Effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain measured by [^{18}F]Mefway PET

Jae-Hoon Lee

Department of Medicine

The Graduate School, Yonsei University

Effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain measured by [^{18}F]Mefway PET

Directed by Professor Young Hoon Ryu

Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Jae-Hoon Lee

December 2016

연세대학교
YONSEI UNIVERSITY

This certifies that the Doctoral Dissertation of Jae-Hoon Lee is approved.

Thesis Supervisor: Young Hoon Ryu

Thesis Committee Member#1: Jong Doo Lee

Thesis Committee Member#2: Hang-Seok Chang

Thesis Committee Member#3: Chul Hoon Kim

Thesis Committee Member#4: Joon Young Choi

The Graduate School
Yonsei University

December 2016

ACKNOWLEDGEMENTS

I would like to express my particular appreciation and gratitude to my supervisor, prof. Young Hoon Ryu, who has been a remarkable mentor to me. I would like to thank him for sharing his knowledge, for his guidance, and for his continuous support throughout the whole research project. His advice on research as well as on my career has been priceless.

I am also very grateful to prof. Jong Doo Lee, prof. Hang-Seok Chang, prof. Chul Hoon Kim, and prof. Joon Young Choi for serving as my committee members even though difficult. I thank them for all their support and advice, which improved the quality of this work.

I would like to extend my gratitude to Jae Yong Choi who provided his expertise in carrying out the research, and in interpreting and publishing the data. I would also like to thank Arthur Cho for helpful discussions and academic assistance.

I further would like to give special thanks to my family. I have a debt of gratitude to my parents and parents-in-law who gave me continuous support and encouragement.

Finally, I would like to express my appreciation to my beloved wife, Yoon Chung Kim, and daughter, Juwon Lee, who always give me joy and give meaning to life.

Jae-Hoon Lee

TABLE OF CONTENTS

ABSTRACT ····· 1

I. INTRODUCTION ····· 3

1. Thyroid hormones and mood modulation ····· 5

2. Transient hypothyroidism during preparation for radioactive iodine treatment ····· 6

3. The serotonergic system in the central nervous system ····· 6

4. Thyroid-Serotonin interaction in neuropsychiatric disorders ····· 8

5. Positron emission tomography ····· 8

6. Radioligands for imaging 5-HT_{1A} receptors ····· 9

7. Purpose of the research ····· 11

II. MATERIALS AND METHODS ····· 12

1. Synthesis of [^{18}F]Mefway ····· 12

2. Experimental animal models ····· 12

3. PET data acquisition ····· 13

4. Image processing ····· 14

5. Kinetic modeling and parametric mapping ····· 15

6. Comparison of regional [^{18}F]Mefway BP_{ND} ····· 17

7. Statistical analysis ····· 17

III. RESULTS ····· 18

1. Validation of the hypothyroid animal model ····· 18

2. Comparison of regional [^{18}F]Mefway uptake ····· 19

3. Changes of [^{18}F]Mefway BP_{ND} in hypothyroidism ····· 21

IV. DISCUSSION ····· 23
1. Measurement of regional [^{18}F]Mefway uptake ····· 23
2. Mechanism of increased [^{18}F]Mefway uptake in the brain after thyroidectomy ····· 25
3. The advantages of [^{18}F]Mefway ····· 27
V. CONCLUSION ····· 29
REFERENCES ····· 30
ABSTRACT (IN KOREAN) ····· 38

LIST OF FIGURES

Figure 1. PET radioligands for imaging 5-HT_{1A} receptors……10

Figure 2. Temporal course of the dynamic PET study ……14

Figure 3. Schematic representation of the construction pathway for functional PET atlases……15

Figure 4. Predefined volumes of interest for time-activity curves and quantitative analyses……16

Figure 5. Representative [^{18}F]Mefway PET images ……19

Figure 6. Time-activity curves of [^{18}F]Mefway uptake in specific regions of the brain ……20

Figure 7. Comparison of the [^{18}F]Mefway BP_{ND} in different brain regions……21

Figure 8. Representative BP_{ND} parametric map……22

LIST OF TABLES

Table 1. Results of thyroid function tests ·····18

ABSTRACT

Effect of hypothyroidism on 5-HT$_{1A}$ receptors in the rat brain measured by [^{18}F]Mefway PET

Jae-Hoon Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Young Hoon Ryu)

This study investigated the effects of hypothyroidism on the brain serotonin (5-HT) 1A receptor using animal disease models and 4-(*trans*-[^{18}F]fluoranylmethyl)-*N*-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-pyridin-2-ylcyclohexane-1-carboxamide ([^{18}F]Mefway) positron emission tomography (PET) imaging.

Five surgically thyroidectomized male Sprague–Dawley rats were assigned to the hypothyroidism group, and five sham-operated male Sprague–Dawley rats were assigned to the control group. Hypothyroid status was confirmed by thyroid function tests. After anesthesia with 2.0% isoflurane in oxygen, fluconazole was infused at a constant rate for one hour to prevent defluorination of the radioligand. Then [^{18}F]Mefway of 8.6–11.1 MBq was administered at a rate of 1 ml/min and dynamic PET scans were performed over the course of 120 min. PET data were reconstructed in user-defined time frames using a two-dimensional ordered-subset expectation maximization algorithm.

All PET data were spatially normalized to T2-weighted magnetic resonance imaging templates, and then time-activity curves of the hippocampus, septum, and cerebellum were extracted using predefined volume of interest templates. Non-displaceable binding values in the hippocampus and septum were calculated using a multilinear reference tissue model, with the cerebellum as the reference tissue, and a ligand-specific parametric map was constructed.

Time-activity curves revealed that the hippocampal and septal uptakes in the hypothyroidism group were 25–52% higher than those in the control group, and non-displaceable binding potentials in the same regions of thyroidectomized rat brains were about 30% higher than those in controls.

In conclusion, hypothyroidism increased the density of postsynaptic 5-HT_{1A} receptors in the hippocampus and septum. Upregulation of postsynaptic 5-HT_{1A} receptors may be responsible for the increased radioligand uptake as an early response to the reduced synaptic concentration of 5-HT caused by hypothyroidism.

Key words: [^{18}F]Mefway, hypothyroidism, positron emission tomography, serotonin 1A receptor, thyroid

Effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain measured by [^{18}F]Mefway PET

Jae-Hoon Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Young Hoon Ryu)

I. INTRODUCTION

Clinical researchers have long recognized the link between the thyroid gland and depression. Dysfunction of the hypothalamic-pituitary-thyroid axis (HPT axis), as well as the hypothalamic-pituitary-adrenal axis, is a known neuroendocrine factor involved in the pathogenesis of depression. Many clinical reports have indicated a high incidence of psychiatric illness in patients with either hypothyroidism or hyperthyroidism.[1-3] The apparent relationship between hypothyroidism and depression has led to the widely accepted hypothesis that decreased thyroid function predisposes an individual to depression, and, conversely, that improved thyroid function favors recovery from the depressed state.[4]

Thyroid dysfunction may be associated with changes in central neurotransmission, and disruptions in thyroid activity have been reported to affect a number of

neurotransmitters. Several lines of investigation have produced evidence indicating that the serotonin (5-HT) system may be involved in the pathophysiology of mood disorders.[5-8] The currently favored hypothesis is that a lack of serotonin in the brain plays a central role in the development and aggravation of such disorders.[9] Anatomically, the 5-HT system projects to many areas in the central nervous system, several of which play a major role in controlling a variety of 'mood-related' behaviors, including appetite, sleep, and attention.[5,7] Such co-occurring clinical and neurophysiological findings in depressed patients with hypothyroidism have raised the possibility that there may be a link between dysfunction of the HPT axis and the 5-HT system.

Multiple different 5-HT receptors have been identified in the brain, and among the various subtypes of 5-HT_1 receptor, that designated 5-HT_{1A} is of particular interest due to its possible involvement in anxiety and depression,[6] as well as in epilepsy, Parkinson's disease, and Alzheimer's disease.[10-14] Therefore, the importance of an *in vivo* molecular imaging agent for 5-HT_{1A} receptors has been increasingly recognized.[15]

Positron emission tomography (PET) imaging is a non-invasive functional imaging modality for *in vivo* interrogation of 5-HT_{1A} receptors, due to its extraordinarily high sensitivity, which allows the detection of interactions between radiopharmaceuticals and their protein targets.[16] Although many PET radioligands have been developed for imaging 5-HT_{1A} receptors, there are no routinely applicable radioligands for use in pre-clinical or clinical studies. Recently, 4-(*trans*-[^{18}F]fluoranylmethyl)-*N*-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-pyridin-2-ylcyclohexane-1-carboxamide ([^{18}F]Mefway) has been developed as a promising radioligand, with highly specific binding to 5HT_{1A} receptors, an excellent signal-to-noise ratio, and favorable metabolic stability.[17,18]

In the present study, we investigated the effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain using animal models of disease and [^{18}F]Mefway PET imaging.

1. Thyroid hormones and mood modulation

It is well established that thyroid hormones are essential for both the development and maturation of the human brain, affecting such diverse events as neuronal processing and integration, glial cell proliferation, myelination, and the synthesis of key enzymes required for neurotransmitter synthesis.[19,20] Thyroid deficiency during the perinatal period results in irreversible brain damage and mental retardation.

There are several lines of evidence suggesting that thyroid hormones affect mature brain function.[21] First of all, thyroid hormone receptors are prevalent in the mature brain. Nuclear receptors for triiodothyronine (T3), the thyroid hormone with the highest biological activity, are widely distributed in the adult rat brain, with higher densities of nuclear T3 receptors in phylogenetically younger brain regions—the amygdala and hippocampus—and lower densities in the brain stem and cerebellum.[22,23] Secondly, deiodinase 2 is expressed primarily in the brain and anterior pituitary gland, where it metabolizes thyroxine (T4) to the active thyroid hormone form, T3. The activity of deiodinase 2 in distinct regions of the brain varies widely, with the highest levels found in cortical areas and lesser activity found in the midbrain, pons, hypothalamus, and brainstem.[24] Finally, thyroid hormones have been detected in relatively high concentrations in cortical tissue.[25] In contrast to peripheral tissue, where T4 levels usually far exceed those of T3, T4 and T3 concentrations are in a similar range in the brain.

