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[18F]-fluorodeoxyglucose positron emission tomography of the cat brain: A feasibility study to investigate osteoarthritis-associated pain

Martin Guillot [a,b], Gabriel Chartrand [c], Ramnada Chav [c], Jacques Rousseau [d,e], Jean-François Beaudoin [d], Johanne Martel-Pelletier [b], Jean-Pierre Pelletier [b], Roger Lecomte [d,e], Jacques A. de Guise [c], Eric Troncy [a,b,*]

[a] Groupe de Recherche en Pharmacologie Animale du Québec (GREPAQ), Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, QC, Canada
[b] Osteoarthritis Research Unit, University of Montreal Hospital Research Centre (CRCHUM), Department of Medicine, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada
[c] Laboratoire de recherche en imagerie et orthopédie (LIO), University of Montreal Hospital Research Centre (CRCHUM), École de Technologie Supérieure, Université du Québec, Montreal, QC, Canada
[d] Centre d'imagerie moléculaire de Sherbrooke (CIMS), Étienne-Le Bel Clinical Research Center, Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC, Canada
[e] Department of Nuclear Medicine and Radiobiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada

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ABSTRACT

The objective of this pilot study was to investigate central nervous system (CNS) changes related to osteoarthritis (OA)-associated chronic pain in cats using [18F]-fluorodeoxyglucose (18FDG) positron emission tomography (PET) imaging. The brains of five normal, healthy (non-OA) cats and seven cats with pain associated with naturally occurring OA were imaged using 18FDG-PET during a standardized mild anesthesia protocol. The PET images were co-registered over a magnetic resonance image of a cat brain segmented into several regions of interest. Brain metabolism was assessed in these regions using standardized uptake values.

The brain metabolism in the secondary somatosensory cortex, thalamus and periaqueductal gray matter was increased significantly ($P \leq 0.005$) in OA cats compared with non-OA cats. This study indicates that 18FDG-PET brain imaging in cats is feasible to investigate CNS changes related to chronic pain. The results also suggest that OA is associated with sustained nociceptive inputs and increased activity of the descending modulatory pathways.

Introduction

In cats, osteoarthritis (OA) develops with aging and causes significant chronic pain expressed as gait abnormalities, decreased activity and various behavioral changes (Bennett and Morton, 2009; Lascelles et al., 2010; Slingerland et al., 2011; Benito et al., 2012; Bennett et al., 2012; Klinck et al., 2012).

Chronicity of pain is associated with changes (plasticity) in the central nervous system (CNS) manifesting as modifications in molecular pathways, cellular function and network activity (Kuner, 2010). Our group previously demonstrated that, when compared with non-OA cats, those with OA pain presented a punctate tactile hypersensitivity (Guillot et al., 2013) and a facilitation of nociceptive temporal summation (Guillot et al., 2014), both recognized to reflect central sensitization (Woolf, 2011). Other central changes can occur with chronic pain such as alterations in CNS activity and in cognition and affect (Kuner, 2010). It is important to characterize those changes, as analgesic treatments have to be developed based on fundamental pain mechanisms. For example, nociceptive temporal summation is N-methyl-D-aspartate (NMDA) receptor-dependent in both animals (Dickenson and Sullivan, 1987) and humans (Price et al., 1994). This phenomenon is reversible, but does not respond to non-steroidal anti-inflammatory drug (NSAID) administration (Mease et al., 2011). Hence, in humans, the inefficiency of NSAID treatments in OA-induced chronic pain highlights the need for the development of drugs targeting central sensitization (e.g. ionic channel or NMDA-receptor blockers, serotonin/noradrenaline reuptake inhibitors) (Mease et al., 2011; Woolf, 2011). Positive results in humans obtained using duloxetine, a dual-reuptake inhibitor of serotonin and noradrenaline, combined with an NSAID (Frakes et al., 2011) is encouraging for us to pursue also in cats.

Central changes associated with chronic pain can potentially be assessed using brain imaging. The use of functional imaging of the CNS to assess pain in animals is a recent and growing field (Borsook

* Corresponding author. Tel.: +1 450 773 8521 ext. 8399.
E-mail address: eric.troncy@umontreal.ca (E. Troncy).

