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Original Article

# Maturational Changes of Gamma-Aminobutyric Acid A Receptors Measured With Benzodiazepine Binding of Iodine 123 Iomazenil Single-Photon Emission Computed Tomography

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ABSTRACT

*Background:* Iodine 123 (I-123) iomazenil is a specific ligand of the central benzodiazepine receptor, which is a part of the postsynaptic gamma-aminobutyric acid A receptor complex. We performed statistical image processing of I-123 iomazenil single-photon emission computed tomography to elucidate maturational changes in the GABAergic system.
*Methods:* Thirty patients (18 boys and 12 girls, aged 17 days to 14 years) with cryptogenic focal epilepsy were enrolled and underwent I-123 iomazenil single-photon emission computed tomography. We used a semiquantitative analytical method consisting of brain surface extraction, anatomic normalization, and a three-parameter exponential model. We then assessed developmental changes in benzodiazepine receptor binding activity in 18 regions of interest in both hemispheres.
*Results:* The highest benzodiazepine receptor binding activity was observed during early infancy in all regions of interest. Benzodiazepine receptor binding activity then decreased exponentially across development. Benzodiazepine receptor binding in the primary sensorimotor cortex, primary visual cortex, cerebellar vermis, and striatum declined more rapidly than that in the cerebellar hemispheres and the frontal cortex. The pons and the thalamus had the lowest benzodiazepine receptor binding activities during the neonatal period, and benzodiazepine receptor binding in these areas declined gradually after infancy toward adolescence. There were no differences in adjusted benzodiazepine receptor binding activity according to laterality or sex.
*Conclusions:* Benzodiazepine receptor binding activity decreased exponentially during infancy in all regions of interest. Binding activity in the primary somatosensory and motor cortices (M1 and S1), the primary and association visual areas, the cerebellar vermis, and the striatum (caudate nucleus and putamen) tended to decline more rapidly than that in the cerebellar hemisphere and the frontal association cortex.


## Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in adult mammalian brains. It plays a key role in regulating central nervous system excitability, as well as the pathophysiology of epilepsy.[1,2] GABA acts as a depolarizing neurotransmitter during early brain development because the higher intracellular concentration of $Cl^-$ ion causes this anion to flow out of the neuron. The expression of the $K^+$-$Cl^-$ cotransporter and that of carbonic anhydrase VII in maturity are key events acting as developmental switches in GABAergic signaling.[3,4] These changes alter "ionic plasticity" and $Cl^-$ and $HCO_3^-$ ion concentrations in postsynaptic neurons.

In addition to its function as a neurotransmitter, GABA is involved in experience-dependent plasticity and synaptogenesis, especially in the immature brain. Synaptic plasticity is bidirectional, resulting in weaker or stronger synapses, depending on experiences and activity.[5] Moreover, the elimination of synapses is essential for learning and memory.[6] Considerable evidence indicates that the GABAergic system also regulates experience-dependent critical-period plasticity in the somatosensory and visual cortices during early development.[7]

Iodine 123 (I-123) iomazenil is a specific ligand of the central benzodiazepine receptor (BZR), which is a part of the postsynaptic GABAA receptor ($GABA_AR$) complex. Benzodiazepine binding sites are located at the interface between α- and γ-subunits of $GABA_AR$.[8,9] Therefore the distribution of I-123 iomazenil is thought to represent the location of $GABA_ARs$. Previous studies analyzed maturational change of $GABA_ARs$ using positron emission topography and

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flumazenil (FMZ), a benzodiazepine antagonist during the neonatal period and after two years of age.[10,11] These studies reported that FMZ binding during the neonatal period was highest in the amygdalohippocampal region and the sensory motor cortex, whereas the cerebellum and most of the cerebral cortex showed relatively low binding. After two years of age, FMZ binding decreased across all brain regions. Beyond the studies of Chugani et al.,[11] however, little is known about how the GABAergic system changes in the developing human brain, from infancy to adolescence. Investigation of developmental changes in BZR binding activity will be useful for understanding the pharmacodynamic action of anticonvulsants and many kinds of pediatric neurological diseases, including epilepsy. Thus we performed statistical image processing of I-123 iomazenil single-photon emission computed tomography (SPECT) images to measure maturational changes of the GABAergic system.

## Materials and Methods

### *Subjects*

We performed 370 I-123 iomazenil SPECT scans at Saitama Children's Medical Center from January 2011 to March 2016. Of these scans, 30 met the following criteria: (1) no evident abnormalities in I-123 iomazenil SPECT, (2) no structural abnormalities of the brain as visualized using magnetic resonance imaging (MRI), (3) no neurological abnormalities except for epilepsy and no developmental delay at the time of SPECT, and (4) no benzodiazepine medications. Thus this study included 30 patients (18 boys) with one SPECT scan each. The underlying disorder was epilepsy in 21 patients, conversion disorder in six, and nonepileptic physiological movement in two. One subject was inadvertently scanned because of Münchausen syndrome by proxy in the caregiver. The patients' median age at scanning was 15.6 months (interquartile range 5.1 to 126.0 months). The patients' age range was 17 days to 14 years. All studies were performed after informed consent had been obtained from the child or the parents. The present study was approved by the Saitama Children's Medical Center Institutional Review Board.

