MicroPET Investigation of Chronic Long-Term Neurotoxicity from Heavy Ion Irradiation

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ABSTRACT

Positron emission tomography (PET) permits imaging of the regional biodistribution and pharmacokinetics of compounds labeled with short-lived positron-emitting isotopes. It has enabled evaluation of neurochemical systems in the living human brain, including effects of toxic substances. MicroPET devices allow studies of the rat brain with a spatial resolution of ~2 mm. This is much poorer resolution than obtained using ex vivo autoradiography. However, animals need not be euthanized before imaging, so repeat studies are possible. This in principle allows the effects of toxic insults to be followed over the lifetime of an individual animal. We used microPET to evaluate brain metabolic effects of irradiation with high-energy heavy ions (HZE radiation), a component of the space radiation environment, on regional glucose metabolism. A significant fraction of neurons would be traversed by these densely ionizing particles during a Mars mission, and there is a need to estimate human neurological risks of prolonged voyages beyond the geomagnetosphere. Rats were irradiated with ^{56}Fe (600 MeV/n) ions at doses up to 240 cGy. At 9 months post-irradiation we did not detect alterations in regional accumulation of the glucose analog [^{18}F]2-deoxy-2-fluoro-D-glucose. This may indicate that damage to the brain from HZE particles is less severe than feared. However, because radiation-induced alterations in some behaviors have been documented, it may reflect insensitivity of baseline cerebral glucose metabolism to HZE radiation. These studies will facilitate design of future studies of chronic, long-term exposure to both therapeutic and abused drugs using microPET.

KEYWORDS: MicroPET, FDG, neurotoxicity, radiation, brain

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INTRODUCTION

Exposure to drugs and other chemicals is often associated with adverse effects including undesired neurological sequelae and in some cases long-term neurotoxicity. Organophosphate ester poisoning is a well-known example.[1] Well-documented examples for therapeutic drugs include tardive dyskinesia after prolonged administration of neuroleptic drugs.[2] There are also established examples of brain damage associated with abused drugs, including encephalopathy in alcoholism,[3] focal infarcts in some cocaine abusers,[4] and in solvent abuse.[5] Problems in relating damage to drug abuse in human subjects can be difficult because of uncertainties in determining the intensity, duration, and pattern of drug consumption. Furthermore, some conditions associated with drug abusers may be predisposing conditions rather than drug effects. This is especially so for relatively subtle psychiatric conditions rather than frank neurological problems.

PET and MicroPET

Positron emission tomography (PET) is a radionuclide imaging modality that was the first technology that enabled direct measurement of components of the neurochemical systems in the living human brain.[6] The most commonly used radionuclides are ^{11}C and ^{18}F. Because of the short half-life of ^{11}C (20 minutes) this has to be produced using a cyclotron and radiochemical laboratory in very close proximity to the PET laboratory. In contrast, ^{18}F has a long enough half-life (110 minutes) for transport of radiotracers over limited distances, and this has proved to be economically viable for radiopharmaceuticals such as [^{18}F]2-deoxy-2-fluoro-D-glucose (FDG) that have a high demand because of extensive clinical use. FDG measures local rates of glucose use since it is metabolically trapped as FDG-6-phosphate, and is therefore a marker of overall metabolic activity.[7] However, developments in medicinal radiochemistry over the past 25 years have brought us to the point where many positron-labeled radiotracers are available that allow various aspects of tissue neurochemistry and biochemistry to be evaluated in humans, primates, and even rats. To take the brain dopamine system as an example,

tracers include ^{18}F-labeled L-dihydroxphenylalanine (DOPA) for measuring the activity of aromatic aminoacid decarboxylase; dihydrotetrabenazine for the vesicular amine transporter, VMAT2; cocaine for the neuronal dopamine transporter; clorgyline for monoamine oxidase A; L-deprenyl for monoamine oxidase B; raclopride for dopamine D2 receptors and SCH23390 for dopamine D1 receptors.[8] Although PET studies of the dopamine system are the most advanced, radiotracers for probing other neurotransmitter systems are also available. For example, in the cholinergic system, nicotinic and muscarinic receptor radioligands are readily accessible, as well as tracers for the vesicular acetylcholine transporter and for acetylcholinesterase.[9]