Disorders of the thyroid gland are frequently associated with severe mental disturbances.[5-8] The observed association between the thyroid system and behavior has prompted investigations of the effects of thyroid hormones in modulating affective illness, and of the role of the HPT axis in the pathophysiology of mood disorders.[26] Thyroid hormones have a profound influence on behavior and mood, and appear to be capable of modulating the phenotypic expression of major affective illness.[26,27] Thyroid hormone replacement is now widely accepted as an effective treatment option for patients with affective disorders.[28,29]

2. Transient hypothyroidism during preparation for radioactive iodine treatment

In the past decade, the prevalence of thyroid cancer has increased rapidly, particularly in Korea,[30,31] and the number of patients who have undergone total thyroidectomy has increased accordingly. After total thyroidectomy, patients receive ^{131}I radioactive iodine treatment either for ablation of the remnant thyroid tissue or for treatment of metastatic or recurrent tumor, depending on their clinical status. To maximize the effectiveness of this treatment, thyroid-stimulating hormone (TSH) levels must be well above the normal range, and withdrawal from thyroid hormone replacement, or induction of hypothyroidism, is the most commonly used means of achieving this goal. Although this induced hypothyroid state is temporary, lasting just a few weeks, it can cause symptoms such as tiredness, weight gain, sleepiness, constipation, muscle aches, and reduced concentration.[32,33] Emotional changes resembling depression are amongst the common symptoms associated with this temporary hypothyroidism. Some people only experience mild symptoms, whilst others experience severe symptoms, including suicidal ideation.

Patients who prepare for radioactive iodine treatment often suffer from depressive symptoms after thyroid hormone withdrawal, and it has been hypothesized that a lack of serotonin in the brain plays a central role in this phenomenon. However, the exact mechanism by which these symptoms occur requires further elucidation, and we hypothesized that PET scanning using [^{18}F]Mefway, a radiotracer for the 5-HT_{1A} receptor, would be of great value in investigating the effect of thyroidectomy on the central serotonin system.

3. The serotonergic system in the central nervous system

Serotonin is found in the gastrointestinal tract, blood, and central nervous system (CNS). About 90% of all serotonin is present in the enterochromaffin cells that are dispersed among the mucosal cells in the gastrointestinal tract. Here, 5-HT promotes

gastrointestinal motility and contraction of isolated strips of intestine. Around 8% of 5-HT is present in platelets and facilitates platelet aggregation. The remaining 5-HT (2%) is found in the CNS, where it regulates various brain functions, such as mood, appetite, sleep, and cognition.

As with the noradrenergic and dopaminergic systems, the bulk of CNS serotonergic nerve terminals originate in the neuronal cell bodies of the brainstem's raphe nuclei, and project, both rostrally and caudally, to neuroanatomically discrete areas throughout the brain, but with extensive innervation of the cerebral cortex and the limbic system, where have been implicated in the pathogenesis of the disorders and thyroid hormone receptors are abundant.[34] Although the serotonin system has been given prominence in recent deliberations about mood modulation, particularly since the advent of drug therapies that specifically interfere with neuronal serotonin reuptake, there has been little investigation of the relationship of this system to the thyroid system.[21]

To date, seven structurally and pharmacologically distinct receptor subtypes ($5\text{-}HT_{1\text{-}7}$) have been identified, and these subfamilies have been further re-assigned into 14 subtypes.[35] All 5-HT receptor subtypes are G-protein-coupled receptors, except for $5\text{-}HT_3$, which is a ligand-gated sodium ion channel. Among the subtypes, the $5\text{-}HT_{1A}$ receptor is the most widely researched, due to its central role in the regulation of 5-HT neurotransmission and its involvement in the pathophysiology of the stress response, aggressive behavior, anxiety, depression, and schizophrenia.[10,36-38] These receptors are distributed universally throughout the CNS. They are localized at high densities in the cerebral cortex, hippocampus, septum, and raphe nucleus, but are found in very low amounts in the striatum, substantia nigra, and the cerebellum. The $5\text{-}HT_{1A}$ receptors in the raphe nuclei are present in the cell bodies and dendrites of 5-HT neurons, and hence are called somatodendritic autoreceptors. At presynaptic sites, they act as reuptake modulators of synaptic 5-HT, causing a decreased release of 5-HT into the terminal fields. $5\text{-}HT_{1A}$ receptors are also located postsynaptically in the cortical and limbic area.

4. Thyroid-Serotonin interaction in neuropsychiatric disorders

Clinical and neuroanatomical studies have suggested that there is an interaction between thyroid hormones and serotonin in the pathophysiology of various neuropsychiatric disorders. It has been established that serotonin plays a major role in mood modulation. Serotonin pathways begin in the brainstem and extend through the midbrain into the limbic system and cortex, modulating the activity of many of the brain regions involved in emotion and memory. Thyroid hormones play an important role in the production of key enzymes for the synthesis of neurotransmitters, including serotonin, norepinephrine, and dopamine.[39] From the few neuro-endocrine challenge studies which have been conducted in humans with thyroid dysfunction, there is some evidence that hypothyroid status is associated with reduced 5-HT responsiveness.[21] Furthermore, this appears to be reversible with thyroid replacement therapy.[40] Results from animal studies have provided strong evidence that thyroid status has a considerable impact on serotonergic neurotransmission in the adult brain. For example, experimentally induced hypothyroid status results in an increase in 5-HT turnover in the brainstem.[41,42] This increased 5-HT turnover in hypothyroid status may lead to an increase in raphe 5-HT_{1A} autoreceptor activity and a decrease in cortical 5-HT concentrations. One animal study has also revealed that 5-HT content is decreased in the cerebral hemisphere and mesodiencephalon in hypothyroidism.[39]

5. Positron emission tomography

PET is a non-invasive imaging technique that provides biological and physiological information about living systems. The PET system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (radiotracer), which is introduced into the body attached to a biologically active molecule. Three-dimensional images of tracer concentration within the body are then constructed by computer analysis. A major advantage of PET is its high sensitivity (10^{-9}-10^{-12} M), which allows for detection of the

interactions between radioligands and their protein targets. This sensitivity is many orders of magnitude greater than the sensitivity achieved with magnetic resonance imaging (MRI) (approx. 10^{-4} M), or computed tomography (approx. 10^{-3} M).[43,44]

Its uses in brain imaging have spanned both research interests and clinical applications. Specific psychiatric disorders in which PET studies may influence patient management include mood and anxiety disorders, attention deficit disorder, schizophrenia, and obsessive compulsive disorder.[45] ^{18}F-fluoro-2-deoxyglucose, a tracer for glucose metabolism, has been most widely investigated for use in clinical PET brain imaging. However, in studies of psychiatric disorders, there has been an increasing demand for more specific radiotracers that bind to the receptors of various neurotransmitters—such as serotonin, dopamine, and opiate—and may play an important role in the study of psychiatric disorders.[46,47]

6. Radioligands for imaging 5-HT$_{1A}$ receptors

In recent decades, extensive investigations have been undertaken to develop an effective PET tracer for imaging 5-HT$_{1A}$ receptors, but only 3 radioligands are currently being used in human subjects: [^{11}C]*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide ([^{11}C]WAY-100635), [^{18}F]*trans*-4-fluoro-N-2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide ([^{18}F]FCWAY), and 4-[^{18}F]-fluoranyl-*N*-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-pyridin-2-ylbenzamide ([^{18}F]MPPF) (Fig. 1).

However, there are several obstacles to the widespread use of these radioligands. The production of [^{11}C]WAY-100635 requires an expensive on-site cyclotron because its half-life is only 20 min. [^{18}F]MPPF overcomes this issue, but it is a substrate for P-glycoprotein in rodents and humans and thus shows poor brain penetration.[48] [^{18}F]FCWAY has a high affinity and selectivity for the target,[49] but suffers from marked defluorination, which increases the skull uptake and hampers the exact quantification of 5-HT$_{1A}$ receptors.[50]

To circumvent the problem of the defluorination of radioligands, the Mukherjee group developed [^{18}F]Mefway.[51] [^{18}F]Mefway is an [^{18}F]-labeled analogue of [^{11}C]WAY-100635 that shows improved metabolic stability *in vivo* by extending the carbon bond on the cyclohexyl ring of [^{18}F]FCWAY. In previous studies, [^{18}F]Mefway has shown high affinity for 5-HT$_{1A}$ receptors, an excellent target-to-reference ratio, 5-HT$_{1A}$-specific images without defluorination in the rhesus monkey, and favorable drug-like properties for 5-HT$_{1A}$ receptors.[17,51,52]

