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Brain Maps on the Go: Functional Imaging During Motor Challenge in Animals

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Abstract

Brain mapping in the freely-moving animal is useful for studying motor circuits, not only because it avoids the potential confound of sedation or restraints, but because activated brain states may serve to accentuate differences that only manifest partially while a subject is in the resting state. Perfusion or metabolic mapping using autoradiography allows one to examine changes in brain function at the circuit level across the entire brain with a spatial resolution (~100 microns) appropriate for the rat or mouse brain, and a temporal resolution (seconds – minutes) sufficient for capturing acute brain changes. Here we summarize the application of these methods to the functional brain mapping of behaviors involving locomotion of small animals, methods for the three dimensional reconstruction of the brain from autoradiographic sections, voxel based analysis of the whole brain, and generation of maps of the flattened rat cortex. Application of these methods in animal models promises utility in improving our understanding of motor function in the normal brain, and of the effects of neuropathology and treatment interventions such as exercise have on the reorganization of motor circuits.

Keywords

Brain Mapping; neuroplasticity; neurorehabilitation; motor activity; sport sciences

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Introduction

Functional brain imaging has in recent years increasingly been applied to studying brain function related to motor activity. Mapping neural circuits aids in understanding the role primary and alternate circuits play in the performance of specific motor tasks, and here represents a promising approach for increasing our understanding in the fields of sports sciences and neurorehabilitative medicine. Recent attention has been drawn to the fact that the type of task and the way it is performed has different effects on the brain. For instance, Lewis et al. (1) and others (2-5) have highlighted the dominance of the cerebellar-thalamic-cortical circuit during the performance of externally guided motor movement, and contrasted this with the dominance of the basal ganglia-thalamic-cortical circuit during the performance of internally guided motor movements. Past studies (6-8), as well as our own work (9) have highlighted the fact, that in response to brain lesions, there is both increased reliance on remaining neurons within damaged circuits, as well as new recruitment of alternate circuits. Imaging can also provide information about neurotransmitter release within specific circuits, and has been extensively developed for the study of dopamine transmission at D2 receptors. For example, Goerendt et al (2003) have used ^{11}C -raclopride positron emission tomography (PET) to investigate levels of striatal dopamine release in healthy volunteers and early unilateral Parkinson's Disease (PD) patients while performing simple sequential finger movements (10).

Functional brain imaging can yield biomarkers for evaluating brain changes over time, either in response to disease or in response to neuroactive interventions, including exercise. During recovery from brain damage, imaging can help evaluate the presence of diaschisis (loss of function due to cerebral lesions in areas remote from the lesion but neuronally connected to it), functional redundancies, sensory substitution and morphological changes. In response to exercise, imaging can help in addressing basic questions such as “Does exercise training result in a neural remapping of cerebral function, and if so, where in the brain is this occurring?” In addition, brain mapping may provide useful endpoints for defining parameters for exercise training as a treatment intervention in specific neurodegenerative (11,12) or mood disorders (13,14). Currently there is no systematic investigation on the relationship between exercise and dynamic brain activation, what parameters constitute ‘effective’ training (type, duration, frequency, intensity, voluntary versus forced), and what is the persistence of any changes upon discontinuing exercise. Future identification of brain regions demonstrating changes in neural activation may provide guidance for establishing specific rehabilitation strategies for patients, as well as providing the basis for studies targeting molecular mechanisms.

A powerful means of testing motor circuits is to examine the effects acute motor challenges have on functional brain activation. Such activated brain states may serve to accentuate differences that only manifest partially while a subject is in the resting state. For instance, brain mapping performed during motor challenges has been performed in early PD (15-17) and in animal models of PD (9), and here has shown utility in unmasking underlying differences between normal and pathological neural circuits.