PET is used extensively in clinical neurological research to study conditions such as parkinsonism.[10] It is also used in psychiatric research, especially schizophrenia and drug abuse.[11-13] In medical diagnosis, cancer applications dominate.[14] PET depends largely on the availability of radiotracers labeled with short-lived isotopes of which FDG is the most commonly employed. The recent development of microPET devices able to measure glucose metabolism and radioligand binding in rat brain has provided a research tool that could give quantitative measures of neurotoxicological effects of drugs and other factors.[15-17] In principle, the great advantage of microPET over traditional methods of imaging the rodent brain is that measurements do not necessitate euthanasia of animals. Thus, individual animals can be imaged for assessment of receptor levels or of metabolic activity either acutely or repeatedly. As a result, longitudinal studies are possible and subjects may be used "as their own control" or imaged sequentially with multiple tracers. MicroPET technology therefore appears to be a promising modality for investigations of long-term neurotoxicity.

To date, however, brain microPET studies in the realm of pharmacology have been limited to evaluation of acute or subchronic neurotoxicity. For example, Brownell et al[18] have evaluated effects on FDG uptake of 3-nitropropionic acid at 1, 28, and 120 days after initial exposure. They found variability in responses of individual animals to this neurotoxin, and reported that decreased FDG accumulation in striatum 1 day after exposure predicted behavioral and neurochemical effects at later times.

The Space Radiation Environment

During the past several years we have been involved in NASA-funded neurobehavioral and neuroimaging studies designed to detect possible effects on the brain of irradiation with high energy heavy ions (HZE radiation). The relevance of this work is that space travel beyond the Earth's protective magnetic field (for example, to Mars) will involve exposure of astronauts to irradiation by high-energy nuclei such as ^{56}Fe, which are a component of galactic cosmic rays. Although the radiation absorbed dose to the brain from this source is not high, these particles have high linear energy transfer (LET) and may irreversibly damage cells they traverse.[19] Exposure to HZE radiation may therefore cause progressive deterioration of brain function, adding to damage involved in normal aging.[20] It has been estimated that at least 7% of neurons in the central nervous system would suffer traversal by heavy ions during a Mars mission using current propulsion technology that would require more than 2 years in interplanetary space.[21] Astronauts cannot be effectively shielded from these densely ionizing particles, so that the risk of adverse effects on the brain are very real. Previous studies support the notion of damage, but are not definitive, especially with regard to long-term effects. For example, an often cited loss of 60% to 80% of the key enzyme in dopamine synthesis, tyrosine hydroxylase, in rat substantia nigra has never been substantiated in a peer-reviewed journal.[22] However, cell loss after HZE irradiation has been documented in well-conducted studies of the retina, which is considered to be part of the central nervous system.[23,24] Recent direct evidence for adverse health effects of heavy ion particles has come from studies of cataracts in members of the US astronaut corps. Cucinotta and colleagues[25] have documented a statistically significant relationship between cataract formation and service on space missions involving higher exposure to HZE radiation. Risk estimates for the impact on various aspects of human health of galactic cosmic rays are associated with large uncertainties.[26,27]

METHODS

Animals

Male Sprague-Dawley (200-225 g) rats were purchased from Taconic Farms (Germantown, NY) and used in this study. Animals were randomly selected to the 0, 120, or 240 cGy irradiation groups on arrival and housed in standard laboratory conditions with a 12-hour dark/light cycle, lights off at 10 am, in a temperature- and humidity-controlled room. The animals were kept in single cages with restriction to food but not water. All procedures related to the use of the animals in this study were conducted based on fundamentals in the Institute of Health Guide for the Use of Animals Laboratory and with all respect to the ethical issues regarding experimenting on animals. The study protocol was approved by the Brookhaven National Laboratory (BNL) Institutional Animal Care and Use Committee.

