

# Brain Pharmacokinetics and Tissue Distribution *In Vivo* of Fluvoxamine and Fluoxetine by Fluorine Magnetic Resonance Spectroscopy

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*This investigation of fluvoxamine and fluoxetine-norfluoxetine distributions in vivo at steady-state and of quantitative kinetics in brain and plasma after drug therapy interruption was performed by fluorine nuclear magnetic resonance spectroscopy ( $^{19}\text{F}$  MRS), spectroscopic imaging (MRSI), and plasma HPLC on 12 subjects treated for depression. MRSI suggests a homogeneous distribution of  $^{19}\text{F}$  MRS visible fluvoxamine mainly in brain. Fluvoxamine steady-state brain concentrations ( $12 \pm 5 \mu\text{M}$ ;  $n = 13$ ) and brain-to-plasma concentration ratios ( $10 \pm 2$ ;  $n = 12$ ) were similar to those of combined fluoxetine-norfluoxetine (CF-norfluoxetine) ( $13 \pm 6 \mu\text{M}$ ;  $n = 4$  and  $10 \pm 6$ ;  $n = 4$ ).*

*Fluvoxamine brain elimination half-life ( $79 \pm 24$  hours;  $n = 4$ ) was significantly shorter than that of CF-norfluoxetine ( $382 \pm 48$  hours;  $n = 2$ ). Fluvoxamine brain-to-plasma-half-life-ratio was  $2.2 \pm 0.3$  ( $n = 4$ ), contrarily to CF-norfluoxetine ( $1.0 \pm 0.3$ ;  $n = 2$ ). This study shows that quantitative pharmacokinetics in target organs by  $^{19}\text{F}$  MRS in vivo should prove useful for understanding and investigating outcome of treatment modifications and side effects.*

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Pharmacodynamic studies mostly rely on plasma kinetics alone because brain concentrations are not easily measured. However, concentrations in the central nervous system (CNS) should be of more interest for understanding the behavioral effects of psychotropic drugs which are expected to be related to their levels in the target organ, i.e., the brain.

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Use of radioisotopes in positron emission tomography (PET) or single photon emission computerized tomography (SPECT) studies may provide a way to estimate the receptor occupancy, but these techniques present major disadvantages because of the inherent hazard related to the radioactivity of the tracer. Repeated studies are limited in order to limit radioactive exposure, thus medium or long term kinetic studies by these techniques are excluded. On the contrary, nuclear magnetic resonance (MR) techniques which have been shown to be harmless to the subject under repetitive recording conditions may be of great interest for estimating tissue concentrations of drugs. Indeed, for some drugs that have one or several fluorine atoms in their active structure, no specific labeling is required since the 100% naturally abundant fluorine-19 nucleus ( $^{19}\text{F}$ ) constitutes a natural non-radioactive, stable label of the

drug detectable by  $^{19}\text{F}$  magnetic resonance spectroscopy (MRS). Thus  $^{19}\text{F}$  MRS provides a means to detect signal from the drug in the tissues *in vivo* (Durst et al. 1990; Komoroski et al. 1990) and also to estimate drug concentrations *in vivo* (Renshaw et al. 1992; Karson et al. 1992) when a calibration method is applied to the measurement. This is the case in particular for the two antidepressant selective serotonin reuptake inhibitors (SSRI) fluvoxamine and fluoxetine, which are widely prescribed for treatment of depression, affective or mood disorders, and for obsessive compulsive disorder.

It has also been shown that brain drug kinetics during treatment modifications such as progressive withdrawal (Bolo et al. 1996) and abrupt withdrawal or uptake (Strauss et al. 1997, 1998) may be obtained in this way. However, at the present time little is known about the distribution at steady state of SSRIs in tissues *in vivo* and their kinetics in target or peripheral tissues. More knowledge on target and peripheral tissue distribution and kinetics should be useful to explain central treatment or secondary effects and could help the clinician for choice of treatment and treatment planning. In this preliminary study, it was our objective to investigate and compare the distribution of the SSRIs fluvoxamine and fluoxetine in tissues and the withdrawal kinetics in brain and in plasma.

## MATERIALS AND METHODS

### Subject Population

This paper reports results from 12 subjects suffering from depression. The study was approved by the CCP-PRB Alsace II Ethics Committee, France. Written informed consent was obtained from all subjects prior to participation. All subjects included in the study underwent a detailed psychiatric interview and were under SSRI treatment for a major depressive episode. The DSM-IV criteria for major depressive disorder was clearly met for 11 of the subjects. For one of the subjects, the major depressive episode concurred with an associated diagnosis of generalized anxiety disorder (DSM-IV).

The average age for the eight women and four men who participated was  $48 \pm 8$  years and ranged from 36 to 67 years. The average age for the eight subjects treated with fluvoxamine (five women and three men) was  $49 \pm 10$  years and ranged from 36 to 67 years. The average age for the four subjects treated with fluoxetine (three women and one man) was  $47 \pm 4$  years and ranged from 42 to 50 years. No significant age difference was found between the fluvoxamine and fluoxetine groups or between the men and women subgroups within each treatment group.

Daily fluvoxamine doses ranged from 100 to 300 mg (one subject on 100 mg, one subject on 200 mg, one sub-

ject on 250 mg, four subjects on 300 mg, and one subject with two treatment periods on 200 mg and 300 mg). Duration of fluvoxamine treatment ranged from one to 12 months (two subjects for one month, two subjects for three months, one subject for seven months, two subjects for 12 months, and one subject for 12 months on 300 mg, and for 3 months on 200 mg).

Daily fluoxetine doses ranged from 10 to 40 mg (one subject on 10 mg, one subject on 20 mg, two subjects on 40 mg). Duration of fluoxetine treatment ranged from three to 12 months (one subject for three months, one subject for four months, one subject for eight months, one subject for 12 months).