### *SPECT scanning protocol*

Patients were scanned three hours after intravenous injection of the tracer using a triple-head gamma camera (Multispect3, Siemens; Malvern, PA). Patients younger than 12 months received 55.5 MBq, patients one to three years old received 74 MBq, patients four to nine years old received 111 MBq, and those 10 years or older received 167 MBq. Triclofos sodium, chloral hydrate, or pentobarbital calcium was administered as a sedative to patients who were unable to remain still in the scanner. A fan beam collimator and a $128 \times 128$ acquisition matrix were used for data acquisition. Images were reconstructed using a Butterworth filter (cutoff frequency: 0.25 Nyquist). Attenuation was corrected according to the Chang technique using an absorption coefficient of 0.05/cm.[12]

### *Image processing*

We performed a semiquantitative analysis using three-dimensional stereotactic surface projections in AZE Virtual Place Hayabusa, as described by Minoshima.[13] The method consists of two steps: brain surface extraction and anatomic normalization. First, linear scaling eliminated the differences in brain size among individuals. Second, regional anatomic differences were minimized using a nonlinear warping technique. As a result, each brain was standardized to match the adult Talairach standard atlas while preserving variation in regional binding activity. The maximum cortical activity was extracted to adjacent predefined surface pixels on a pixel-by-pixel basis using the three-dimensional stereotactic surface projection techniques. For each predefined contour pixel, a search for the peak cortical pixel was conducted in a standardized individual's image set on a predefined line vertical to the standard atlas contour, with a search depth of six pixels. The maximum search depth was 13.5 mm, which approximately covers all peak gray matter activity in the SPECT images. The peak pixel value was assigned to the corresponding surface pixel (surface projection). We used the stereotactic volume of interest template to calculate the averaged BZR binding activity in each region of interest (ROI). A predefined set of volumes of interest covering these areas is shown in Fig 1.

### *Data analysis*

Averaged BZR binding activities were normalized to the injected dose of tracer and the collection time, as described further. Averaged BZR binding activities in each ROI were plotted by age.

$$\text{Normalized BZR binding activity} = \frac{\text{Averaged BZR binding activity in ROI}}{\text{Injected dose of tracer } (MBq) \times (\text{Collection time (min)}/30 \text{ min})}$$

We used a three-parameter exponential equation to model the data. First, we calculated the average BZR binding activity in children over 10 years of age. We tested the hypothesis that there would be little change in BZR binding activity once children reached 10 years of age by performing a regression slope test to determine whether the slope equals zero. A *t* test confirmed our hypothesis, as it showed that the slope is not statistically different from zero in any of the ROIs. This finding indicates that BZR binding activity does not significantly change after 10 years of age. We then calculated the rate of decline of BZR binding activity in each ROI using the natural logarithm for patients younger than 10 years of age. We finally adjusted the data using adolescent BZR binding activity. Data were analyzed using SPSS (SPSS Inc, Chicago, IL) software.

The Mann-Whitney *U* test was used to assess differences in BZR binding activity between the two hemispheres and between different sexes in each ROI. Differences were defined as significant at a probability level of $P < 0.05$.

## Results

Table 1 shows the normalized BZR binding activity in each ROI, the rate of decline with age, the BZR binding activity during the neonatal period, and the BZR binding activity during adolescence. Figure 2 shows binding activity versus age in all ROIs, including the cerebral cortex, cerebellum, striatum, amygdala, thalamus, and pons. The figure plots the normalized BZR binding activities versus age, the logarithmic approximation formula, and the logarithmic approximation curve of normalized BZR binding activity of all ROIs from patients younger than 10 years of age.

In all brain regions, the normalized BZR binding activity was highest in the neonatal period, after which they showed exponential declines until adolescence. Binding activity in the primary somatosensory and motor cortices (M1 and S1), the primary and association visual areas, the cerebellar vermis, and the striatum (caudate nucleus and putamen) appeared to decline more rapidly than that in the cerebellar hemisphere and frontal association cortex. The pons and thalamus appeared to have the lowest BZR binding activity in the neonatal period. However, like other areas, BZR binding activity declined exponentially from infancy into childhood to adolescence.

We noticed a variation in the timing of the initial decline in BZR binding activity. The decline in BZR binding activity appeared to first