

Imaging Serotonin and Dopamine Transporters with ^{123}I - β -CIT SPECT: Binding Kinetics and Effects of Normal Aging

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^{123}I - β -carbomethoxy-3- β -(4-iodophenyl)-tropane (CIT) is a useful ligand for dopamine transporters (DATs) and serotonin transporters (5-HTTs). Previous SPECT studies have shown a state of sustained equilibrium in the striatum on day 2 after injection that allows quantification of striatal DATs using a simple ratio of specific-to-nondisplaceable binding. The aim of this study was to investigate the kinetics of ^{123}I - β -CIT uptake in the thalamus, hypothalamus, and midbrain, areas known to contain 5-HTTs in high densities. **Methods:** SPECT with a triple-head camera was performed on 16 healthy volunteers (13 women, 3 men; mean age \pm SD, 32 ± 11 y) after intravenous bolus injection of 130 ± 20 MBq (3.5 ± 0.5 mCi) ^{123}I - β -CIT. Two individuals were scanned 1, 2, 4, 7, 10, 13, 16, and 24 h after injection, and the remaining 14 were scanned 4, 7, 10, 20, and 24 h after injection. Values from 19 previously examined healthy volunteers (8 women, 11 men; mean age, 52 ± 20 y) were included in the analysis to study the age dependency of β -CIT binding in striatal and 5-HTT-rich brain areas in a larger control sample. **Results:** Peak uptake 4 h after injection, followed by stable uptake until 10 h and a slow decrease until 24 h, was observed in the thalamus-hypothalamus region. Activity in the midbrain-pons region peaked 2 h after injection. Because of a concomitant slow but steady decline of uptake in reference regions starting 4 h after injection, a higher stability of binding ratios for 5-HTT-rich brain areas was observed on day 2, suggesting that a state of transient equilibrium is reached between 20 and 24 h but that conditions are only close to transient equilibrium between 4 and 10 h after injection for 5-HTT-rich brain areas. In addition to an age-related decline of striatal ^{123}I - β -CIT binding of 6.6% per decade, a significant age-associated decrease of β -CIT binding of 3–4% per decade was found in 5-HTT-rich brain areas. The decline of β -CIT binding in these regions may be explained, at least in part, by a loss of monoamine transporters with age but may also be related to age-associated morphologic changes. **Conclusion:** ^{123}I - β -CIT appears to be a suitable ligand for imaging serotonin transporters with SPECT. However, careful age matching is warranted for ^{123}I - β -CIT SPECT studies of 5-HTT changes in patients with neuropsychiatric disorders.

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Changes in serotonin transporter function have been implicated in a variety of neuropsychiatric disorders including major depression (1,2), Parkinson's disease (3,4), and Alzheimer's disease (5). Moreover, the serotonin transporter (5-HTT) is the primary site of action of several antidepressant drugs, including the so-called selective serotonin reuptake inhibitors (6).

2- β -carbomethoxy-3- β -(4-iodophenyl)-tropane (CIT) is a cocaine derivative with high affinity for the dopamine transporter (DAT) (7) as well as for 5-HTT (8). ^{123}I - β -CIT has been shown to visualize DATs and 5-HTTs in rodent (9), monkey (10–12), and human brains (13–15). In vivo displacement studies in primates have shown that striatal β -CIT binding is mainly associated with DAT, whereas binding in the hypothalamus and midbrain is mainly associated with 5-HTT (10,12). In an autoradiographic study of the postmortem human brain, ^{125}I - β -CIT binding in the thalamus, hypothalamus, and midbrain, with the exception of the substantia nigra, could be completely displaced by addition of the selective serotonin reuptake inhibitor citalopram, indicating that β -CIT binding in these areas is almost exclusively to 5-HTTs (16). Because of the different localization of DATs and 5-HTTs, the possibility of studying both transporter types using β -CIT as a single ligand has been suggested (10).

Moreover, a different time course of ^{123}I - β -CIT uptake in the striatum and in 5-HTT-rich regions such as the hypothalamus and the midbrain has been shown in SPECT studies in monkeys (10,12) and humans (14,17). Whereas uptake in the striatum appears to be slow, reaching a maximum approximately 16 h after tracer injection, followed by stable binding for up to 24 h after injection in humans, peak uptake in the

hypothalamus and midbrain was observed approximately 4 h after injection (14,17). Uptake in the occipital cortex and cerebellum, which are used as reference regions, is fast, peaking before 1 h after injection and stabilizing approximately 4 h after injection. The stability of β -CIT uptake in the striatum and in reference regions on day 2 (from approximately 18 to 24 h after injection) indicates a state of sustained equilibrium and allows quantification of striatal DATs using a simple ratio of specific-to-nondisplaceable binding (17,18).

Because of relatively stable uptake in the cerebellum and in 5-HTT-rich brain areas approximately 4 h after injection (14,17), we have previously used this time point for measuring 5-HTTs with [^{123}I] β -CIT SPECT, assuming equilibrium conditions for the 5-HTT (19). However, most subjects in earlier kinetic studies have been scanned only until 6 or 8 h after injection on the first study day (14,17). Thus, a possibility remained that maximal uptake in the hypothalamus and midbrain may be reached at a later time or that washout more than 4 h after injection may be more pronounced than expected from previous studies. Moreover, these studies included only a few subjects.

Our aim in this study was to investigate the kinetics of [^{123}I] β -CIT uptake in 5-HTT-rich brain areas in a larger group of healthy volunteers and to revalidate our previous assumption of stable regional uptake in 5-HTT-rich brain areas approximately 4 h after injection. Moreover, by including data from previously studied healthy volunteers, we examined the age dependency of striatal β -CIT binding in a larger control sample and investigated possible age correlations for β -CIT binding in 5-HTT-rich brain areas.

