

High Levels of Serotonin Transporter Occupancy With Low-Dose Clomipramine in Comparative Occupancy Study With Fluvoxamine Using Positron Emission Tomography

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Context: Serotonin transporters (5-HTT) are regarded as one of the major therapeutic targets of antidepressants. However, there have only been a few studies about 5-HTT occupancy, and in particular, data concerning classical antidepressants are still limited.

Objective: To investigate the relationship between 5-HTT occupancy and a wide range of antidepressant dosing protocols.

Design, Setting, and Participants: Antidepressant occupancies of 5-HTT were measured using positron emission tomography (PET) with [¹¹C](+)McN5652. Twenty-seven healthy volunteers were measured with and without pretreatment with single low doses of antidepressants, and long-term doses were evaluated in 10 patients. Scan data were collected between December 12, 1995, and August 7, 2002, and data were analyzed during the 2001-2002 period at the National Institute of Radiological Sciences (Chiba, Japan).

Intervention: Four different doses of clomipramine hydrochloride (5-50 mg) and 3 different doses of fluvoxamine maleate (12.5-50 mg) were used for single administration. Long-term doses were 20 to 250 mg per day for clomipramine hydrochloride, and 25 to 200 mg per day for fluvoxamine maleate.

Main Outcome Measure: Occupancies in the thalamus were calculated using the individual baseline of [¹¹C](+)McN5652 for single-dose studies and 2 long-term-dose studies, and the mean value of healthy volunteers as the baseline for 8 long-term-dose studies. The average data from inactive enantiomers [¹¹C](−)McN5652 were used for the estimation of nonspecific binding.

Results: Occupancy of 5-HTT increased in a curvilinear manner. Even 10 mg of clomipramine hydrochloride showed approximately 80% occupancy, which was comparable with that of 50 mg of fluvoxamine maleate. Estimated median effective dose (ED₅₀) of clomipramine hydrochloride was 2.67 mg for oral dose and 1.42 ng/mL for plasma concentration; those of fluvoxamine maleate were 18.6 mg and 4.19 ng/mL, respectively.

Conclusions: Clinical doses of clomipramine and fluvoxamine occupied approximately 80% of 5-HTT, and dose escalation would have minimal effect on 5-HTT blockade. Ten milligrams of clomipramine hydrochloride was enough to occupy 80% of 5-HTT in vivo.

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SEROTONIN TRANSPORTERS (5-HTT) are located at presynaptic serotonergic neurons and have a key role in the regulation of serotonin concentration in the synapse. They are believed to be the primary target for selective serotonin reuptake inhibitors (SSRIs) and several tricyclic antidepressants such as clomipramine hydrochloride.¹ In clinical practice, SSRIs are commonly used, and clomipramine is also one of the most widely used antidepressants and drugs for obsessive compulsive disorders.²⁻⁴

The recent neuroimaging techniques of positron emission tomography

(PET)⁵⁻⁷ and single photon computed emission tomography (SPECT)^{8,9} have introduced several ligands for the measurement of 5-HTT in vivo. However, there have been limited data about the effect of antidepressants on 5-HTT in the living human brain.⁸⁻¹¹ Occupancy of 5-HTT as the percentage reduction of specific binding during the treatment of SSRIs has been reported in 2 SPECT studies^{8,9} and 1 PET study.¹² Using β-CIT labeled with iodine 123, 40% to 50% of 5-HTT occupancies were reported during the treatment with 60 mg/d of fluoxetine hydrochloride⁹ or 20 to 60 mg/d of citalopram hydrobromide.⁸ However, the detectability of oc-

cupancy by less selective ligands of 5-HTT, such as [¹²³I]β-CIT, requires some circumspection,⁵ and the baseline of the calculation of occupancy also needs to be addressed with some caution because of the possible difference in nonspecific binding.^{11,13} A recent study using [¹¹C]DASB [3-¹¹C-amino-4-(2-dimethylaminomethylphenylsulphanyl)benzonitrile] reported approximately 80% 5-HTT occupancy during treatment with 10 to 20 mg/d of paroxetine hydrochloride and 20 mg/d of citalopram hydrobromide.¹² However, the relationship with low doses is not yet clear, and no data are as yet available on 5-HTT occupancy with classic tricyclic antidepressants like clomipramine.