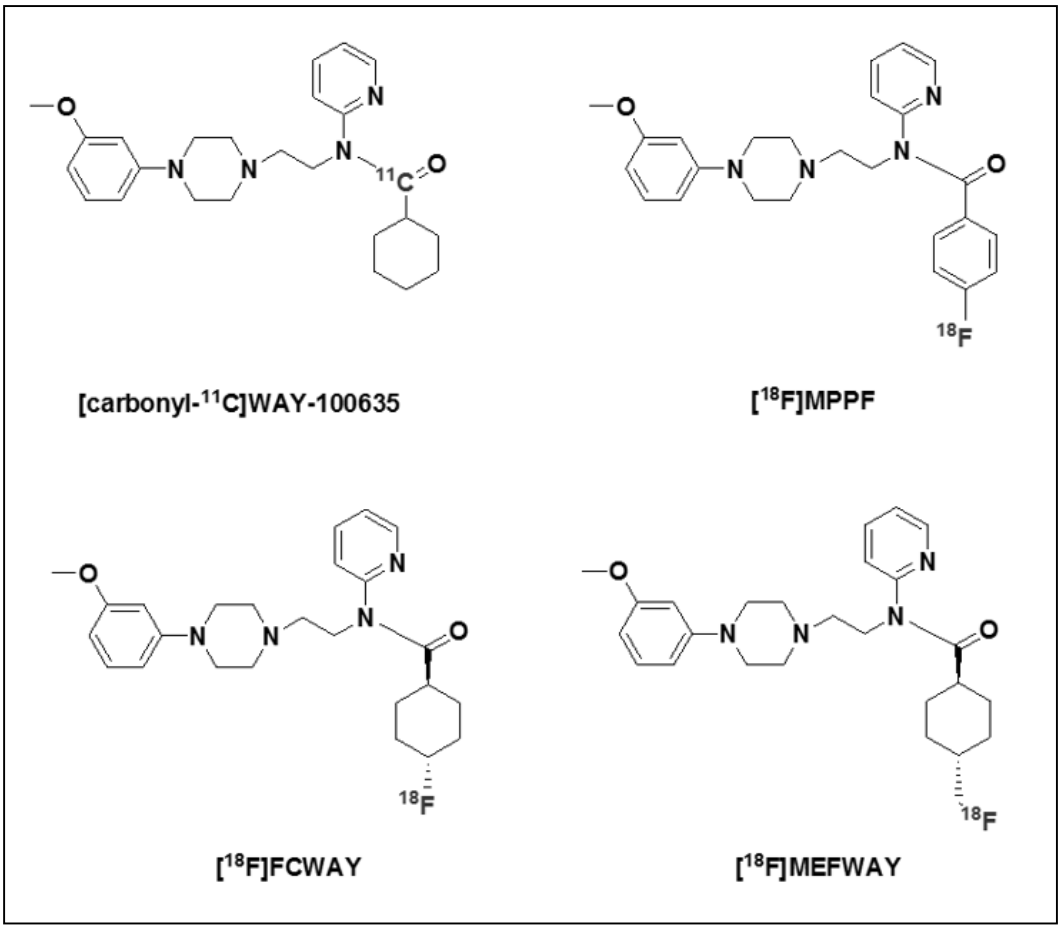

Figure 1. PET radioligands for imaging 5-HT$_{1A}$ receptors.

7. Purpose of the research

The purpose of this study was to evaluate the effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain, specifically in the hippocampus and septum, using hypothyroidism animal models and [^{18}F]Mefway PET imaging.

II. MATERIALS AND METHODS

1. Synthesis of [^{18}F]Mefway

[^{18}F]Mefway was synthesized by a nucleophilic substitution reaction of a *p*-tosylated precursor, as has been previously described.[53] The specific activity after completion of synthesis as assessed by high-performance liquid chromatography was higher than 90 GBq/μmol, and the radiochemical purity exceeded 99%.

2. Experimental animal models

Surgically thyroidectomized 6-week-old male Sprague–Dawley rats (n = 5) (Central Lab. Animal Inc., Seoul, Korea), initially weighing 175–200 g, were used in this experiment and were assigned to the disease animal group. The thyroidectomized rats were housed for two weeks, with four per cage, at uniform temperatures, with alternating 12 hour cycles of light and dark. They received a rat chow diet and had free access to 0.5% $CaCl_2$ drinking water.

Sham-operated male Sprague–Dawley rats of the same age (n = 5) were assigned to the control group. The sham-operated rats were also housed for two weeks, with four per cage, at uniform temperatures, with alternating 12 hour cycles of light and dark. Sham rats received rat chow and had free access to normal drinking water.

To verify the hypothyroid status of the thyroidectomized rats, T3, T4, and TSH levels were determined using the RTHYMAG-30K Milliplex MAP Rat Thyroid Magnetic Bead Panel (Millipore Corporation, Billerica, MA, USA). The detailed procedure was as follows: The plate was wet with a 150 μL wash buffer for 10 min, and the buffer was then decanted. Next, 25 μL of standards or samples, 25 μL of beads, and 25 μL of assay buffer were added to the plate, and the plate was incubated overnight at 4°C. The beads were then washed three times, after which 50 μL of biotinylated detection antibody cocktail

was added, and the plate was incubated at room temperature for one hour. Subsequently, 50 μL of streptavidin-phycoerythrin was added and the plate was incubated at room temperature for a further 30 min. Lastly, the beads were washed three times, 150 μL of sheath fluid was added, and the plate was then read on a Luminex® plate-reader (Luminex Corporation, Austin, TX, USA). Analyses of thyroid hormones were performed at KOMABIOTECH Inc. (Seoul, Korea).

3. PET data acquisition

PET scanning was performed with a Siemens Inveon small animal PET scanner (Siemens Medical Solutions, Erlangen, Germany). This scanner has a peak absolute system sensitivity of ≥ 10% in the 250–750 KeV energy window, an axial field of view of 12 cm, a 1.4 mm full width at half maximum spatial resolution at the center of the field of view, and a transaxial field of view of 10 cm.

Anesthesia was induced with 2.5% isoflurane and was maintained during the PET scan with 1.5% isoflurane for 3 h (Fig. 2). After cannulation in a tail vein, rats were positioned in the center of a gantry. Then, to suppress the *in vivo* defluorination, rats were pretreated with fluconazole (60 mg/kg, One Flu injection, JW Pharmaceutical Co., Ltd., Seoul, Korea).[52] Tracer accumulation in the brain was investigated by dynamic PET scanning over a period of 120 min after injection of 8.6–11.1 MBq of [^{18}F]Mefway. Paired Sprague–Dawley rats that received sham surgery were used as controls. Thereafter attenuation corrections were performed using data from a 10 min transmission scan with a ^{57}Co point source.

PET data were reconstructed in user-defined time frames (10 sec × 6 frames, 30 sec × 8 frames, 300 sec × 5 frames, 1800 sec × 3 frames) with a voxel size of 0.86 × 0.86 × 0.80 mm^3, using a 2-dimensional ordered-subset expectation maximization algorithm (4 iterations and 16 subsets).

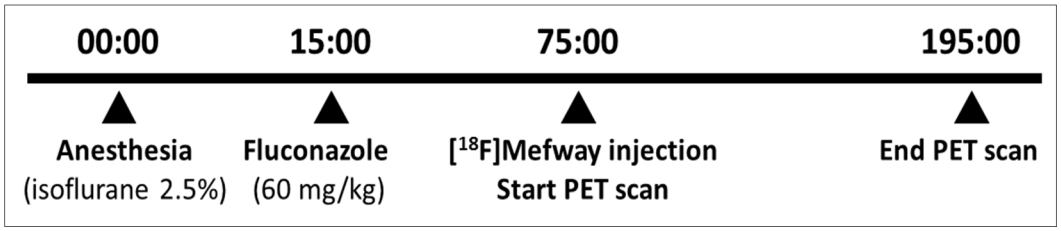

Figure 2. Temporal course of the dynamic PET study. Rats were pre-treated with fluconazole to prevent defluorination of the radiotracer. Dynamic brain PET scanning was performed after administration of [^{18}F]Mefway, over a period of 120 min.

4. Image processing

The procedure used for the template construction was based on work by Casteels et al.,[54,55] but revised to obtain symmetrical templates.[56] The procedure for the construction of functional templates was divided into three steps (Fig. 3). First, a representative image of each set of tracers was selected as a "standard" for that specific tracer. Next, each of the individual scans was normalized into the space of the representative scan. This within-modality affine registration was achieved by minimizing the sum of squares differences between the image to be normalized and the reference image. Secondly, a symmetrical voxel-wise averaged template was obtained from the previously aligned images. To this end, a left–right flipped duplicate of the previously obtained average image was created, and this was normalized into the original average template. Thirdly, a cross-modality registration was performed between the symmetrical averaged image and the reference MRI that was in the same space as the PET templates. A T2-weighted MRI template was used for spatial normalization and co-registration.[57] This procedure was performed using a rigid-body transformation based on the normalized mutual information maximization algorithm. Thereafter, the transformation matrix obtained in the co-registration was applied to all of the images used in the construction of the template, for further use in the study.

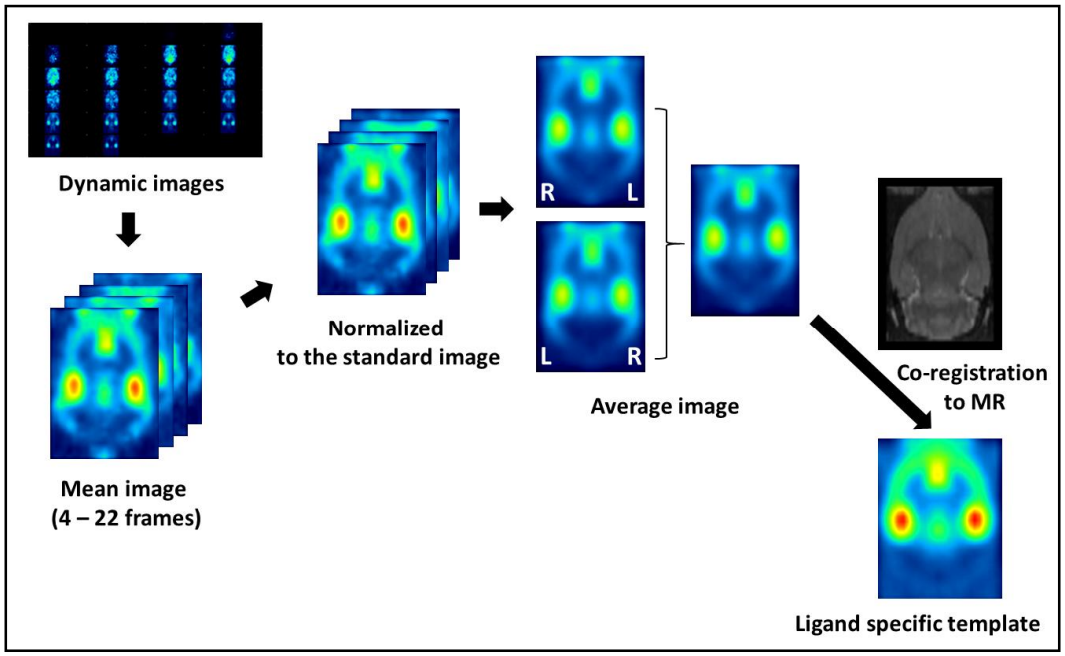

Figure 3. Schematic representation of the construction pathway for functional PET atlases. MR, magnetic resonance.