MATERIALS AND METHODS

Healthy Volunteers

The study was approved by the local ethics committee, and informed consent was obtained from every healthy volunteer. Sixteen healthy volunteers (13 women, 3 men; age range, 21–60 y; mean age [\pm SD], 32 ± 11 y) participated. They were examined by an experienced psychiatrist using a structured clinical interview to exclude any present or past neuropsychiatric illness and abuse of alcohol or illicit drugs. Additionally, a history negative for psychiatric disease in first-degree relatives was required. Physical examination showed normal findings in all participants, and no history of any serious medical condition was reported. No individuals were taking prescribed medication, with the exception of oral contraceptives in 6 women.

To study the age dependency of β -CIT binding in striatal and 5-HTT-rich brain areas in a larger control sample, values from 19 previously studied subjects (8 women, 11 men; age range, 24–71 y; mean age, 52 ± 20 y) were included in the analysis. Mean age was not significantly different between women and men for the combined control group of 35 subjects (44 ± 4 y for women versus 42 ± 5 y for men). The historical control group consisted of 13 healthy volunteers and 6 patients with peripheral neurologic disorders. None of the healthy volunteers or patients with peripheral neurologic disorders had a history of present or past psychiatric disease or drug abuse. The healthy volunteers did not take any prescribed medication. The patients did not take antidepressants or

other drugs known to interact with β -CIT binding to dopamine or serotonin transporters. The results from 13 subjects from this historical control group were reported previously (20).

Radlpharmaceutical Preparation

[^{123}I] β -CIT was synthesized according to the method of Neumeyer et al. (8), with several modifications that were described earlier (14).

Data Acquisition

After blockade of thyroid uptake with 600 mg sodium perchlorate orally 30 min before tracer application, the healthy volunteers received a mean dose of 130 ± 20 MBq (3.5 ± 0.5 mCi) (range, 89–167 MBq [2.4–4.5 mCi]) [^{123}I] β -CIT intravenously as a single bolus. SPECT was performed using a triple-head rotating scintillation camera (Multispect 3; Siemens, Knoxville, TN) with a resolution of 9 mm full width at half maximum in the transaxial plane. Camera heads were equipped with medium-energy collimators. To ensure consistency in positioning for repeated scans, each subject's head was placed in the head holder by means of a laser beam system with the infraorbitomeatal plane aligned perpendicular to the rotational axis. A chin strap was used to minimize head movement during scanning. Two individuals were repeatedly scanned with image acquisition starting 1, 2, 4, 7, 10, 13, 16, and 24 h after injection. The remaining 14 individuals were repeatedly scanned with image acquisition starting 4, 7, 10, 20, and 24 h after injection. For each scan, a total of 180 frames were collected in a step-and-shoot mode. SPECT lasted 20 min (20 s per frame) for data acquisitions starting up to 10 h after injection and 40 min (40 s per frame) for data acquisitions starting later.

The injected dose in the historical control group ($n = 19$) was slightly higher (mean, 147 ± 31 MBq [4.0 ± 0.8 mCi]) [^{123}I] β -CIT; range, 97–222 MBq [2.6–6 mCi]). Fifteen subjects of this group had been scanned at 4 h after injection; 16 subjects, at 20 h; and 7 subjects, at 24 h.

Data Analysis

Cross sections 3.5 mm thick and oriented parallel to the canthomeatal plane were reconstructed by filtered backprojection (Butterworth filter; cutoff frequency, 0.7; order, 7) in 128×128 matrices. Attenuation correction was performed assuming uniform attenuation within an ellipse drawn around the head contour (attenuation coefficient, 0.120/cm). Circular regions of interest (ROIs) were placed in the midline in areas corresponding to the thalamus and hypothalamus (32 pixels each), midbrain (26 pixels each), and pons (21 pixels each). Irregular ROIs were drawn in areas corresponding to the left and right striatum (40 pixels each), in the frontal and occipital cortex at the level of maximal striatal binding (180 pixels each), and in the left and right cerebellar hemispheres (60 pixels each). All ROIs were drawn with the help of a brain atlas by a single examiner. Values of the 4 circular midline ROIs with the highest counting rate (usually from 1 slice below the maximum of striatal binding downward, the area corresponding to thalamus and hypothalamus) were pooled to form a thalamus-hypothalamus region. Values of the next 6 midline ROIs below (the area corresponding to midbrain and pons) were pooled to form a midbrain-pons region, and the average counts per pixel were calculated. Counts in striatal regions were calculated in several consecutive single-slice views, and the values of the slices with the highest counting rate for each striatum were used to avoid tilting errors. Activities from 3 adjacent frontal and 3 adjacent occipital cortex ROIs at the level of maximal striatal binding were pooled.

The values of both cerebellar ROIs in 3 consecutive axial slices of maximal cerebellar activity were averaged.

Brain regional uptake was calculated as cpm/MBq injected dose, corrected for decay and body mass (cpm/pixel/MBq × kg). Individual washout rates (%/h) between 4 and 10 h after injection and between 20 and 24 h after injection were calculated as $\{(uptake_{10h}/uptake_{4h}) \text{ minus } 1\} \times 100/6$ and $\{(uptake_{24h}/uptake_{20h}) \text{ minus } 1\} \times 100/4$. Cerebellar activity was assumed to represent nonspecific bound and free activity, because the density of DATs and 5-HTTs in the cerebellum is known to be low (21,22). Ratios between mean counts in target regions (striatum, thalamus, and hypothalamus; midbrain and pons; frontal cortex) and the cerebellum were calculated. These ratios minus 1 represent specific-to-nondisplaceable binding and are assumed to be directly related to the binding potential at the time of binding equilibrium (17,18).