McN5652 (trans-1,2,3,5,6, 10-β-hexahydro-6-[4 (methylthio) phenyl] pyrrolo [2,1-a] isoquinoline) is a selective serotonin reuptake inhibitor that has nanomolar affinity for serotonin 5-HT transporters. Its pharmacologically active enantiomer, (+)McN5652, has at least 2 orders of magnitude with higher affinity than its pharmacologically inactive enantiomer, (-)McN5652.^{6,14} [¹¹C](+)McN5652 is currently being used as a PET tracer for 5-HTT, with good test-retest reproducibility.^{6,10,11,15} However, a relatively high fraction of nonspecific binding and possible differences in nonspecific binding among regions has made it difficult to evaluate specific bindings and occupancies accurately.¹¹ Nevertheless, by the use of [¹¹C](-)McN5652, we have been able to calculate 5-HTT occupancy despite the regional differences of nonspecific binding.¹¹

As for pharmacological evidence regarding the clinical dose of antidepressants, there have been only limited in vivo data, especially in relationship to 5-HTT occupancy. The purpose of this study was to investigate the precise relationship between 5-HTT occupancy and the dose of the classic tricyclic antidepressant clomipramine, or one of the SSRIs, fluvoxamine maleate, with a wide range of dosing protocols using [¹¹C](+)McN5652.

METHODS

SUBJECTS

Twenty-seven healthy male volunteers (mean ± SD age, 22.0 ± 2.3 years; range, 20-29 years) participated in single low-dose studies, and 10 patients (mean ± SD age, 35.7 ± 12.1 years; range,

21-55 years) with psychiatric disorders who were receiving long-term treatment with antidepressants were included in this study (Table 1 and Table 2). All healthy volunteers were recruited from among university students and hospital employees. They filled out a questionnaire about their medical history and medications, and were then interviewed by a medical staff member. They had no history of present or past psychiatric, neurological, or somatic disorders, and no alcohol- or drug-related problems. They had not taken any kind of medication for at least 2 weeks prior to the start of the study. Six patients (age range, 21-50 years) had been taking clomipramine, 4 patients (age range, 24-55 years) had been taking fluvoxamine, and 6 of the 10 patients were also taking 1 or 2 benzodiazepines as anxiolytic or hypnotic treatments. The doses, duration of treatment, and diagnoses are presented in Table 2.

This study was approved by the ethics and radiation safety committees of the National Institute of Radiological Sciences (Chiba, Japan). Written informed consent was obtained from each subject.

PET MEASUREMENT

[¹¹C](+)McN5652 and [¹¹C](-)McN5652 were synthesized by 5-methylation of the corresponding desmethyl precursor, which was stabilized by adding a protecting agent for mercapto groups, dithiothreitol, into the reaction medium immediately after the demethylation of McN5652 by an automated procedure.¹⁶ The radiochemical purity was higher than 95%.

For 34 of the 37 subjects, PET scans were performed using a Siemens ECAT47 system (CTI-Siemens, Knoxville, Tenn), which provides 47 slices with 3.375-mm (center-to-center) thickness. Radioactivity was measured in 2-dimensional mode, and the data were reconstructed with a ramp filter with a cut-off frequency of 0.5 (full-width half-maximum [FWHM], 6.3 mm). The other 3 subjects were measured using an EXACT HR+ scanner (CTI-Siemens, Knoxville, Tenn), which pro-

Table 1. Summary of Single-Dose Studies

Drug	Dose, mg	No. of Doses	Mean (SD) Occupancy, %
Clomipramine hydrochloride	5	2	67.2 (8.3)
	10	2	81.1 (6.9)
	25	3	84.9 (12.3)
	50	6	94.0 (4.7)
Fluvoxamine maleate	12.5	3	28.4 (31.2)
	25	7	56.8 (18.9)
	50	4	84.9 (2.2)

Table 2. Summary of Long-term Dose Studies With Patients

Drug	Doses, mg/d	Duration of Treatment	Concentration, ng/mL	Diagnosis	Occupancy, %
Clomipramine hydrochloride	250 (qid)	3 mo	660	OCD	100.0
	100 (qid)	8 mo	84	MDD	83.9
	70 (tid)	3 mo	53	OCD	100.0
	55 (qid)	2 mo	NA	MDD	86.4
	20 (bid)	2 wk	17	AD	97.1
	20 (qd)	1 wk	11	AD	89.3
Fluvoxamine maleate	200 (bid)	6 mo	143	MDD	86.8
	100 (bid)	3 wk	NA	MDD	79.7
	75 (tid)	6 mo	54	BPD	93.6
	25 (qd)	3 wk	NA	AD	76.6

Abbreviations: AD, anxiety disorder; bid, twice daily; BPD, bipolar disorder; MDD, major depressive disorder; NA, not applicable; OCD, obsessive-compulsive disorder; qd, daily; qid, 4 times daily; tid, 3 times daily.