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and Becerra, 2011). The major advantages of these techniques are the possibility to collect objective measures of pain, to compare data across several species, to target specific central mechanisms, and to perform study designs with repeated data (Borsook et al., 2010; Borsook and Becerra, 2011; Davis and Moayedi, 2013). [^{18}F]-fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET) imaging is the modality of choice to investigate modifications in brain metabolism related to chronic pain (Clarke and Lawrence, 2013). As brain metabolism is coupled with cerebral function, ^{18}FDG is an excellent surrogate of cerebral function (Kessler, 2003). ^{18}FDG-PET provides hyper- or hypo-metabolism patterns related to chronic pain, which are currently difficult to obtain using functional magnetic resonance imaging (MRI) techniques in animals (Thompson and Bushnell, 2012; Clarke and Lawrence, 2013). In particular, several regions involved in pain modulation have shown hyperactivity in chronic pain states in rodents (the periaqueductal gray (PAG), amygdala, thalamus, primary somatosensory cortex, secondary somatosensory cortex, and anterior cingulate cortex) (Thompson and Bushnell, 2012; Clarke and Lawrence, 2013) and in humans (secondary somatosensory cortex, cingulate cortex, thalamus, amygdala, and PAG) (Shiraishi et al., 2006; Kulkarni et al., 2007; Gwilym et al., 2009).

The objective of the present study was to investigate if OA-associated chronic pain in cats is associated with central changes as detected by ^{18}FDG-PET. OA pain in cats is characterized by structural (radiography) and functional (orthopedic examination) assessments. Our hypothesis was that several brain regions involved in pain modulation also present change in brain metabolism (detection of brain plasticity secondary to chronic pain).

Materials and methods

Cat selection

The Institutional Animal Care and Use Committee approved the study protocol (protocol Rech-1482, 29 November 2012), and the Canadian Council on Animal Care Guidelines were followed with respect to the care and handling of the cats.

Housing and selection of cats

This study included five normal, healthy (non-OA) cats (mean age, 4.7 years; range, 2–9 years) and seven cats with pain associated with naturally occurring OA (mean age, 8.3 years; range, 6–10 years) from a research facility (ArthroLab) cat colony. The cats were fed a standard certified commercial cat food (Hill's Prescription Diet w/d Feline, Hill's Pet Nutrition) once daily in the afternoon according to the manufacturer's recommendations. Water was supplied ad libitum. The cats were loose-housed, with free access to toys, raised platforms and a large window. Beds in a quiet area were also freely accessible. All cats presented a normal neurological evaluation, complete blood count (CBC), blood biochemical profile (including T4), and urine analysis. A thorough orthopedic examination was performed by a blinded observer to detect changes in gait, posture, and the presence of joint pain.

Cats were categorized as having changes or no changes in gait or posture, and as experiencing subjective pain or no pain. OA was diagnosed using a thorough radiographic evaluation: mediolateral and caudocranial projections of the stifle, ventrodorsal projection of the coxofemoral joints, dorsopalmar projection of the carpal joints and dorsoplantar projection of the tarsal joints, as well as the mediolateral projection of shoulders and elbows were performed. These radiographs were conducted under sedation using medetomidine (0.02 mg/kg; Domitor 1 mg/mL, Pfizer Canada Animal Health) and morphine (0.1–0.2 mg/kg; Morphine Sulfate Injection 10 mg/mL, Sandoz), administered intramuscularly (IM).

The extent of radiographic OA was graded by a veterinary radiologist: osteophytes and enthesiophytes were scored together according to their global size (0–3); subchondral sclerosis was scored according to its extent (0–3); and joint effusion was identified and was scored as normal (0) or increased (1) (Guillot et al., 2012, 2013). Each joint was graded and the radiographic score was defined for forelimbs and hind limbs as the sum of the score of their joints (Table 1). Cats designated as 'non-OA' had no abnormalities detected during all the examinations described above, and the selected 'OA cats' were considered subjectively to be in pain and to present radiographic changes related to OA.

Table 1
Radiographic features of the cats.