Statistical Analysis

Descriptive statistical analyses were performed on the calculated washout rates of brain regional uptake between 4 and 10 h after injection and 20 and 24 h after injection. Likewise, descriptive statistical analyses were performed on the calculated specific-to-nondisplaceable binding ratios. The results are expressed as mean ± SD. Washout rates for the cerebellum and occipital cortex were compared using an unpaired, 2-tailed Student *t* test. A possible age dependency of [¹²³I]β-CIT binding in the striatum (striatum-to-cerebellum ratio minus 1 at 20 h after injection) and in 5-HTT-rich brain areas (thalamus-hypothalamus-to-cerebellum and midbrain-pons-to-cerebellum ratios minus 1 at 4 and 20 h after injection) was examined separately for this study population and for the combined control group. Because the control group of this study included only two older individuals, the Spearman rank correlation was used to rule out any leverage effects by so-called influential points. The Pearson product moment correlation and linear regression analyses were used for the combined control group, which included this group and the 19 individuals previously studied. All statistical analyses were performed using version 7.5 SPSS software (SPSS, Inc., Chicago, IL). *P* < 0.05 was considered statistically significant.

RESULTS

Kinetic Study

Figure 1 shows time-activity curves of regional brain uptake in 2 healthy volunteers scanned repeatedly from 1 to 24 h after injection of 130 MBq (3.5 mCi) [¹²³I]β-CIT. Striatal uptake increased until 16 h after injection. Uptake in the thalamus-hypothalamus region reached a maximum 4 h after injection and remained stable until 10 h after injection. Activity in the midbrain and pons peaked 2 h after injection and remained relatively stable until at least 10 h after injection. Frontal and occipital cortex time-activity curves were similar. A steep decrease in cortical uptake between 1 and 2 h after injection was followed by a moderate decline between 2 and 4 h and relatively stable binding thereafter. Cerebellar uptake decreased moderately between 1 and 2 h after injection and then paralleled the cortical uptake curves.

Figure 2 shows the time course of averaged regional [¹²³I]β-CIT uptake in the remaining 14 healthy volunteers, studied from 4 to 24 h after injection. A plateau of binding between 4 and 10 h after injection, followed by a slow

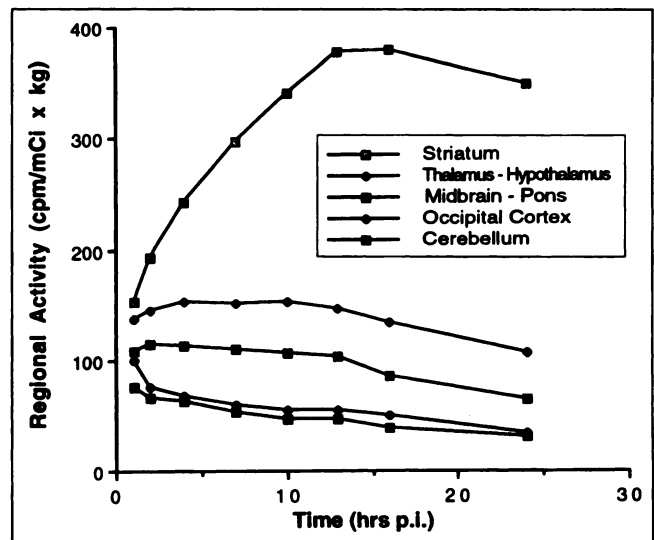


FIGURE 1. Time-activity curves of averaged brain regional uptake from 1 to 24 h after injection of 130 MBq [¹²³I]β-CIT in 2 healthy volunteers. Striatal uptake increased until 16 h. Uptake in thalamus-hypothalamus region peaked 4 h after injection and remained stable until 10 h. Midbrain and pons activity peaked at 2 h and remained relatively stable until at least 10 h. Steep decrease in cortical uptake between 1 and 2 h after injection was followed by moderate decline between 2 and 4 h and relatively stable binding thereafter. Cerebellar uptake decreased moderately between 1 and 2 h after injection and then paralleled occipital uptake curve. p.i. = after injection.

decrease thereafter, was seen in the thalamus and hypothalamus. A slow but steady decline of uptake was evident in the brain stem, cortex, and cerebellum starting 4 h after injection.

Table 1 gives mean washout rates of brain regional uptake

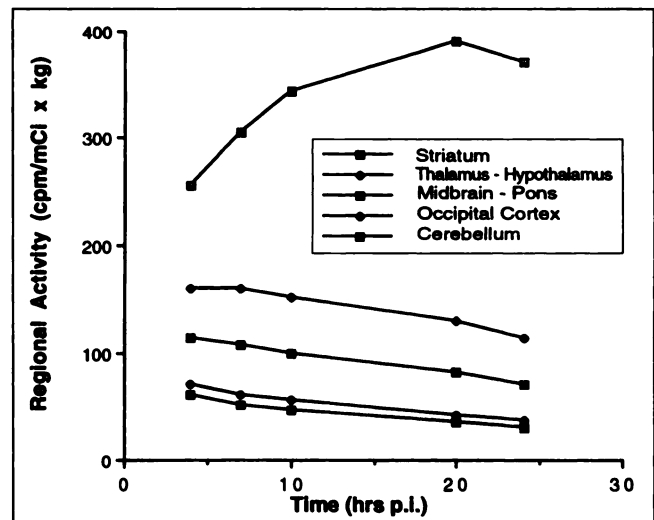


FIGURE 2. Time-activity curves of averaged brain regional uptake from 4 to 24 h after injection of 130 MBq [¹²³I]β-CIT in 14 healthy volunteers. Plateau of uptake between 4 and 10 h in thalamus and hypothalamus was followed by slow decrease. Slow but steady decline of uptake was evident in brain stem, cortex, and cerebellum starting 4 h after injection. p.i. = after injection.