vides 63 planes and a 15.5-cm field-of-view, and the data were reconstructed with a Hanning filter cut-off frequency 0.4 (FWHM, 7.5 mm). The subjects were placed in the supine position with eyes closed and ears unplugged. To minimize head movement during each scan, head fixation devices (Fixster Instruments, Stockholm, Sweden) and thermoplastic attachments made to fit the individuals were used. A transmission scan of 10 minutes with a germanium 68–gallium 68 source was followed by a dynamic scan for 90 minutes with a bolus injection of 581 to 20.51 mCi (215–759 MBq) of [¹¹C](+)McN5652. The specific radioactivities ranged from 0.31 to 5.25 Ci/μmol (11.6–194.3 GBq/μmol) (mean ± SD radioactivity, 2.61 ± 1.19 Ci/μmol [96.4 ± 44.1 GBq/μmol]) at the time of injection. A dynamic scan for 90 minutes with a bolus injection of 12.30 to 19.86 mCi (455–735 MBq) (mean ± SD injection, 18.11 ± 2.68 mCi [670–99 MBq]) of [¹¹C](–)McN5652 was performed on 14 healthy volunteers. The specific radioactivities were 0.69 to 3.66 Ci/μmol (25.6–135.6 GBq/μmol) (mean, 2.11 ± 0.95 [77.9 ± 35.2]) at the time of injection.

To measure the occupancy by a single administration of antidepressants, 13 healthy volunteers took part in the clomipramine study. Initial PET scans were carried out to establish the baseline data. Second PET scans were performed 5 hours after orally taking 5 mg to 50 mg of clomipramine hydrochloride (2 volunteers for 5 mg; 2 for 10 mg; 3 for 25 mg; and 6 for 50 mg) (Table 1). Blood samples (10 mL) were taken to measure the plasma concentration of clomipramine just before tracer injection. Fourteen healthy volunteers took part in the fluvoxamine occupancy study. Initial PET scans were carried out to attain the baseline data. Second PET scans were performed 5 hours after oral administration of different doses of fluvoxamine maleate (3 volunteers at 12.5 mg; 7 at 25 mg; 4 at 50 mg) (Table 1). Three subjects were scanned with the EXACT HR+ scanner for first and second scans having been given 25 mg of fluvoxamine maleate. Blood samples (10 mL) were taken to measure the plasma concentration of fluvoxamine just before tracer injection.

The plasma concentration of clomipramine was measured using high-performance liquid chromatography (HPLC) with ultraviolet detection, and that of fluvoxamine was measured by HPLC and a mass spectrometer.

To measure 5-HTT occupancy by long-term doses of antidepressants, the 10 patients were measured for 5-HTT binding during long-term treatment with clomipramine or fluvoxamine (Table 2). The PET scans were performed 7 hours after intake of fluvoxamine for 2 patients; 4 hours for 1 patient; 1 hour for 1 patient. Scans were performed 10 hours after intake of clomipramine for 1 patient; 4 hours for 3 patients; and 1 hour for 2 patients. Blood samples (10 mL) were taken to measure their plasma concentrations just before tracer injection. One of the patients taking clomipramine and 2 of the patients taking fluvoxamine refused the blood sampling.

DATA ANALYSIS

Positron emission tomographic images summated for 90 minutes were coregistered to magnetic resonance imaging (MRI) data with SPM99 (Wellcome Department of Cognitive Neurology, London, England), and regions of interest were defined over the cerebellum and thalamus based on these coregistered MRI and PET images. Because the specific binding of [¹¹C](+)McN5652 in the cerebral cortex is too low for quantitative analysis,^{10,11} we focused on the thalamus to quantify its specific binding. Binding potential (BP) was used for the quantification that is expressed as k_3/k_4 in compartment models. In the 3-compartment model, K_1 was used to describe the uptake of the tracer across the blood-brain barrier; k_2 represented back diffusion from tissue to the vascular space; and k_3 and k_4 described the binding and dissociation of the radioli-

gand at the specific binding site. The ratio k_3/k_4 is expressed by the equation

$$(1) \quad k_3/k_4 = \text{DVR} - 1,$$

where DVR is the distribution volume ratio based on the assumption that K_1/k_2 in the target tissue is equal to that in the reference tissue. However, the amount of nonspecific binding in the target tissue (the thalamus) was different from that in the reference tissue (cerebellum) with [¹¹C](+)McN5652.¹¹ The DVR of the target tissue to the reference tissue is given by the following equations for [¹¹C](+)McN5652 and the pharmacologically inactive enantiomer [¹¹C](–)McN5652:

