Journal of Nuclear Medicine, published on November 9, 2017 as doi:10.2967/jnumed.117.198317

Imaging synaptic density: A different look at neurological diseases

Eugenii A. Rabiner[1, 2]

[1] Imanova Ltd, London UK

[2] Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology and Neuroscience, King's College, London, UK

Correspondence to:

Eugenii A. Rabiner

Imanova Ltd, Burlington Danes Building

Hammersmith Hospital, Du Cane Road

London, W12 0NN,

UK

Word Count: 1683

The loss of synapses and neurons is the primary feature of neurodegenerative pathology. The development of novel, disease modifying medication requires the ability to identify patients in very early (even pre-symptomatic) stages of disease, and follow the progress of the neurodegenerative process within individual subjects. Current methods used to evaluate synaptic loss in the human brain are limited to histology or electron microscopy of post-mortem tissue. Such cross-sectional examination, has obvious drawbacks for drug development, is unsuitable for examining subjects in the early phases of the disease process, and lacks the facility to follow the longitudinal pathology within individuals. Non-invasive molecular imaging techniques provide the obvious route for addressing these shortcomings. A number of candidate molecular targets have been suggested for this role in the past, based on the availability of suitable PET radioligands and the presence of the target molecules on glutamatergic and/or GABA-ergic neurons that comprise large proportions of the total neuronal populations. These included the GABA-A receptor ([^{11}C]flumazenil), the 5-HT$_{1A}$ and the 5-HT$_{2A}$ receptors ([^{11}C]WAY-100635 and [^{11}C]MDL-100907) and the histamine-3 receptor ([^{11}C]GSK-189254 and [^{11}C]MK-8278).

The use of molecular imaging methods to quantify synaptic loss requires the identification of a suitable molecular target, possessing a number of suitable characteristics. In order to be useful as a general marker of synaptic density, such a target should be present on all synapses (rather than just a subset). A stable stoichiometry should exist between the target and the synapse, a relationship that should remain consistent in the presence of a disease process. Finally, medications widely used in these patient populations should not have significant affinity for the molecular target in question. Hence, the recent development of PET ligands for the synaptic vesicle glycoprotein 2A (SV2A), a target present in the vast majority (if not all) presynaptic terminals (1), represents an exciting breakthrough in our ability to monitor synaptic status in the brain of pre-clinical species (2) and in humans (3). SV2A belongs to the family of synaptic vesicle glycoproteins, which includes also SV2B and SV2C, but SV2A is the only member of the family that is present ubiquitously in the adult brain, has a stoichiometry (with estimated ~1.5 molecules per vesicle), and correlates well with classical markers of pre-synaptic terminals, such as synaptophysin and synaptotagmin. These characteristics make SV2A an excellent candidate marker of synaptic density.

A number of SV2A selective compounds have been labelled with PET isotopes and evaluated as radioligands for the SV2A. [11C]Levetiracetam, (4) demonstrated poor brain uptake and low signal. [11C]UCB-A has been tested in mini-pigs, rats (5) and rhesus monkeys (Nabulsi, unpublished data, 2012), but has slow brain kinetics limiting its utility as a PET imaging tool. Hence, most of the work in this area was performed with [11C]UCB-J (2) and [18F]UCB-H (6), with both ligands demonstrating kinetics suitable for robust quantification of SV2A density. [11C]UCB-J has favourable dosimetry (4.5 uSv/MBq) (2) and an excellent test-retest variability in the human brain (3-5% across the various regions) (7). [18F]UCB-H displays an adequate signal and variability higher than [11C]UCB-J, but similar to other radioligands in use (6) with estimated human radiation dosimetry of 19.7 uSv/MBq. (8).

A head-to-head comparison of [11C]UCB-J and [18F]UCB-H in the non-human primate brain (2) demonstrated [11C]UCB-J *in vivo* affinity approximately 9-fold higher than [18F]UCB-H, and a much higher specific signal for [11C]UCB-J. As no reference region exists for SV2A, the volume of distribution (V_T) has to be used as an outcome parameter. For [18F]UCB-H, approximately 50% of the V_T comprises non-displaceable binding (V_{ND}), while the corresponding value for [11C]UCB-J is closer to 20%, providing a significant advantage for [11C]UCB-J over [18F]UCB-H. PET ligands labelled with 11C have significant advantages over 18F labelled, such as the facility to perform more than 1 scan per day in the same subject (particularly valuable for where radial artery cannulation is required, or in studies involving

pharmacological or behavioural challenges) and significantly lower dosimetry allowing multiple scanning for individuals. The superior imaging characteristics combined with a 11C-label make [11C]UCB-J the current ligand of choice for investigating synaptic density in the brain, but [18F]UCB-H provides a viable option for smaller PET centres, relying on radioligand distributed from central production sites.

