Development of a medium-throughput method to screen the effect of test articles on mouse brain activity using ^{14}C-2-deoxyglucose (^{14}C-2DG) 3D autoradiography

Jenna M. Sullivan[1], Mohammed Qutaish[1], Ildiko Polyak[1], Mark Aldridge[1], Jack Heimann[1], Inna Gertsenshteyn[1], Marc Seaman[1], Paige Czarnecki[1], Matthew D. Silva[1], Jacob Hesterman[1], Andrew Giovanni[2], Sharon Engel[2], Carrie Bowen[2], Ryan Terry-Lorenzo[2], Nina Dedic[2], Larry Hardy[2]

[1]Invicro, Boston, MA, USA, [2]Sunovion Pharmaceuticals Inc., Marlborough, MA, USA

Introduction

^{14}C-2-deoxglucose (^{14}C-2DG) is a radioactive glucose analogue. As with ^{18}F-FDG, ^{14}C-2DG is taken up into cells via the glucose transporter and trapped within the cell after phosphorylation. Both ^{14}C-2DG and ^{18}F-FDG have been used to measure changes in brain function and neuronal activity [1-3].

^{18}F is a positron-emitter and is used in PET imaging, ^{14}C is a beta emitter and is often used in autoradiography. Autoradiography is typically performed on individual tissue sections. It offers high in-plane resolution but very limited spatial information. PET provides spatial information but relatively limited resolution. To bridge the gap between traditional autoradiography and PET, we have implemented *ex vivo* autoradiography techniques in 3D to produce high resolution, quantitative, nuclear tomographic images.

The objective of this study was to apply 3D autoradiography methods to develop a medium-throughput approach to assess the effect of test articles on brain activity using ^{14}C-2DG in mice.

Materials & Methods

C57BL/6 mice were separated into groups of nine animals. After an overnight fast, each mouse was administered one of 12 test articles while awake via intraperitoneal injection. Fifteen minutes after test article administration, each mouse was administered ^{14}C-2DG intravenously (100 µCi/kg). Mice were euthanized at 45-minutes post-injection of ^{14}C-2DG, and their brains were harvested.

Each brain was resected whole and frozen. Frozen brains were embedded in optimal cutting temperature (OCT) compound blocks, 54 brains/block (**Fig 1**), in grid-like configurations for autoradiography. Each block contained radioactive and white light visible fiducial markers. Transverse, 30-µm thick, brain sections were obtained using a cryomacrotome. High resolution optical (white light) images were acquired prior to each section being taken from the block. Sections were exposed to phosphor imaging plates with radioactive standards to measure radioactivity in the tissue and to produce autoradioluminograms.