Feature		Non-OA cats	OA cats
Median radiographic score (range)	Forelimbs	0 (0-0)	2 (0–4)
	Hind limbs	0 (0-0)	2 (1–11)
Median of the number of OA-affected joint of a cat (range)	Forelimbs	0 (0-0)	2 (0–2)[a]
	Hind limbs	0 (0-0)	2 (1–4)[b]

[a] On seven cats, affected joints were shoulder (3/7 = 42.9%) and elbow (2/7 = 28.6%) joints.
[b] On seven cats, affected joints were coxofemoral (4/7 = 57.1%), tarsal (3/7 = 42.9%), and stifle (3/7 = 42.9%) joints.
OA, osteoarthritis.

^{18}FDG-PET brain imaging

High-resolution small animal PET scanners can provide precise functional brain images in animals such as cats (Shimada et al., 2000; Kim et al., 2008). Hence, PET imaging was performed using a LabPET small animal scanner (Gamma Medica) with a 7.5-cm axial × 10-cm diameter field of view. Imaging sessions were performed under standardized anesthesia induced using a 4 mg/kg IM injection of alfaxolone and maintained with isoflurane and oxygen using an endotracheal tube (end tidal isoflurane maintained at 1.6–1.7%) and a Bain anesthetic system with fresh oxygen gas flow set at 1 L/min. Body temperature (actively maintained between 36.5 and 37.5 °C), respiratory rate, arterial pressure (oscillometric method), blood oxygen saturation (pulse oximeter), and inhalation and exhalation (capnography) were monitored continuously.

The cats were placed in dorsal recumbency, with the head in the center of the field of view. The imaging session started immediately after a 13–16 MBq/kg intravenous (IV) injection of ^{18}FDG. Static three-dimensional images of the cat brains were acquired for 35 min starting 35 min post-injection. The images were reconstructed using 15 iterations of a maximum likelihood expectation maximization algorithm implementing a physical description of the detectors in the system matrix (Fig. 1a) (Selivanov et al., 2000).

Semi-quantitative analysis

Manual delineation of 11 regions of interest (ROIs) of the brain was performed using datasets from ex vivo 7-Tesla magnetic resonance (MR) images of a cat brain (euthanased for a reason unrelated to our study) and anatomical brain atlases (Reinoso-Suarez, 1961; Scannell et al., 1995) (Fig. 1b). These ROIs consisted of the whole right and left visual cortex (including the primary, secondary and association visual cortex), superior temporal cortex, mesencephalon, PAG, thalamus (including epi- and meta-thalamus), secondary somatosensory cortex, insula, anterior cingulate cortex, primary somatosensory cortex, motor cortex, and prefrontal cortex (including orbitofrontal cortex).

PET imaging, which is related to biological functions and not structure, presents few landmarks in the brain. However, MR images present complementary structural details. Locations of collected PET data have to be assigned with the use of a brain atlas (Hjornevik et al., 2007). Because there are variations in the brain region anatomy between different animals, wrapping the section from different animals to a common coordinate space is a prerequisite to compare its functional properties (Jupp and O'Brien, 2007). The PET images were co-registered over the above brain atlas for this purpose. A surface mesh of the cortex, created from the segmented MR images, was interactively positioned inside the PET images by applying a rigid transformation. Then, an iterative rigid to elastic deformation scheme was used to deform the mesh toward the brain cortex of the PET images. Once the surface mesh was fitted on the outer border of the cortex, a match for every vertex of the mesh was obtained between its original and final position, giving a set of translation vectors.

An interpolation based on Shepard's inverse distance weighting was used to generate a translation vector field over the entire PET dataset. This translation vector field was applied to a deformable grid having the same dimension as the MRI dataset. Once the rigid and non-rigid transformations were applied to the grid, sampling the PET scan at every final node position by cubic interpolation successfully overlaid a deformed PET image over the segmented MR images (Fig. 1c). Finally, a correction factor was computed from the FDG-PET signal contained inside the whole brain prior to and after co-registration.

For each cat brain, mean signal values were computed from defined ROIs of the co-registered PET data, and expressed as standardized uptake values (SUV). For each imaging session, a calibration phantom was used to convert image counts to radioactivity concentration.

Statistical method

Data are reported in reference to a control region as a Ratio = $SUV_{ROI}/SUV_{Superior\ temporal\ cortex}$, and analyzed two-sided using a Wilcoxon–Mann–Whitney exact test with the α value set at 0.005 (Bonferroni correction for multiple comparisons).