Blood samples for HPLC measurement of either serum fluvoxamine, or serum fluoxetine and norfluoxetine concentrations were taken within 1 hour prior to the MRS measurement. Estimations of steady state or washout concentrations were calculated from MRS recordings performed after a minimum of 30 days treatment for fluvoxamine and three months treatment for fluoxetine. These minimum treatment durations taken for establishment of steady state are compatible with reported brain uptake pharmacokinetic data for fluvoxamine (Strauss et al. 1997) and fluoxetine (Karson et al. 1993). Steady state assessments were performed from one to four times per subject during the steady state period, with the reported  $n$  being the number of assessments. One subject had the MRS measurement without the corresponding blood sample for plasma assessment and another had a blood sample taken without having a corresponding MRS exam for brain assessment: data from these unpaired brain or plasma assessments at steady state for fluvoxamine treatment were included in the respective brain or plasma steady state average calculation, but not in the brain-to-plasma ratio or correlation calculations which require paired data points.

Repeated washout pharmacokinetic recordings were performed on four subjects after abrupt withdrawal from fluvoxamine, and on two subjects after abrupt withdrawal from fluoxetine. Abrupt withdrawal means that the subjects stopped taking the drug entirely on the first day of withdrawal. Time points for kinetic data are reported in hours after the last drug administration. None of the subjects reported any undesirable withdrawal effects.

### Plasma Concentrations by HPLC

Plasma fluvoxamine or fluoxetine and norfluoxetine concentrations were measured from serum of blood samples which were taken within one hour of the of MRS recordings.

A high performance liquid chromatography (HPLC) method was developed in-house to separate and dose fluvoxamine in human serum. The method uses clovox-

amine as an internal standard. An initial extraction of the serum in alkaline medium (NaOH 2M), using a heptane-isopropanol mixture, is followed by a hydrochloric acid (0.1N) extraction. An amount of 30  $\mu$ l of the aqueous phase is then injected on a Nucleosil C8 5  $\mu$  column (150  $\times$  4.6 mm). A phosphate buffer-acetonitril mixture is used for the mobile phase which has a flow of 1.1 ml/minute. The absorption wavelength used is 190 nm. The retention time is 4.7 minutes for the internal standard and 6.2 minutes for fluvoxamine.

A HPLC method was also developed in-house to separate and dose fluoxetine and norfluoxetine in human serum. The method uses desmethyldomipramine as an internal standard. An initial extraction of the serum in alkaline medium (NaOH 2M), using a hexane-isoamyl alcohol mixture, is followed by a phosphoric acid (0.05%) extraction. An amount of 30  $\mu$ l of the aqueous phase is then injected on an Ultrasphere ODS 5  $\mu$  column (250  $\times$  4.6 mm). A phosphate buffer-acetonitril mixture is used for the mobile phase which has a flow of 2.0 ml/minute. The absorption wavelength used is 214 nm. The retention time is 7 minutes for norfluoxetine, 9.1 minutes for fluoxetine, and 12.1 minutes for the internal standard. The calibration range is performed with an O serum loaded with fluoxetine and norfluoxetine with the final fluoxetine and norfluoxetine concentrations reaching 50, 100, 250, and 500  $\mu$ g/l, respectively. Measurement accuracy was assessed during the dosing procedure in a concentration range from 0 to 50  $\mu$ g/l.

### Proton and Fluorine MR Recordings

MR spectroscopy and imaging were performed on a Medspec 3 Tesla spectrometer (Bruker Spectrospin SA, Wissembourg, France) using UXNMR/Paravision acquisition software (Bruker Medizintechnik GMBH, Ettlingen, Germany). Separate proton and fluorine head coils were used for recordings in two steps after subject positioning. Both coils had identical geometry and design, i.e., they were linear cylindrical bird cage head coils with a 28-cm inner diameter. For the duration of the recordings, the subject lay supine with head resting at the center of the magnet on a head support around which the RF coils were placed. First shimming and frequency adjustments were performed using the proton head coil tuned to 125 MHz. With the proton coil in place, pilot images in the three planes were recorded to verify adequate positioning, and a proton single pulse-acquire spectrum was recorded to verify adequate shimming.

The shimming was considered acceptable if the line width at half-height of the water peak from the whole head was not larger than 25 Hz. Proton 3-dimensional (3D) images with a matrix size of 128  $\times$  128  $\times$  128, and a field of view (FOV) of 25.6 cm in each dimension were acquired for anatomical referencing of head spectro-

scopic images. A gradient echo imaging sequence with a non-selective square excitation pulse preceded by a non-selective adiabatic inversion pulse, was used for the 3D T<sub>1</sub> weighted images. After removal of the proton coil from the back of the magnet without movement of the subject, the identically positioned linear fluorine head coil was used for fluorine spectroscopy at 117 MHz. A 10 ml cylindrical sealed vial of a solution of 10 mM trifluoroethanol (TFE) in water attached to the head support was used as an external quantitative reference.

For estimations of brain concentration, fluorine pulse-acquire spectroscopy of the whole head was performed with a repetition time (TR) of 1s, a flip angle of 90°, a spectral width (SW) of 20000 Hz, and data size of 16384 points. For localization of signal from the drug *in vivo*, two-dimensional fluorine MR spectroscopic images (MRSI) (Brown et al. 1982) of the head were performed at steady state before the washout period as described by Bolo et al. (1997): at the nominal fluorine resonance frequency 117 MHz with RF transmitter frequency centered between the fluvoxamine and TFE resonances, in the axial (x-y) plane with no slice selection, with a matrix size of 8  $\times$  8 for 2-D phase encoding. Since there was no slice selection, the MRSI images can be considered as peak area projections in the axial plane from the volume inside the head coil. Other parameters of the MRSI were: raw data free induction decay (FID) size = 2048 points, SW = 15151 Hz, TR = 1s, FOV = 25.6  $\times$  25.6 cm, NS = 16 averages for a total experiment time of 17 minutes.

Spectral processing was performed with UXNMR/Paravision software (Bruker Medizintechnik GMBH, Ettlingen, Germany) blindly to any information concerning subjects, treatments, doses, or plasma concentrations. Image processing for segmentation, volume measurements, and tissue density projections was done with software developed in-house. For the whole head non localized recordings used to estimate brain concentrations, resolution enhancement by zero filling was applied in order to qualitatively separate fluoxetine and norfluoxetine peaks *in vivo* without introducing artefacts, notwithstanding the experimental conditions which did not allow sufficient separation for quantitation purposes. Apodization with a matched filter was applied in order to increase the signal-to-noise ratio. The raw data FIDs from the whole head recordings were zero filled to 32768 points and were apodized with an exponential filter resulting in 50 Hz line broadening before Fourier transformation to spectra. In order to increase the accuracy of numerical integration, minor manual interactive spectral baseline corrections were performed on a small number of spectra for which the noise in the signal free portion was not evenly scattered around the zero line. Numerical integration of the TFE peak and the fluvoxamine peak or the combined fluox-

etine-norfluoxetine (CF-norfluoxetine) peak was performed using 18 ppm-wide (2106 Hz) integration regions.