$$(2) \quad \text{DVR}(+) = \frac{\frac{K_1^+}{k_2^+} \left(1 + \frac{k_3^+}{k_4^+} \right)}{\frac{K_1^{r+}}{k_2^{r+}}},$$

$$(3) \quad \text{DVR}(-) = \frac{\frac{K_1^-}{k_2^-}}{\frac{K_1^{r-}}{k_2^{r-}}},$$

respectively, where K_1^+ , k_2^+ , k_3^+ , and k_4^+ are the estimated parameters of [¹¹C](+)McN5652 for the target tissue; K_1^{r+} and k_2^{r+} are the estimated parameters of [¹¹C](+)McN5652 for the reference tissue without a specific binding site, and K_1^- , k_2^- , K_1^{r-} and k_2^{r-} are those of [¹¹C](–)McN5652. Then k_3^+/k_4^+ is expressed as

$$(4) \quad \frac{k_3^+}{k_4^+} = \frac{\text{DVR}(+)}{\frac{K_1^+/k_2^+}{K_1^{r+}/k_2^{r+}}} - 1$$

If it is assumed that $(K_1^+/k_2^+)/(K_1^{r+}/k_2^{r+})$ is equal to $\text{DVR}(-)$, then BP can be derived from the equation

$$(5) \quad \text{BP} = \text{DVR}(+)/\text{DVR}(-) - 1,$$

where the DVR of the thalamus to the cerebellum was calculated without arterial input function by the graphical method.^{11,16} This assumption is based on the result that the value of $\text{DVR}(+)$ in the saturation study of [¹¹C](+)McN5652 was almost equal to $\text{DVR}(-)$.¹¹

CALCULATION OF 5-HTT OCCUPANCY

Serotonin transporter occupancy (Occu [%]) using [¹¹C](+)McN5652 is expressed as

$$(6) \quad \text{Occu}(\%) = 100 \cdot (\text{BP}_{\text{baseline}} - \text{BP}_{\text{drug}})/\text{BP}_{\text{baseline}} = 100 \cdot [1 - (\text{DVR}(+)_{\text{drug}} - \text{DVR}(-))/(\text{DVR}(+)_{\text{baseline}} - \text{DVR}(-))],$$

where $\text{DVR}(+)$ and $\text{DVR}(-)$ are the distribution volume ratios of [¹¹C](+)McN5652 and [¹¹C](–)McN5652 as estimated by graphical method with the reference tissue¹⁷; the test-retest variability and reliability (intraclass correlation coefficient) of [¹¹C](+)McN5652 for this graphical method were 10.8% and 0.83, respectively, based on

the 10-subject data (unpublished data). In this study, the mean±SD value of DVR(-) was estimated as 1.16±0.06 based on the data of 14 healthy volunteers (mean±SD age, 23.4±4.1 years), and it was used as a fixed value for the calculation of occupancy since not all subjects underwent the PET scans with [¹¹C](-)McN5652.

In 8 of the 10 patients studied, the mean±SD value of DVR(+)_{baseline} for the 27 healthy volunteers (1.89±0.21 [range, 1.59-2.40]) was used for the calculation because the individual DVR(+)_{baseline} values were not available for these 8 patients.

The relationship between 5-HTT occupancy and the dose or plasma concentration of antidepressants was modeled by the equation

$$(7) \quad 5\text{-HTT}_{\text{occu}} = 100 \cdot D / (ED_{50} + D),$$

where 5-HTT_{occu} is 5-HTT occupancy, ED₅₀ (the in vivo median effective dose) is a constant, and D is the concentration of the drug near the 5-HTT. Dose and plasma concentration were used as functional surrogates of D.¹⁸