NASA Space Radiation Laboratory

Animals were irradiated at 0, 120, or 240 cGy of 600 MeV/n ^{56}Fe at the NASA Space Radiation Laboratory (NSRL)

during September 2004. The beam of heavy ions was collimated to focus primarily on the head region. The dose rate was such that animals were in the beam for approximately 2 minutes. Before irradiation, anesthesia was induced using a 4% isoflurane/oxygen mixture and maintained on a 2% mixture. Unconsciousness was then maintained on 2% isoflurane in oxygen.

MicroPET

At 6 and 9 months after irradiation, each rat was imaged using a microPET R4 scanner manufactured by Concorde MicroSystems (Knoxville, TN). Animals were given IV injections of FDG (~0.7 mCi) via the lateral tail vein and placed in a novel environment (a plexiglass box) for 40 minutes. During this uptake period FDG enters the brain and is converted to FDG-6-phosphate, which is metabolically trapped and reflects regional rates of glucose consumption. Animals were then anesthetized with ketamine/xylazine (100 mg/kg/10 mg/kg) and positioned in the microPET for 20 minutes. Following the scan, blood plasma was prepared and a 10-µL aliquot was assayed for radioactivity in a well counter. Images were coregistered and uptake in regions of interest were evaluated using PMOD version 2.6 software (PMOD Technologies Ltd, Zurich, Switzerland) according to a manually constructed template.

Autoradiographic Imaging

In addition to microPET, brain uptake of FDG in some animals was evaluated autoradiographically. After decapitation of anesthetized rats, brains were removed and 300-µm sections were prepared using a vibratome. These were transferred to microscope slides and placed on a slide warmed for 60 minutes. The dried slides were apposed to phosphor imaging plates for 2 hours. The plates were scanned and images displayed using a Perkin-Elmer Cyclone phosphorimager (Wellesley, MA).

RESULTS

Representative microPET images are shown in Figure 1. Mean standardized uptake values (SUVs) at 6 months and 9 months for striatum, thalamus, and hippocampus for the 3 irradiation groups, together with averaged tissue-to-global uptake ratios, are displayed graphically in Figure 2. SUVs for a larger list of brain regions at the 9-month time point are given in Table 1. There were no significant differences across radiation dose (0, 120, or 240 cGy) or time of scanning (6 or 9 months). Furthermore, there were no significant ($P < .05$) group differences for global FDG uptake. There was a very weak trend toward higher global FDG uptake in the 240-cGy group at 9 months compared with 6 months (21% increase; $P < .2$).

Autoradiographic images of sections of brains from representative animals did not show obvious group differences in patterns of FDG uptake. Sections are shown for each of the dose groups in Figure 3. Although of higher spatial resolution than microPET, these images have much poorer resolution than classical [^{14}C]2-deoxyglucose studies.

DISCUSSION

Behavioral studies by ourselves and others have not revealed large or obvious long-term effects on open-field behavior of moderate doses of HZE radiation. However, we have detected reductions in cocaine-stimulated ambulation and in performance in light-dark and auditory discrimination conditioning.[28-31] Other workers have also documented behavioral effects of HZE radiation.[32-36] Although one might expect behavioral effects to have neurochemical correlates, such as altered local rates of glucose metabolism, our microPET studies did not reveal any regional changes in