Undertaking to answer these questions in an animal model, rather than in a clinical population, provides distinct advantages. It obviates problems related to recruitment, compliance and retention of patients. Functional neuroimaging can also be performed in the freely-moving animal during a locomotor challenge, which presents significant technical challenges in clinical studies. Finally, animal studies allow correlation between motor functional testing, functional brain activation and histologic outcomes.

In the past, functional brain activation has been studied in nontethered, freely moving animals with a variety of modalities. Brain electrical recordings using radiotelemetry offer the

advantage of detailed information regarding the temporal and spatial synchronization of neural processes, however, only limited cortical and subcortical areas can be mapped electrophysiologically in a single subject. Brain functional activation in animals has also been explored at the cellular level using measurement of changes in c-fos, an early gene product (18-20), 5-³H-uridine uptake (21,22), a nucleic acid important in neuronal RNA synthesis, or cytochrome oxidase, a mitochondrial enzyme (23-26). These indirect markers of neural activity provide excellent spatial resolution, however, the temporal resolution is very poor, requiring long-lasting stimulation. Spatial resolution, with micro-PET and advanced image reconstruction software, remains at best ~1.6 mm at the center of the field of view. This represents ~10% and 18% of the width of the rat and mouse brain, respectively and is poorly suited for the detection for all but the broadest changes in regional cerebral blood flow (CBF) or metabolism (CMR). Functional magnetic resonance imaging (fMRI) and single photon emission computed tomography (SPECT, microSPECT), though they provide whole brain analysis and adequate spatial and temporal resolution, require sedation of the animal, limiting the behaviors that can be examined. We have made use of perfusion or metabolic mapping using autoradiography for the brain mapping in small animals. Autoradiographic methods allow one to examine changes in brain function at the circuit level across the entire brain, with a spatial resolution (~100 microns) appropriate for the rat or mouse brain, and a temporal resolution (seconds – minutes) sufficient for capturing acute brain changes.

To facilitate brain mapping during locomotor activity, our laboratory has developed a self-contained, fully implantable miniature infusion pump (MIP) that in small animals (27,28) allows bolus injection of pharmacologic agents by remote activation. The ability to trigger this pump by remote activation has allowed us to rapidly inject cerebral blood flow tracers in the nonrestrained, nontethered animal, thereby allowing the brain mapping of behaviors involving locomotion. Currently, no such device exists on the market, with osmotic (Alzet, Durect Corp., Cupertino, CA), elastomeric (VIP, Advanced Neuromodulation Systems, Plano, TX), electrolytic (Infu-Disk, Med-e-cell, San Diego, CA), peristaltic (iPRECIO, Primetech Corp., Tokyo, Japan; Pegasus, Instech Laboratories, Inc., Plymouth Meeting, PA) minipumps providing only slow infusion rates, without the ability for user-initiated remote activation. We have validated the MIP as a tool for functional neuroimaging in rats in which a perfusion tracer such as ¹⁴C-iodoantipyrine is administered with the animal in the freely-moving state, and brain mapping of the regional distribution of cerebral blood flow occurs in the cryosectioned brain using autoradiography. Generation of functional images of motor tasks (29,30), of auditory center activation in response to an acoustic challenge (31) and limbic areas during conditioned fear memory (32) has provided strong evidence of the unique ability of the MIP to produce “snapshots” of the brain functional activation during a behavior. The MIP has provided a new tool for functional neuroimaging, and opened opportunities for performing “brain scans on the move” (<http://www.nibib.nih.gov/publicPage.cfm?pageid=657>). To allow us to analyze and display autoradiographic data obtained with the MIP, we have developed methods for the three dimensional reconstruction of the rat brain from autoradiographic sections, voxel based analysis of the whole brain, and generation of perfusion maps of the flattened rat cortex. Below, we summarize methods related to use of the MIP in mapping locomotor behavior, as well as methods for the subsequent data analysis.