TABLE 1
Washout Rates of Brain Regional Uptake of [¹²³I]β-CIT in 16 Healthy Volunteers

Region	4–10 h after injection	20–24 h after injection*
Striatum	+6.1 ± 1.7 (+3.7 to +8.7)	-1.1 ± 1.2 (-3.1 to +2.4)
Thalamus–hypothalamus	-0.5 ± 1.3 (-2.9 to +2.1)	-2.9 ± 1.8 (-5.8 to 0.0)
Midbrain–pons	-1.7 ± 1.4 (-4.5 to +1.3)	-2.9 ± 2.5 (-7.2 to +1.7)
Frontal cortex	-3.2 ± 1.5 (-5.3 to +0.5)	-3.5 ± 1.5 (-5.5 to -1.1)
Occipital cortex	-3.0 ± 1.4 (-5.0 to -0.7)	-3.3 ± 2.4 (-6.4 to +0.6)
Cerebellum	-3.5 ± 1.5 (-5.3 to -0.9)	-3.9 ± 1.4 (-6.6 to -2.2)

*In two subjects, washout between 16 and 24 h after injection.
Values are mean ± SD (range) of percentage washout per hour.

between 4 and 10 h after injection and between 20 and 24 h after injection. Table 2 gives mean striatum-to-cerebellum, thalamus–hypothalamus-to-cerebellum, midbrain–pons-to-cerebellum, and frontal cortex-to-cerebellum ratios minus 1. Whereas the average changes in thalamus–hypothalamus and midbrain–pons uptake between 4 and 10 h after injection were low ($-0.5\% \pm 1.3\%/h$ and $-1.7\% \pm 1.4\%/h$, respectively), washout in the occipital cortex and in the cerebellum ($-3.0\% \pm 1.4\%/h$ and $-3.5\% \pm 1.5\%/h$, respectively) was slightly more pronounced. Consequently, the thalamus–hypothalamus-to-cerebellum and midbrain–pons-to-cerebellum ratios minus 1 increased by $6.8\% \pm 3.0\%/h$ and $5.5\% \pm 3.9\%/h$, respectively. Because of relatively higher washout rates in the thalamus–hypothalamus and brain stem from 20 to 24 h after injection ($-2.9\% \pm 1.8\%/h$ and $-2.9\% \pm 2.5\%/h$, respectively) and comparable cerebellar washout during this period, the thalamus–hypothalamus-to-cerebellum and midbrain–pons-to-cerebellum ratios minus 1 increased at lower rates from 20 to 24 h after injection (by $1.5\% \pm 3.6\%/h$ and $2.5\% \pm 5.3\%/h$, respectively). Uptake in the striatum was relatively stable between 20 and 24 h after injection ($-1.1\% \pm 1.2\%/h$), but the moderate degree of washout in the cerebellum ($-3.9\% \pm 1.4\%/h$) led to an increase in the striatum-to-cerebellum ratio minus 1 of $3.8\% \pm 1.8\%/h$.

No statistically significant difference between washout in the cerebellum and washout in the occipital cortex was seen from 4 to 10 h after injection ($-3.5\% \pm 1.5\%$ versus

$-3.0\% \pm 1.4\%/h$, respectively) or from 20 to 24 h after injection ($-3.9\% \pm 1.4\%$ versus $-3.3\% \pm 2.4\%/h$, respectively).

Age Dependency of [¹²³I]β-CIT Binding

A negative correlation between age and the striatum-to-cerebellum ratio minus 1 was seen only at 20 h in this study, which included only 2 older individuals (rank correlation coefficient [r_s] = -0.51 ; $P = 0.061$). A significant negative correlation was found between age and the thalamus–hypothalamus-to-cerebellum ratio minus 1 at 4 h after injection ($r_s = -0.53$; $P = 0.035$). No significant correlations between age and the thalamus–hypothalamus-to-cerebellum ratio minus 1 at 20 h after injection and the midbrain–pons-to-cerebellum ratio minus 1 at 4 and 20 h after injection were observed (thalamus–hypothalamus 20 h after injection, $r_s = -0.05$; brain stem 4 h after injection, $r_s = -0.01$; brain stem 20 h after injection, $r_s = -0.05$).

Table 3 shows the mean striatum-to-cerebellum, thalamus–hypothalamus-to-cerebellum, and midbrain–pons-to-cerebellum ratios minus 1 in the combined control group. A highly significant age-related decline in striatal [¹²³I]β-CIT binding was evident (Fig. 3). The striatum-to-cerebellum ratios minus 1 at 20 h after injection ranged from 5.7 to 13.0 (mean, 8.6 ± 1.8). Regression analysis revealed a decrease of 35.8% over the age range of 21–75 y, or 6.6% per decade. A significant age-related decrease in binding was found as well in the thalamus–hypothalamus region at 4 and 20 h after

TABLE 2
[¹²³I]β-CIT Binding Ratios in 16 Healthy Volunteers

Region	4 h after injection	10 h after injection	20 h after injection*	24 h after injection
Striatum	3.3 ± 0.7 (2.5–5.0)	6.6 ± 1.0 (5.0–9.1)	9.9 ± 1.4 (7.7–13.0)	11.4 ± 1.7 (8.2–16.1)
Thalamus–hypothalamus	1.67 ± 0.27 (1.25–2.38)	2.32 ± 0.33 (1.81–2.82)	2.61 ± 0.29 (2.05–2.96)	2.74 ± 0.42 (1.86–3.47)
Midbrain–pons	0.91 ± 0.11 (0.76–1.05)	1.19 ± 0.18 (0.82–1.43)	1.27 ± 0.24 (0.91–1.59)	1.36 ± 0.27 (0.98–1.87)
Frontal cortex	0.21 ± 0.08 (0.06–0.40)	0.26 ± 0.11 (0.12–0.52)	0.24 ± 0.08 (0.10–0.39)	0.26 ± 0.14 (0.07–0.51)

*Values from 14 individuals.
Values are mean ± SD (range) of target region-to-cerebellum ratios minus 1.