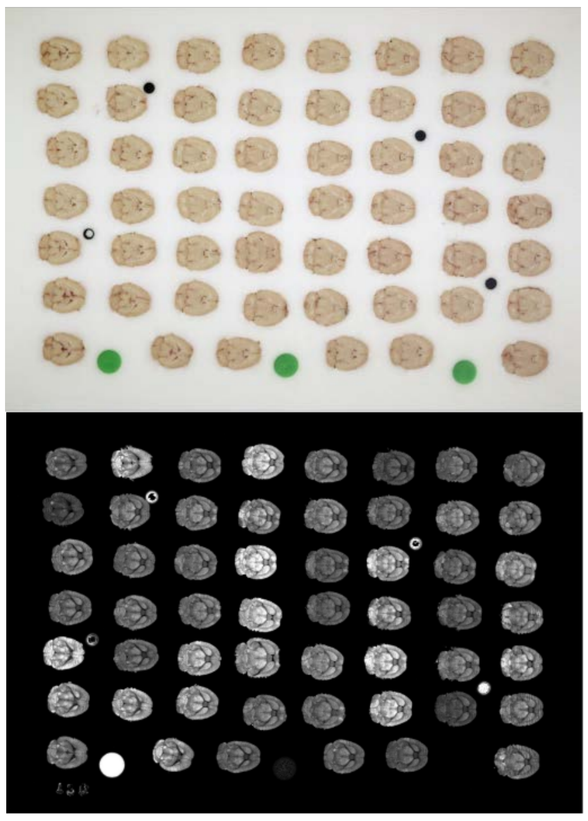

Figure 1. *(Top) White light image of one block showing 54 brains being sectioned in the horizontal plane. (Bottom) Autoradioluminogram of the brains shown in top image. Registration and QC fiducials filled with radioactive ink (black and green circles as seen on white light image) and are included in each block.*

All 2D white light images and autoradioluminograms (~225 per block) were reconstructed into 3D volumes. Individual 3D brain volumes were digitally extracted and all brains (white light and autoradiography data) were registered to a common space**.** Qualitative and quantitative statistics (vehicle vs. test articles) were performed on radioactivity concentration normalized to whole brain activity at the voxel-level and the region-level using a custom 67-region brain atlas. Voxel-level parametric maps of significant differences between vehicle and test article after false discovery rate (FDR) correction were produced.

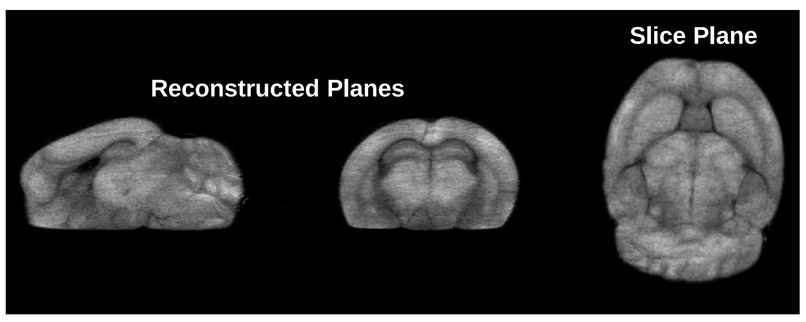

Figure 2. *Example of a 3D reconstructed ^{14}C-2DG image volume. The reconstructed planes (sagittal and coronal) are shown on the left and the slice plane (transverse plane) is shown on the right.*

Results

Relative changes in ^{14}C-2DG signal in brain subregions are detectable with 3D autoradiography

By collecting every 30-µm thick section, we were able to build 3D brain volumes (**Fig 2**) and analyze the autoradiography data in 3D using conventional *in vivo* image processing methods. The pattern of ^{14}C-2DG distribution agreed with published results for similar ^{14}C-2DG or ^{18}F-FDG studies [1,2]. Statistical maps demonstrated significant group-level effects **(Fig 3).** The group that received haloperidol (1 mg/kg) showed significantly higher normalized ^{14}C-2DG signal in the dorsal striatum and lateral habenula and significantly lower normalized ^{14}C-2DG signal in the sensory-motor cortex related part of the thalamus (as compared to vehicle group).

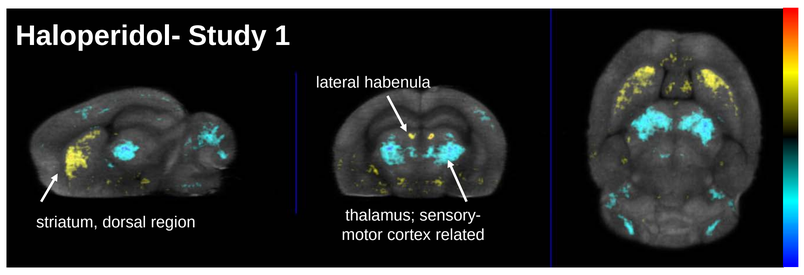

Figure 3. *Voxel-level statistical map of significant voxels from comparison of ^{14}C-2DG whole brain normalized concentration, haloperidol (1 mg/kg, n=9) vs. vehicle (water, n=9). T-stat values in significant voxels (after FDR correction with q = 0.1) are shown in color on top of an autoradiography image from a single brain for anatomical reference. T-stat map has been smoothed with a 0.1 mm gauss filter and is scaled from -12 to 12. Warm colors indicate significantly higher signal in haloperidol group than control and cool colors indicate significantly lower signal in haloperidol group than control.*