5. Kinetic modeling and parametric mapping

Making use of the stereotactic rat brain atlas, we defined a volume of interest (VOI) map on the MRI template that represented the major cortical and subcortical structures of the rat brain. Each region was primarily defined on coronal slices, with sagittal and axial slices used to confirm regional boundaries. VOIs were defined for the rat brain hippocampus, septum, and cerebellum. After VOIs were superimposed on the normalized PET image (Fig. 4), time-activity curves (TACs) were extracted from each VOI and presented in units of standardized uptake value to normalize the differences in rat weight and administered dose.[57]

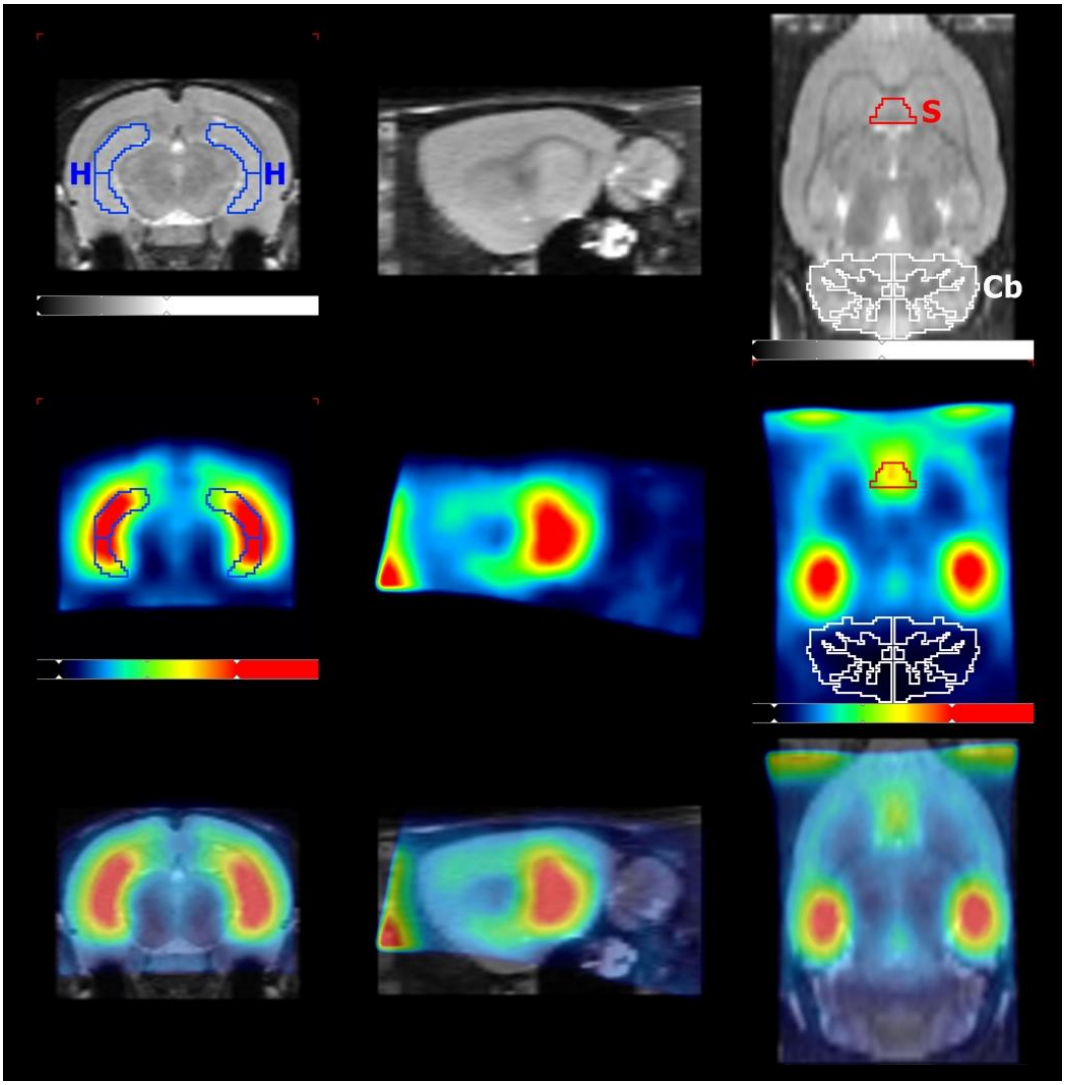

Figure 4. Predefined volumes of interest for time–activity curves and quantitative analyses. PET images were spatially co-registered to the T2-weighted magnetic resonance imaging template. Predefined volumes of interest of the septum, hippocampus, and cerebellum were overlaid on the time-averaged dynamic PET data (20–60 min) for time-activity curves and for quantitative analyses. H, hippocampus; S, septum; Cb, cerebellum.

For quantitative analysis, we used kinetic modeling and generated parametric images of [^{18}F]Mefway PET. Non-displaceable binding potential (BP_{ND}) was used to evaluate receptor density. The cerebellum was used as the reference region because it contains very few 5-HT_{1A} receptors in adult rats.[58] Individual binding value estimations in the hippocampus and septum were computed based on a multilinear reference tissue model, and the estimated BP_{ND} values were calculated.[59]

Kinetic analyses and voxel-based BP_{ND} mapping were performed using PMOD 3.3 (PMOD Technologies Ltd., Adliswil, Switzerland). Parametric BP_{ND} maps were smoothed with a Gaussian filter of 1.2 mm full width at half maximum.

6. Comparison of regional [^{18}F]Mefway BP_{ND}

We determined the [^{18}F]Mefway BP_{ND} of predefined VOIs, including the hippocampus, septum, and cerebellum, in thyroidectomized as well as control rats. Using spatially normalized BP_{ND} maps and the VOIs, regional BP_{ND} was determined in both the animal disease models and controls. Regional BP_{ND} in the thyroidectomized rats was compared with that in controls.

7. Statistical analysis

All data were presented as mean ± standard deviation or standard errors of the mean. The differences in BP_{ND} were analyzed using the Mann-Whitney U test. All statistical analyses were performed with GraphPad Prism version 5.04 (GraphPad Software, Inc., San Diego, CA, USA).

III. RESULTS

1. Validation of the hypothyroid animal model

T3 and T4 levels in the thyroidectomized rats were 7.9 ± 0.1 ng/ml and 235.6 ± 17.2 ng/ml, respectively, and were significantly lower than those in controls (12.5 ± 0.8 ng/ml, 496.4 ± 48.0 ng/ml, respectively; both $p < 0.01$; Table 1). The thyroidectomized group showed significantly higher levels of serum TSH than did the control group (21.1 ± 1.7 ng/ml vs. 1.9 ± 0.1 ng/ml; $p < 0.01$). Finally, hypothyroid status was confirmed based on the lower T3 and T4 levels and higher TSH levels in thyroidectomized rats as compared with those in the control rats.

Table 1. Results of thyroid function tests[1] Hypothyroidism was verified in the thyroidectomized rats by low serum T3 and T4 values and high TSH values

Group	TSH (ng/ml)	T3 (ng/ml)	T4 (ng/ml)
Thyroidectomized	21.1 ± 1.7	7.9 ± 0.1	235.6 ± 17.2
Control	1.9 ± 0.1	12.5 ± 0.8	496.4 ± 48.0

[1]Values are expressed as mean ± standard deviation (n = 5 for each group).

2. Comparison of regional [^{18}F]Mefway uptake

Dynamic PET images were averaged between 20 and 60 min time-periods. Representative PET images showed globally increased [^{18}F]Mefway in the whole brain, including the hippocampus and septum (Fig. 5).

Time-activity curves revealed that the thyroidectomized rats showed a significant increase in [^{18}F]Mefway uptake in the hippocampus and septum, compared to the sham-surgery group. The degrees of [^{18}F]Mefway uptake in these two regions were 25–52% higher than those of the control group (Fig. 6).

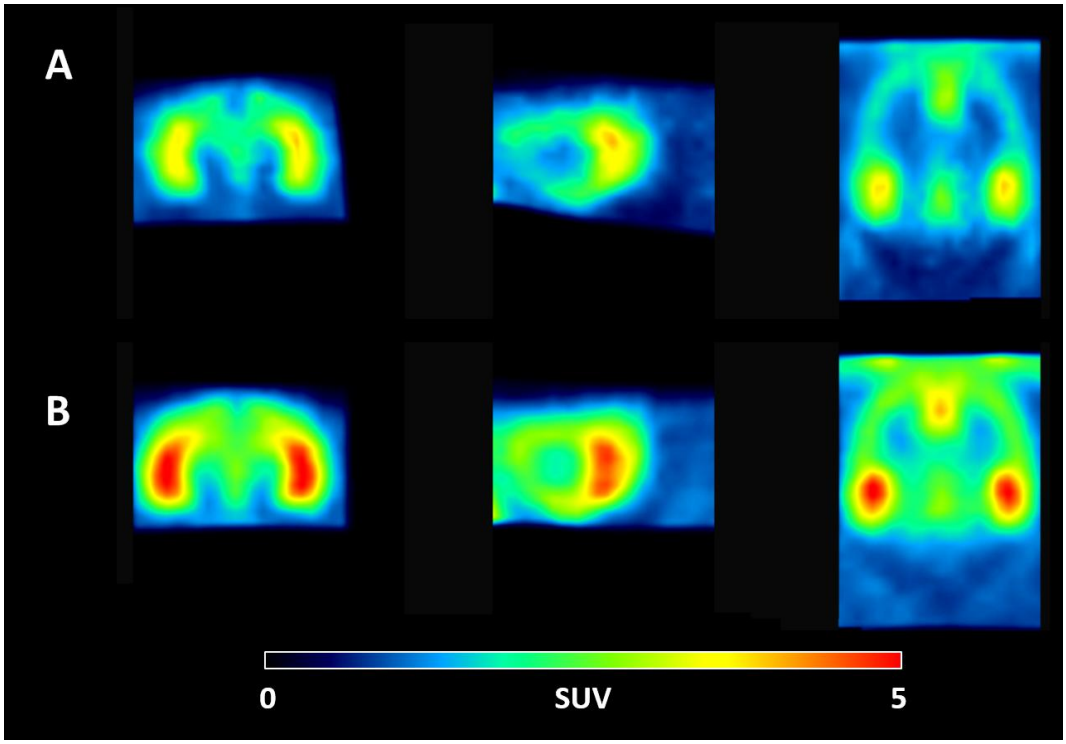

Figure 5. Representative [^{18}F]Mefway PET images. PET images were averaged between 20 and 60 min. When compared to the control group (A), both septal and hippocampal uptakes were significantly higher in the thyroidectomized group (B).