TABLE 3
 $[^{123}\text{I}]\beta\text{-CIT}$ Binding Ratios in Combined Control Group

Region	4 h after injection (n = 31)	20 h after injection (n = 30)	24 h after injection (n = 23)
Striatum	3.1 ± 0.6 (2.0–5.0)	8.6 ± 1.8 (5.7–13.0)	10.6 ± 2.2 (5.9–16.1)
Thalamus–hypothalamus	1.59 ± 0.28 (1.03–2.38)	2.28 ± 0.42 (1.58–2.96)	2.56 ± 0.56 (1.25–3.47)
Midbrain–pons	0.88 ± 0.14 (0.56–1.10)	1.15 ± 0.25 (0.60–1.59)	1.29 ± 0.32 (0.57–1.87)

Values are mean ± SD (range) of target region-to-cerebellum ratios minus 1.

injection (3.9% and 3.2% per decade, respectively) (Figs. 4A and B). A significant age-related decrease in $\beta\text{-CIT}$ binding of 4.5% per decade was observed in the brain stem 20 h after injection (Fig. 5B). Only a trend for an age-related decrease (3.1% per decade) in binding in the midbrain and pons was found 4 h after injection (Fig. 5A).

DISCUSSION

Kinetics of $[^{123}\text{I}]\beta\text{-CIT}$ Binding in 5-HTT-Rich Brain Areas

$[^{123}\text{I}]\beta\text{-CIT}$ is a high-affinity ligand for both dopamine and serotonin transporters (7,8). Studies have shown that $\beta\text{-CIT}$ binding in the striatum is almost exclusive to DATs whereas binding in the hypothalamus and midbrain is mainly associated with 5-HTT (10,12,16,19). Kinetic studies have shown a different time course of $[^{123}\text{I}]\beta\text{-CIT}$ uptake in the striatum and in 5-HTT-rich brain areas (14,17). Whereas uptake in the striatum appears to be slow, reaching a maximum approximately 16 h after injection, followed by stable binding for up to 24 h after injection, peak uptake in the hypothalamus and midbrain occurs approximately 4 h after injection. Uptake in the occipital cortex and in the cerebellum, which are used as reference regions, is fast,

peaking before 1 h after injection and stabilizing approximately 4 h after injection (14,17). The stability of $\beta\text{-CIT}$ uptake in the striatum and in reference regions on day 2 after injection indicates a state of binding equilibrium and allows for quantification of striatal DATs using a simple ratio of specific-to-nondisplaceable binding (17,18).

A period of stable $\beta\text{-CIT}$ uptake approximately 4 h after injection was observed in 5-HTT-rich brain areas in previous studies (14,17). Because uptake appeared to be relatively stable at 4 h in reference regions as well, we have used 4 h after injection as the time point for measuring 5-HTTs with $[^{123}\text{I}]\beta\text{-CIT}$ SPECT, assuming equilibrium conditions for the 5-HTT (19). However, few subjects were included in these kinetic studies, and data were available only up to 8 h after injection on the first study day (14,17). A major finding of the kinetic study that we are now reporting was a remarkable stability of $\beta\text{-CIT}$ uptake in the thalamus–hypothalamus area from 4 to 10 h after injection, followed by a steady but slow decrease until 24 h after injection. Activity in the midbrain and pons peaked 2 h after injection, remained relatively stable until 10 h, and washed out at slightly higher rates on day 2. A slow but steady decline of uptake was evident in cortical areas and in the cerebellum starting 4 h after injection. Because of slightly more pronounced washout in the cerebellum than in the thalamus–hypothalamus and midbrain–pons between 4 and 10 h after injection, the thalamus–hypothalamus-to-cerebellum and midbrain–pons-to-cerebellum ratios minus 1 increased by $6.8\% \pm 3.0\%/h$ and $5.5\% \pm 3.9\%/h$, respectively. Because of relatively higher washout rates in the thalamus–hypothalamus and brain stem from 20 to 24 h after injection and comparable cerebellar washout during this period, the thalamus–hypothalamus-to-cerebellum and midbrain–pons-to-cerebellum ratios minus 1 increased at lower rates from 20 to 24 h after injection (by $1.5\% \pm 3.6\%/h$ and $2.5\% \pm 5.3\%/h$, respectively). This finding suggests that despite stable uptake in the thalamus, hypothalamus, and brain stem between 4 and 10 h after injection, conditions may be closer to a state of transient equilibrium (23) between 20 and 24 h after injection than between 4 and 10 h after injection for 5-HTT-rich brain areas. Another method of estimating the binding potential at true equilibrium is to measure the ratio of specific-to-nondisplaceable binding at the peak uptake of

