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Harderian Gland Adenectomy: A Method to Eliminate Confounding Radio-opacity in the Assessment of Rat Brain Metabolism by ^{18}F-fluoro-2-deoxy-D-glucose Positron Emission Tomography

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The ^{18}F isotope of fluoro-2-deoxy-D-glucose (FDG) is a radiotracer commonly used in positron emission tomography (PET) for determining regional metabolic activity in the brain. However, in rats and many other species with nictitating membranes, harderian glands located just behind the eyes aggressively incorporate ^{18}F-FDG to the extent that PET images of the brain become obscured. This radioactive spillover, or 'partial volume error,' combined with the limited spatial resolution of microPET scanners (1.5 to 2 mm) may markedly reduce the ability to quantify neuronal activity in frontal brain structures. Theoretically, surgical removal of the harderian glands before ^{18}F-FDG injection would eliminate the confounding uptake of the radioactive tracer and thereby permit visualization of glucose metabolism in the frontal brain. We conducted a pilot study of unilateral harderian gland adenectomy, leaving the contralateral gland intact for comparison. At 1 wk after surgery, each rat was injected intravenously with ^{18}F-FDG, and 40 min later underwent brain microPET for 20 min. Review of the resulting images showed that the frontal cortex on the surgical side was defined more clearly, with only background ^{18}F-FDG accumulation in the surgical bed. Activity in the frontal cortex on the intact side was obscured by intense accumulation of ^{18}F-FDG in the harderian gland. By reducing partial volume error, this simple surgical procedure may become a valuable tool for visualization of the frontal cortex of rat brain by ^{18}F-FDG microPET imaging.

Abbreviations: FDG, fluoro-2-deoxy-D-glucose; FWHM, full width half maximum; PET, positron emission tomography

The glucose analog [^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is a radioactive tracer used in positron emission tomography (PET) to estimate brain activity. In the brain, cold or radiolabeled FDG is incorporated into neurons at a rate proportional to energy demand. Once inside the cell, FDG is metabolized by hexokinase to form glucose 6-phosphate but, unlike glucose, is not further phosphorylated, and it therefore accumulates in the cytosolic compartment. Thus, after systemic injection, ^{18}F-FDG will accumulate quickly over a period of minutes and can be imaged and quantified by use of PET. At typical radiotracer concentrations, FDG does not impair glucose-mediated ATP production during glycolysis. In highly metabolic structures such as muscle and brain, ^{18}F-FDG PET is a very useful tool to profile regional brain activity patterns as well as changes in neuronal activity after drug challenge or physiologic manipulation or during altered behavioral states. This activity often is calculated as the 'regional cerebral metabolic rate of glucose' (rCMRglc). Numerous PET imaging studies in humans have reported regional blood flow and activity changes within specific regions of the brain that correlate with drug administration and somatosensory stimulation. Moreover, ^{18}F-FDG PET has been used as both a diagnostic tool and a biomarker for therapeutic evaluation in neurologic (for example, stroke, Alzheimer disease, Parkinson disease) and psychiatric disorders (for example, schizophrenia, anxiety, depression).

The ability of PET to resolve small differences in activity between adjacent structures or changes within structures is affected not only by the theoretical limits of PET, termed the 'positron range limit' (approximately 1.5 mm for low-energy positrons) and the efficiency of scanner detectors but is also strongly influenced by spillover of radioactivity in neighboring tissue. If sufficiently large, this 'partial volume error' can distort PET images and increase the probability of obtaining false positives and negatives during hypothesis testing. In animal species with nictitating membranes, such as rodents, the harderian glands located just behind the eyes are a noteworthy contributor to spillover artifacts. They lie deep within the orbit and encircle the bulbus oculi and optic nerve on the medial, superior, and inferior sides. Although harderian glands are reported to have a range of incompletely described photoreceptive, endocrine, and immunologic functions, they appear to serve primarily as an exocrine secreting accessory to the lacrimal gland for eye lubrication[11,13] (for example, chromodacryorrhea is a condition in which an over-abundance of blood-colored porphyrins are released from the harderian glands). Harderian glands are more avid than any other bodily tissue at incorporating ^{18}F-FDG from the blood during PET experiments. Because the glands are located just rostral to the cranium, the frontal brain structures are particularly susceptible to partial volume errors.