Unique patterns of response in voxel-level results

Patterns of response in voxel-level statistical maps varied by test article (**Fig 3, 4,** and **7**). Atomoxetine (10 mg/kg) produced a significant bilateral decrease in normalized ^{14}C-2DG signal in the somatosensory areas. Both guanfacine (1 mg/kg) and clonidine (0.03 mg/kg) produced significant bilateral decreases in normalized signal in the sensory-motor cortex related area of the thalamus. Clonidine also produced significant bilateral increases in the basolateral and lateral amygdalar nuclei (LA/BLA), striatum-like amygdalar nuclei (sAMY), and the dentate gyrus in the hippocampus. The similarities in ^{14}C-2DG uptake between guanfacine and clonidine may be explained by the underlying pharmacology of the drugs, which are both α_{2A} receptor agonists. However, while guanfacine is selective for α_{2A}, clonidine has activity at all three α_2 receptor subtypes. Atomoxetine is a selective norepinephrine reuptake inhibitor.

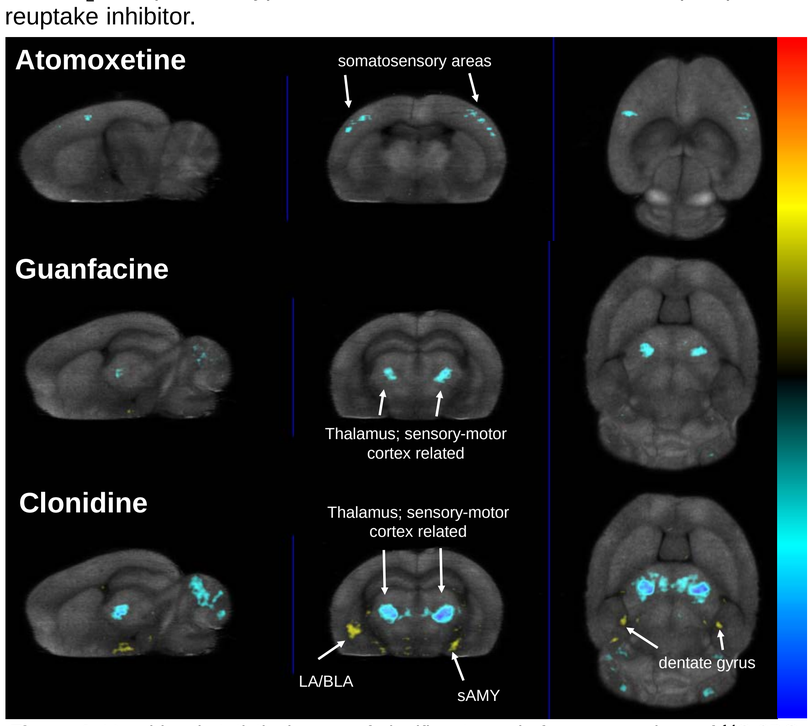

Figure 4. *Voxel-level statistical maps of significant voxels from comparison of ^{14}C-2DG whole brain normalized concentration, atomoxetine (10 mg/kg, n=9) vs. vehicle (water, n=9), guanfacine (1 mg/kg, n=8) vs. vehicle (water, n=8), and clonidine (0.03 mg/kg, n=8) vs. vehicle (water, n=8). Maps are presented in the same format and scaling as in Fig 3.*

Results are replicable

The effect of haloperidol (1 mg/kg) on ^{14}C-2DG signal was replicated in 2 additional experiments, conducted in separate cohorts of mice and conducted months apart (**Fig 5** and **6**)**.**

