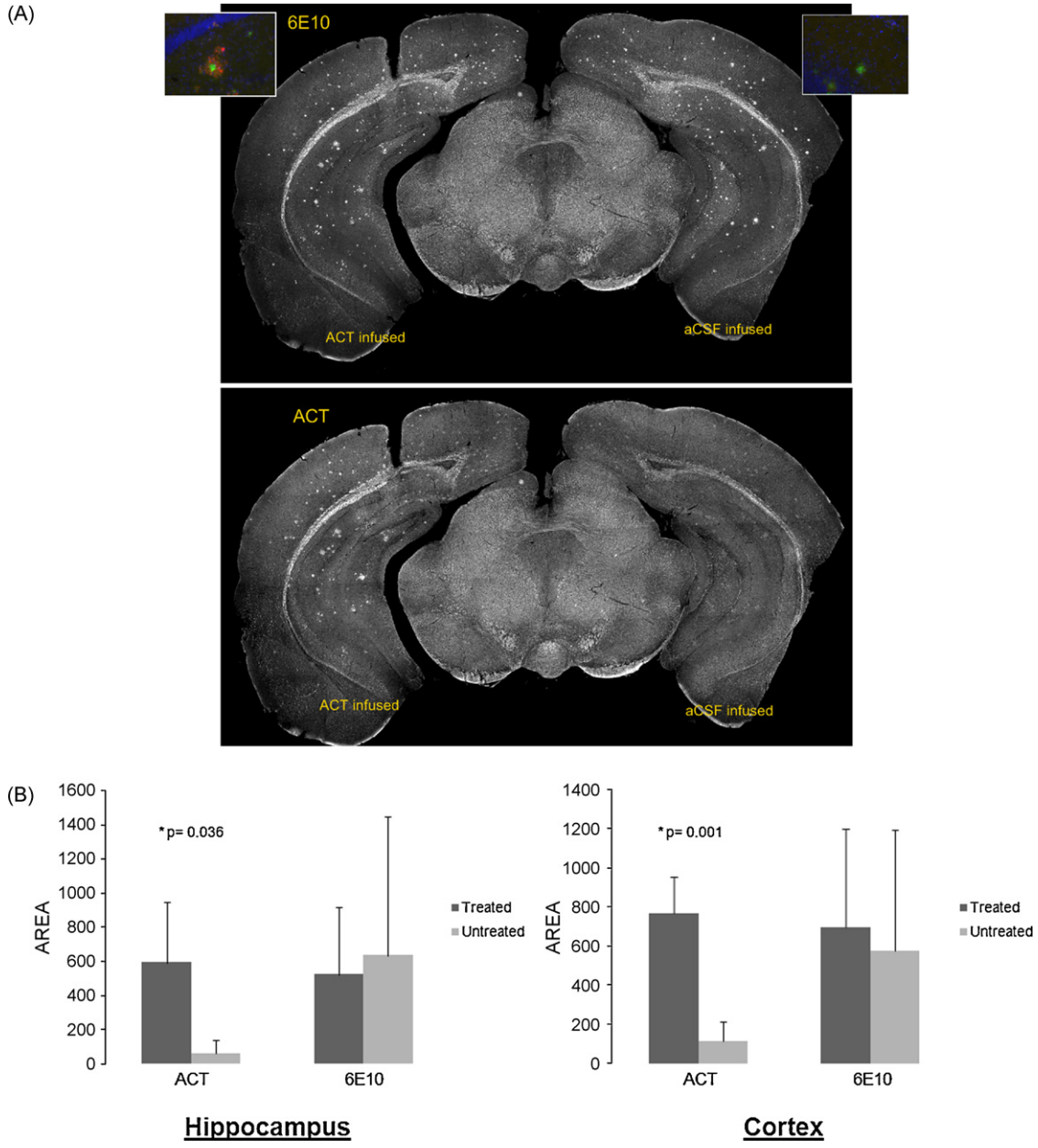

Fig. 4. (A) A montage of images taken at 10× from 25 μm sections of an animal treated with ACT on the left and aCSF control on the right. The top section is immuno-labelled with the Aβ antibody 6E10 and the bottom with ACT antibodies. Inlays (40×) show Aβ in green, ACT in red, and Hoechst nuclear stain in blue. (B) Semi-quantitative analyses of ACT and Aβ immunostaining in the hippocampus and cortex of PS/APP mice ($n = 4$) infused with ACT into the parenchyma of one hemisphere and aCSF in the contralateral hemisphere. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

