[11C]SB207145: Development and application of a tool for measuring endogenous serotonin levels in humans

A core theme of Cimbi is to develop our understanding of the neurobiological mechanisms that contribute to the emergence of inter-individual differences in features of behavior and personality. The development, validation and implementation of novel tools for measuring relevant neurobiological mechanisms provides unique opportunities to better observe these processes and understand their role in the neural architecture that shapes behavior and contributes to risk for neuropsychiatric illnesses. Positron emission tomography (PET) provides unmatched resolution of the living human brain as a molecular neuroimaging tool and offers a window into the distribution of receptors, transporters and enzymes. This is an extremely valuable tool because it is uniquely able to quantitatively measure, with outstanding precision, features of the serotonin system including its various receptors and the serotonin transporter. Additionally, molecular neuroimaging with PET makes it possible to measure release of endogenous neurotransmitter in brain. However, this is easier said than done and in Cimbi we have focused on promising PET radioligands for measuring endogenous serotonin release in the living human brain. This is exemplified by the detailed description in the Cimbi annual report 2012 (also available on www.cimbi.org) of the development of [11C]Cimbi-36 as a potential tool for measuring endogenous serotonin release. Here we will share with you how Cimbi has completed a set of studies aimed at systematically characterizing a PET radioligand known as [11C]SB207145, which binds to the serotonin type 4 receptor (5-HT4R). From the determination of how to quantify the signal from [11C]SB207145 PET scans to initial evidence that it may also represent a proxy for inter-individual differences in brain serotonin levels, the collaborative effort across research groups within Cimbi underscore its abilities to move biomedical research forward.

Radioligand development and validation

The compound SB207145 was first developed at GlaxoSmithKline. The radio-labeling of the compound with a carbon-11 positron emitter, making [11C]SB207145, was described in 2008 along with the determination that it was a suitable radioligand for measuring 5-HT4R with PET (Gee et al., 2008). The prospect of a novel radioligand capable of measuring a previously unavailable feature of the serotonin system, which had also been associated with antidepressant actions, sparked a collaboration between Cimbi and GlaxoSmithKline.

Following initial studies in pig here in Cimbi, supporting the promise of [11C]SB207145 as a tool for measuring 5-HT4R binding (Kornum et al., 2009), this radioligand was taken into humans. The human studies were led by the former Cimbi researcher Dr. Lisbeth Marner. Building on earlier work describing [11C]SB207145 in both animal models and human, Dr. Marner and colleagues completed a set of studies at the PET and Cyclotron Unit at Rigshospitalet, a core Cimbi institution, further characterizing the [11C]SB207145 PET signal (Marner et al., 2009, 2010). The specificity of [11C]SB207145 to 5-HT4R in the human brain was affirmed with this set of studies along with a suitable method for quantification of the PET signal, providing compelling evidence for its continue study in humans.

Genetic and pharmacological studies in mice

Despite its intense study for decades, the ability to measure endogenous serotonin release in humans has remained elusive. Dr. Marner and colleagues reported that 5-HT4R in humans was not sensitive to putative increases in serotonin levels following an acute pharmacological challenge with a selective serotonin reuptake inhibitor (SSRI) and serotonin 1A receptor antagonist. However, alternative evidence suggesting that 5-HT4R binding measured with SB207145 may be a proxy for differences in serotonin levels was suggested by a set of studies within the NRU animal laboratory and international collaborators.

While working with a rat model for depression, the former Cimbi researcher Dr. Cecilie Löe Licht showed that an acute challenge of the serotonin system did not affect 5-HT4R levels, but a statistically significant 16-47% down-regulation of 5-HT4R levels was observed following 14-21 day treatment with the SSRI, paroxetine (Licht et al., 2009). Dr. Licht and colleagues also presented evidence that depletion of serotonin levels in this rat model significantly increased 5-HT4R levels in some brain regions. Following this study and in collaboration with Dr. Trevor Sharp's laboratory at Oxford University, we reported that genetic manipulation of the gene coding for the serotonin transporter (5-HTT), a key regulator of serotonin levels, affected 5-HT4R levels (Jennings et al., 2012). Together, these data converge in support of a model wherein 5-HT4R levels are inversely linked to alterations in brain serotonin levels. That is, increases in brain serotonin levels result in decreased 5-HT4R binding while decreases in brain serotonin levels result in increased 5-HT4R binding. These findings added to our interest in pursuing the applications of this novel PET molecular neuroimaging tool.

Imaging genetics and behavioral relevance

In parallel with the on-going characterization of SB207145 and its potential application for measuring long-term changes or differences in brain serotonin levels, we also pursued studies supporting its behavioral relevance. In 2012, former Cimbi researcher Dr. Mette Haahr completed a study evaluating the association between 5-HT4R binding in humans and memory performance. This study was motivated by evidence that 5-HT4R signaling critically affected learning and memory and our newly developed capacity to quantitatively measure 5-HT4R binding in humans, *in vivo*. Consistent with this association, Dr. Haahr and colleagues reported a significant negative association between 5-HT4R binding in the hippocampus and short term memory performance (Haahr et al., 2012). The hippocampus is an essential brain structure for learning and memory and these results reinforced a link between serotonin signaling and memory, alluding to 5-HT4R as an important mediator of this process.