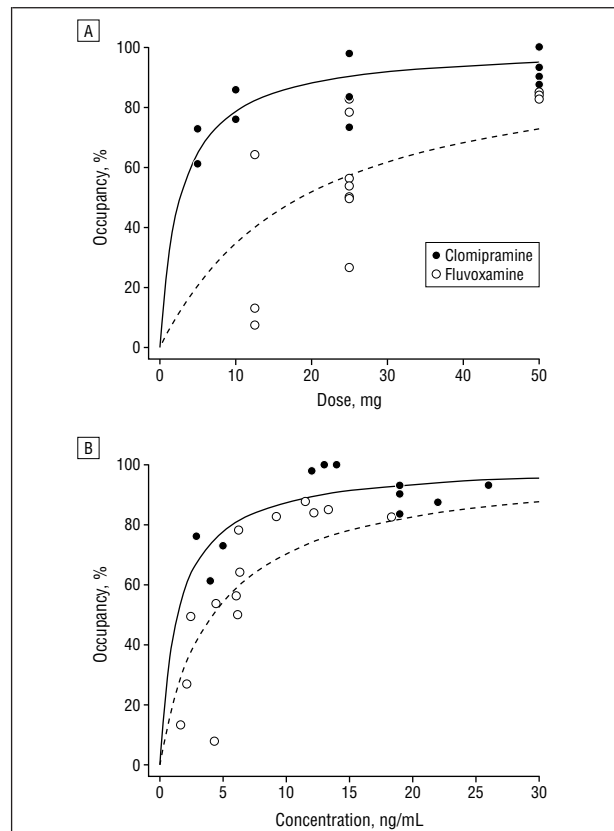
RESULTS

The occupancy of 5-HTT in the thalamus was dose-dependently increased both by clomipramine and fluvoxamine (Table 1, **Figure**). In the single-dose studies, clomipramine occupied more than 60% of 5-HTT even with the lowest dose of 5 mg (Table 1 and Figure). The ranges of occupancy were from 61.3% to 100% between 5 mg and 50 mg of clomipramine hydrochloride and from 7.7% to 87.7% between 12.5 mg and 50 mg of fluvoxamine maleate. There were hyperbolic relationships between 5-HTT occupancy and oral dose ($r=0.780$) and plasma concentration of clomipramine ($r=0.752$) (Figure) (2 of the plasma samples were under the detection limit and are not shown in the Figure). There were also hyperbolic relationships between 5-HTT occupancy and oral dose ($r=0.686$) and plasma concentration of fluvoxamine ($r=0.808$) (Figure). The calculated ED₅₀ was 2.67 mg for oral dose and 1.42 ng/mL for plasma concentration of clomipramine, and that of fluvoxamine was 18.6 mg for oral dose and 4.19 ng/mL for plasma concentration.

In this study, we used the patient data with long-term dose to estimate 5-HTT occupancy. The occupancies from long-term dose ranged from 83.9% to 100% for clomipramine and 76.6% to 93.6% for fluvoxamine (Table 2). Including the patient data, the saturation curves did not significantly deviate against the dose or plasma concentration (clomipramine: $r=0.724$; fluvoxamine: $r=0.722$) or plasma concentration (clomipramine: $r=0.726$; fluvoxamine: $r=0.835$). The calculated ED₅₀ including the patient data was 2.62 mg for oral dose and 1.40 ng/mL for plasma concentration of clomipramine. The calculated ED₅₀ of fluvoxamine was 17.4 mg for oral dose and 4.20 ng/mL for plasma concentration including the patient data.

COMMENT

The present results demonstrated that 5-HTT was occupied by clomipramine or fluvoxamine in dose-



Serotonin transporter occupancy of healthy volunteers by single oral administration of clomipramine hydrochloride and fluvoxamine maleate. Occupancies are plotted against oral dose (A) and plasma concentration (B) of clomipramine and fluvoxamine.

dependent manners (Table 1), and curvilinear functions against drug concentration were clearly demonstrated (Figure). The present results showed marked high 5-HTT occupancy even with a small dose of clomipramine (Table 1 and Figure). Approximately 80% of 5-HTT was occupied with 10 mg of clomipramine hydrochloride (Table 1 and Figure). On the other hand, the 5-HTT occupancy of fluvoxamine increased slowly compared with that of clomipramine. The ED₅₀ values of fluvoxamine were several times larger than those of clomipramine. Although several different values using different methods have been reported, this order of ED₅₀ was generally consistent with the binding parameters in vitro.^{19,20} However, the daily clinical doses of those antidepressants were not so different between clomipramine hydrochloride (at 75-300 mg/d [50-100 mg/d in Japan]) and fluvoxamine maleate (at 100-300 mg/d [50-100 mg/d in Japan]).⁴

The present results indicated that the variations in 5-HTT occupancy with low doses (12.5 mg and 25 mg) of fluvoxamine maleate were relatively large, but 50 mg of fluvoxamine maleate consistently occupied approximately 80% of 5-HTT (Table 1 and Figure). This suggested that at least 50 mg of fluvoxamine maleate was necessary to obtain certainly high 5-HTT occupancy (Table 2). These results of 5-HTT occupancy were concordant with those reporting that a dose of 50 to 150 mg of fluvoxamine maleate per day was therapeutically effective and that the minimally effective dose was 50 mg/d.²¹

A recent study indicated that the clinical doses of paroxetine and citalopram occupy 70% to 80% of 5-HTT in living human brain.¹² It seems that close to 80% of 5-HTT occupancy is necessary to obtain therapeutic effects. That is a value of occupancy similar to that reported for the dopamine D2 receptor with a clinical dose of antipsychotics.²² Furthermore, saturated 5-HTT with high-doses of antidepressants (Table 2) can explain the fact that there was no relationship between the plasma concentration of fluvoxamine and clinical response during the treatment with a relatively high oral dose (200-300 mg/d²³ or 150-300 mg/d²⁴).