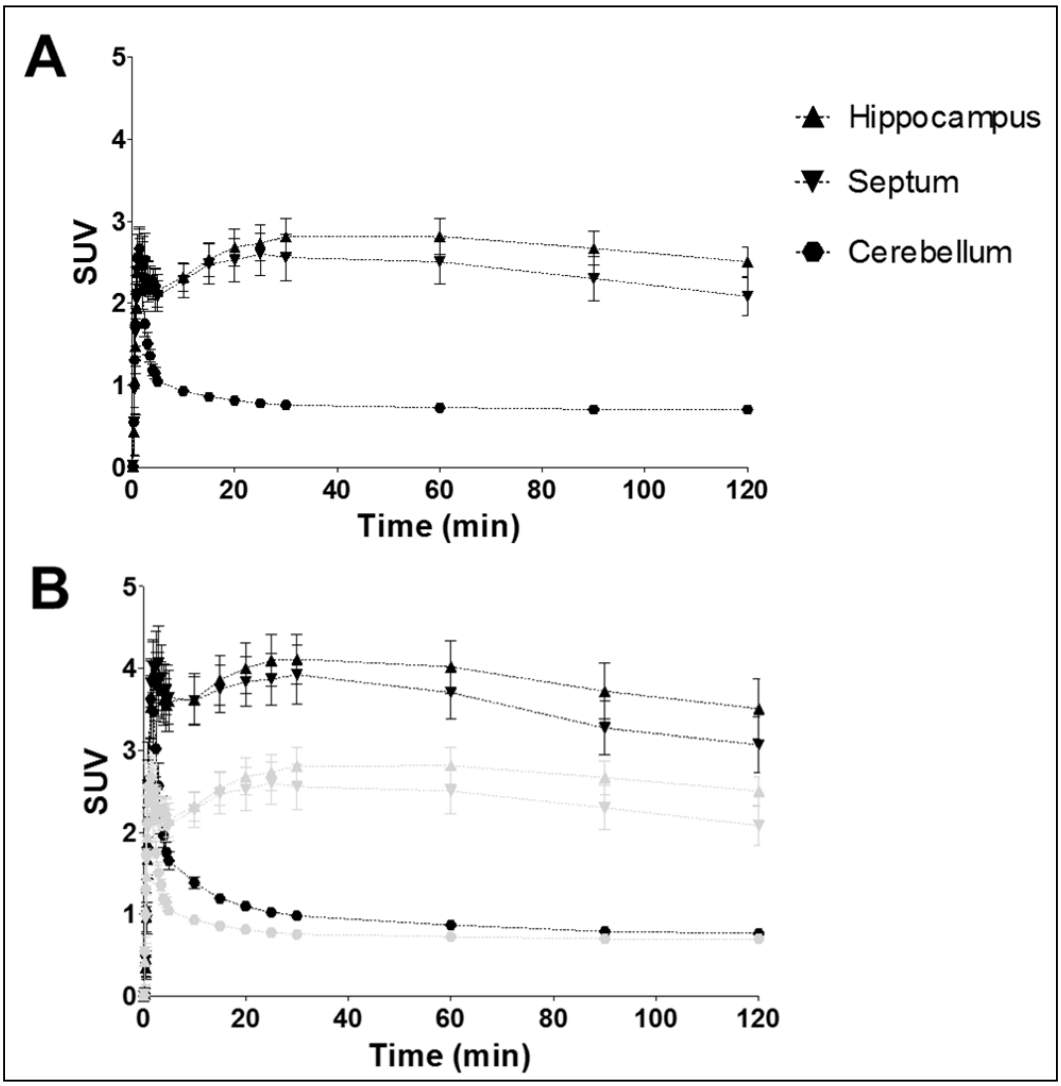

Figure 6. Time-activity curves of [^{18}F]Mefway uptake in specific regions of the brain. The degree of regional radiotracer uptake was expressed as a standardized uptake value. When compared to the control group (A), [^{18}F]Mefway uptake in the hippocampus and septum was 25–52% higher in the thyroidectomized group (B). In contrast, cerebellar uptake was similar in both groups, characterized by a rapid washout followed by a plateau at a low concentration in the later phase.

3. Changes of [^{18}F]Mefway BP_{ND} in hypothyroidism

Using predefined VOIs, the [^{18}F]Mefway BP_{ND} of each brain region of the rats in the hypothyroidism model group was compared with that of the rats in the control group. As shown in Fig. 7, the [^{18}F]Mefway BP_{ND} of the hippocampus was increased in the thyroidectomized group (3.4 ± 0.4) as compared to the control group (2.7 ± 0.4; $p < 0.01$). In the septum, the [^{18}F]Mefway BP_{ND} of the thyroidectomized rats demonstrated a significantly higher uptake than that seen in the controls (3.0 ± 0.4 *vs.* 2.5 ± 0.4; $p < 0.01$).

Parametric images of [^{18}F]Mefway BP_{ND} were generated using a multilinear reference tissue model. In the hypothyroidism model group, BP_{ND} was significantly increased in the hippocampus and septum, as compared to the control group (Fig. 8).

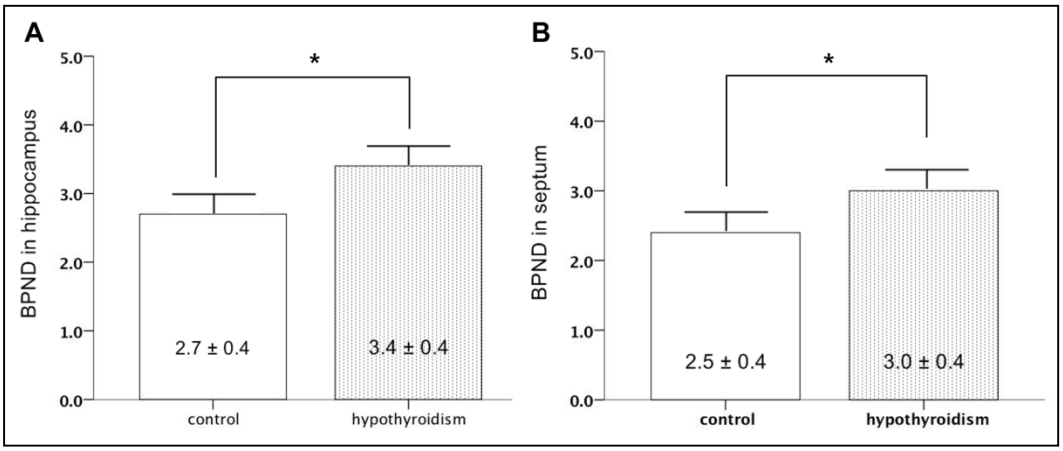

Figure 7. Comparison of the [^{18}F]Mefway BP_{ND} in different brain regions. The thyroidectomized group showed significantly higher BP_{ND} in both the hippocampus (A) and the septum (B) (*$p < 0.01$) compared with that seen in the controls. Values are expressed as mean ± the standard error of the mean.

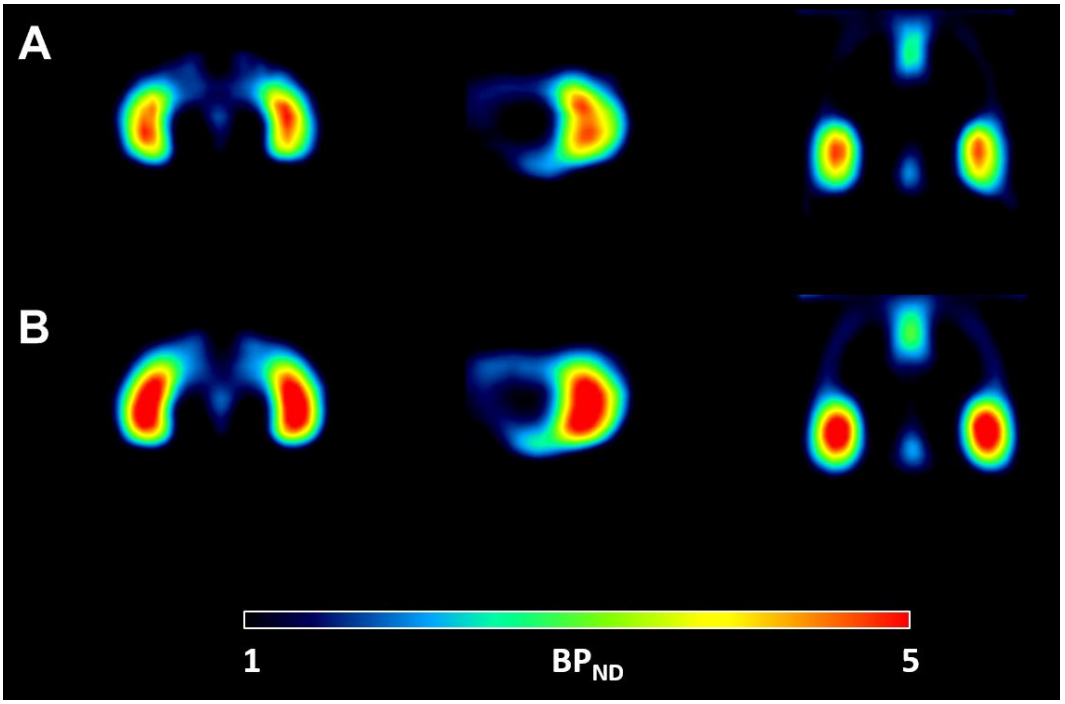

Figure 8. Representative BP_{ND} parametric map. Compared to the control group (A), the thyroidectomized group (B) shows higher BP_{ND} values in the hippocampus and septum, which is consistent with the result of the volume-of-interest-based analysis.

IV. DISCUSSION

This was the first non-invasive functional imaging study of the effect of hypothyroidism on 5-HT_{1A} receptors in the rat brain, specifically in the limbic system. In thyroidectomized rats, both [^{18}F]Mefway uptake and BP_{ND} were significantly higher than in the sham-operated rats. The results of the present study may facilitate understanding of the pathophysiology of neuropsychiatric disorders in terms of HPT axis dysfunction, and may show the translational possibility of using [^{18}F]Mefway to image 5-HT_{1A} receptors in patients with neuropsychiatric disorders.

