Getting Buzzed on Sugar: Proof-of-Principle that the Brain of a Bee can be imaged using ^{18}FDG

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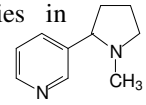
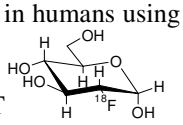
Introduction: Plants enjoy a rich evolutionary benefit in their ability to synthesize thousands of compounds both for defense against biotic and abiotic stressors, and for sexual reproduction and outcrossing. In the genus *Nicotiana,* nicotine, a powerful alkaloid, is produced in the plant's roots and delivered to the aboveground tissues where it acts as a defense toxin. However, recent findings show that floral nectar can contain up to 2.5 ppm of this compound and modify the feeding behavior of pollinators such as the honey bee.[1,2] Nicotine is well known for its strong addictive qualities in mammals, yet little is known about the effects it can have on the brain chemistry of the bee. The purpose of this project was to determine whether the brain of a bee could be imaged using short-lived radiotracers.



2-[^{18}F]fluoro-2-desoxy-glucose (^{18}FDG), a radioactive analog of glucose first developed at BNL in mid-70's, is used to image sugar metabolism *in vivo* in humans using Positron Emission Tomography (PET). ^{18}FDG's popularity has grown world-wide for its use in diagnostic PET imaging, and has become commercially available to most hospitals. However, commercial ^{18}FDG is formulated in isotonic saline. To render the tracer in a state suitable for insect ingestion, we developed a rapid desalination method.



Experimental: *Desalination of ^{18}FDG using Ion Retardation:* 'Cold' functionality test of the resin: 1 mL of a saline solution (containing 2 mg/mL glucose) was passed through a column containing 3 grams of ion retardation resin (BioRad, AG®11A8, Richmond, Calif.), then rinsed with DI water. The conductivity of eluting fractions was measured and tested for glucose levels using a colorimetric assay (p-anisidine reagent) (Table 1). Prepared columns were used with commercial ^{18}FDG-formulation (10 mCi in 0.5 mL saline) and rinsed with and additional 2 mL of DI water to elute the sugar. The extract was refluxed to dryness at 100°C under an argon sweep and the ^{18}FDG reconstituted in 200 µL of 40% aqueous sucrose. This process took roughly 30 minutes to complete enabling 5.75 mCi of reconstituted ^{18}FDG for insect feeding.

Administration of Tracer: A free-flying bumble bee (*Bombus terrestris*) was caught the day prior to the study and deprived of food for 12 hours. The bee was allowed to free-feed from drops of the prepared ^{18}FDG sucrose solution (50µl of initial 200µL, ~ 1.43 mCi) for 3 hours prior to sacrificing the insect and imaging. The drops of radioactive sugar were consumed after 2 hours and were replaced with a source of non-radioactive sucrose water (40%) to rinse proboscis and mouth of the bee.

Dissection and Imaging: After feeding, the bee was sacrificed using liquid nitrogen, thawed and washed with water to remove surface contamination from feeding. Legs and wings were removed from the body. The bee was imaged using positron autoradiography exposing plates for 25 minutes in order to gain sufficient sensitivity for radioactivity distribution. The body was then dissected and counted using a 4-π gamma counter sensitive to the annihilation gammas.

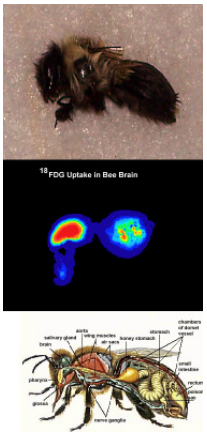
Results: *Desalination of ^{18}FDG*

Table 1: Ion Retardation Resin

Sample	Conductivity	Sugar Test
Saline/glucose solution (2mg/ml) 1mL	3.09mS	positive
sample flow-through 1 mL	283µS	positive
1st water fraction 1mL	800µS	positive
2nd water fraction 1mL	200µS	positive, but less intense

complete desalination was not achieved, but sufficient for insect ingestion.

Bee Image



The radioactivity in the head area was 24% higher than that in the lower body section according to dissection and static count data.

Image resolution wasn't sufficient to enable us to distinguish fine structure within the insect's brain region.

However, we were able to distinguish between the insect's crop and stomach area and the general brain region. This study demonstrates the potential to measure total bee brain activity using ^{18}FDG.

References

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