On the other hand, ED₅₀ of clomipramine was much smaller compared with the usual clinical dose. The present results showed that 10 mg of clomipramine hydrochloride would be enough for nearly 80% of 5-HTT occupancy. It has been reported that there was no relationship between clinical effects and plasma concentration of clomipramine at a fixed dose of 150 mg/d^{25,26} or different doses between 30 and 75 mg/d.²⁷⁻²⁹ These reports support the present results that clomipramine occupied close to 80% of 5-HTT with an oral dose of 10 mg and that only a minimal increase in 5-HTT occupancy could be expected with a high dose. However, some reports have suggested a relationship between clinical response and dose (25-200 mg³⁰) or plasma concentration of clomipramine and its metabolite desmethylclomipramine.^{30,31} Other studies showed a relationship between clinical response and the plasma concentration of desmethylclomipramine only.^{25,32} Since desmethylclomipramine is a potent noradrenaline reuptake inhibitor and clomipramine has relatively high affinities to several receptors,³³ mechanisms other than 5-HTT blockade can be supposed after sufficient saturation of 5-HTT.

Although the clinical merits of therapeutic drug monitoring of SSRIs are controversial,³⁴ 5-HTT occupancy correlates well with the plasma concentration as compared with the dose especially in the case of fluvoxamine (Figure). This suggested the possible use of therapeutic drug monitoring of SSRIs. If a patient does not respond well to an SSRI despite a high plasma concentration, this would be a good reason to switch antidepressants since there are many SSRI nonresponders.³⁵ There would be no merit in a dose escalation in a highly saturated dose range.³⁶ The beneficial alternative would be switching to a non-SSRI with different pharmacological properties.^{35,37,38} Clomipramine has been reported to show a better clinical outcome compared with citalopram or paroxetine in severely depressed hospitalized patients.^{39,40} A high dose of clomipramine can work on multiple neurotransmitter systems.

There are several limitations to the present study, especially with regard to the 5-HTT occupancy of the 8 patients being treated with antidepressants long-term. We used the mean DVR(+)_{baseline} of the 27 healthy volunteers as the baseline. The variance of the calculated occupancy of 83.9% would be 73.3% to 90.7% using the extreme values of DVR(+) (1.59 and 2.40). This variance was similar to that of dopamine receptor occupancy using the average value as a baseline.²² However, since BPs of [¹¹C](+)McN5652 in the thalamus were higher in patients with mood disorders⁴¹ and no data are

available for the 5-HTT of obsessive-compulsive disorders in vivo, some of the occupancy values calculated in this study might be underestimated or overestimated. In addition, the age effect of BPs was not considered in this study, since the effect of age⁴² in these different diseases was not clear. In this study we used the mean value of DVR(-) (1.16±0.06) from 14 healthy volunteers as an estimate of nonspecific binding. The estimation of nonspecific binding can affect the absolute value of occupancy.¹¹ For example, the calculated occupancy of 82.6% can vary between 78.9% and 86.7% based on the deviation of DVR(-) value. Furthermore, if the nonspecific binding of the patients was different from that of the healthy volunteers, the estimated value of occupancy might be inaccurate.

In this study we have demonstrated that clinical doses of clomipramine and fluvoxamine occupied about 80% of 5-HTT, and that a small dose (such as 10 mg) of clomipramine can occupy about 80% of 5-HTT in vivo. However, further studies are needed to investigate the relation between clinical effects and occupancy especially for the use of a long-term small dose of clomipramine.