To obtain images of drug localization (Figure 1), the MRSI data were zero filled to a matrix size of  $64 \times 64$  in the spatial dimensions which were apodized with a sine-squared filter in order to increase the spatial resolution of the images without introducing artefacts as was verified by inspecting images of noise regions. Before MRSI image formation the FIDs were apodized in the spectral dimension with an exponential filter resulting in 20 Hz line broadening in order to increase the signal-to-noise ratio. Spectroscopic images were obtained over 118 Hz wide spectral regions centered on the resonances of interest. To obtain localized spectra (Figure 2), the MRSI data were zero filled to 16384 points in the spectral dimension which was apodized with an exponential filter resulting in 50 Hz line broadening in order to increase the signal-to-noise ratio. Localized spectra were then obtained by Fourier transformation followed by a magnitude calculation. Tissue density images in the axial plane (Figures 1e-f) were obtained by operator interactive segmentation of the 3D  $T_1$  weighted images using in-house software; in these images the relative density of a given structure (e.g., brain tissue) at each pixel location is defined as the number of voxels of the segmented structure that project onto the pixel location in the axial plane divided by the total number of voxels in the 3D image set. Density of head tissue other than brain excluding cerebrospinal fluid (CSF) was obtained by density image subtraction. Spectroscopy and MRSI of the legs was also performed in this study in a manner similar to the head recordings, but results are not reported in this paper.

### MRS Estimations of Tissue Concentrations

Spectra obtained from solutions *in vitro* of fluoxetine dissolved in physiological saline with concentrations ranging from 0.11 to 1.76 mM demonstrated linearity between drug concentration and signal intensity for the hardware configuration used in this study ( $r^2 = 1.00$ ). Estimations of the amount of brain fluvoxamine or CF-norfluoxetine in micromoles were obtained by taking the ratio of the drug peak integral to the TFE peak integral times the amount of TFE in the external reference and correcting for  $T_1$  decay effects. Thus, the absolute concentration  $C$  in  $\mu\text{M}$  was estimated using the following formula:

$$C = \frac{I_{\text{drug}}}{I_{\text{TFE}}} \times \frac{A_{\text{TFE}}}{V_{\text{brain}}} \times \text{Corr}_{T_1}$$

with  $I_{\text{drug}}$  and  $I_{\text{TFE}}$  being, respectively, the drug and TFE integrals,  $A_{\text{TFE}}$  the amount of TFE in the external reference (100  $\mu\text{m}$ ), and  $V_{\text{brain}}$  the brain volume.  $\text{Corr}_{T_1}$  cor-

rects for differences in saturation between the brain and the external reference and is given by the following for 90° pulses:

$$\text{Corr}_{T_1} = \frac{(1 - \exp(-TR/T_{1\text{TFE}}))}{(1 - \exp(-TR/T_{1\text{drug}}))}$$

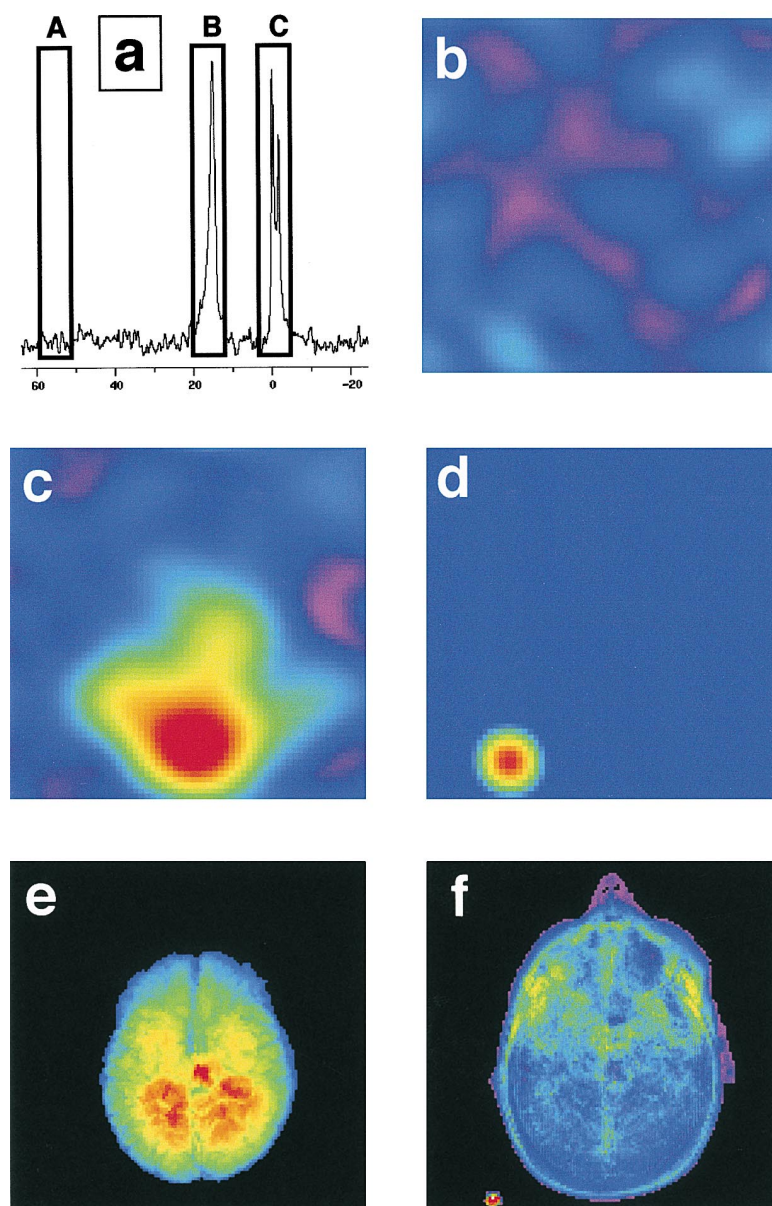
with  $T_{1\text{TFE}}$  and  $T_{1\text{drug}}$  being, respectively, the TFE and drug  $T_1$  values. In this study we have not attempted to measure the relaxation times *in vivo* of fluvoxamine or fluoxetine. The  $T_1$  of fluvoxamine  $^{19}\text{F}$  in brain tissue at a field strength of 1.5 T has been estimated in a group of subjects with obsessive compulsive disorder to be in the range of ca. 140 to 230 msec (Strauss et al. 1997). The  $T_1$  of fluoxetine  $^{19}\text{F}$  in brain tissue at a field strength of 1.5 T has been estimated to be ca. 190 ms (Karson et al. 1993). We assume that the  $T_1$ 's are in a similar range in this study, and have taken an approximate intermediate value of 180 ms for the drug  $T_1$  *in vivo* to calculate the  $T_1$  correction term. The  $T_1$  of the TFE external reference was measured with an inversion recovery 180° – TI – 90° sequence, with an adiabatic 180° inversion pulse followed by a spoiler gradient pulse to destroy residual transverse components during the inversion delay TI.