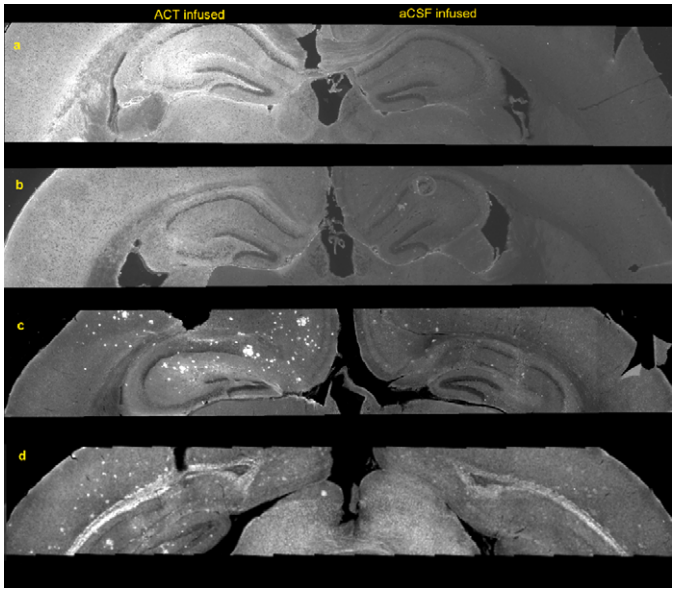

Fig. 5. Montage images of 25 μm coronal sections of 10-month-old non-transgenic (A and B) and PS/APP mice (C and D) immuno-labelled with and antibody against α-1-antichymotrypsin following 28-day infusion demonstrating that ACT stays on the infused hemisphere with or without the presence of plaques.

hemispheres and will facilitate further examination of the mechanistic involvement of ACT and other molecules in the pathology of Alzheimer's disease.

4. Discussion

The variability of plaque load (i.e. size, density, and number) between brain hemispheres in PS/APP mice, though a factor to consider in these types of experiments, is substantially less than plaque variability from mouse to mouse (Fig. 1A and B). Based on our analysis of 6 untreated, unrelated animals in our colony, we determined the plaque load data from 5 brain sections through 6 ten-month-old PS/APP mice and compared the standard deviation (plaque variance) between sides of the same slice (11%), between total hemispheres of the same animal (28%), and between individual animals (78%). All of the data from the left hemisphere were compared with the right for each mouse (Hemi–Hemi, 1 data point for each mouse); the left half of a single slice is compared with the right side (Left–Right, 1 data point for each slice); and a permuted comparison of each mouse with every other mouse (Mouse–Mouse, 1 data point for each pair of mice). Where data were aggregated (Hemi–Hemi and Mouse–Mouse), the mean of the aggregated counts was used to make the standard deviation comparable to the single slice data (standard deviation of two numbers). The data indicate that evaluation of whole hemispheres in the same mouse is about 4 times less varied than comparing different ani-