Because the rat is the most universally popular research species used in the emerging technology of microPET imag-

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Figure 1. The nictitating membrane of the right eye of the rat was retracted laterally with forceps. An incision was made between the nictitating membrane and medial canthus of the eye. The harderian gland is visible through the thin tissues at the incision site.

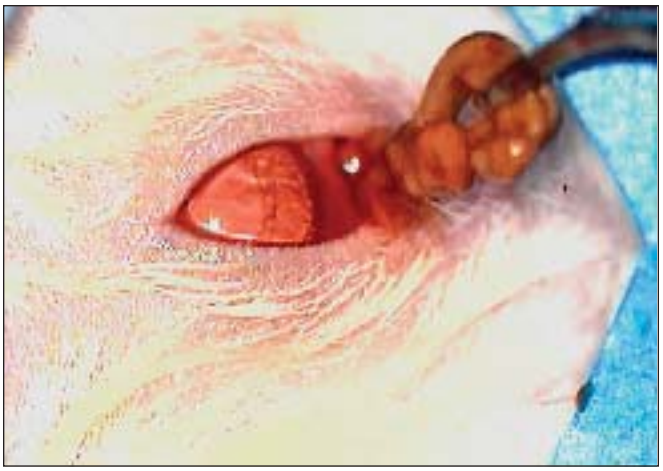

Figure 2. After the initial incision, the harderian gland was grasped with forceps and retracted out of the orbit. Care was taken to remove the lobed gland from the dorsal and ventral aspects of the eye. No sutures were needed to close the incision site.

ing, we sought here to develop a surgical procedure to resect the harderian glands in an attempt to reduce this confounding artifact. Surgical removal of the rat harderian glands before ^{18}F-FDG PET imaging may provide an improved method for more accurately tracking brain glucose metabolism as a function of neural activity. The purpose of this study was to determine whether unilateral harderian gland adenectomy changes the visualization of glucose metabolism in rat forebrain on the side that underwent surgery compared with the intact contralateral side.

Harderian gland adenectomy surgeries have been described for the rat, hamster, and gerbil.[1,3,11,17] The procedures vary greatly: for example, the surgery in the rat is accomplished after enucleating of the eye,[4,11] in the hamster by incising the medial corner of the eye and removing the glands,[3] and in the gerbil by reflecting the nictitating membrane medially and then incising the bulbar conjunctiva to expose the harderian gland.[1] This study will describe a different approach, in which the incision is located between the medial canthus of the eye and the nictitating membrane, does not require suturing, and allows the animal to recover quickly with little discomfort and a low risk of infection. The effectiveness of this procedure in reducing radiation spillover into the brain was evaluated postsurgically by use of ^{18}F-FDG PET.

Materials and Methods

The Pfizer Institutional Animal Care and Use Committee approved the surgical and imaging procedures. The Ann Arbor division of Pfizer Global Research and Development is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Two Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) each weighing approximately 350 g were anesthetized by chamber induction with 5% isoflurane (Abbott Laboratories, Abbott Park, Illinois) and an O_2 flow rate of 1 l/min. Once anesthetized, rats were maintained on 2% isoflurane and an O_2 flow rate of 1 l/min by use of a Mapleson E breathing circuit during surgery prep. The surgical site was not shaved, because we found it was less irritating to the eye. The whiskers were clipped to prevent them from being in the surgical field. A 1:50 dilution of povidone–iodine solution in saline was used to disinfect the periocular skin and to flush the conjunctival sac.

Each rat then was placed laterally for surgery on a Gaymar warm-water pad (VetEquip, Pleasanton, CA), and anesthesia was maintained (ADS 1000, Engler Engineering, Hialeah, FL) at 2% isoflurane and an O_2 flow rate of 0.6 l/min. After the rat was deeply anesthetized, the nictitating membrane was grasped and retracted out of the conjunctival sac. With the aid of a surgical microscope, a small incision was made rostral to the base of the nictitating membrane cartilage through the conjunctival tissue until the harderian gland could be seen (Figure 1). The harderian gland is an irregularly lobed glandular mass that is subdivided laterally by 3 clefts, occupies a large part of the posterior surface of the orbit, and extends medially into the space between the eye and orbital rim.[6] The gland then was grasped at the medial side with a pair of serrated eye dressing forceps and gently extracted while being dissected away from the conjunctival tissue (Figure 2). Care was taken to avoid introducing trauma to the optic nerve, because the gland interdigitates with it and the intraorbital lacrimal gland.