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REFERENCES

- Schloss P, Williams DC. The serotonin transporters: a primary target for antidepressant drugs. *J Psychopharmacol.* 1998;12:115-121.
- Koran LM, McElroy SL, Davidson JRT, Rasmussen SA, Hollander E, Jenike MA. Fluvoxamine versus clomipramine for obsessive compulsive disorder: a double-blind comparison. *J Clin Psychopharmacol.* 1996;16:121-129.
- DeWilde JE, Doogan DP. Fluvoxamine and chlorimipramine in endogenous depression. *J Affect Disord.* 1982;3:249-259.
- Rush JA. Mood disorders: treatment of depression. In: Sadock BJ, Sadock VA, eds. *Kaplan & Sadock's Comprehensive Textbook of Psychiatry.* 7th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2000:1377-1385.
- Farde L, Hallidin C, Muller L, Suhara T, Karlsson P, Hall H. PET study of [¹¹C]β-CIT binding to monoamine transporters in the monkey and human brain. *Synapse.* 1994;16:93-103.
- Szabo Z, Kao PF, Scheffel U, Suehiro M, Mathews WB, Ravert HT, Musachio JL, Marengo S, Kim SE, Ricaurte GA, Wong DF, Wagner HN Jr, Dannals RF. Positron emission tomography imaging of serotonin transporters in the human brain using [¹¹C](+)McN5652. *Synapse.* 1995;20:37-43.
- Houle S, Ginovart N, Hussey D, Meyer J, Wilson A. Imaging the serotonin transporter with positron emission tomography: initial human studies with [¹¹C]DAPP and [¹¹C]DASB. *Eur J Nucl Med.* 2000;27:1719-1722.