To date, a number of pilot studies examined SV2A density in a variety of clinical conditions. Hypothesised reductions in SV2A binding were seen around the epileptic focus in temporal lobe epilepsy patients (9), and in the cortical areas of patients with mood disorders (10) and schizophrenia (11). [11C]UCB-J has also been used to characterise the relationship between plasma concentration and SV2A occupancy of the anti-epileptic drugs levetiracetam and brivaracetam (12). Of particular interest are the pilot data indicating reductions in SV2A density, consistent with synaptic loss in a patient with Anlzheimer's disease. (13) (Figure 1). These early results suggest a broad applicability in for SV2A PET ligands in the study of brain pathology, and a number of large PET programmes are underway, utilizing SV2A as a molecular marker of neurodegeneration pathology in the human brain (e.g. MIND MAPS - https://mitochondrialdiseasenews.com/2017/04/05/imanova-mrc-funding-mind-maps-study/).

Future utility of SV2A radioligands as markers of synaptic density, will depend largely on the demonstration of signal change correlating with disease progression. A better understanding of the the underlying biology of the SV2A will be essential for the appropriate interpretation of changes seen in PET studies. While the SV2A has a very consistent stoichiometry with individual synaptic vesicles, information on the variability of vesicle numbers per individual pre-synaptic terminal, and the effects of pathology on the number of SV2A per vesicle, the number of vesicles per synapse, or the effective affinity of the PET ligands for SV2A, is to be obtained. In addition, it would be important to understand whether acute changes in neuronal activity lead to a change in [11C]UCB-J or [18F]UCB-H PET signal.

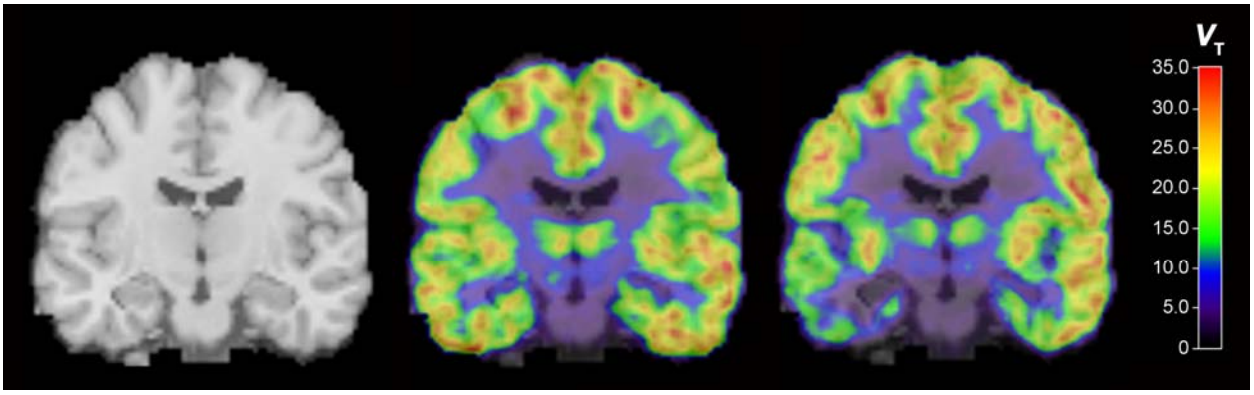
Methodological improvements will enable access to these methods for a wider community of clinical PET researchers. While the lack of a reference region necessitates the use of arterial blood input function for the quantification of the SV2A PET ligands, the potential exists for implementation of pseudo-reference region approaches that may obviate the need for the collection of arterial blood for some specific study designs (though more work is required to achieve this (14). While the utility of such methods remains to be demonstrated, they may be usefully applied in specific experimental situations where relative regional, rather than global absolute binding values are of interest, or where useful assumptions may be made about the levels of non-displaceable binding (see (15) for an example of such an approach). Alternative 18F-labelled ligands are in development (16, 17) and may provide higher-signal options to [18F]UCB-H for groups restricted to 18F-labelled compounds.

In conclusion, the development of good quality PET ligands for the SV2A opens up an exciting field of molecular imaging with great prospects for the investigation and monitoring of brain pathology involving synaptic dysfunction.