Building on work suggesting a role for 5-HT4R signaling in feeding and food-related reward and addiction, Dr. Haahr and Cimbi colleagues evaluated its association with body weight. Consistent with a relation between 5-HT4R and feeding related behaviors, we reported that body-mass index, a measure of size relative to height, was significantly positively associated with 5-HT4R binding in humans (Haahr et al., 2012). Notably, this positive association was observed within key reward-related brain regions including the ventral striatum and prefrontal cortex. These findings further link serotonin signaling and risk for obesity, pointing toward a possible effect on reward-processing related to feeding behavior.

In light of our observation in mice that genetic manipulation of serotonin levels affected 5-HT4R levels, which is consistent with SB207145 as a potential proxy for measuring endogenous serotonin levels, we evaluated a similar effect using imaging genetics from data contained in the Cimbi database. Current Cimbi post doc Dr. Patrick Fisher and Cimbi colleagues applied an imaging genetics framework, which is an approach for identifying genetic factors that significantly contribute to inter-individual differences in neurobiological mechanisms. In the case of 5-HT4R, we evaluated a commonly studied genetic polymorphism known as 5-HTTLPR, which affects the gene coding for the serotonin transporter. The shorter S-allele of this polymorphism shows relatively decreased serotonin transporter expression and this we used as a model for elevated brain serotonin levels. Consistent with this model, we found that individuals with at least one copy of the S-allele showed 9% lower 5-HT4R binding in the neocortex compared to individuals with two copies of the longer L-allele (Fisher et al., 2012). This finding is particularly intriguing because the 5-HTTLPR polymorphism has been widely studied and related to aspects of brain function, behavior and risk for neuropsychiatric illnesses including depression and responsiveness to common antidepressant treatments. Thus, our findings provide evidence for a molecular mechanism through which this polymorphism may contribute to these behavioral and clinical phenotypes.

Evidence for sensitivity to endogenous serotonin levels

2013 at Cimbi witnessed a culmination of extensive work aimed at understanding and applying our understanding of 5-HT4R binding with [11C]SB207145 PET. Drs. Patrick Fisher and Mette Haahr within NRU led a double-blind, placebo-controlled study evaluating the effects of pharmacologically increased serotonin levels on 5-HT4R binding (Haahr, 2014). This study randomly assigned 32 healthy males to receive daily doses of either placebo or the SSRI, fluoxetine, which increases brain serotonin levels. Building on the animal work that was just described, we hypothesized that fluoxetine, by increasing brain serotonin levels, would significantly decrease 5-HT4R binding, supporting this PET measure as a proxy for brain serotonin levels in humans.

To determine this effect we included 5-HT4R binding data estimated in multiple regions throughout the brain, including the hippocampus, amygdala, neocortical brain regions and the striatum. Comparing the receptor binding levels before and after intervention, using what is known as an "occupancy plot", we estimated the change in 5-HT4R binding following SSRI intervention. Consistent with SSRIs inducing an increase in brain serotonin levels, we observed (**Figure 1**) that 5-HT4R binding was decreased 5.2% in individuals who received fluoxetine. Importantly, the group that received placebo did not show a significant change in 5-HT4R binding, consistent with a specific effect resulting from fluoxetine modulating brain serotonin levels. In addition to these results being published in the prestigious journal, *Molecular Psychiatry*, these findings garnered public attention and were highlighted in different media.

Future directions

The progression in characterizing [11C]SB207145 as a PET radioligand for measuring 5-HT4R in humans and its application as a proxy for endogenous serotonin levels highlights many core themes of Cimbi. The systematic characterization and validation of [11C]SB207145 contributes the arsenal of tools available to scientific research for probing the serotonin system in humans, *in vivo*. The serotonergic manipulation studies in animal models underscore the translational capabilities of Cimbi and the fostering of fruitful collaborations extending outside Cimbi's borders. Critical to the application of novel molecular neuroimaging tools is identifying behavioral relevance, which has been reinforced for 5-HT4R with studies related to memory function and body-weight, bringing together data collected through the Cimbi neuropsychology test battery and molecular neuroimaging. The imaging genetics study in humans was facilitated by the well-organized and rich Cimbi database, providing access to potentially relevant measures

across Cimbi disciplines. Also, related findings provide critical insight into how genetic variability shapes serotonin signaling and implicates a molecular mechanism mediating established links between genetic variation, brain function and behavior. Finally, the pharmacological challenge study provides novel evidence that this molecular neuroimaging tool may be a useful proxy for endogenous serotonin levels, a measure which has remained elusive, despite great effort. Within the following pages we will highlight other exciting findings that during 2013 have emerged from the various Cimbi-related research projects. Building on these findings and collaborations, 2014 holds great promise for Cimbi and we look forward to sharing those findings within next year's report.

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