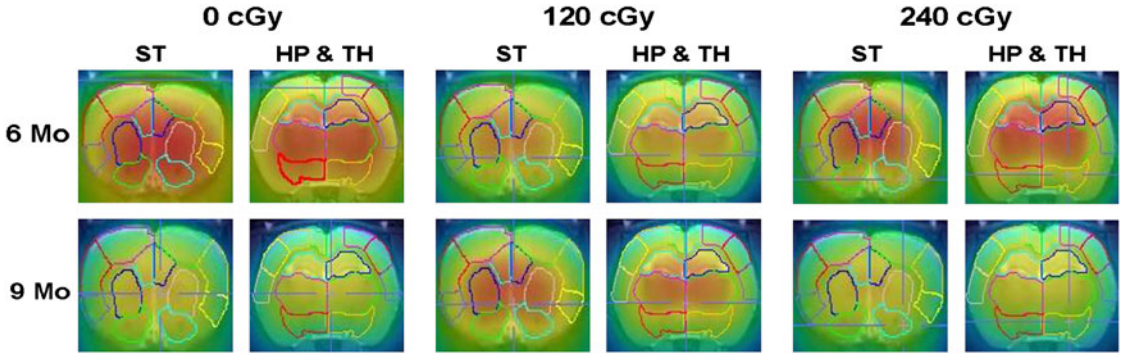

Figure 1. Representative coronal microPET images at the level of the striatum (ST) and of the thalamus (TH), and hippocampus (HP) after administration of [^{18}F]2-deoxy-2-fluoro-D-glucose. Animals were anesthetized with ketamine/xylazine and scanned 6 months and 9 months after irradiation with a 600-MeV 56Fe particle beam at doses of 0 cGy, 120 cGy, and 240 cGy.

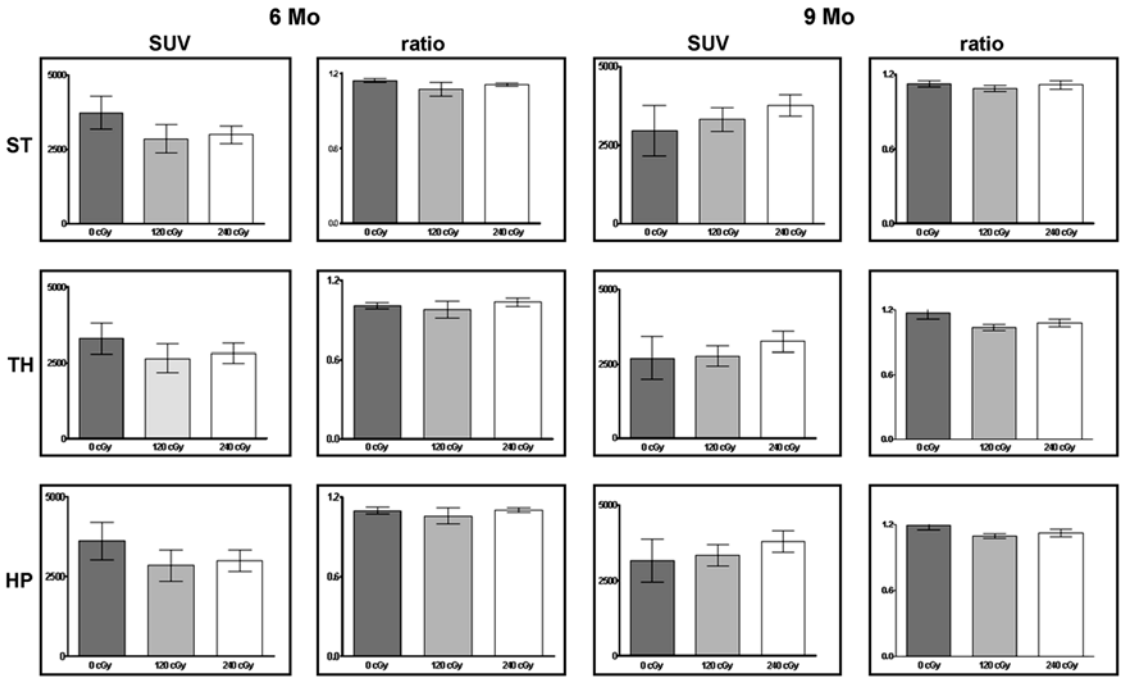

Figure 2. Regional brain standardized uptake values (SUVs) and tissue-to-global radioactivity ratios for [^{18}F]2-deoxy-2-fluoro-D-glucose in striatum (ST), thalamus (TH), and hippocampus (HP) at 6 months and 9 months post-irradiation. Each panel shows SUV or uptake ratio for (left-to-right) 0, 120, and 240 cGy groups.