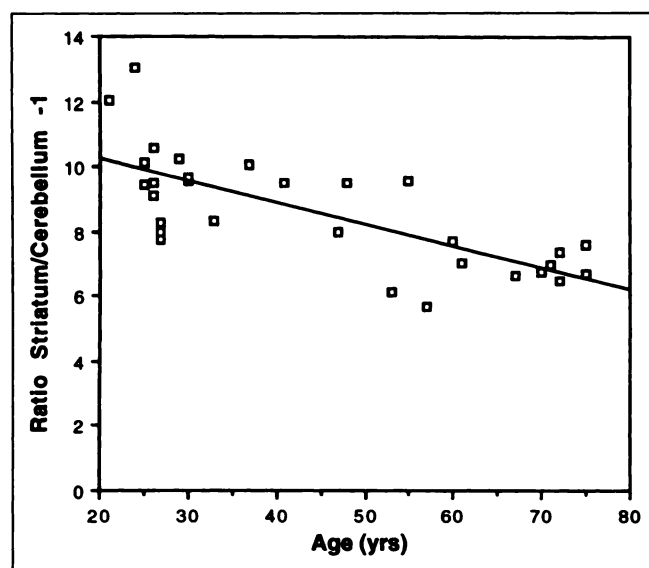


FIGURE 3. Correlation between striatal $[^{123}\text{I}]\beta\text{-CIT}$ binding (striatum-to-cerebellum ratio minus 1 at 20 h after injection) and age in 30 healthy volunteers. Striatum-to-cerebellum ratio minus 1 = $-0.0674 \times \text{age} + 11.576$; $r = -0.736$; $P = 0.0001$.

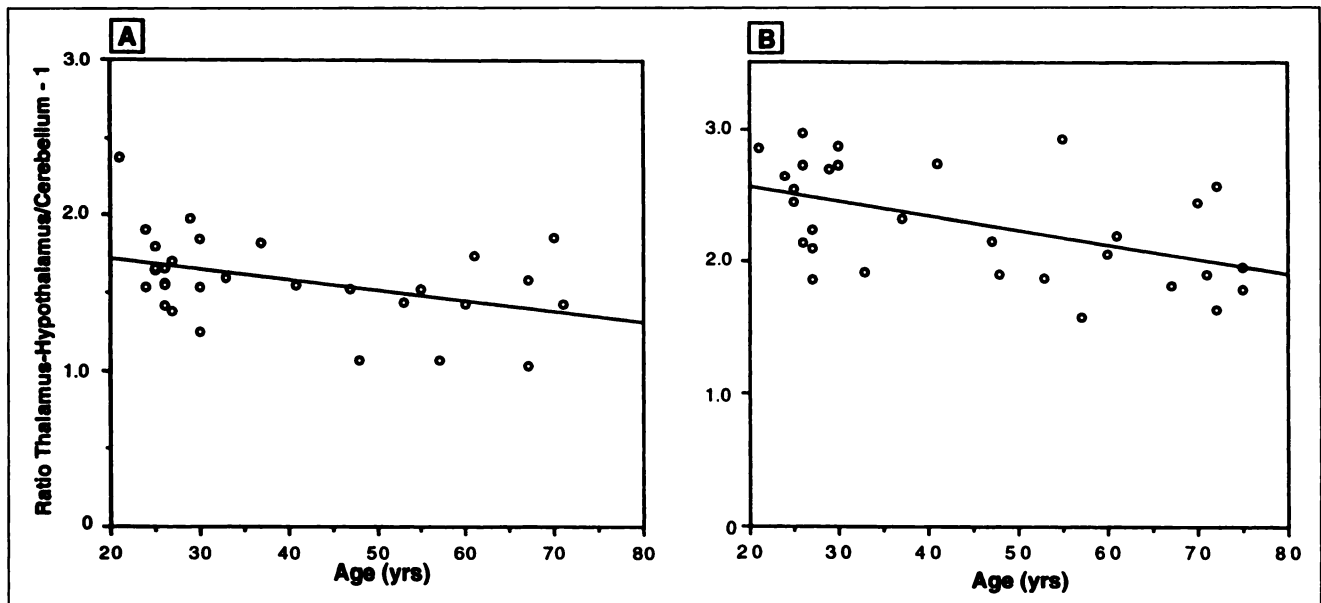


FIGURE 4. (A) Correlation between age and [^{123}I] β -CIT binding in thalamus and hypothalamus (thalamus–hypothalamus-to-cerebellum ratio minus 1) 4 h after injection in 31 healthy volunteers. Thalamus–hypothalamus-to-cerebellum ratio minus 1 = $-0.0066 \times \text{age} + 1.846$; $r = -0.395$; $P = 0.028$. (B) Correlation between age and [^{123}I] β -CIT binding in thalamus and hypothalamus 20 h after injection in 30 healthy volunteers. Thalamus–hypothalamus-to-cerebellum ratio minus 1 = $-0.0112 \times \text{age} + 2.781$; $r = -0.512$; $P = 0.004$.

specific binding, as defined by subtraction of uptake in the reference region from uptake in the target region (24). The peak of specific (target minus cerebellum) uptake in 5-HTT-rich brain areas in our group of healthy volunteers varied between 4, 7, and 10 h after injection. Because a proper identification of the peak of specific uptake is required for the peak-equilibrium analysis, the kinetic profile of [^{123}I] β -CIT in 5-HTT-rich brain areas does not seem to be

compatible with this method. In a previous study, we compared β -CIT binding in the thalamus–hypothalamus area in depressed patients treated with the selective serotonin reuptake inhibitor citalopram with a group of healthy volunteers of comparable age and could show a highly significant binding reduction in the patients (19). A parallel time course of tracer uptake was observed in patients and healthy volunteers, both studied 2, 4, 16, 20, and 24 h after