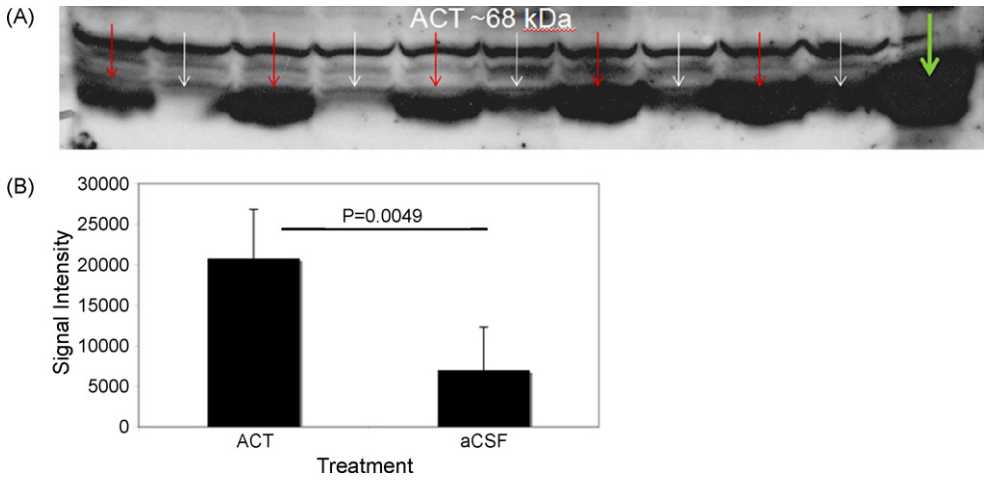

Fig. 6. (A) Western Blot of PS/APP mouse hemispheres receiving ACT (red arrows) or aCSF (white arrows) into lateral ventricles. The green arrow denotes ACT taken from an osmotic pump implanted for 28 days to show minimal degradation of the protein during the experiment. (B) Quantification of ACT in hemispheres infused with ACT or aCSF. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

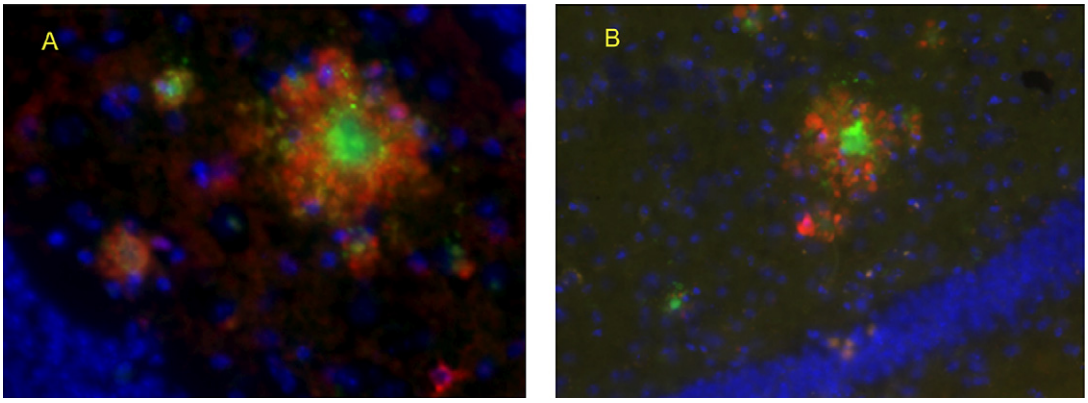

Fig. 7. Comparison of ACT associated with amyloid plaques (40×) from a PS/APP/ACT transgenic mouse (A) and a PS/APP ACT-infused mouse (B) both at 9 months of age. Immuno-labelling of Aβ is shown in green (6E10 antibody), ACT in red, and Hoechst nuclear stain in blue. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

mals to each other. Because of this variability, it was advantageous to develop a technique for the delivery of drugs and small molecules that would allow the treatment of individual brain hemispheres. By exposing one hemisphere to an experimental compound and the contralateral hemisphere to the vehicle only, our novel technique described here easily allows each animal to serve as its own histological and biochemical control.