FDG accumulation as a result of irradiation with ^{56}Fe particles. Possible reasons why no changes in microPET/FDG images were seen include the following:

1. The spatial resolution of microPET is approximately 2 mm, so that differences in small brain structures between control and irradiated rats might not be detected. One such structure is the nucleus accumbens, which is implicated in cocaine-stimulated locomotion.
2. The imaging studies were conducted under open-field conditions, where no overt behavioral effects of radiation were seen. It is possible that FDG patterns might differ between controls and irradiated rats if the animals were injected with cocaine, or conducted operant tasks during the 45-minute uptake period—conditions where behavioral differences were seen.
3. The studies may have been technically suboptimal. Indeed, rigorous FDG studies in human research subjects involve placement of arterial catheters, so that the local brain accumulation of FDG/FDG-6-phosphate can be quantified in terms of the input of FDG to the brain in arterial blood plasma. The autoradiographic [^{14}C]2-deoxyglucose method developed by Sokoloff[37] uses arterially cannulated rats. In our hands, however, there was unacceptable mortality associated with cannulation, probably in part because of the age of our animals. This was especially problematical when it was desired to cannulate animals 2 or more times to conduct longitudinal studies.
4. Other potentially confounding issues were present, including anesthesia, which was necessary both for head-only irradiation and for microPET imaging (and also for cannulation in some cases). Some studies have documented long-term effects of anesthesia on cognitive abilities of both animals and humans.[38-40] It is possible therefore that deficits from anesthesia could mask deficits as a result of radiation. Another possibility is that lack of a behavioral enrichment program for our animals could have resulted in research subjects in both control and irradiated groups whose motor and cognitive abilities are well below their potential. In other words, behavioral and imaging deficits might be better exposed if all animals were maintained in a more challenging environment. This possibility is supported by the recent microPET/FDG studies of Barbarich-Marsteller et al.[41] These workers showed that rats subject to moderate food deprivation and access to an exercise wheel exhibited altered patterns of FDG accumulation relative to control animals housed under standard conditions.

To date there are only a few published microPET studies that assessed neuroreceptor levels after chronic, long-term administration of drugs or other substances.[42-44] Thanos et al[42] reported in a microPET/[^{11}C]raclopride study that after 7 weeks of ethanol self-administration, ethanol-preferring rats maintained lower D2 receptor levels as compared with rats that drank very little ethanol over the same period of time. Rodriguez et al[43] using a rat model of Parkinson's

Table 1. Standardized Uptake Values at 9 Months Post-irradiation*

Brain Region	0 cGy (n = 5)	120 cGy (n = 8)	240 cGy (n = 8)
Right olfactory bulb	3900 ± 474	3510 ± 422	3840 ± 333
Left olfactory bulb	3886 ± 461	3486 ± 416	3853 ± 321
Right frontal region	2978 ± 321	2765 ± 342	2977 ± 275
Left frontal region	2914 ± 319	2670 ± 311	2918 ± 263
Right cingulate gyrus	4106 ± 361	3682 ± 424	4170 ± 376
Left cingulate gyrus	4070 ± 379	3667 ± 413	4160 ± 373
Right orbital cortex	4252 ± 440	3898 ± 453	4243 ± 420
Left orbital cortex	4068 ± 453	3849 ± 441	4154 ± 391
Right parietal cortex	2587 ± 279	2636 ± 348	2607 ± 243
Left parietal cortex	2562 ± 385	2413 ± 259	2582 ± 218
Right insular rhinal cortex	2823 ± 281	2783 ± 308	2871 ± 284
Left insular rhinal cortex	2692 ± 346	2667 ± 262	2878 ± 278
Right nucleus accumbens	3471 ± 350	3157 ± 376	3587 ± 330
Left nucleus accumbens	3457 ± 371	3148 ± 362	3504 ± 288
Left striatum	3854 ± 402	3468 ± 371	3932 ± 354
Right striatum	3493 ± 319	3174 ± 370	3593 ± 320
Right granular cortex	3256 ± 265	2990 ± 369	3356 ± 297
Left granular cortex	3257 ± 247	2989 ± 368	3373 ± 307
Right occipital cortex	2663 ± 247	2628 ± 341	2752 ± 259
Left occipital cortex	2658 ± 262	2483 ± 274	2742 ± 244
Right temporal motor cortex	2827 ± 170	2664 ± 231	2805 ± 182
Left temporal motor cortex	2703 ± 292	2537 ± 170	2759 ± 182
Right hippocampus	3684 ± 306	3337 ± 362	3826 ± 355
Left hippocampus	3688 ± 326	3344 ± 357	3782 ± 344
Right thalamus	3565 ± 303	3155 ± 335	3659 ± 348
Left thalamus	3527 ± 326	3143 ± 330	3612 ± 335
Right hypothalamus	2020 ± 187	1700 ± 174	2035 ± 167
Left hypothalamus	1977 ± 196	1710 ± 177	2010 ± 160
Right cerebellum	3057 ± 253	2788 ± 341	2878 ± 284
Left cerebellum	3005 ± 316	2730 ± 313	2953 ± 277