Methods

Standardizing complex behaviors during brain mapping

Animals of uniform age, strain and gender are chosen. Because motor behaviors involve multisensory input to the animal, standardization of not only the motor paradigm (e.g. speed, duration, etc.), but also of the experimental environment is necessary. Ambient light levels and sound levels should be kept low and standardized using a hand-held light meter (Control Co.,

TX) and sound meter (Radioshak, TX). Olfactory cues are minimized by wiping the experimental arena with water and alcohol. Time of day, duration of testing, room temperature is standardized. No entry in and out of the experimental room by staff should be permitted during the imaging.

Care must be taken to define appropriate controls such that group differences in brain activation can be ascribed to the motor paradigm itself, and not to differences in the animal's mental state (e.g. habituation, novelty, anxiety) that could be eliminated through standardization of the protocol. To overcome the animal's natural fear and anxiety, rats are equally handled prior to behavioral testing, and are familiarized with the paradigms prior to the start of the experiments. "Novelty" effects are avoided by prior exposure to the behavioral paradigm on several occasions. On the day of experimentation, animals are habituated to the experimental room for 1 hour prior to testing, and then again for 45 minutes after loading of the radiotracer into the MIP. Use of detailed kinematic scoring may be useful for providing an independent measure of the uniformity of the motor challenge and in evaluating behavioral compensation strategies that may occur during recovery from damage to the nervous system. Such measures can be introduced as covariates in the brain mapping analysis to evaluate the role alternate behavioral strategies may play in eliciting alternate patterns of activation. Kinematic measures can be obtained by video recordings with a high speed video camera (1000 frames/sec) during the period of tracer injection (10 seconds), as well as during an earlier baseline period. Offline analysis of such behaviors can occur using the Observer (Noldus, Inc.), a software program that could be used for the manual coding of behaviors, for instance, footslips, dominance of hindlimb versus forelimb, and carry of the limbs and posture of the animal. Alternatively, more detailed kinematic analyses can be provided by programs such as Motus (Vicon, CA), Catwalk or Ethovision (Noldus, Inc., VA) depending on the application.

Microbolus infusion pump

Details of the design and fabrication of the first and second generation of the MIP have been published (27,33). The MIP consists of an intravenous catheter, a silicone embedded electronics controller remotely activated by a photodetector that responds to trains of light pulses (30 KHz frequency) and controls a normally-closed miniaturized solenoid valve, an ejection chamber containing the radiotracer, and a silastic reservoir containing a euthanasia solution. The photodetector with peak spectral sensitivity in the near infrared (NIR) spectrum of wavelengths allows for transcutaneous triggering of the pump with trains of light pulses from external NIR LEDs in the experimental room. Upon triggering, the microvalve opens, allowing the hydraulic pressure from the reservoir to release first the radiotracer into the animal's circulation, followed a few seconds thereafter by a euthanasia agent (1.0 ml pentobarbital 50 mg/kg, 3 M potassium chloride). A batteryless design (24.5 g implant weight) allows electrical power to be applied to the pump within a behavioral cage surrounded by an emitter inductive coil driven by a novel Class E oscillator (Figs. 1C, 1D), while a rechargeable battery operated pump (32.5 g implant weight) allows activation of the pump outside the inductive coil. Efficacy of the rechargeable pump has been demonstrated with animals moving freely in a large 1.25 m diameter circular maze (Barnes maze), with optical triggering occurring by means of five LEDs mounted on each of the surrounding walls and ceiling of the room (personal communication).

Surgical implantation of the MIP

Rats are anesthetized with isoflurane (3.0% induction, 1.2% maintenance). The ventral skin of the neck is aseptically prepared and the right external jugular vein is catheterized with a blunt-tipped, 3.5-5 French silastic catheter, advanced 3.5 cm into the superior vena cava. Placement of the catheter into a large vessel allows blood flow to be maintained around the catheter tip (important for minimizing thrombosis), while advancing the tip to approximately 5 mm above