The effect of relaxation during RF pulse and receiver dead-time ( $T_d$ ) on concentration measurement error was assessed and was found to be negligible in our case. The 90°  $^{19}\text{F}$  RF pulses were 100  $\mu\text{s}$  long and thus short enough to neglect the  $T_1$ -like relaxation occurring during the pulse. The dead-time was 250  $\mu\text{s}$  long. The  $T_2^*$ 's of fluvoxamine, combined fluoxetine-norfluoxetine and the external reference were estimated by spectral fitting for a sample of data points and found to be  $9 \pm 3$  ms,  $9 \pm 3$  ms, and  $10 \pm 2$  ms, respectively. The  $T_2^*$  correction factor:

$$\text{Corr}_{T_2^*} = \frac{\exp(-T_d/T_{2\text{TFE}}^*)}{\exp(-T_d/T_{2\text{drug}}^*)}$$

thus yielded a value ranging from 1.003 to 1.02 and was neglected for all concentration calculations.

The fluvoxamine signal originates from all fluvoxamine inside the volume of the fluorine RF coil. To calculate the brain concentration, it is assumed to originate primarily from the brain, which is consistent with the  $^{19}\text{F}$  MRSI results obtained in this study. Brain volumes for each subject were estimated from the 3D proton images after segmentation of brain tissue (excluding CSF). Since the external reference was present next to the head in each experiment, variations in signal amplitude with coil loading were considered to affect both the external reference and tissue drug signals identically therefore eliminating the need to make corrections for coil loading. Since the fluorine MRS peaks of the two drugs and the external reference arise from a  $\text{CF}_3$  group, no correction for number of fluorine nuclei per molecule ( $n = 3$  for the  $\text{CF}_3$  group) was needed either.



**Figure 1.** Spectroscopic imaging and tissue density images of the head of fluvoxamine treated subject km. **(a)** Spectral regions used for <sup>19</sup>F MRSI images: boxes are delimited on a spectrum *in vivo* of the whole head with the external reference present. A = noise region (for Figure 1b), B = fluvoxamine region (for Figure 1c), C = trifluoroethanol (TFE) external reference region (for Figure 1d). **(b)** MRSI of noise region. **(c)** MRSI of fluvoxamine region. **(d)** MRSI of TFE region. **(e)** distribution of brain tissue density in the axial plane (identical to MRSI plane). **(f)** distribution of head tissues other than brain in the axial plane (identical to MRSI plane).

Individual ratios of brain to plasma concentrations at steady state were calculated for each measurement on each subject and the means and standard deviations of these ratios are reported. The error on the concentration value was estimated for each measurement from the calculated relative errors on the values of the integrals (drug and external reference) due to signal to noise according to Weiss and Ferretti (1983). For the entire fluvoxamine study the relative error on the concentration value was on average 2.9% and ranged from 0.9% to 8.6% for 121 measurements. For the entire fluoxetine study the relative error on the concentration value was on average 2.9% and ranged from 1.0% to 6.5% for 39 measurements. The paired t-test  $r^2$  and the Pearson correlation coefficient were used to statistically evaluate differences and correlation between brain and plasma

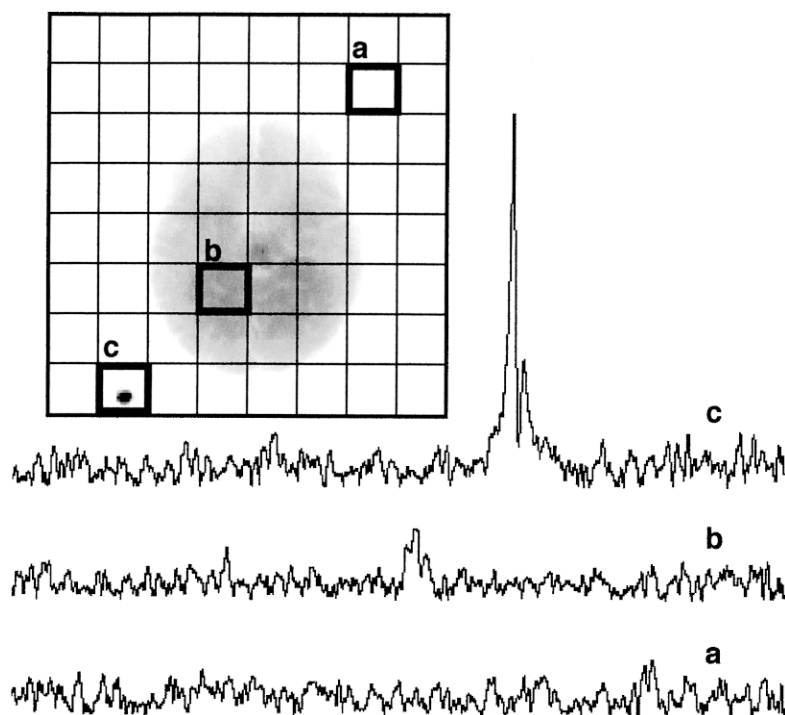
concentrations and also correlation between age and plasma, brain concentrations or brain-to-plasma ratios.