1. Measurement of regional [^{18}F]Mefway uptake

The 5-HT system is known to regulate various brain functions, such as mood, appetite, sleep, and cognition. Two major pathways have been described for the 5-HT projections from the dorsal raphe nucleus to the forebrain.[60] Although there is extensive overlap between raphe projections, the dorsal raphe projects to the frontal cortex and striatum, while the median raphe innervates the hippocampus and the septum. 5-HT_{1A} receptors, key players in the CNS 5-HT system, are heterogeneously distributed throughout the rat brain. The dorsal raphe nucleus and limbic system have abundant 5-HT_{1A} receptors, while these receptors are scarce in the cerebellum and brainstem. In the presynapses of the raphe nucleus, they act as autoreceptors that modulate the reuptake of synaptic 5-HT, causing a decrease in the release of 5-HT into the terminal fields. 5-HT_{1A} receptors are also located postsynaptically in the cortical and limbic area.[61]

In the present study, the hippocampus and septum were selected as regions of interest because they were known to comprise major afferent pathways of 5-HT neurotransmission within the limbic system, which supports a variety of functions including emotion, behavior, motivation, long-term memory, and olfaction. The high receptor density in these regions indicated that the 5-HT_{1A} receptor played an important role in mood, behavior modulation, and cognitive functions. For example, several MRI

studies have revealed a lower hippocampal volume in patients suffering from depression.[62] A morphometric analysis has also shown a decreased volume of septal nuclei in schizophrenia and affective disorders.[63]

In our study, the time-activity curves for [^{18}F]Mefway in the cerebellum were characterized by rapid washout, followed by low radioactivity concentrations over 2 h (Fig. 6). This cerebellar uptake pattern is similar for other 5-HT$_{1A}$ radioligands, resulting in low counting statistics in the cerebellum at later stages. Low cerebellar uptake is largely due to the scanty amount of 5-HT$_{1A}$. However, unlike the metabolites of [^{11}C]WAY-100635, the metabolites of [^{18}F]Mefway do not cross the blood–brain barrier and therefore do not contribute to an increase in nonspecific radioactivity in the brain, thereby facilitating biomedical modeling of PET data. One potential cause of the low cerebellar uptake could have been the presence of a P-glycoprotein transporter, but this requires further investigation in the future.

In this study, quantitative analysis was not performed for the raphe nucleus, which also has abundant 5-HT$_{1A}$ receptors. The raphe nucleus has already been a region of interest in the field of 5-HT receptor studies because presynaptic 5-HT receptors in this region function as a point of control for activity in 5-HT neurons. Furthermore, one animal study has indicated that it is feasible to delineate the raphe nucleus in rat brain PET images.[17] As shown in Fig. 6, there was a distinct area of increased radiotracer uptake against the background of scanty uptake in the brainstem that probably corresponded to the raphe nucleus; however, reliable segmentation of radioactivity in the raphe nucleus seems to be difficult without accurate anatomic information, and becomes more cumbersome when the receptor density changes in the region. Manual segmentation of raphe nucleus radioactivity is somewhat arbitrary and is susceptible to both overestimation and underestimation, specifically in animal models of disease. For this reason, the raphe nucleus was not included in the quantitative analysis.

2. Mechanism of increased [^{18}F]Mefway uptake in the brain after thyroidectomy

The density of 5-HT_{1A} receptors has been studied most in experimentally induced hypothyroid animals. Studies of the density of 5-HT_{1A} (postsynaptic) receptors outside the brainstem, however, have yielded contradictory results. An increase in cortical and hippocampal 5-HT_{1A} receptors was observed by Tejani-Butt et al.,[64] but not by Hong et al.[65] and Kulikov et al.,[66] who found no significant differences as compared to euthyroid adult rodents. Furthermore, clinical studies of patients with major depressive disorder have consistently detected a decrease in 5-HT_{1A} receptor binding in the raphe nucleus and limbic and cortical regions.[67-69]

In this study, BP_{ND} was used as a measure of receptor density, and there was greater binding in the limbic regions of thyroidectomized animals relative to those of the control animals. One mechanism suggested for this increased radioligand uptake is the upregulation of postsynaptic 5-HT_{1A} receptors as an early response to a local decrease in 5-HT in the synaptic region.

Previous studies have demonstrated that hypothyroidism alters the function of the hippocampus, resulting in cognitive impairment, anxiety, and depressive symptoms in rodent models and humans.[70,71] It has also been reported that reduced neurogenesis is responsible for neurocognitive problems, anxiety, depression,[72] and neurodegenerative diseases.[73] Recently, the subgranular zone of the hippocampal dentate gyrus and the subventricular zone have been considered to be the two main places where neurogenesis occurs in the adult mammalian brain.[74] Thyroid hormone, specifically T3, was reported to regulate cell proliferation and neural stem cell commitment toward the neuroblast in both the rodent subgranular zone and subventricular zone.[75,76] In one study, hypothyroidism in adult rats induced a decrease of proliferation by 30% in the adult subgranular zones that was restored by thyroid hormone replacement.[77] Thus, it is suggested that neuropsychiatric disorders associated with hypothyroidism may be related to reduced hippocampal neurogenesis.

Studies have supported changes in the brains of hypothyroid adult rats as follows: an increase in 5-hydroxyindoleacetic acid, the main 5-HT metabolite, in the brainstem;[42,78,79] a reduced 5-HT concentration in the cortex;[39] and a decreased concentration of serotonin precursor (5-hydroxytryptophan) throughout the brain.[80] On the basis that there is increased 5-HT turnover in the brainstem, and decreased levels of 5-HT and its precursors in other brain regions, it can be concluded that hypothyroidism causes a decrease in 5-HT release in the cortical projection area by increasing 5-HT turnover in the brainstem and consequently activating the raphe 5-HT_{1A} autoreceptors.

When there is a prolonged lack of stimulation due to reduced amounts of neurotransmitter, there is often an increase in the number of cell receptors, hence the postsynaptic neurons become hypersensitive. We hypothesized that this hypersensitive response to a decreased 5-HT concentration may be responsible for the increased [^{18}F]Mefway uptake in the limbic region after thyroidectomy. Such a result would be consistent with a 5-HT receptor study by Tejani-Butt et al.[64] In addition, if a decrease in 5-HT concentration persists for a long time, this may eventually result in the downregulation of 5-HT_{1A} receptors in the cortex and limbic system, causing a reduced radiotracer uptake in the corresponding regions. The temporal change in the postsynaptic cellular response to decreased 5-HT concentration may account for the contradictory results from previous and present studies. However, this assumption requires further investigation and validation. If the dissociation constant is the same in the experimental and control groups, the increase in the binding potential can be attributed to an increase in the available 5-HT_{1A} receptor on the plasma membrane. When the 5-HT concentration is lowered in the synapse, the 5-HT_{1A} receptor is initially upregulated in response to it, but it is expected to be down-regulated later. The change in the later time should be further validated.

In the present study, we did not further confirm the reversal of increased 5-HT_{1A} receptor density produced by the thyroidectomy via thyroid hormone replacement. It was because the rest-retest reliability of [^{18}F]Mefway PET has not been validated in the rodents; that is, PET results from different time points in the same animal may give

different results. And we did not evaluate the changes in the 5-HT_{1A} transporter, either. It is well known that regulation of the transporter is also essential in the modulation of neurotransmission at the synapse. A few animal studies have reported that the 5-HT_{1A} transporter density remained unchanged in hypothyroidism based on the Wistar rat model,[66,81] but it is possible that a difference in species may yield different results, as found in the 5-HT_{1A} receptor studies.

3. The advantages of [^{18}F]Mefway

With regard to the applicability of these findings to clinical research, [^{18}F]Mefway has several advantages over the currently available radioligands for 5-HT_{1A} receptor imaging, that is, [^{11}C]WAY-100635, [^{18}F]FCWAY, and [^{18}F]MPPF. First of all, [^{18}F]Mefway has a practical advantage in its use of ^{18}F. [^{11}C]-radioligands have limited utility due to the short half-life of ^{11}C ($t_{1/2}$ = 20 min). Thereby, PET facilities that lack a cyclotron have difficulty in acquiring such radioligands from distant cyclotron centers. For accurate quantification of hippocampal 5-HT_{1A} levels, longer scan times (> 2 h) are required because the equilibrium time of the radioligand to the 5-HT_{1A} receptors appears to exceed 2 h, thus further limiting the utility of [^{11}C]-radioligands.

Secondly, [^{18}F]Mefway circumvents the defluorination issue that is the main barrier for the clinical application of [^{18}F]FCWAY. Defluorination hampers the exact quantification of receptor density due to a spillover effect in which radioactivity in the skull can also be detected in nearby brain regions, such as the cortex. Besides defluorination, another practical drawback of [^{18}F]FCWAY is that it is difficult to synthesize due to the low yield of 4-fluorocyclohexane carboxilic acid. A radioprobe and its precursor must have a sufficient and reproducible synthetic yield for use in PET experiments.

Thirdly, the metabolites of [^{18}F]Mefway do not cross the blood–brain barrier. The main advantage of [^{11}C]WAY-100635 is its high target-to-background ratio, but its rapid

metabolism in plasma–with consequent crossing of the blood–brain barrier into the brain–makes quantification more challenging and BP_{ND} estimates unreliable when the cerebellum is used as a reference tissue.[82] In contrast, the metabolites of [^{18}F]Mefway do not re-enter the brain, while showing comparable specific binding to the receptors.

Finally, [^{18}F]Mefway PET can serve as a useful tool in animal research, making it possible to reduce the number of smaller animals required in longitudinal studies. In special situations, such as unilateral lesion studies, the reproducibility of data from these imaging studies may actually be superior to that obtained from traditional invasive techniques, because each animal serves as its own control. [^{18}F]Mefway PET provides a bridge from animal research to human research and clinical applications.

V. CONCLUSION

In the present study, we investigated [^{18}F]Mefway uptake in the hippocampus and septum of experimental animal models of hypothyroidism, and VOI-based and voxel-wise analyses showed concordant differences.