the right atrium allows delivery of the infusate close to the heart (important for rapid euthanasia), without the added risk of catheter fibrosis if the tip is placed inside the contracting organ. The catheter is tunneled through the subcutaneous space to the back and connected to the MIP situated subcutaneously in the infrascapular region. To prevent shifting of the MIP, the pump's lateral edges are sewn to the overlying skin by means of four o-rings and the skin is closed over the implant, except around the pump's percutaneous access port. The percutaneous port allows for postoperative flushing of the catheter to ensure patency, and it allows for loading of the radiotracer 40 minutes prior to imaging, which avoids the housing of radioactive animals. After implantation of the MIP, rats are returned to single housing. Postoperatively catheters are flushed every 2 days with 0.8 ml of 5 U/ml heparin (0.9% normal saline) followed by a replacement of the dead volume of the catheter (~ 50 μ l) with taurolidine-citrate (Strategic Applications, Inc., IL), a catheter lock solution that promotes catheter patency by acting as an anticoagulant and antimicrobial.

Loading the radiotracer into the MIP

On postoperative day 4-7, the animal is immobilized for 5 minutes in a rodent restrainer (Decapicone, Kent Scientific, CT). The MIP is loaded through the percutaneous port with the perfusion tracer, [14 C]-iodoantipyrine (100-125 μ Ci/kg in 300 μ l of 0.9% saline, American Radiolabelled Chemicals, MO) (Fig. 1A). Thereafter, a euthanasia solution (1.0 mL of pentobarbital 75 mg/kg, 3 mol/L potassium chloride) is loaded transcutaneously into the reservoir. After removal from the restraining device, animals are allowed to recover undisturbed in a quiet environment for 40 minutes in a transport cage.

Injection of the CBF radiotracer, [14 C]-iodoantipyrine

Rats are then exposed to the behavioral paradigm (e.g. treadmill walking, free running in a behavioral maze). Triggering of the pump with a sequence of infrared light pulses occurs after a 0.5-2 minute exposure. Immediately after bolus injection of [14 C]-iodoantipyrine, the euthanasia solution is injected into the circulation system (Fig. 1B). Rapid euthanasia is needed to prevent nonspecific diffusion of the tracer (34). Injection results in cardiac arrest within ~10 seconds, a precipitous fall of arterial blood pressure, termination of brain perfusion, and death. This 10 second time window provides the temporal resolution during which the distribution of CBF-TR is mapped (27). Cerebral blood flow related tissue radioactivity (CBF-TR) is measured by the classic [14 C]-iodoantipyrine method (35-37). In this method there is a strict linear proportionality between tissue radioactivity and cerebral blood flow when the radioactivity data is captured within a brief interval (~10 sec.) after the radiotracer injection (38,39).

Autoradiography

Brains are rapidly removed, flash frozen in dry ice/methylbutane (-55°C), embedded in OCT™ compound (Miles Inc., Elkhart, IN), and stored in a freezer at -70°C. Brains are subsequently cut in a cryostat (Microm HM550, Microm International, Walldorf, Germany) at -18°C in 20 micron coronal sections with a uniform interslice distance determined by the structures to be examined (usually 100 to 300 microns). Slices are thaw mounted on glass slides, heat dried at 55 °C and exposed for 2 days at room temperature to Kodak Biomax MR film in spring-loaded x-ray cassettes along with radioactive 14 C standards (Amersham Biosciences, Piscataway, NJ). Important in the digitizing of autoradiographs (Fig. 2) is that the field of illumination is homogeneous and stable. Autoradiographs are placed on a voltage stabilized light box with diffuser plate and concentric circular 'natural daylight' fluorescent bulbs (Northern Lights Illuminator, InterFocus Ltd, England). Images are recorded with a Retiga 4000R charge-coupled device monochrome camera (Qimaging, Canada) and a 60 mm Micro-Nikkor macro lens (Nikon Inc., USA), digitized on an 8-bit gray scale using Qcapture Pro 5.1 (Qimaging,

Canada) using an ATI FireGL V3100 128 MB digitizing board on a microcomputer. Alternate methods include scanning in of autoradiographs, preferably with a scanner that illuminates film from above by a moving line of LEDs while scanning film from below (e.g. Epson 1000 XL, Epson, USA). However, the user needs to be aware of the potential for distortions caused by Newton rings (interference patterns caused by the reflection of light between the film/glass interface) and film warping (i.e. film not flat on scanner bed).