#### Analysis of Washout Data

For <sup>19</sup>F signal from the head, analysis of washout data was performed on the estimated brain concentrations. No correction was made for signal arising from circulating blood which constitutes approximately 3% of brain volume *in vivo*. Experimental washout data points of estimated drug concentration vs. time were analyzed by fitting a three parameter monoexponential decay curve:

$$C(t) = A \cdot \exp(-K \cdot t) + B$$

with A = span in  $\mu\text{M}$ , K = rate constant in  $(\text{hours})^{-1}$ , t = time in hours, and B = plateau in  $\mu\text{M}$ , to the data points



**Figure 2.** Localized  $^{19}\text{F}$  spectra from the MRSI (subject km). Localizations of the selected  $^{19}\text{F}$  magnitude spectra are spatially referenced by overlaying the MRSI voxel grid on images of the relative densities of brain and external reference in the axial plane. (a)  $^{19}\text{F}$  spectrum from voxel not containing any structure. (b)  $^{19}\text{F}$  spectrum from voxel containing brain tissue. (c)  $^{19}\text{F}$  spectrum from voxel containing the external reference vial.

by a non-linear regression method minimizing sum-of-squares (GraphPad-Prism 1995). Plasma concentration decays were analyzed in the same manner by omitting the plateau B. Bi-exponential decay curves fit the data more poorly than the mono-exponential ones and were therefore not considered for the analysis. Half-lives reported are  $0.69/K$ . Values of estimated curve parameters are reported with the curve goodness-of-fit parameter  $r^2$ . The validity of the model was verified with the test of runs in each case. The ratios of kinetic parameters (half-life and decay constant  $K$ ) were calculated for each subject and the means and standard deviations of these ratios are reported.

## RESULTS

### MRSI Results

Figure 1 (a-f) shows typical  $^{19}\text{F}$  MR spectroscopic images recorded in the axial plane without slice selection of the head of a fluvoxamine treated subject compared to proton 3D  $T_1$  weighted imaging data recorded in the same session. The subject km whose data is shown had been taking 300 mg a day of fluvoxamine for one year prior to the MR recordings. Her serum fluvoxamine concentration measured by HPLC within an hour of the MRSI exam was  $0.72\ \mu\text{M}$ , and the estimated brain concentration was  $8.5\ \mu\text{M}$ . The spectral regions which were used for the MRSIs are shown in Figure 1a on a spectrum *in vivo* of the whole head with the external reference present. Head MRSIs of the spectral regions of noise (spectral region A in Figure 1a), fluvoxamine

(spectral region B in Figure 1a), and TFE (spectral region C in Figure 1a) recorded at steady state are shown in Figures 1b, c, and d, respectively. Figure 1e shows brain tissue density whereas Figure 1f shows the density of head tissue other than brain (excluding CSF and bone) plus the density of the external TFE reference. All densities are shown in the axial plane. Comparison of the head fluvoxamine MRSI (Figure 1c) to the brain density (Figure 1e) demonstrates a similarity of intensity distribution. Conversely, there is a poor correspondence between the fluvoxamine MRSI and the densities of other tissues (skin and muscle) excluding brain (Figure 1f). The external reference MRSI intensity distribution corresponds to its position as is seen by comparing Figures 1d and f. The MRSI of the noise spectral region (Figure 1b) showed no high intensity areas, indicating that the MRSIs were free of artefacts.

Localized magnitude spectra selected from the MRSI data set are shown in Figure 2. The MRSI axial plane spatial grid in Figure 2 is overlaid on the sum of brain and external reference vial relative density images in order to spatially reference the localization of the spectra relative to these structures. In the MRSI grid each voxel for spectral localization has a volume of approximately 260 ml. Sample spectra shown were selected from voxels localized outside of either head or external reference (2a), through the head (2b), and containing the external reference (2c). The spectrum from the voxel not containing any structure shows only noise, which is consistent with an artefact free MRSI data set. The spectrum from the voxel localized in the head shows a single peak at the fluvoxamine frequency. The spectrum

**Table 1.** Steady-State Brain and Plasma Estimated Concentrations

Drug	Brain Conc. ( $\mu\text{M}$ )	<i>n</i>	Plasma Conc. ( $\mu\text{M}$ )	<i>n</i>	Brain/Plasma Ratio	<i>n</i>
Fluvoxamine	12 $\pm$ 5	13	1.12 $\pm$ 0.31	13	10 $\pm$ 2	12
Fluoxetine*	13 $\pm$ 7	4	1.73 $\pm$ 1.00	4	10 $\pm$ 6	4

Average values are reported in  $\mu\text{M}$  ( $\pm$  standard deviation) of fluvoxamine or combined fluoxetine-norfluoxetine with brain-to-plasma ratios. *n* = number of measurements. Fluoxetine\* = fluoxetine + norfluoxetine.

from the voxel containing the external reference shows a single peak at the TFE frequency.

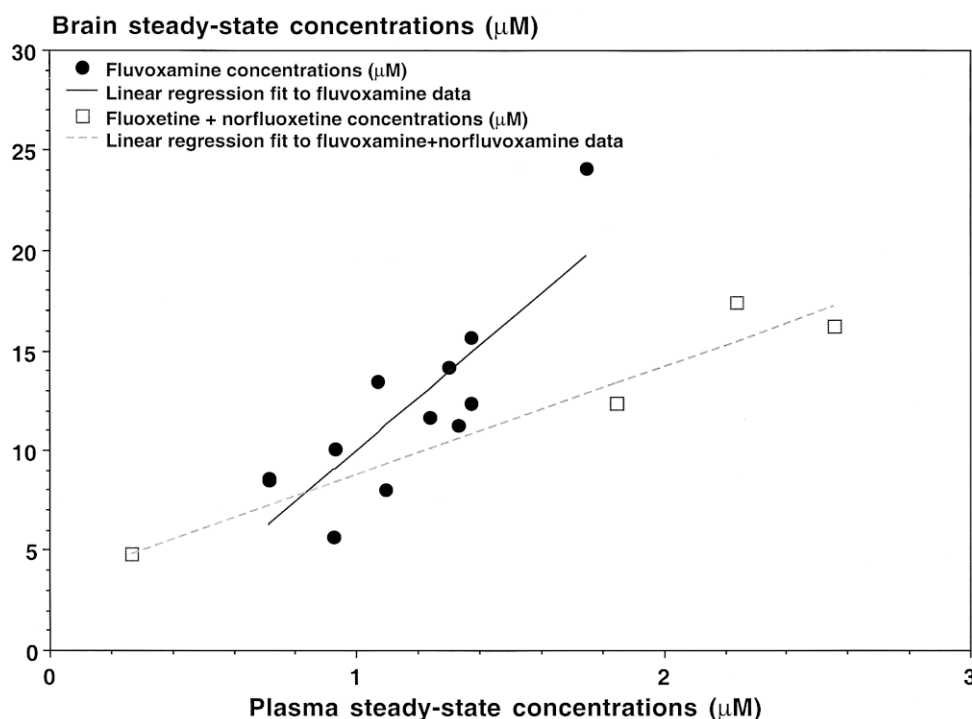
### Steady State Brain and Plasma Concentrations