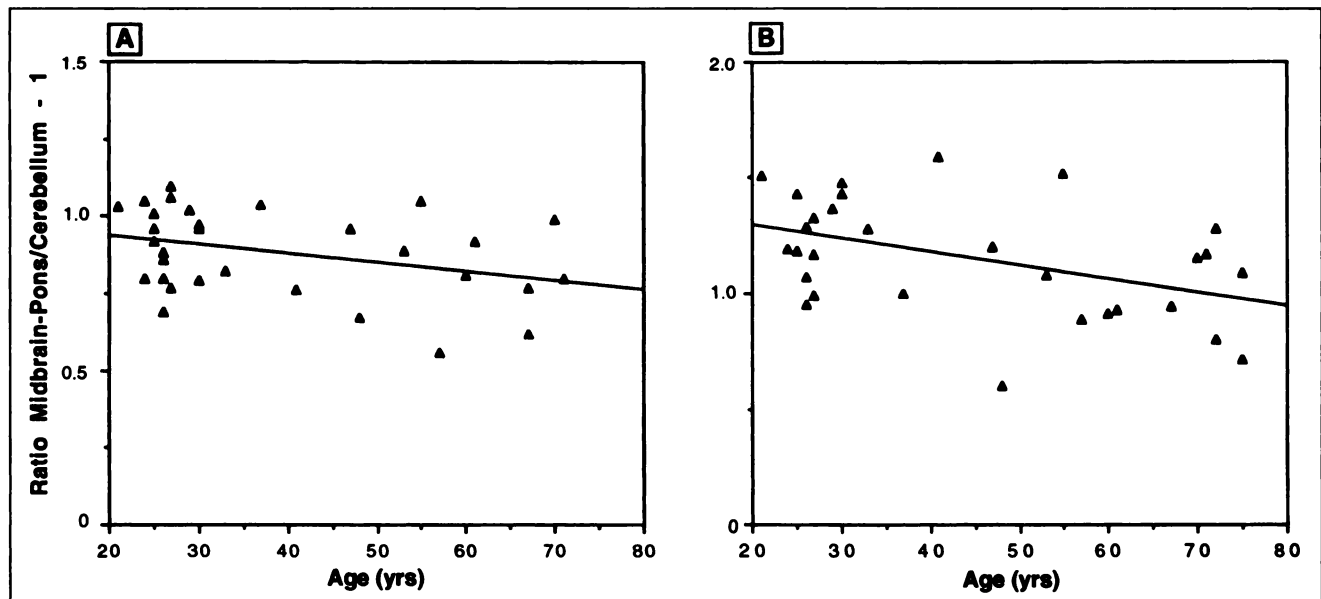


FIGURE 5. (A) Correlation between age and [^{123}I] β -CIT binding in midbrain and pons (midbrain–pons-to-cerebellum ratio minus 1) 4 h after injection in 31 healthy volunteers. Midbrain–pons-to-cerebellum ratio minus 1 = $-0.0029 \times \text{age} + 0.995$; $r = -0.342$; $P = 0.06$. (B) Correlation between age and [^{123}I] β -CIT binding in midbrain and pons 20 h after injection in 30 healthy volunteers. Midbrain–pons-to-cerebellum ratio minus 1 = $-0.00585 \times \text{age} + 1.412$; $r = -0.449$; $P = 0.013$.

injection, with maximum binding 4 h after injection and slightly lower uptake on day 2. The binding reduction of [¹²³I]β-CIT in the thalamus–hypothalamus area in patients treated with citalopram was virtually the same at 4 and 20 h after injection (thalamus–hypothalamus-to-cerebellum ratios minus 1, –47% versus –45%, respectively) (19). This finding suggests that both time points are feasible for the measurement of 5-HTTs with [¹²³I]β-CIT SPECT.

Kinetics of [¹²³I]β-CIT Binding in Striatum

Our finding of stable uptake in the striatum between 20 and 24 h after injection (washout rate, $-1.1\% \pm 1.2\%/h$) is in line with the results of previous studies (17,18). However, the moderate degree of washout in the cerebellum ($-3.9\% \pm 1.4\%/h$) in this study led to a $3.8\% \pm 1.8\%/h$ increase in the striatum-to-cerebellum ratio minus 1. In comparison, van Dyck et al. (18) reported a remarkable stability in [¹²³I]β-CIT uptake in the striatum (average change, 0.29%/h) and occipital cortex (average change, $-1.19\%/h$) between 18 and 24 h after injection, resulting in an average 1.72%/h increase in the ratio of specific-to-nondisplaceable binding. Stable occipital uptake and stable plasma concentrations of [¹²³I]β-CIT with a decline of less than 1%/h were observed between 4 and 8 h after injection in 5 healthy volunteers by Laruelle et al. (17). In contrast to these workers, we have used the cerebellum as a reference region because the density of DATs and 5-HTTs in the cerebellum are known to be negligible whereas 5-HTTs are prevalent in low densities throughout the cerebral cortex (16,21).

Washout rates in the cerebellum were not significantly different from those in the occipital cortex. Thus, use of the cerebellum as opposed to the occipital cortex as a reference region cannot alone account for the lower stability of the specific-to-nondisplaceable ratios. One possible explanation for the higher washout in the reference regions may be much lower administered doses of radiotracer than those used by van Dyck et al. (18) (average, 130 versus 366 MBq). Biodistribution studies in healthy volunteers showed high initial uptake in the lung, with subsequent distribution to the liver, intestine, and brain (25). One could therefore speculate that a higher injected dose may result in protracted redistribution of the tracer from extracerebral compartments to plasma and brain, resulting in prolonged stability of tracer uptake in reference regions. In practical terms, the instability of the ratios of specific-to-nondisplaceable binding indicates that measurements should be performed at one specific time and not within a longer window, e.g., between 18 and 24 h after injection. Even the low washout rates in the occipital cortex reported by van Dyck et al. (18) would lead to an average increase of 10% in the specific striatum-to-cerebellum ratio from 18 to 24 h after injection.