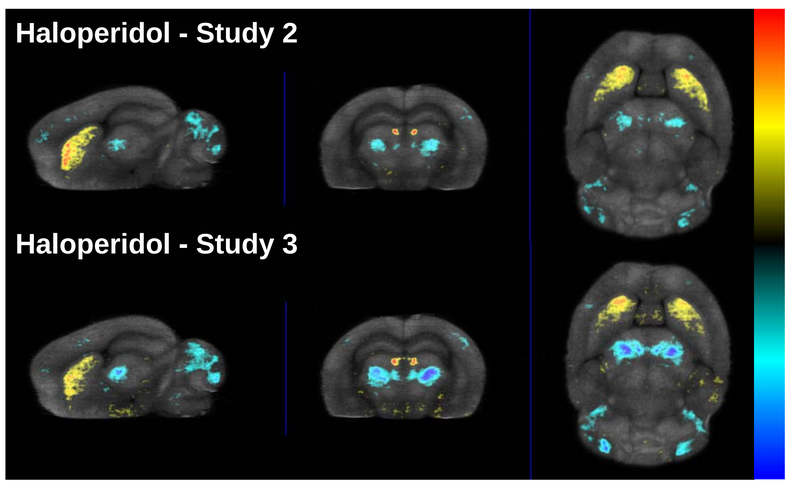

Figure 5. *Voxel-level statistical map of significant voxels from comparison of ^{14}C-2DG whole brain normalized concentration, haloperidol (1 mg/kg) vs. vehicle (water) in second experiment (top, n=8 animals/group) and third experiment (bottom, n=9 animals/group). Maps are presented in the same format and scaling as in Fig 3.*

Results (continued)

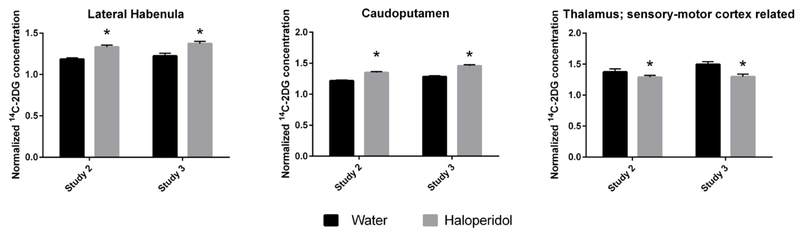

Figure 6. *Region-level results demonstrating replicability of haloperidol effect across studies, as well as agreement between region-level and voxel-level results. Results from Study 2 (n=8 animals per group) and Study 3 (n=9 animals per group) are shown. Bars are group mean and error bars are standard deviation. ^{14}C-2DG concentration was normalized to whole brain. * indicates significant difference between vehicle (water) and haloperidol groups ($p < 0.05$).*