Previously, bilateral treatment, either into lateral ventricles or brain parenchyma was difficult to accomplish with mice. Because transgenic mice provide an important experimental system for disease studies, such as Alzheimer's disease, it was imperative to develop a durable and reliable technique for simultaneous bilateral delivery into the brain that is well-tolerated by mice. Commercially available bilateral delivery devices for mice are pedestal cannulae, which have proven problematic for a variety of reasons: (1) The pedestals are bulky and require large amounts of adhesive to fix into place because the cannula surface that comes into contact with the skull is flat while the skull is curved. This design requires that enough adhesive be applied to fill the gaps and to secure the applicator in place. Frequently, the adhesive does not have a chance to cure completely and reacts with surrounding tissue, causing irritation and the need for animals to be removed from the experiment. (2) The pedestals and tubing remain partially exposed, allowing the animals access to the catheters potentially compromising experiments and also causing concern for their health (inflammation, cachexia, death). (3) The bilateral pedestals have limited flexibility for the spacing of the catheters. If they are designed with fixed distances and fixed depths, their placement in a 25 g mouse is very different compared to a 35 g mouse, leading to improper placement of the cannula. (4) Other systems require specialized arms to lower the catheter into place, whereas these are best manipulated individually by hand to be reliably and reproducibly positioned. (5) Moreover, the entire catheters and pumps we used can be completely removed without terminating the life of the animal. This is generally not true of commercially available catheters, which, when removed cause damage to the skull and do not leave the scalp in healthy enough condition to be sutured. (6) Lastly, since these catheters remain completely subcutaneous, behavioral testing can be safely performed while an animal is being treated, which is not a possibility with pedestals. Overall, the stereotaxic placement of pedestal cannulae, particularly bilateral pedestal cannulae, into a mouse brain is very restrictive and problematic.

Our catheter system described here facilitates targeted compound delivery to individual mouse hemispheres and is remarkably adaptable to reaching any region of the mouse brain and overcomes the challenges listed above. Because the stainless steel catheters can be fashioned to any depth and are implanted individually, variability in the size of the mouse brains can be easily accounted for and accurate catheter placement and proper contouring is ensured. Most importantly, the entire system is implanted subcutaneously with little if any protrusion. The use of the non-reactive nitrile cap to secure the catheters in place requires only a fraction of the adhesive compared to the pedestal method, thereby giving ample time for it to cure properly before the scalp is sutured. Our observations demonstrate that, in most cases, the animals re-grow hair over the scalp in a very short time and never scratch or over-groom the site or damage the catheters. The mortality in experiments using this method is closer to 2–3% as opposed to 30–40% using conventional techniques over the 28-day infusion period.

Here we demonstrated that ACT binds to pre-existing amyloid plaques in APP transgenic mice only in the infused hemisphere. We also showed that ACT could be delivered both to the parenchyma of the mouse hippocampus and into lateral ventricles using our new catheters. Changes in total plaque load, however, in the presence of ACT were not observed over the 28-day infusions. Although ACT is known to be involved in the process of fibrillization of Aβ both *in vitro* and *in vivo*, we did show that mature plaques retain binding capacity for ACT. The morphology of the amyloid plaques after ACT infusion strongly resembles the plaque morphology in PS/APP/ACT transgenic mice (Fig. 7). Our observation suggests that our delivery system is an effective method to test various compounds in mouse models of neurodegenerative and other diseases. Ongoing investigations with inhibitors of the Aβ/ACT interaction will help to determine ACT's role in AD both during and beyond plaque promotion.

In sum, using our novel catheter system, we have studied the infusion of a protein known to be a component of AD pathology into a mouse model of AD. The potential application of this system, however, is quite broad. While we have focused on intrahippocampal and intracerebroventricular (ICV) delivery, our catheters can conceivably be inserted in any depth and be bent to any angle to deliver compounds to any region of the brain in other mouse models of neurodegenerative disease. Bilateral pedestal cannulae do not provide users with such adaptability, since accounting for variability in animal size poses a problem. Likewise, using 2 independent pedestal catheters to achieve bilateral delivery in mice is prohibited due to the large size of the pedestals. The technique described here even allows for the implantation of 2 cannulae per hemisphere (4 per brain), which would allow delivery of multiple agents to either hemisphere in the same mouse with minimum mortality.

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