References

1. Bajjalieh SM, Frantz GD, Weimann JM, McConnell SK and Scheller RH. Differential expression of synaptic vesicle protein 2 (SV2) isoforms. *J Neurosci*. 1994; 14: 5223-35.
2. Nabulsi N, Mercier J, Holden D, et al. Synthesis and preclinical evaluation of 11C UCB J as a PET tracer for imaging the synaptic vesicle glycoprotein 2A in the brain, *J Nucl Med*. 2016; 57:777-784.
3. Finnema SJ, Nabulsi NB, Eid T, et al. Imaging synaptic density in the living human brain. *Sci Transl Med*. 2016 8:348ra96.
4. Cai H, Mangner TJ, Muzik O, Wang MW, Chugani DC, and Chugani HT. Radiosynthesis of 11C-Levetiracetam: A Potential Marker for PET Imaging of SV2A Expression. *ACS Medicinal Chemistry Letters.* 2014; 5 (10): 1152-1155.
5. Lubberink M, Estrada S, Thibblin A, et al. [11C]UCB-A, a novel PET tracer for synaptic vesicle protein 2A. *Nucl Med Biol.* 2016; 43: 325–332.
6. Warnock GI, Aerts J, Bahri MA, et al. Evaluation of 18F-UCB-H as a novel PET tracer for synaptic vesicle protein 2A in the brain. *J Nucl Med*. 2014; 55: 1336–1341.
7. Finnema SJ, Nabulsi NB, Mercier J, et al. Kinetic evaluation of 11C UCB-J, a novel radioligand for PET imaging of synaptic vesicle glycoprotein 2A in humans, *J Cereb Blood Flow Metabol*, in press.
8. Bretin F, Bahri MA, Bernard C, et al. Biodistribution and radiation dosimetry for the novel SV2A radiotracer [18F]UCB-H: first-in-human study. *Mol Imaging Biol*. 2015;17: 557–564.
9. Finnema S, Detyniecki K, Chen M-K, et al. Reduced SV2A binding in the seizure onset zone in temporal lobe epilepsy patients-A PET study with 11C-UCB-J. *J Nucl Med*. 2017;58:632-632.
10. Esterlis I, Pietrzak R, DellaGioia N, et al. In Vivo Evidence of Lower Synaptic Density in Depression and Associated Mood and Cognitive Deficits: A [11C] UCB-J PET Imaging Study. *Biol Psychiatry*. 2017;81:S159.
11. Radhakrishnan R, Skosnik P, Finnema S, et al. In-vivo Evidence of Decreased Synaptic Density in Schizophrenia: A [11C] UCB-J PET Imaging Study. *Biol Psychiatry*. 2017;81:S389.
12. Naganawa M, Finnema S, Mercier J, et al. Drug characteristics derived from PET kinetic modeling studies: Combined 11C-UCB-J human PET with levetiracetam and brivaracetam occupancy of SV2A. *J Nucl Med*. 2017;58:358-358.
13. Bao W, Jia H, Finnema S, Cai Z, Carson RE, Huang Y. PET Imaging for Early Detection of Alzheimer's Disease. From Pathologic to Physiologic Biomarkers. *PET Clin*. 2017; 12:329–350.
14. Rossano S, Finnema S, Fontaine K, Carson R. Optimizing OSEM reconstruction parameters for imaging synaptic density in humans with 11C-UCB-J PET. *J Nucl Med.* 2017;58:576-576
15. Gunn RN, Murthy V, Catafau A, et al. Translational characterization of [^{11}C]GSK931145, a PET Ligand for the Glycine Transporter Type 1. *Synapse*. 2011; 65:1319–1332.
16. Li S, Cai Z, Zhang W, et al. Synthesis and in vivo evaluation of 18F-UCB-J: Radiotracer for PET imaging of synaptic density. *J Nucl Med.* 2017;58:851-851.
17. Cai Z, Li S, Finnema S, et al. Imaging synaptic density with novel 18F-labeled radioligands for synaptic vesicle protein-2A (SV2A): synthesis and evaluation in nonhuman primates. *J Nucl Med.* 2017;58:547-547.

Figure 1



SV2A density (expressed as [^{11}C]UCB-J V_T) - in the brain of a single patients with AD (*middle*), and an age matched healthy control (*right*). A template MRI is provided to aid anatomical localization (*left*) (Courtesy of R Carson, Yale University PET Centre)