Data Analysis

Significant group differences in regional CBF-related tissue radioactivity (CBF-TR) are determined by statistical parametric mapping (SPM) and region-of-interest (ROI) analysis, which provide complementary approaches for the exploration of brain function of complex behaviors.

Effectiveness of SPM analysis of whole brain data sets is limited by the accuracy of proper reconstruction prior to analysis of the brain's coronal sections into a three-dimensional volume (40). These include potential errors introduced by artifacts in the coronal sections, as well as the propagation of errors due to misregistration of preceding slices. Prior to slice registration it is important that in the data set all brains have the same number of slices anterior to an internally defined landmark (e.g. fusion of the anterior commissures), and likewise posterior to this landmark. Several registration methods and algorithms have been developed, which differ mainly in the image features used to establish a measure of similarity between corresponding slices and the extent of required user-interaction. Registration methods for autoradiographs have used, for example, artificial landmarks (41), external section contour (42), principle axes transformation (43,44), consistent matrix transformation (43,45), or multi-modal warping based on mutual information metric as a mapping cost function (46). We have chosen to employ TurboReg (<http://bigwww.epfl.ch/thevenaz/turboreg/>) an automated pixel based registration algorithm (47) in which each section is sequentially registered to the previous section and then used as a reference for the subsequent section. This non-warping geometric model includes rotations and translations (rigid-body transformation), and nearest-neighbor interpolation. Since performance of the automated registration is imperfect in the anterior and posterior-most brain, in these regions alignment is performed manually using Adobe Photoshop CS (Adobe Systems Inc., USA). Small misalignments are in part also adjusted for by spatial normalization of each brain to a template using affine and nonlinear transformations, and smoothing of the data within SPM. In addition, proper adjustment of the minimum cluster-size (i.e. contiguous voxels) required for significance helps prevent false positives related to slice misalignment. Detailed methods on the alignment of the serial coronal slices into a 3D brain, and subsequent creation of the brain template, spatial normalization, optical density normalization and implementation of SPM in the rat brain have been previously published (30).

Statistical Parametric Mapping (SPM)—SPM (48,49), which was introduced in 1991, (Wellcome Dept. of Cognitive Neurology, Institute of Neurology, London, UK), is a collection of MatLab tools available in the public domain for basic visualization and analysis of neuroimages (<http://www.fil.ion.ucl.ac.uk/spm/>). It includes tools for automated non-linear spatial normalization, coregistration, spatial smoothing, as well as others. SPM was developed for analysis of imaging data in humans and has been recently adapted by us for use in rat brain autoradiographs (30) and confirmed by others (50,51). This non-biased, semi-automated, voxel-by-voxel analysis of whole-brain activation is used for detection of significant changes in functional brain activation that might be difficult to predict apriori. For brain structures that display irregular borders or a mosaic organization of their afferent and efferent projections, defining appropriate regions-of-interest (ROIs) is typically difficult, and exact placement of the ROI can be challenging. SPM, because it establishes significance at the voxel level, rather

than with a user-defined ROI, allows improved detection of activation in geometrically complex regions. Experimental designs including categorical, parametric and multifactorial designs, can be accommodated, as well as small volume corrections. Significance is established at the level of individual voxels or clusters of contiguous voxels. Because SPM is a whole-brain analysis, a correction for multiple comparisons is applied using Gaussian Random Field Theory (52). This correction is less conservative than a Bonferroni correction for independent statistical tests, and for an anatomically open hypothesis (i.e. a null hypothesis that there is no effect anywhere in the brain), is much more sensitive. Results are typically displayed as color-coded statistical parametric maps superimposed on the brain coronal, transverse and sagittal slices, or else visualized with three dimensional viewing programs such as MRICron (<http://www.sph.sc.edu/cmd/rorden/>) (Fig. 3). Brain regions are identified using an anatomical atlas of the rat brain (53).