The steady state concentrations and brain/plasma ratios of fluvoxamine and combined fluoxetine-norfluoxetine are similar as is shown in Table 1. For both drugs, the brain and plasma concentrations were significantly correlated as is shown in Figure 3, in which brain concentrations are plotted vs. plasma concentrations.

For fluvoxamine, the estimated brain tissue concentration at steady state for these subjects was 12  $\pm$  5  $\mu\text{M}$

(mean  $\pm$  SDV, *n* = 13) and ranged from 6 to 24  $\mu\text{M}$ . The steady state plasma concentration was 1.12  $\pm$  0.31  $\mu\text{M}$  (mean  $\pm$  SDV, *n* = 13) and ranged from 0.71 to 1.75  $\mu\text{M}$ . Brain concentrations were significantly higher than plasma concentrations (*p* = .000005). The brain to plasma concentration ratio was 10  $\pm$  2 (mean  $\pm$  SDV, *n* = 12) and ranged from 6 to 14. Furthermore, brain fluvoxamine concentrations were positively correlated with plasma concentrations ( $r^2$  = 0.69, *n* = 12) (Figure 3). No significant difference between men and women was found for either brain or plasma concentrations. No correlation was found between brain concentrations and either treatment dose, treatment duration, or cumulated dose (product of the daily dose with the duration of treatment in days). No correlation was found between age and either plasma concentrations, brain concentrations, or brain-to-plasma ratios.

Since the MR resonances of fluoxetine and its active metabolite norfluoxetine cannot be discriminated in the experimental conditions of this study, brain concentrations were estimated for their combination and were compared to the combined plasma concentrations. The estimated brain tissue concentration of combined fluoxetine-norfluoxetine (CF-norfluoxetine) at steady state for these subjects was 13  $\pm$  6  $\mu\text{M}$  (mean  $\pm$  SDV, *n* = 4) and ranged from 5 to 17  $\mu\text{M}$ . The steady state plasma



**Figure 3.** Correlation of steady state brain concentrations to plasma concentrations for fluvoxamine and combined fluoxetine-norfluoxetine. Filled circles represent fluvoxamine concentration data points and the full line represents the linear regression fit to fluvoxamine data. Empty squares represent combined fluoxetine-norfluoxetine concentration data points and the broken line represents the linear regression fit to combined fluoxetine-norfluoxetine data.



concentration was  $1.73 \pm 1.00 \mu\text{M}$  (mean  $\pm$  SDV,  $n = 4$ ) and ranged from 0.3 to  $2.6 \mu\text{M}$ .

Brain concentrations were significantly higher than plasma concentrations ( $p = .004$ ). The brain to plasma concentration ratio was  $10 \pm 6$  (mean  $\pm$  SDV,  $n = 4$ ) and ranged from 6 to 18. Furthermore brain CF-norfluoxetine concentrations were positively correlated with plasma concentrations ( $r^2 = 0.94$ ,  $n = 4$ ) (Figure 3). Since steady state values for CF-norfluoxetine brain and plasma were obtained on a smaller population sample size (three women and one man) than for fluvoxamine a statistical comparison between men and women was not performed and other correlations tested should be interpreted with caution. No significant correlation was found between brain concentrations and either treatment dose, treatment duration, or cumulated dose (product of the daily dose with the duration of treatment in days). Positive correlations were found between age and plasma concentrations ( $r^2 = 0.86$ ,  $n = 4$ ), brain concentrations ( $r^2 = 0.92$ ,  $n = 4$ ), and brain-to-plasma ratios ( $r^2 = 0.93$ ,  $n = 4$ ).

#### Washout After Abrupt Discontinuation of Treatment

The results of the analysis of the fluvoxamine and CF-norfluoxetine washout pharmacokinetics in brain tissue and plasma are summarized in Table 2 which presents initial concentrations ( $C_0$ ), plateaus (B), half-lives, and brain-to-plasma half-life ratios for each subject. For all of the fits, the test of runs did not detect any deviation from the model of monoexponential decay. Figure 4 presents typical results of estimated brain and plasma

concentrations and the exponential decay fits to the data during fluvoxamine washout after abrupt discontinuation for one subject. The brain tissue fluvoxamine washout half-life of  $79 \pm 24$  hours ( $n = 4$ ) was found to be  $2.2 \pm 0.3$  times longer ( $n = 4$ ) than its plasma half-life of  $35 \pm 8$  hours ( $n = 4$ ). The brain tissue fluoxetine half-life of  $382 \pm 48$  hours ( $n = 2$ ) was found to be close its plasma half-life of  $406 \pm 172$  hours (ratio of half-lives =  $1.0 \pm 0.3$ ) with only two subjects being studied for their tissue kinetics. Fluvoxamine brain and plasma half-lives were significantly shorter than those of fluoxetine.

## DISCUSSION

In this preliminary study we were able to compare the steady state and pharmacokinetic values of fluvoxamine and combined fluoxetine-norfluoxetine in brain *in vivo* using  $^{19}\text{F}$  MRS, and in plasma using HPLC in a group of subjects under treatment for a major depressive episode. Quantitative estimations by  $^{19}\text{F}$  MRS were obtained using an external reference and by integrating over broad spectral regions thus taking into account variations of the experimental conditions of coil loading and magnetic field inhomogeneities. Localization of the tissue from which the drug signal *in vivo* primarily originates was also possible from MRSI recordings performed in the head.