Effects of Normal Aging on [¹²³I]β-CIT Binding

Our data from a larger control sample show a highly significant reduction of striatal [¹²³I]β-CIT binding with age. The striatum-to-cerebellum ratios minus 1 at 20 h after injection decreased by 35.8% over the age range of 21–75 y,

or 6.6% per decade. This finding is in line with the results of previous PET and SPECT studies (18,26–28). The rate of decline of striatal binding is similar to the rates observed with [¹¹C]cocaine and PET (7% per decade) (27), [¹¹C]d-threo-methylphenidate and PET (6.6% per decade) (28), and another SPECT study using [¹²³I]β-CIT (8% per decade) (18). Postmortem studies have consistently shown age-related reductions of striatal dopamine transporters (29–31) and of the content of dopamine transporter mRNA in the substantia nigra (32), suggesting that the reduction of striatal β-CIT binding shown by in vivo studies in fact reflects a loss of striatal dopamine transporters with age. The age-related decline of striatal dopamine transporter binding found in this study and in previous in vivo studies is slightly larger than the estimated age-related decline of dopaminergic neurons in the normal substantia nigra (4.7% per decade) (33). This finding agrees with in vitro studies showing that the decline of dopamine transporter mRNA with age appears to exceed the rate of dopamine cell loss in the substantia nigra (32).

In addition to a decline in striatal β-CIT binding, a significant age-related decrease was found in 5-HTT-rich brain areas. Thalamus–hypothalamus-to-cerebellum ratios minus 1 decreased by 3.9% (4 h after injection) and 3.2% (20 h after injection) per decade. A significant age-related decline in β-CIT binding of 4.5% per decade was observed in the brain stem 20 h after injection. A trend for an age-related decline (of 3.1% per decade) in binding in the midbrain and pons was found 4 h after injection. This decrease of β-CIT binding in 5-HTT-rich brain areas may be explained, at least in part, by an age-related loss of 5-HTTs. However, no consistent age-related changes of 5-HT concentrations or of 5-HTT density have been observed in the postmortem human brain (5,34). Thus, when interpreting our results, one must consider other potential contributing factors. Recent studies have shown a relatively high affinity of [¹²³I]β-CIT for norepinephrine transporters (NETs) (35). In a PET study in nonhuman primates, 50% of [¹¹C]β-CIT binding in the thalamus could be displaced by the 5-HTT ligand citalopram. However, a displacement of about 50% of [¹¹C]β-CIT binding in this area was also observed with the NET ligands desipramine and mazindol. The combined administration of citalopram and desipramine reduced binding in the thalamus by about 80%, indicating that uptake in this area represents binding to both 5-HTTs and NETs (36). High densities of NETs are present in the thalamus, hypothalamus, locus coeruleus, and other brain stem nuclei including the dorsal and median raphe in the rat brain, whereas NET binding is low in the caudate nucleus and putamen (37). An autoradiographic study of the postmortem human brain using the selective NET ligand [³H]nisoxetine showed a significant age-related decline of NETs in the locus coeruleus, which is the most important source of noradrenergic projections to the forebrain (38). Consequently, the age-related changes of [¹²³I]β-CIT binding in the thalamus, hypothalamus, and brain stem evident in

our SPECT study may be linked to a decline of NETs with age.

In contrast to NETs and 5-HTTs, which appear to be widely distributed throughout the brain, DATs seem to be restricted to the striatum and the substantia nigra (39). Thus, DAT binding is unlikely to contribute significantly to β -CIT binding in the thalamus and hypothalamus. Based on the results of two different membrane-binding studies, the ratio of DATs to 5-HTTs in the substantia nigra would be approximately 0.3 (21,22), suggesting a minor contribution of DATs to β -CIT binding in the midbrain. The age-related decline of dopaminergic neurons and the even more pronounced decline of DAT mRNA in the substantia nigra (32,33) are likely to be associated with a reduction of DAT density in the substantia nigra. The age-related decline of β -CIT binding in the midbrain may therefore be explained, at least in part, by a loss of DATs in the substantia nigra. In light of the overlapping localization of all 3 monoamine types in the brain stem and of 5-HTTs and NETs in the thalamus and hypothalamus, the use of selective ligands would be a clear advantage to elucidate the contribution of each monoamine transporter type to the observed aging effects.

Morphologic changes with age are another potential confounding factor in the interpretation of our results. A volumetric MRI study of healthy male volunteers showed a significant reduction of the striatum volume with age, exceeding the age-associated atrophy of the cerebral hemispheres (40). A less marked reduction of the thalamus volume with age was observed in that study. However, because of the concomitant enlargement of the third ventricle, a contribution of atrophy to the age-associated decline of β -CIT binding in the thalamus and hypothalamus has to be considered (partial-volume effect). The use of unpaired circular ROIs for the thalamus and hypothalamus in that study may not be optimal because of the inclusion of the third ventricle. However, because of the limited spatial resolution of SPECT, the use of paired ROIs for these areas cannot overcome the problem of partial-volume effects caused by ventricular enlargement. Future studies with MRI coregistration and the use of functional imaging systems with higher spatial resolution may be able to overcome these limitations.

CONCLUSION

$[^{123}\text{I}]\beta$ -CIT is a useful ligand for imaging serotonin transporters with SPECT. The high stability of specific-to-nondisplaceable binding ratios in 5-HTT-rich brain areas between 20 and 24 h after injection suggests that 5-HTTs can be imaged under conditions of transient equilibrium during this period. The age-associated decrease of β -CIT binding in 5-HTT-rich brain areas indicates that careful age matching is warranted for $[^{123}\text{I}]\beta$ -CIT SPECT studies of changes of 5-HTTs in patients with neuropsychiatric disorders. Studies using selective ligands should help to clarify to what degree NETs and DATs contribute to uptake of β -CIT in 5-HTT-

rich brain areas and to the observed effects of aging in these regions.

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