*Values for brain concentration of ^{18}F are the mean ± SEM and have units of Bq per m^3per Bq injected per kg body weight (equivalent to nCi per cc per mCi injected per kg body weight).

disease assessed dopamine transporter and D2 receptor levels before and after embryonic stem cell transplantation into the substantia nigra. They found that grafted animals exhibited decreased striatal D2 receptor levels, consistent with increased release of dopamine from nerve terminals in caudate-putamen. In another study, using a rat model of obesity and both microPET/[^{11}C]raclopride and autoradiography, Michaelides et al[44] found that D2 levels in striatum were inversely related to body weight and also dependent on genetic profile and feeding regimen. More recently, effects of 6-hydroxydopamine lesioning have been evaluated in the rat brain using microPET. Inaji et al[45] used [^{11}C]raclopride and the dopamine transporter radioligand [^{11}C]PE2I to study attempted tissue repair using transplanted fetal mesencephalic tissue. Ishida et al[46] studied the kinetics of altered dopamine synthesis and D1 and D2 dopamine receptors in lesioned animals. Also, Schiffer et al[47] recently used both FDG and [^{11}C]raclopride to investigate the consequences of striatal placement of a microdialysis cannula as an example of a chronic brain implant. FDG accumulation was significantly reduced throughout the hemisphere in which the cannula was placed, for at least 3 weeks, although neither striatal D2 receptor binding nor extracellular dopamine was affected. These studies demonstrate the potential of microPET used in conjunction with neuroreceptor radioligands in neurotoxicological studies.

Conclusions

MicroPET has great advantages over traditional small-animal imaging methods that can compensate for its high cost and limited resolution. These stem from the fact that the animal need not be euthanized before imaging. Therefore, repeated studies are possible in an individual animal,

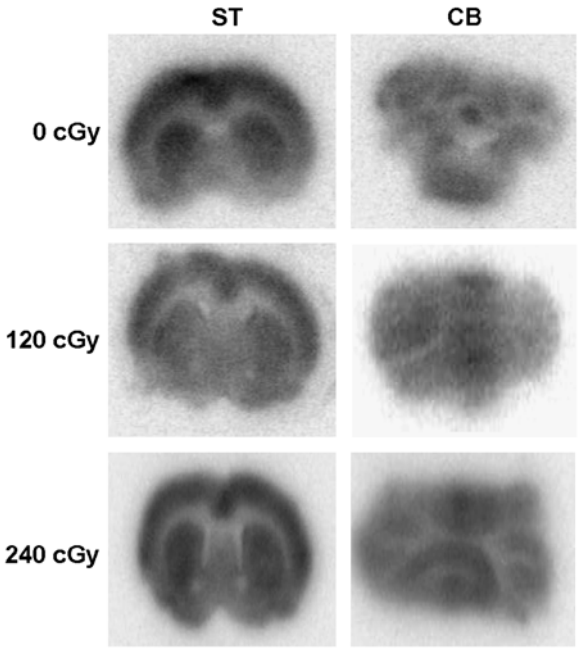

Figure 3. Representative ^{18}F imaging plate autoradiographs of vibratome-cut sections of rat brain at the level of striatum (ST) and cerebellum (CB).

using either the same or different radiotracers. This in principle allows the effects of toxic insults to be followed over the lifetime of an individual animal, greatly reducing the number of animals needed to establish effects. Furthermore, microPET imaging can be used in ongoing experiments to determine whether and when other assays are appropriate. Despite the great potential for longitudinal and long-term PET studies of toxicity in animal models, there are important practical issues that have to be considered. Among these are the effects of repeated induction of anesthesia, the need for blood vessel cannulation in some studies, and animal care issues when animals are maintained for a large fraction of their life spans.

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