#### Drug Localization and Distribution

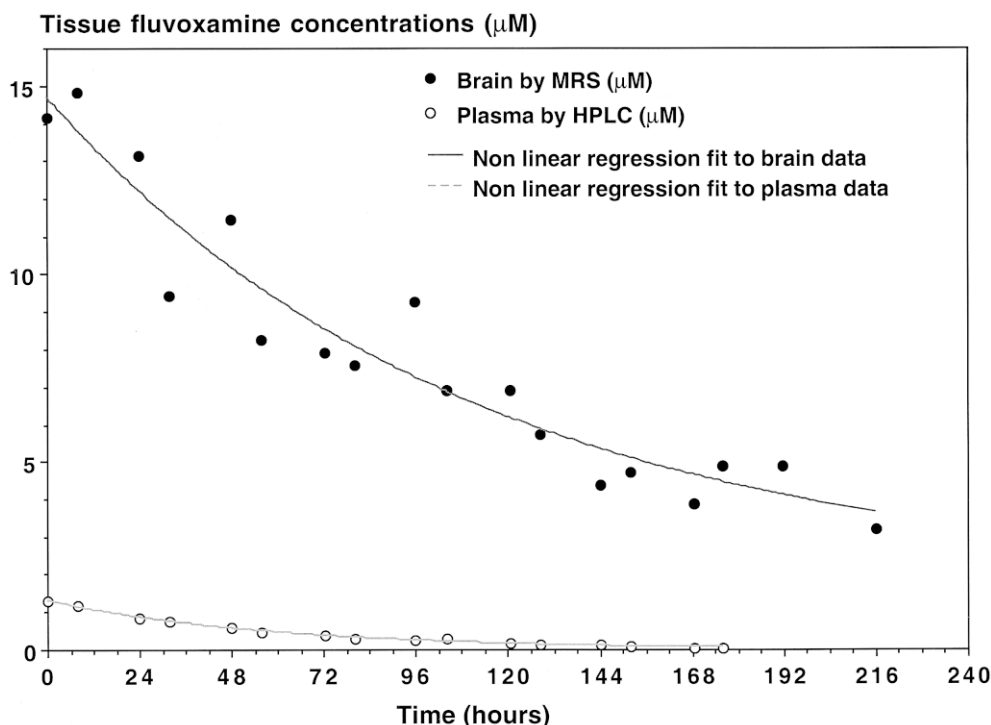
In agreement with localization studies of fluoxetine (Komoroski et al. 1994), we found that fluvoxamine sig-

**Table 2.** Brain and Plasma Pharmacokinetic Parameters from Washout Results

Subject	Results from Brain Data					Results from Plasma Data			Brain/Plasma Ratios	
	Initial Conc. C(0) ( $\mu\text{M}$ )	Plateau B ( $\mu\text{M}$ )	Half-life (Hours)	$r^2$	P(runs)	Span A ( $\mu\text{M}$ )	Half-Life (Hours)	$r^2$	B/P Ratio Half-Lives	B/P Ratio C(0)
FLUVOXAMINE										
dg	$15 \pm 4$	$2 \pm 2$	77	0.92	0.84	$1.32 \pm 0.02$	40	0.99	1.94	11.13
vn	$12 \pm 3$	$4 \pm 1$	49	0.89	0.70	$1.24 \pm 0.01$	25	0.99	1.97	9.80
km	$16 \pm 1$	$0 \pm 1$	107	0.99	0.71	$1.37 \pm 0.02$	44	0.99	2.44	11.39
lc	$9 \pm 1$	$2 \pm 1$	83	0.99	0.80	$0.42 \pm 0.05$	32	0.98	2.56	20.68
Mean	13	2	79			1.09	35		2.23	13.25
SDV	3	2	24			0.45	8		0.32	5.00
FLUOXETINE-NORFLUOXETINE										
dj	$16 \pm 4$	$0 \pm 3$	349	0.92	0.19	$2.52 \pm 0.09$	284	0.98	1.23	6.36
lb	$18 \pm 1$	$0 \pm 3$	416	0.99	0.50	$2.28 \pm 0.19$	528	0.89	0.79	7.90
Mean	17	0	382			2.40	406		1.01	7.13
SDV	2	0	48			0.17	172		0.31	1.09

Fluvoxamine and fluoxetine-norfluoxetine washout non-linear regression fitted curve parameters. The exponential decay function  $C(t) = A \exp(-K/t) + B$  was fitted to the estimated tissue concentration curves, with A and B, respectively, the span and plateau of the decay. The initial concentration C(0) is the sum A+B, the half life is  $0.69/K$ ,  $r^2$  is the goodness of fit, and P(runs) is the P value for the runs test. Parameters K, A and B are presented with their standard error ( $\pm$  SE). SDV = standard deviation.





**Figure 4.** Fluvoxamine tissue pharmacokinetics for subject dg. Filled and empty circles represent the estimated concentrations in  $\mu\text{M}$  for brain tissue (estimated by  $^{19}\text{F}$  MRS) and plasma (measured by HPLC), respectively. The solid and dotted lines represent the respective exponential decay curves fitted to the data.

nal recorded *in vivo* from the whole head originates primarily from brain tissue. Comparison of the drug signal intensity distribution in the MRSI to the brain density in the same imaging plane furthermore suggests that the  $^{19}\text{F}$  MRS visible drug, which is the unbound form, is homogeneously distributed in the brain. This homogeneous distribution does not preclude a heterogeneous distribution on specific binding sites, since specific binding represents at the therapeutic dose a negligible fraction of the total drug concentration detected by MRS.

Our finding that the steady state brain SSRI concentrations were about 10 times higher than plasma concentrations is compatible with its pharmacokinetic profile, with a reported apparent distribution volume of approximately 20 in humans for fluvoxamine (Palmer and Benfield 1994; Wilde et al. 1993) and 12 to 43 for fluoxetine (Altamura et al. 1994), indicating considerable uptake of the drug into tissue spaces. The result that no correlation was found between the steady state brain or plasma concentrations and the daily dose indicates that there can be a considerable variability of plasmatic levels relative to treatment dose. Furthermore, the lack of correlation of brain or plasma concentrations with either treatment duration or cumulated dose indicates that once steady state is attained for a subject further treatment does not affect the established state of bal-

ance. These results confirm the low reliability of only taking into account drug dosage to appreciate the real impact of the drug on the brain. For the clinician, if the treatment is failing, the question is whether to increase the drug dosage or to change the treatment altogether. The good correlation found between brain and plasma concentrations comforts the view that the plasma level is a better indicator than dosage to help the clinician for the therapeutic decision.