Region-of-interest analysis—Significance differences on the SPM analysis, are confirmed using an ROI analysis. This analysis is free of the potential error that may be introduced by the spatial normalization and three-dimensional slice alignment required for the SPM analysis, though it may introduce the user's subjective judgment in making ROI selections. The average optical density is measured for individual predefined or user-circumscribed ROIs (e.g. Image Pro Plus, Media Cybernetics, Bethesda, MD, or other software) within a slice. A Z-score transformation is performed on the tissue radioactivity data to produce patterns of regional tracer concentrations for each animal (54). This transformation eliminates variations in mean tracer distribution between subjects and experimental groups created by global effects on vascular smooth muscle and systematic experimental error. Group differences in regional CBF-TR are compared using *t* tests (unpaired, two-tailed, $P < 0.05$). In addition to the analysis on the transformed data, analysis is also done on the nontransformed data for each location, each slice (all locations in a given slice) and globally (all locations in all slices). For cerebral cortical data, we have developed a method for measurement, analysis and display (55). In this method, regions-of-interest (ROI) are selected in the cortical mantle in a semiautomated fashion using a radial grid overlay, spaced in 15° intervals from the midline. ROI measurements of intensity are mapped on a flattened two-dimensional surface (Fig. 4). Topographic maps of statistical significance at each ROI allow for the rapid viewing of group differences. Cortical z-scores are displayed on the cortical flat maps using a continuous colorimetric scale, with superimposed boundaries of brain regions defined according to a standard atlas of the rat brain (53).

Discussion

Many fundamental mammalian behaviors involve locomotion. Use of the MIP allows brain mapping to proceed in freely-moving, small animals without sedation, restraint or tethers. This is a substantial advantage in studying certain animal behaviors such as climbing, maze running, movement within an 'enriched' environment, as well as aggression, mating or social behaviors. In these paradigms use of the MIP to administer a radiotracer avoids risks associated with administration via tethered catheters, in particular entanglement of the animal with its tether, as well as reshaping by the tether of the behavior itself. However, for motor behaviors that are more restricted or repetitive (e.g. Rotarod treadmill running, reaching and grasping tasks) injection of the tracer through a tethered catheter may be an alternate solution.

Given the dynamic nature of behavior (e.g. (56-58)), an important variable in brain mapping is the timing of the injection of the tracer. Thus, for instance, in our own studies with [¹⁴C]-iodoantipyrine, we have noted that in rats during treadmill walking when the tracer was injected at 1 minute (Fig. 4) versus 2 minutes (29) or 6 minutes after exposure, differences in the functional brain activation appeared. While activation of motor circuits remained robust at all times, increased activation was observed in the early compared to the late administration in

olfactory, piriform and secondary motor cortex, as well as the amygdaloid area. The increased activation suggests that immediately following placement of the animal on the treadmill, there is increased attention being focused on olfactory novelty, its associated exploratory behavior and emotional arousal. Possible attenuation of the activation of motor cortex may also have occurred at the later time due to habituation.