As the gland was extracted, there was some occasional bleeding from the inferior ophthalmic artery, which supplies the gland, or the opthalmic veins, which drain it. In these cases, the eyelids were closed, and slight pressure was applied with a cotton-swabbed applicator to the medial canthus of the eye until bleeding subsided. With practice, the surgeon could remove the entire lobed gland intact. If the gland tore, however, the ventral and dorsal aspects of the orbit were examined carefully by use of a surgical microscope to ensure complete removal of the superior and inferior lobe. Suturing the incision was unnecessary. An ophthalmic antibiotic ointment was applied immediately postoperatively and twice daily for the next 3 d. Buprenorphine (0.01 mg/kg; Reckitt Benckiser Pharmaceuticals, Richmond, VA), a postoperative analgesic, was administered immediately after surgery and once daily for 3 d thereafter.

At 1 wk after harderian gland adenectomy, rats underwent PET to evaluate potential changes in ^{18}F-FDG distribution. The rats were food-deprived overnight, and a blood glucose level was obtained the next day. They then were anesthetized with isoflurane (initial dose, 5%; maintenance, 2%) and injected intravenously through the tail vein with 30 MBq of [^{18}F]-FDG (IBA Molecular, Oakwood Village, OH) as a tracer. After a 30-min absorption interval, rat was anesthetized with ketamine–xylazine (90 and 10 mg/kg, respectively) and positioned on the

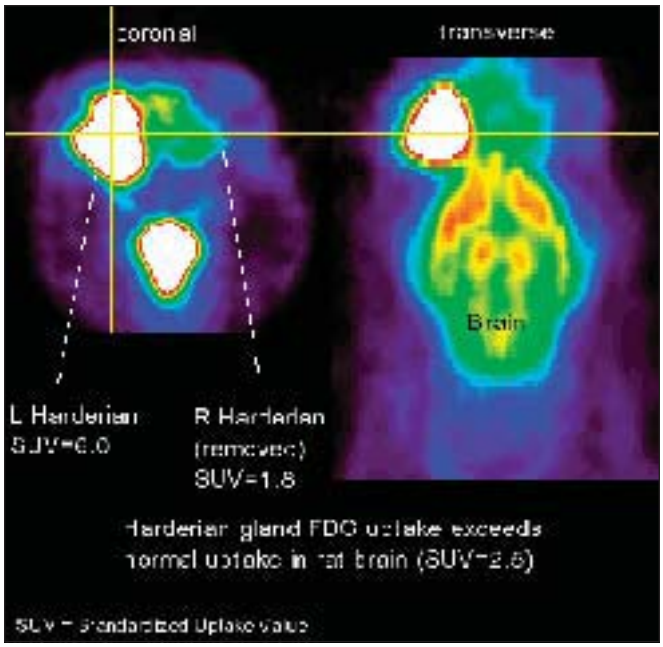

Figure 3. Pseudocolored PET images of a rat head (left, coronal section; right, transverse section) showing intense ^{18}F-FDG accumulation in the left, intact harderian gland (white) in contrast to background-level ^{18}F-FDG accumulation in the vicinity of the right, resected gland (blue-green). The left forebrain shows evidence of the presence of radioactive spillover between the left harderian gland and the brain, but similar spillover is not seen on the right side. The transverse image also shows intense (red), but normal, physiologic regional activity in more caudal brain regions, such as the cortex and striatum.

scanning bed. The level of anesthesia achieved with this cocktail is sufficient to immobilize the animal for 40 min. The head was secured gently into an acrylic holder, being held in position by use of blunt ear bars and a plastic bite bar, similar to a stereotaxic apparatus. Rats were static-scanned for 20 min (40 to 60 min after ^{18}F-FDG injection) centered over the brain in microPET scanner (field of view, 10 cm; resolution, 2 mm; CTI-Concorde Microsystems R4, Seimens, (Siemens Preclinical Solutions, Knoxville, TN). The PET scan was reconstructed into single-timeframe images by use segmented attenuation and scatter correction using OSEM2D software (span, 3; ring difference, 31; 16 subsets and 4 OSEM iterations; (Siemens Preclinical Solutions, Knoxville, TN). Images then were scaled to a standardized uptake value and coregistered to a stereotaxic brain atlas by use of a mutual information algorithm. Regions of interest were defined for harderian gland volume and whole brain. Images of intact and harderianectomized animals were compared pixel-by-pixel by use of the SPM2 statistical package (SPM, University College London, London, UK). Upon completion of scanning, rats were euthanized by CO_2 overdose.