#### Estimation of Steady State Concentrations and Pharmacokinetic Parameters

One goal of importance for this type of study is for the  $^{19}\text{F}$  MRS measurements to attain general clinical usefulness as an aid in explaining treatment and side effects. For this to occur, results between different studies using different methodologies should be reproducible. The steady state concentrations of fluvoxamine in brain tissue obtained in this study are in the same range as those found in other studies of the drug by  $^{19}\text{F}$  MRS notwithstanding differences in the methodology used for calibrating the estimated concentrations, i.e., external reference present during acquisition (this study) vs. precalibration with similarly loaded external reference (Strauss et al. 1997). Likewise, the fluvoxamine brain half-lives and ratios of brain to plasma half-lives were

also in the same range. This suggests that if the calibration procedures take into account the factors affecting signal intensity (e.g.,  $T_1$ s and coil loading) in order to obtain absolute concentrations then reproducibility of results between laboratories can be attained. Interestingly enough, the average steady state brain to plasma ratio was two times lower than that found in Strauss's (1997) study not because brain concentrations were lower but because plasma concentrations were about two times higher. This could be explained by differences in the subject populations: the subjects in this study were being treated for a major depressive episode, as opposed to obsessive compulsive disorder (OCD).

The steady state brain concentrations found for combined fluoxetine-norfluoxetine were also in the range of results found in the literature for  $^{19}\text{F}$  MRS studies (Renshaw et al. 1992; Miner et al. 1995) which also use calibration methods (precalibration with an external loading reference) different from this study. To our knowledge, washout kinetics of brain fluoxetine and norfluoxetine have not been studied by  $^{19}\text{F}$  MRS, but the values we found are compatible with uptake kinetics data (Renshaw et al. 1992). Since this study uses the same methodology to compare kinetics of the two SSRIs, and fluvoxamine results were consistent with those of other studies, it is suggested that fluoxetine results should be reproducible as well. An obvious shortcoming of the kinetic data is that the reported values result from the combination of fluoxetine and norfluoxetine data which are not separable under these conditions. As the two compounds have different plasma half-lives: 24 to 96 hours for fluoxetine vs. 168 to 360 hours for norfluoxetine, respectively (Altamura et al. 1994), their brain half-lives could as well present such a large discrepancy. A relatively large variability was found between the two subjects for which the brain fluoxetine-norfluoxetine half-life ranged from 349 to 416 hours. Brain half-lives were comparable to plasma half-lives which demonstrated an even larger variability (284 and 528 hours, respectively). The kinetic results should be interpreted with caution, and considering the particularly small number of subjects studied under washout conditions in each treatment group, no definitive conclusion should be drawn for the brain pharmacokinetics.

Other important factors such as subject age and duration of treatment should be taken into account to compare the results of this study to others. However this study is limited by the small population size and caution should be applied in the interpretation of its results. In this study, the age range as well as the treatment duration range were large with several subjects under treatment for about a year. No correlation was found between age and the steady state plasma or brain concentrations for the eight subjects treated with fluvoxamine whereas one was found for the four sub-

jects under fluoxetine. If this preliminary result is confirmed with further studies, this would suggest different age related changes in metabolism for these two drugs. It appears of particular interest to continue this type of investigation of these SSRIs under long term treatment conditions.

As is with other studies, our data suffer from some of the usual shortcomings of  $^{19}\text{F}$  MRS investigations of this type *in vivo*. The inability to distinguish *in vivo* the SSRIs from their metabolites is related to chemical shift differences which are too small relative to the linewidth *in vivo*. This was the case for fluoxetine and norfluoxetine which, although visibly separated in the spectrum were not sufficiently so for quantitation purposes. Our measurements were made at a field strength of 3 Tesla for which  $^{19}\text{F}$   $T_1$  values could be different than those measured at 1.5 Tesla. Since we did not measure  $T_1$  values, we cannot know if the similarity of our results to those of other  $^{19}\text{F}$  MRS studies is related to the fact that we used the brain  $T_1$  values from those studies to which we are comparing. Further investigation of  $^{19}\text{F}$   $T_1$  values *in vivo* at high fields is still needed. Some of the dispersion in estimated concentration values could be due to the fact that for head measurements, since shimming optimizes field homogeneity preferentially on the head rather than on the external reference, the TFE signal was often distributed on a relatively wide frequency range. However, the large integral ranges used in this study were considered to take this into account.

### Subject Population and Clinical Usefulness

In this study, plasma steady state concentrations obtained were found to be somewhat higher than the values from the literature for fluvoxamine (Palmer and Benfield 1994; De Vries et al. 1993) and fluoxetine (Altamura et al. 1994) studies. The discrepancies can be accounted for by the difference between single dose and long term multiple dose studies (Kelly et al. 1989) for which the values are comparable. It is notable that a relatively large variability has been found in the plasma parameters and some  $^{19}\text{F}$  MRS studies show distinctive outliers for brain concentrations or kinetic parameters.

In this study, the selection of subjects could have affected these parameters if a particular subset of the general population has been selected. The subjects in this study were all under treatment for a major depressive episode with the majority (11/12) clearly diagnosed with major depressive disorder, whereas most data from the literature has been from subjects diagnosed with OCD. This could explain the differences found between our study and others. If confirmed by further investigations, these preliminary results would suggest that for major depressive disorder treated with these two SSRIs, plasma concentrations are higher than but brain concentrations are similar to those found in OCD,

thus pointing to different metabolic profiles for these disorders.

## CONCLUSION

The ability to track the individual pharmacodynamic parameters of target tissues as shown by this study leads us to believe that the  $^{19}\text{F}$  MRS investigative technique presents a great promise for providing both the clinician and the pharmaceutical industry with objective markers relevant to understanding drug therapeutic and side effects.

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