Rat and human motor systems share many features, including connectional similarities and similarities in the general distribution of chemical markers, as well as similarities in development (59-61). Although quadrupedal gait may in principle involve activation of different neural circuits than bipedal gait, our previous work mapping CBF during treadmill walking in normal, nonlesioned rats showed activation during the task of motor circuits (primary motor cortex, dorsolateral striatum, ventrolateral thalamus, midline cerebellum), in primary somatosensory cortex mapping the forelimbs, hindlimbs and trunk, as well as in secondary visual cortex (5,9,29,30). These results in rats concur with work in humans that demonstrates during walking increases of regional cerebral blood flow in the supplementary motor area, medial primary sensorimotor area, the striatum, visual cortex and the cerebellar vermis using single photon emission computed tomography (62,63). Nevertheless, differences do exist and need to be considered in extrapolating from rats to humans. For example, there are differences in the proportional volume of individual brain structures, differences in the anatomic distribution of tyrosine hydroxylase staining neurons within the dopaminergic substantia nigra (SN), and differences in the anatomic continuity of the globus pallidus (59). Furthermore, there are interspecies differences in the morphology of dopaminergic SN neurons, which may result in differences in input resistance and firing patterns between primates and rats (64).

One of the disadvantages of autoradiography is that it is a terminal procedure. Euthanasia must be rapid to prevent nonspecific diffusion of the [^{14}C]-iodoantipyrine (34), and by necessity cryosectioning of the brain is required. For studies choosing to use PET/microPET or SPECT/microSPECT to image brain function, the problem of behavioral restraint can be solved and euthanasia avoided if radiotracers are administered by non-agitating means, and regional brain activation is imaged after completion of the behavioral task and capture of the tracer (65). Since imaging of brain metabolism using a tracer such as radiolabelled 2-deoxy-D-glucose (2-DG) typically takes place after tracer uptake is complete and relatively imperturbable, this method is suitable for neuroimaging in nontethered, ambulatory subjects. This approach has been applied to functional brain mapping in humans during treadmill walking using [^{18}F]-fluoro-2-deoxyglucose ([^{18}F]-FDG) PET (66,67). The primary drawback of radiolabelled 2-DG is that the duration of the uptake and capture of the tracer is around 25-45 minutes (68), which is suboptimal given the fact that many behaviors are more short-lived, and that prolonged exposure may elicit habituation in functional brain activation. In principle, delivery of a radiotracer that reaches a cerebral equilibrium in a shorter time frame than 2-DG, would allow imaging of behaviors with a greater temporal resolution.

A tracer extracted and captured intracellularly, similar to 2-DG for PET applications is the copper(II) complex of pyruvaldehyde bis(N4-methylthiosemicarbazone), Cu-PTSM. This tracer is extracted and captured intracellularly, similar to [^{18}F]-FDG but in a substantially shorter time frame (2 minutes versus 45 minutes for [^{18}F]-FDG). When labeled with ^{61}Cu ($t_{1/2}=3.3$ h), ^{64}Cu ($t_{1/2}=12.7$ h), or ^{67}Cu ($t_{1/2}=58.5$ h), Cu-PTSM might be appropriate for neuroimaging in freely moving subjects (65). Relevant tracers for SPECT applications include Technetium-99m-hexamethylpropyleneamine oxime ($\text{Tc}^{99\text{m}}$ -D,L-HMPAO or exametazime; radioactive $t_{1/2}=6.03$ h), a commercially available tracer (Ceretek, Nycomed-Amersham, Little Chalfont, UK) that has been used to study brain activation in freely-moving human subjects during walking (62) and during cycling on a stationary bicycle (69). Here uptake of the tracer is within 1 minute, and imaging can occur within 2 minutes to 4 hours after injection.

An alternate flow tracer for SPECT applications is the ethylcysteinate dimer [$\text{Tc}^{99\text{m}}\text{-L,LECD}$] bicesate ($\text{Tc}^{99\text{m}}\text{-ECD}$, Neurolite, Dupont-Pharma, Stevenage, UK). With this agent imaging can take place between 5 min – 2 hours following injection. ECD has been applied to functional neuroimaging during cognitive testing (70,71) and bicycling (72), in which administration of the tracer occurred outside of the scanner environment, and subjects were taken to the scanner after uptake of the tracer was complete.