8. Pirker W, Asenbaum S, Kasper S, Walter H, Angelberger P, Koch G, Pozzera A, Deecke L, Podreka I, Brücke T. β - CIT SPECT demonstrates blockade of 5-HT-uptake site by citalopram in the human brain in vivo. *J Neural Transm Gen Sect*. 1995;100:247-256.
9. Tauscher J, Pirker W, De Zwaan M, Asenbaum S, Brücke T, Kasper S. In vivo visualization of serotonin transporters in the human brain during fluoxetine treatment. *Eur Neuropsychopharmacol*. 1999;9:177-179.
10. Parsey RV, Kegeles LS, Hwang DR, Simpson N, Abi-Dargham A, Mawlawi O, Slifstein M, Van Heertum RL, Mann JJ, Laruelle M. In vivo quantification of brain serotonin transporters in human using [^{11}C]McN5652. *J Nucl Med*. 2000;41:1465-1477.
11. Ikoma Y, Suhara T, Toyama H, Ichimiya T, Takano A, Sudo Y, Inoue M, Suzuki K. Quantitative analysis for estimating binding potential of brain serotonin transporters with [^{11}C]McN5652. *J Cereb Blood Flow Metab*. 2002;22:490-501.
12. Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K, Houle S. Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [^{11}C]DASB PET imaging study. *Am J Psychiatry*. 2001;158:1843-1849.
13. Logan J, Volkow N, Fowler JS, Wang G-J, Fischman MW, Foltin RW, Abumrad NN, Vitkun S, Gatley SJ, Pappas N, Hitzemann R, Shea CE. Concentration and occupancy of dopamine transporters in cocaine abusers with [^{11}C]cocaine and PET. *Synapse*. 1997;27:347-356.
14. Shank RP, Vaught JL, Pelley KA, Setler PE, McCormsey DF, Maryanoff BE. McN5652: a highly potent inhibitor of serotonin uptake. *J Pharmacol Exp Ther*. 1988;247:1032-1038.
15. Lombardo I, Parsey RV, Hwang DR, Huang Y, Hashimoto T, Simpson N, Mawlawi O, Slifstein M, Arevalo A, Van Heertum R, Mann JJ, Laruelle M. Test/retest reproducibility of measurement of 5-HT transporter parameters in humans with (+) [^{11}C]McN5652: comparison between methods [abstract]. *J Nucl Med*. 2000;41:211.
16. Sasaki M, Suhara T, Kubodera A, Suzuki K. An improved automated synthesis and in vivo evaluation of PET radioligand for serotonin re-uptake sites: [^{11}C]McN5652X. *Kaku Igaku*. 1996;33:1319-1327.
17. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab*. 1996;16:834-840.
18. Kapur S, Zipursky RB, Jones C, Remington GJ, Wilson AA, DaSilva J, Houle S. The D2 receptor occupancy profile of loxapine determined using PET. *Neuropsychopharmacology*. 1996;15:562-566.
19. Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol*. 1997;340:249-258.
20. Hyttel J. Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int Clin Psychopharmacol*. 1994;9(suppl 1):19-26.
21. Walczak DD, Apter JT, Halikias JA, Borison RL, Carman JS, Post GL, Patrick R, Cohn JB, Cunningham LA, Rittberg B, Preskorn SH, Kang JS, Wilcox CS. The oral dose-effect relationship for fluvoxamine: a fixed-dose comparison against placebo in depressed outpatients. *Ann Clin Psychiatry*. 1996;3:139-151.
22. Farde L, Nordström A-L, Wiesel F, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. *Arch Gen Psychiatry*. 1992;49:538-544.
23. Kasper S, Dotsch M, Kick H, Vieira A, Moller HJ. Plasma concentrations of fluvoxamine and maprotiline in major depression: implications on therapeutic efficacy and side effects. *Eur Neuropsychopharmacol*. 1993;3:13-21.
24. De Wilde JEM, Doogan DP. Fluvoxamine and clomipramine in endogenous depression. *J Affect Dis*. 1982;4:249-259.
25. Broadhurst AD, James HD. Clomipramine plasma level and clinical response. *Postgrad Med J*. 1977;53(suppl 4):139-145.
26. Montgomery SA, Montgomery DB, McAuley R, Dawling S, Braithwaite RA. Plasma concentration of clomipramine and clinical response in depressed patients. *Postgrad Med J*. 1980;56(suppl 1):130-133.
27. Gringras M. A comparison of a low and high dosage regime of clomipramine (Anafranil). *J Int Med Res*. 1975;3(suppl 1):47-54.
28. Frank P. Comparisons of various clomipramine (Anafranil) dosage regimes. *J Int Med Res*. 1977;5(suppl 1):11-15.
29. Miller P, Luscombe DK, Jones RB, Seldrup J, Beaumont G, John V. Relationships between clinical response, plasma levels, and side-effects of clomipramine (Anafranil) in general practitioner trials. *J Int Med Res*. 1977;5(suppl 1):108-118.
30. Danish University Antidepressant Group (DUAG). Clomipramine dose-effect study in patients with depression: clinical end points and pharmacokinetics. *Clin Pharmacol Ther*. 1999;66:152-165.
31. Faravelli C, Ballerini A, Ambonetti A, Broadhurst AD, Das M. Plasma levels and clinical response during treatment with clomipramine. *J Affect Dis*. 1984;6:95-107.
32. Corte LD, Broadhurst AD, Sgaragli GP, Filippini S, Heeley AF, James HD, Faravelli C, Pazzagli A. Clinical response and tricyclic plasma levels during treatment with clomipramine. *Br J Psychiatry*. 1979;134:390-400.
33. Mctavish D, Benfield P. Clomipramine: an overview of its pharmacological properties and a review of its therapeutic use in obsessive compulsive disorder and panic disorder. *Drugs*. 1990;39:136-153.
34. Lundmark J, Bengtsson F, Nordin C, Reis M, Wålander J. Therapeutic drug monitoring of selective serotonin reuptake inhibitors influences clinical dosing strategies and reduces drug costs in depressed elderly patients. *Acta Psychiatr Scand*. 2000;101:354-359.
35. Fredman JS, Fava M, Kienke AS, White CN, Nierenberg AA, Rosenbaum JF. Partial response, nonresponse, and relapse with selective serotonin reuptake inhibitors in major depression: a survey of current "next-step" practices. *J Clin Psychiatry*. 2000;61:403-408.
36. Benkert O, Azegedi A, Wetzell H, Staab HJ, Meister W, Philipp M. Dose escalation vs continued dose of paroxetine and maprotiline: a prospective study in depressed out-patients with inadequate treatment response. *Acta Psychiatr Scand*. 1997;95:288-296.
37. Fava M. Management of nonresponse and intolerance: switching strategies. *J Clin Psychiatry*. 2000;61(suppl 2):10-12.
38. Withersty DJ, Vanin JR. Guidelines for treating depression using non-selective serotonin reuptake inhibitors. *W V Med J*. 1999;95:20-23.
39. Danish University Antidepressant Group. Citalopram: clinical effect profile in comparison with clomipramine: a controlled multicenter study. *Psychopharmacology*. 1986;90:131-138.
40. Danish University Antidepressant Group. Paroxetine: a selective serotonin reuptake inhibitor showing better tolerance, but weaker antidepressant effect than clomipramine in a controlled multicenter study. *J Affect Disord*. 1990;18:289-299.
41. Ichimiya T, Suhara T, Sudo Y, Okubo Y, Nakayama K, Nankai M, Inoue M, Yasuno F, Takano A, Maeda J, Shibuya H. Serotonin transporter binding in patients with mood disorders: a PET study with [^{11}C](+)-McN5652. *Biol Psychiatry*. 2002;51:715-722.
42. Yamamoto M, Suhara T, Okubo Y, Sudo Y, Ichimiya T, Sudo Y, Inoue M, Takano A, Yasuno F. Age-related decline of serotonin transporter binding in living human brain measured by PET with [^{11}C](+)-McN5652. *Life Sci*. 2002;71:751-757.