Effect of hypothyroidism on 5-HT$_{1A}$ receptors in the rat brain

A. The thyroid hormone assay verified the hypothyroid status of the thyroidectomized rats.
B. TACs showed that [^{18}F]Mefway uptake in the hippocampus and septum of the thyroidectomized rats was 25–52% higher than the uptake in the sham-operated rats.
C. VOI and voxel-based analyses also showed a significant increase in the regional brain uptake in the hypothyroidism group, as compared with the control group.

Quantitative analyses using VOI templates and a parametric map of BP$_{ND}$

A. [^{18}F]Mefway-specific brain templates were constructed by spatial co-registration of PET data to T2-weighted MRI templates for the Sprague-Dawley rat brain.
B. Both VOI and voxel-wise analyses showed enhanced brain uptake in the thyroidectomized group, as compared to the sham-surgery group.

These results suggest that hypothyroidism increases the density of postsynaptic 5-HT$_{1A}$ receptors in the rat brain.

REFERENCES

1. Joffe RT. A perspective on the thyroid and depression. Can J Psychiatry 1990;35:754-8.
2. Loosen PT. Hormones of the hypothalamic-pituitary-thyroid axis: a psychoneuroendocrine perspective. Pharmacopsychiatry 1986;19:401-15.
3. Wilson W, Jefferson JW. Thyroid disease, behavior, and psychopharmacology. Psychosomatics 1985;26:481-92.
4. Whybrow PC, Prange AJ, Jr. A hypothesis of thyroid-catecholamine-receptor interaction. Its relevance to affective illness. Arch Gen Psychiatry 1981;38:106-13.
5. Willner P. Antidepressants and serotonergic neurotransmission: an integrative review. Psychopharmacology (Berl) 1985;85:387-404.
6. Hamon M, Gozlan H, el Mestikawy S, Emerit MB, Bolanos F, Schechter L. The central 5-HT_{1A} receptors: pharmacological, biochemical, functional, and regulatory properties. Ann N Y Acad Sci 1990;600:114-29.
7. Gothert M. 5-Hydroxytryptamine receptors. An example for the complexity of chemical transmission of information in the brain. Arzneimittelforschung 1992;42:238-46.
8. Van de Kar LD. Neuroendocrine aspects of the serotonergic hypothesis of depression. Neurosci Biobehav Rev 1989;13:237-46.
9. Prange AJ, Jr., Wilson IC, Lynn CW, Alltop LB, Stikeleather RA. L-tryptophan in mania. Contribution to a permissive hypothesis of affective disorders. Arch Gen Psychiatry 1974;30:56-62.
10. Akimova E, Lanzenberger R, Kasper S. The serotonin-1A receptor in anxiety disorders. Biol Psychiatry 2009;66:627-35.
11. Savitz J, Lucki I, Drevets WC. 5-HT_{1A} receptor function in major depressive disorder. Prog Neurobiol 2009;88:17-31.

12. Assem-Hilger E, Lanzenberger R, Savli M, Wadsak W, Mitterhauser M, Mien LK, et al. Central serotonin 1A receptor binding in temporal lobe epilepsy: a [carbonyl-^{11}C]WAY-100635 PET study. Epilepsy Behav 2010;19:467-73.
13. Bara-Jimenez W, Bibbiani F, Morris MJ, Dimitrova T, Sherzai A, Mouradian MM, et al. Effects of serotonin 5-HT_{1A} agonist in advanced Parkinson's disease. Mov Disord 2005;20:932-6.
14. Schechter LE, Dawson LA, Harder JA. The potential utility of 5-HT_{1A} receptor antagonists in the treatment of cognitive dysfunction associated with Alzheimer s disease. Curr Pharm Des 2002;8:139-45.
15. Kumar JS, Mann JJ. PET tracers for 5-HT_{1A} receptors and uses thereof. Drug Discov Today 2007;12:748-56.
16. Rudin M, Weissleder R. Molecular imaging in drug discovery and development. Nat Rev Drug Discov 2003;2:123-31.
17. Saigal N, Bajwa AK, Faheem SS, Coleman RA, Pandey SK, Constantinescu CC, et al. Evaluation of serotonin 5-HT_{1A} receptors in rodent models using [^{18}F]mefway PET. Synapse 2013;67:596-608.
18. Choi JY, Kim BS, Kim CH, Kim DG, Han SJ, Lee K, et al. Translational possibility of [^{18}F]Mefway to image serotonin 1A receptors in humans: Comparison with [^{18}F]FCWAY in rodents. Synapse 2014;68:595-603.
19. Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development--current perspectives. Endocr Rev 1993;14:94-106.
20. Bernal J, Nunez J. Thyroid hormones and brain development. Eur J Endocrinol 1995;133:390-8.
21. Bauer M, Heinz A, Whybrow PC. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. Mol Psychiatry 2002;7:140-56.
22. Schwartz HL, Oppenheimer JH. Nuclear triiodothyronine receptor sites in brain: probable identity with hepatic receptors and regional distribution. Endocrinology 1978;103:267-73.

23. Ruel J, Faure R, Dussault JH. Regional distribution of nuclear T_3 receptors in rat brain and evidence for preferential localization in neurons. J Endocrinol Invest 1985;8:343-8.
24. van Doorn J, Roelfsema F, van der Heide D. Concentrations of thyroxine and 3,5,3'-triiodothyronine at 34 different sites in euthyroid rats as determined by an isotopic equilibrium technique. Endocrinology 1985;117:1201-8.
25. Campos-Barros A, Hoell T, Musa A, Sampaolo S, Stoltenburg G, Pinna G, et al. Phenolic and tyrosyl ring iodothyronine deiodination and thyroid hormone concentrations in the human central nervous system. J Clin Endocrinol Metab 1996;81:2179-85.
26. Bauer M, Whybrow PC. Thyroid hormone, neural tissue and mood modulation. World J Biol Psychiatry 2001;2:59-69.
27. Bauer M, Priebe S, Berghofer A, Bschor T, Kiesslinger U, Whybrow PC. Subjective response to and tolerability of long-term supraphysiological doses of levothyroxine in refractory mood disorders. J Affect Disord 2001;64:35-42.
28. Altshuler LL, Bauer M, Frye MA, Gitlin MJ, Mintz J, Szuba MP, et al. Does thyroid supplementation accelerate tricyclic antidepressant response? A review and meta-analysis of the literature. Am J Psychiatry 2001;158:1617-22.
29. Aronson R, Offman HJ, Joffe RT, Naylor CD. Triiodothyronine augmentation in the treatment of refractory depression. A meta-analysis. Arch Gen Psychiatry 1996;53:842-8.
30. Jung KW, Won YJ, Kong HJ, Oh CM, Shin A, Lee JS. Survival of korean adult cancer patients by stage at diagnosis, 2006-2010: national cancer registry study. Cancer Res Treat 2013;45:162-71.
31. Jung KW, Won YJ, Kong HJ, Oh CM, Seo HG, Lee JS. Cancer statistics in Korea: incidence, mortality, survival and prevalence in 2010. Cancer Res Treat 2013;45:1-14.
32. Reiners C, Lassmann M, Luster M. Recombinant human thyrotropin: safety and quality of life evaluation. J Endocrinol Invest 2012;35:30-5.

33. Borget I, Corone C, Nocaudie M, Allyn M, Iacobelli S, Schlumberger M, et al. Sick leave for follow-up control in thyroid cancer patients: comparison between stimulation with Thyrogen and thyroid hormone withdrawal. Eur J Endocrinol 2007;156:531-8.
34. Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. Physiol Rev 1992;72:165-229.
35. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 1994;46:157-203.
36. Kepe V, Barrio JR, Huang SC, Ercoli L, Siddarth P, Shoghi-Jadid K, et al. Serotonin 1A receptors in the living brain of Alzheimer's disease patients. Proc Natl Acad Sci U S A 2006;103:702-7.
37. Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, et al. Reduced serotonin-1A receptor binding in social anxiety disorder. Biol Psychiatry 2007;61:1081-9.
38. Haleem DJ, Samad N, Perveen T, Haider S, Haleem MA. Role of serotonin-1A receptors in restraint-induced behavioral deficits and adaptation to repeated restraint stress in rats. Int J Neurosci 2007;117:243-57.
39. Ito JM, Valcana T, Timiras PS. Effect of Hypo- and Hyperthyroidism on Regional Monoamine Metabolism in the Adult Rat Brain. Neuroendocrinology 1977;24:55-64.
40. Cleare AJ, McGregor A, Chambers SM, Dawling S, O'Keane V. Thyroxine replacement increases central 5-hydroxytryptamine activity and reduces depressive symptoms in hypothyroidism. Neuroendocrinology 1996;64:65-9.
41. Henley WN, Vladic F. Hypothyroid-induced changes in autonomic control have a central serotonergic component. Am J Physiol 1997;272:H894-903.
42. Savard P, Merand Y, Di Paolo T, Dupont A. Effects of thyroid state on serotonin, 5-hydroxyindoleacetic acid and substance P contents in discrete brain nuclei of adult rats. Neuroscience 1983;10:1399-404.

43. Grasby PM, Bench C. Neuroimaging of mood disorders. Current Opinion in Psychiatry 1997;10:73-8.

44. Rabiner EA, Bhagwagar Z, Gunn RN, Sargent PA, Bench CJ, Cowen PJ, et al. Pindolol augmentation of selective serotonin reuptake inhibitors: PET evidence that the dose used in clinical trials is too low. Am J Psychiatry 2001;158:2080-2.

45. Newberg AB, Moss AS, Monti DA, Alavi A. Positron emission tomography in psychiatric disorders. Ann N Y Acad Sci 2011;1228:E13-25.

46. Phelps ME, Mazziotta JC, Baxter L, Gerner R. Positron emission tomographic study of affective disorders: problems and strategies. Ann Neurol 1984;15 Suppl:S149-56.

47. Brody AL, Saxena S, Silverman DH, Alborzian S, Fairbanks LA, Phelps ME, et al. Brain metabolic changes in major depressive disorder from pre- to post-treatment with paroxetine. Psychiatry Res 1999;91:127-39.

48. Aznavour N, Zimmer L. [^{18}F]MPPF as a tool for the in vivo imaging of 5-HT$_{1A}$ receptors in animal and human brain. Neuropharmacology 2007;52:695-707.