We believe that imaging during motor challenge tests, analogous to a ‘stress test’ in cardiology, is a powerful tool for mapping motor circuits and in unmasking deficits early in the course of neurodegenerative diseases. Clinically, brain imaging may be helpful for the study of brain activation during motor activity in deficits syndromes such as Parkinson's disease (15-17), or in related fields such as for the study of abnormal motor movements (e.g. akathisia, tardive dyskinesia, chorea, tics, stereotopies). Future application might include the exploration of primary and alternate motor and sensory circuits in the brain during different types of exercise (isotonic, isometric, endurance, skilled training, etc.), the definition of what parameters constitute effective exercise (duration, frequency, intensity, passive versus active, discontinuation, variability, etc.), the role of unilateral motor interventions in producing contralateral adaptations (cross education)(73), constraint-induced movement therapy (74), or the cross-modality transfer of motor learning in multisensory training programs (75). Here the MIP may serve as a useful adjunct to allowing tracers to be injected in animals free of restraint and tethers.

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Fig. 1. Implanted Microbolus Infusion Pump

(A) With the valve of the electronics module (blue) in the closed position the radiotracer (green) is backloaded through the percutaneous port. Thereafter, the euthanasia agent (yellow) is loaded transcutaneously into the implanted elastomeric reservoir. (B) Triggering of the valve allows serial forward injection (i.v) of first, the radiotracer, then the euthanasia agent, (C) Rat cage with with surrounding inductive primary coil for use in a fear conditioning paradigm, (D) The implanted pump is triggered by inductive power transfer. An LED gives visual feedback through the skin of the opening of the valve.

Fig. 2. Representative coronal autoradiographs of a rat with a unilateral striatal lesion or a sham-lesion during walking (bottom row) or at rest (top row)

Optical densities (pseudocolored) reflect variations in cerebral blood flow related regional tissue radioactivity (rCBF). The left side of the autoradiographs represents the lesioned side of the brain. Whereas sham-lesioned animals show a robust bilateral activation of motor cortex (arrows), lesioned rats show diminished rCBF in the motor cortex in the lesioned hemisphere, as well as some more subtle attenuation in the dorsal striatum (double arrows).

Fig. 3. Effects of 6 weeks of treadmill training on subsequent functional brain activation during a locomotor challenge

Regions of statistically significant activations (red scale) and deactivations (blue scale) are shown between exercised ($n = 10$) and nonexercised ($n = 10$) rats in the three-dimensionally reconstructed brain (reproduced with permission from (5)). 2Cb, 3Cb (2nd and 3rd cerebellar lobules), CA3 (hippocampus CA3 region), Cg1 (cingulate cortex area 1), CPu (striatum), DG (dorsal geniculate of the hippocampus), M1, M2 (primary, secondary motor cortex), MD (medial thalamic nucleus), RS (retrosplenial cortex), S2 (secondary somatosensory cortex), SC (superior colliculus), Sim A (simple lobule of the cerebellum).

Fig. 4. Cortical flat maps of the color-coded average Z-score differences for rats during treadmill walking (n = 9) or rest (n = 9)

Functional brain activation is shown during treadmill walking, i.e. $Z\text{-score}_{\text{Motor}}$ minus $Z\text{-score}_{\text{Rest}}$, when the tracer, [^{14}C]-iodoantipyrine, was injected 1 minute after exposure to the paradigm. Superimposed on the maps are the borders of the main cortical areas: A, cortical amygdaloid nucleus; Au, auditory; FrA, frontal association; I, insular; Ent, entorhinal; M1, primary motor; M2, secondary motor; OF, orbital frontal; PtA, parietal association; Pir, piriform; PRh, perirhinal; RSA, retrosplenial. Primary somatosensory mapping: S1FL, the forelimbs; S1HL, the hindlimbs; S1TR, the trunk; S1BF, the barrel fields; S1J/S1UL, the jaw, lip, and oral region; S2, secondary somatosensory; TeA, temporal association; Tu, olfactory; V1, primary visual; V2, secondary visual (53).