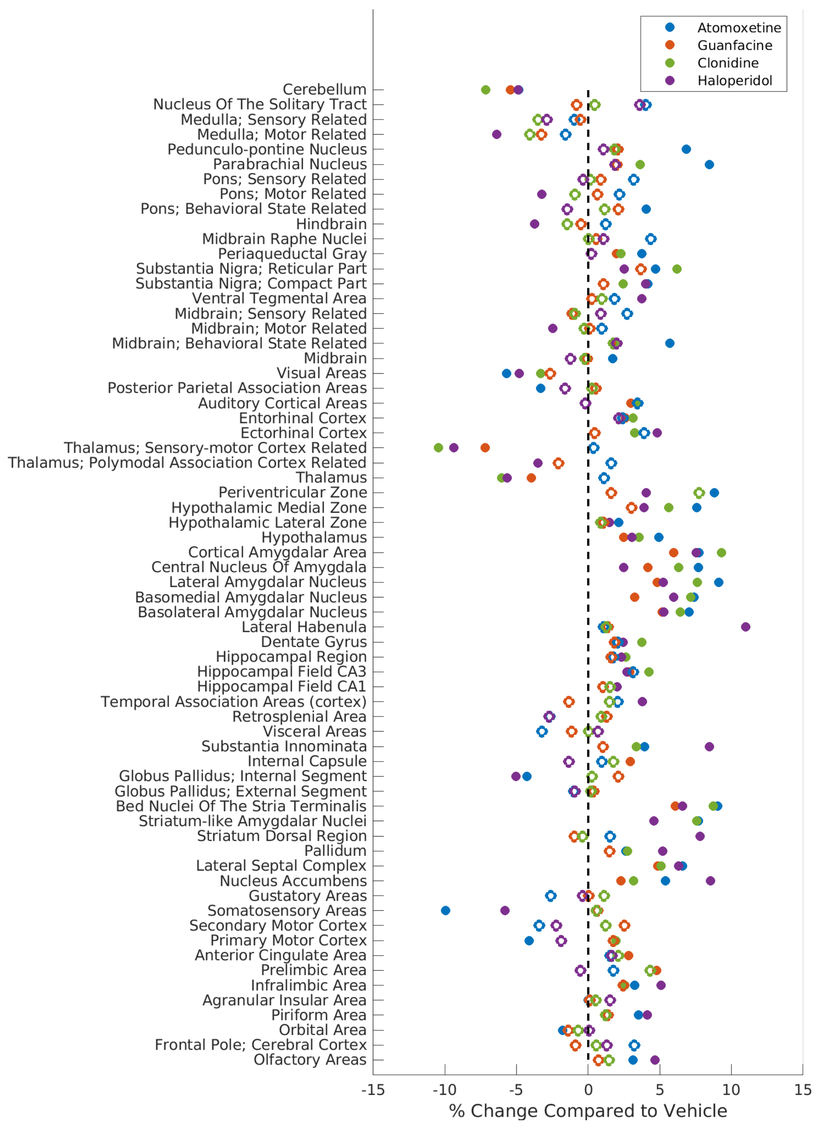

Figure 7. *Effects of atomoxetine (10 mg/kg), guanfacine (1 mg/kg), clonidine (0.03 mg/kg), and haloperidol (1 mg/kg; study 1) on ^{14}C-2DG signal in regions-of-interest (ROI). Group mean ^{14}C-2DG concentration normalized to whole brain was calculated for each ROI then the percent change relative to vehicle was calculated as a measure of effect. Percent change was calculated as (test article-vehicle) / test article * 100. Filled markers indicate that there was significant difference relative to vehicle group (after FDR correction with q = 0.05) in that region for the test article indicated by the marker color.*

Discussion/Conclusions

A medium-throughput 3D autoradiography method was developed and applied to measure the effect of test articles on ^{14}C-2DG radioactivity in the mouse brain. As the resolution of quantitative autoradiography is on the order of 100 µm, this method provides a higher-resolution complement to preclinical *in vivo* PET imaging.

Replicable results were produced with this method, as demonstrated by the consistent haloperidol response between studies. Unique patterns of response were seen in both the voxel-level and region-level results for different compounds. Similar response was seen for compounds with similar mechanism of action (guanfacine and clonidine). Together with the high resolution and replicability of this approach, this suggests that ^{14}C-2DG autoradiography may be a useful assay to measure drug-induced modifications of brain activity.

Acknowledgements

We'd like to thank the laboratory and image analysis teams at Invicro for their precision and expert execution of these studies.

References

[1] Barlett EJ, Brodie JD, Simkowitz P, et al. [1994], American Journal of Psychiatry, 151:681-686.
[2] Pizzolato G, Soncrant TT, Holloway HW, et al. [1985], Journal of Neuroscience, (5)11:2831-2838.
[3] Wyckhuys T, Wyffels L, Langlois M, et al. [2014], Journal of Pharmacology and Experimental Therapeutics, (350)2:375-386.