Results

The rats quickly recovered from surgery, and postoperative complications (infection, pain) were not noted. Topical antibiotic treatment and systemic analgesia were suspended after 3 d. The whole-brain standardized uptake value did not differ significantly between intact and unilaterally harderianectomized animals (data not shown). The most dramatic change in the operated rats involved gross asymmetry of ^{18}F-FDG uptake in the head, with much lower than normal activity surrounding the orbit of the right eye, near where the harderian gland would normally be located (Figure 3). The region between the right transorbital surgical bed area and the brain was easily distinguishable from adjacent structures, as compared with the left, intact side, which showed a continuum of high activity (spillover) with little contrast. The most rostral brain features (forebrain and olfactory lobes) showed greater activity on the intact side compared with the surgery side, clear demonstration of the confounding effect the harderian glands have in introducing partial volume error.

Discussion

The harderian gland adenectomy surgery described here is a quick and simple procedure. The small incision site does not require suturing and likely will heal very readily by second intention. Postadenectomy care of rats includes topical antibiotic treatment and systemic analgesia. We have not seen any postoperative complications with this procedure, and bilateral harderian gland adenectomy surgery since has become routine in our laboratory.

In its exocrine role, the harderian gland primarily secretes lipids and porphyrins as an eye lubricant, although several other ill-defined functions have been ascribed to this gland.[11,12,13] Importantly, little published evidence suggests that removal of the harderian glands of rats produces adverse conditions. For example, few morphometric changes in endocrine parameters have been noted after harderianectomy, and although postsurgical increased uterine weight has been reported, vaginal cyclicity and ovarian, adrenal, hypophysial, and body weights were normal[11,12] In addition, harderian gland removal was specifically shown not to negatively alter pituitary–adrenal function.[11]

The problem of radioactive spillover from the harderian glands during the use of ^{18}F-FDG as a PET tracer in rats and mice brain studies has been reported by multiple groups.[2,5,6,8,15,16,18] Although the specific mechanism by which FDG is incorporated into the glands is unclear, one hypothesis is that glucose plays a rate-limiting role in lipid synthesis.[16] Kuge and coworkers[5,6] were the first to describe this image-degrading phenomenon in rats and went so far as to state that "Quantitative PET determination of [regional cerebral glucose metabolism] is unreliable in rats because of the extracranial radioactivity and the limited resolution of scanners." The authors noted that ex vivo autoradiographic methods appear to be superior to PET in this regard. Since that work, some researchers have argued that advances in microPET technology have resulted in improved spatial resolution (currently approximately 1.5 mm full width half maximum [FWHM]) to the point that partial volume effects contributed by the harderian glands are minimized.[14] Other groups, however, continue to find that spillover masks accurate measurement of regional cerebral metabolism of glucose in the frontal lobe of the rat[2,15] and mouse[18] brain. The theoretical limit of resolution for PET is dependent on the positron range limit for any particular isotope energy and may not get much higher than approximately 1 mm FWHM unless further advancements in equipment and reconstruction algorithms emerge. The utility of performing harderianectomy during rodent PET studies would appear also to be applicable for other ^{18}F- based PET radiotracers that have been reported to accumulate in the harderian gland, such as ^{18}F-(3-N-methyl)benperidol,[9] as well as other PET and single photon emission computed tomography (SPECT) isotopes, including [^{11}C]octanoate,[4] [^{123}I]FP-CIT,[11] and ^{99m}Tc-tetrofosmin.[15]

Our findings suggest that harderian gland adenectomy in the rat would provide a beneficial effect in reducing unwanted obscuring of closely spaced structures due to spillover of ^{18}F-

FDG activity into frontal regions of the brain. This surgical procedure likely will improve the quality of images and reduce variability during quantification of tissue tracer uptake values. Rat harderian gland adenectomy will be of particular value in studies requiring high spatial resolution PET imaging.

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