49. Lang L, Jagoda E, Schmall B, Vuong BK, Adams HR, Nelson DL, et al. Development of fluorine-18-labeled 5-HT$_{1A}$ antagonists. J Med Chem 1999;42:1576-86.

50. Ryu YH, Liow JS, Zoghbi S, Fujita M, Collins J, Tipre D, et al. Disulfiram inhibits defluorination of ^{18}F-FCWAY, reduces bone radioactivity, and enhances visualization of radioligand binding to serotonin 5-HT$_{1A}$ receptors in human brain. J Nucl Med 2007;48:1154-61.

51. Saigal N, Pichika R, Easwaramoorthy B, Collins D, Christian BT, Shi B, et al. Synthesis and biologic evaluation of a novel serotonin 5-HT$_{1A}$ receptor radioligand, ^{18}F-labeled mefway, in rodents and imaging by PET in a nonhuman primate. J Nucl Med 2006;47:1697-706.

52. Choi JY, Kim CH, Jeon TJ, Kim BS, Yi CH, Woo KS, et al. Effective microPET imaging of brain 5-HT$_{1A}$ receptors in rats with [^{18}F]MeFWAY by suppression of radioligand defluorination. Synapse 2012;66:1015-23.

53. Choi JY, Kim CH, Ryu YH, Seo YB, Truong P, Kim EJ, et al. Optimization of the radiosynthesis of [^{18}F]MEFWAY for imaging brain serotonin 1A receptors by using the GE TracerLab FXFN-Pro module. J Labelled Comp Radiopharm 2013;56:589-94.

54. Casteels C, Vermaelen P, Nuyts J, Van Der Linden A, Baekelandt V, Mortelmans L, et al. Construction and evaluation of multitracer small-animal PET probabilistic atlases for voxel-based functional mapping of the rat brain. J Nucl Med 2006;47:1858-66.

55. Casteels C, Vunckx K, Aelvoet SA, Baekelandt V, Bormans G, Van Laere K, et al. Construction and evaluation of quantitative small-animal PET probabilistic atlases for [^{18}F]FDG and [^{18}F]FECT functional mapping of the mouse brain. PLoS One 2013;8:e65286.

56. Vallez Garcia D, Casteels C, Schwarz AJ, Dierckx RA, Koole M, Doorduin J. A standardized method for the construction of tracer specific PET and SPECT rat brain templates: validation and implementation of a toolbox. PLoS One 2015;10:e0122363.

57. Schiffer WK, Mirrione MM, Biegon A, Alexoff DL, Patel V, Dewey SL. Serial microPET measures of the metabolic reaction to a microdialysis probe implant. J Neurosci Methods 2006;155:272-84.

58. el Mestikawy S, Riad M, Laporte AM, Verge D, Daval G, Gozlan H, et al. Production of specific anti-rat 5-HT_{1A} receptor antibodies in rabbits injected with a synthetic peptide. Neurosci Lett 1990;118:189-92.

59. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 2003;23:1096-112.

60. Kosofsky BE, Molliver ME. The serotoninergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. Synapse 1987;1:153-68.

61. Popova NK, Naumenko VS. 5-HT_{1A} receptor as a key player in the brain 5-HT system. Rev Neurosci 2013;24:191-204.
62. Campbell S, Marriott M, Nahmias C, MacQueen GM. Lower hippocampal volume in patients suffering from depression: a meta-analysis. Am J Psychiatry 2004;161:598-607.
63. Brisch R, Bernstein HG, Dobrowolny H, Krell D, Stauch R, Trubner K, et al. A morphometric analysis of the septal nuclei in schizophrenia and affective disorders: reduced neuronal density in the lateral septal nucleus in bipolar disorder. Eur Arch Psychiatry Clin Neurosci 2011;261:47-58.
64. Tejani-Butt SM, Yang J, Kaviani A. Time course of altered thyroid states on 5-HT_{1A} receptors and 5-HT uptake sites in rat brain: an autoradiographic analysis. Neuroendocrinology 1993;57:1011-8.
65. Hong TP, Huang TY, Qiu XC. Effects of different thyroid states on 5-HT1A receptor in adult rat brain. Sheng Li Xue Bao 1992;44:75-80.
66. Kulikov AV, Jeanningro R. The effects of hypothyroidism on 5-HT_{1A} and 5-HT_{2A} receptors and the serotonin transporter protein in the rat brain. Neurosci Behav Physiol 2001;31:445-9.
67. Bhagwagar Z, Rabiner EA, Sargent PA, Grasby PM, Cowen PJ. Persistent reduction in brain serotonin1A receptor binding in recovered depressed men measured by positron emission tomography with [^{11}C]WAY-100635. Mol Psychiatry 2004;9:386-92.
68. Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ, Mathis C. Serotonin type-1A receptor imaging in depression. Nucl Med Biol 2000;27:499-507.
69. Sargent PA, Rabiner EA, Bhagwagar Z, Clark L, Cowen P, Goodwin GM, et al. 5-HT_{1A} receptor binding in euthymic bipolar patients using positron emission tomography with [carbonyl-^{11}C]WAY-100635. J Affect Disord 2010;123:77-80.
70. Dugbartey AT. Neurocognitive aspects of hypothyroidism. Arch Intern Med 1998;158:1413-8.
71. Fernandez-Lamo I, Montero-Pedrazuela A, Delgado-Garcia JM, Guadano-Ferraz A, Gruart A. Effects of thyroid hormone replacement on associative learning and

hippocampal synaptic plasticity in adult hypothyroid rats. Eur J Neurosci 2009;30:679-92.

72. Mirescu C, Gould E. Stress and adult neurogenesis. Hippocampus 2006;16:233-8.
73. Fu AL, Zhou CY, Chen X. Thyroid hormone prevents cognitive deficit in a mouse model of Alzheimer's disease. Neuropharmacology 2010;58:722-9.
74. Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011;70:687-702.
75. Lopez-Juarez A, Remaud S, Hassani Z, Jolivet P, Pierre Simons J, Sontag T, et al. Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche. Cell Stem Cell 2012;10:531-43.
76. Kapoor R, Desouza LA, Nanavaty IN, Kernie SG, Vaidya VA. Thyroid hormone accelerates the differentiation of adult hippocampal progenitors. J Neuroendocrinol 2012;24:1259-71.
77. Montero-Pedrazuela A, Venero C, Lavado-Autric R, Fernandez-Lamo I, Garcia-Verdugo JM, Bernal J, et al. Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. Mol Psychiatry 2006;11:361-71.
78. Savard P, Merand Y, Di Paolo T, Dupont A. Effect of neonatal hypothyroidism on the serotonin system of the rat brain. Brain Res 1984;292:99-108.
79. Henley WN, Bellush LL, Tressler M. Bulbospinal serotonergic activity during changes in thyroid status. Can J Physiol Pharmacol 1998;76:1120-31.
80. Jacoby JH, Mueller G, Wurtman RJ. Thyroid state and brain monoamine metabolism. Endocrinology 1975;97:1332-5.
81. Kulikov A, Moreau X, Jeanningros R. Effects of experimental hypothyroidism on 5-HT_{1A}, 5-HT_{2A} receptors, 5-HT uptake sites and tryptophan hydroxylase activity in mature rat brain1. Neuroendocrinology 1999;69:453-9.
82. Billard T, Le Bars D, Zimmer L. PET radiotracers for molecular imaging of serotonin 5-HT_{1A} receptors. Curr Med Chem 2014;21:70-81.

ABSTRACT (IN KOREAN)

[^{18}F]Mefway 양전자방출단층촬영술로 측정한 갑상선기능저하증이 쥐 뇌의 세로토닌 1A 수용체에 미치는 영향

<지도교수 유영훈>

연세대학교 대학원 의학과

이 재 훈

이 연구는 동물 질병 모델 및 4-(*trans*-[^{18}F]fluoranylmethyl)-*N*-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-pyridin-2-ylcyclohexane-1-carboxamide ([^{18}F]Mefway) 양전자방출단층촬영술(PET)을 사용하여 갑상선기능저하증이 뇌의 세로토닌 1A 수용체에 미치는 영향을 연구하였다.

갑상선절제술을 받은 Sprague-Dawley 수컷 쥐 5마리를 갑상선기능저하증 집단에 배정하였고, 모의 수술(sham operation)을 받은 Sprague-Dawley 수컷 쥐 5마리를 대조군으로 설정하였다. 갑상선기능저하 상태는 갑상선 기능 검사로 확인하였다. 산소에 2%의 아이소플루레인을 혼합하여 마취를 시킨 후, [^{18}F]Mefway의 탈불소화를 방지하기 위하여, 1시간 동안 플루코나졸을 투여하였다. 그 다음 8.6–11.1 MBq의 [^{18}F]Mefway를 1 ml/min 속도로 투여한 후, PET 영상을 120분간 연속적으로 획득하였다. PET 영상은 2차원 정칙화된 기댓값 최대화 알고리즘(ordered subsets expectation maximization algorithm)을

이용하여 재구성하였다.

모든 PET 영상은 T2 강조 자기공명영상 틀(template)에 공간정규화 한 후 사전 정의된 관심영역 틀을 이용하여 해마, 사이막(septum), 소뇌의 시간방사능곡선을 추출하였다. 해마와 사이막의 수용체 결합능(non-displaceable binding potential)은 소뇌를 참조조직으로 삼아 다중선형 참조조직 모형(multilinear reference tissue model)을 통하여 계산하였고 리간드-특정 모수지도(parametric map)를 작성하였다.

시간방사능곡선에서 갑상선기능저하증 집단의 해마와 사이막 섭취는 대조군보다 25-52% 더 많았고 이 부위의 결합능은 갑상선절제술을 받은 쥐에서 대조군에 비하여 30% 가량 더 높았다.

결론적으로, 갑상선기능저하증은 해마와 사이막의 시냅스후 세로토닌 1A 수용체 밀도를 증가시켰다. 이는 갑상선기능저하증으로 인해 시냅스의 세로토닌 농도가 감소하고 이에 따른 초기 반응으로써 시냅스후 세로토닌 1A 수용체가 상향조절 되었기 때문일 것이다.

핵심되는 말: [^{18}F]Mefway, 갑상선기능저하증, 양전자방출단층촬영술, 세로토닌 1A 수용체, 갑상선