In vivo ^{7}Li and ^{19}F NMR studies of drugs in the brain

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For various reasons, it is advantageous to measure the concentration of a psychoactive drug in the brain *in vivo*. Many drugs contain the element fluorine. Using ^{19}F NMR spectroscopy, we have studied the psychoactive drugs trifluoperazine and fluoxetine in the brain *in vivo*. Using ^{7}Li NMR, it is possible to detect lithium ion, used to treat manic depressive illness. We have measured the concentration and distribution of lithium in both human and rat brain *in vivo*. Measurement of drug levels in the human brain may provide a measure of therapeutic or toxic effects, as well as insight into drug metabolism and mechanism of action.

IN VIVO NMR spectroscopy of endogenous metabolites is being developed as a clinical diagnostic tool[1,2]. Here we describe a less common use of *in vivo* NMR spectroscopy, namely, to monitor drugs and their metabolites directly[3,4], particularly in the brain. Work in our laboratory is emphasized.

The magnitude of the pharmacological or toxic effect of a drug depends on the concentration at the receptor sites, in the tissue cells of the target organ. Measuring drug levels in the blood plasma is a reasonable method for monitoring drug therapy. However, the plasma concentration may not reflect the concentration at the active site. Individual variations in pharmacokinetics and metabolism may arise from differences in physiological function, disease state, diet, and other factors. NMR spectroscopy can measure drug concentration in tissue *in vivo* and can potentially probe drug metabolism. NMR spectroscopy can be used repetitively on the same individual, permitting pharmacokinetic and longitudinal studies.

Few isotopes are well suited for *in vivo* studies of drugs. Table 1 lists isotopes of interest, compared to those used for endogenous metabolites. The major limitation of *in vivo* NMR studies of drugs is low sensitivity. In normal therapeutic use, most drugs do not reach sufficiently high concentrations for detection by NMR *in vivo*. The intrinsic sensitivity, background signal, magnetic field strength, and volume of tissue will determine the minimum detectable concentration *in vivo*.

Tissue heterogeneity, magnetic field inhomogeneity, and restricted molecular mobility will produce substantially larger *in vivo* line widths than in homogeneous solution. Compounds of similar molecular structure, such as metabolites, may not be resolved. NMR spectroscopy samples all NMR-visible (i.e. liquid state) spins of a given isotope in the active volume. NMR can only provide an average concentration over the relatively large volume sampled. For drugs, the compartmental (i.e. intra/extra-cellular) distribution will be important in that the pertinent receptors probably reside in a single compartment. However, the overall drug concentration in the tissue should be a better measure of plasma concentration. Measurement of absolute concentration requires calibration to a signal of known concentration, such as tissue water or an external standard phantom.

The above considerations assume that all of a given species is contributing to the NMR signal. However, low-molecular weight metabolites and drugs can bind strongly to macromolecules or the cell membrane, greatly restricting molecular mobility, broadening the NMR signal, and rendering the compound invisible to high resolution NMR.

Restricting the spatial region giving rise to an NMR signal *in vivo* is critical to clinical utility. The simplest method of spatial localization is restriction of the radiofrequency coil size and shape. Spatially localized spectroscopy using field gradients to define the active region has been undergoing rapid development[1,2]. However, most *in vivo* studies of drugs have been performed with only crude localization because of inadequate signal strength from smaller regions. Care must be exercised in interpreting such signals, which may arise from several anatomic regions in varying proportion. Figure 1 shows a home-built, quadrature birdcage head coil used in our ^{19}F studies of the human brain.[5] Coils for detection of lithium (Li) were similar[6].

^{19}F studies

An isotope that has seen widespread use is ^{19}F, which has very favourable NMR properties (Table 1). Because it is not present in biological systems to any significant extent, there is no endogenous background signal. Many drugs contain fluorine as a part of their molecular

Table 1. NMR isotopes for *in vivo* studies of drugs

Isotope	% Natural isotopic abundance	Relative NMR receptivity	Resonance frequency (MHz at1.5 T)	Natural concentration (mM)	Minimum detectable concentration (mM)
^{1}H	100.0	100.00	63.9	0.5–10	0.1
^{7}Li	92.6	27.2	24.8	–	0.1
^{19}F	100.0	83.4	60.1	–	0.05
^{31}P	100.0	6.65	25.9	1–20	1

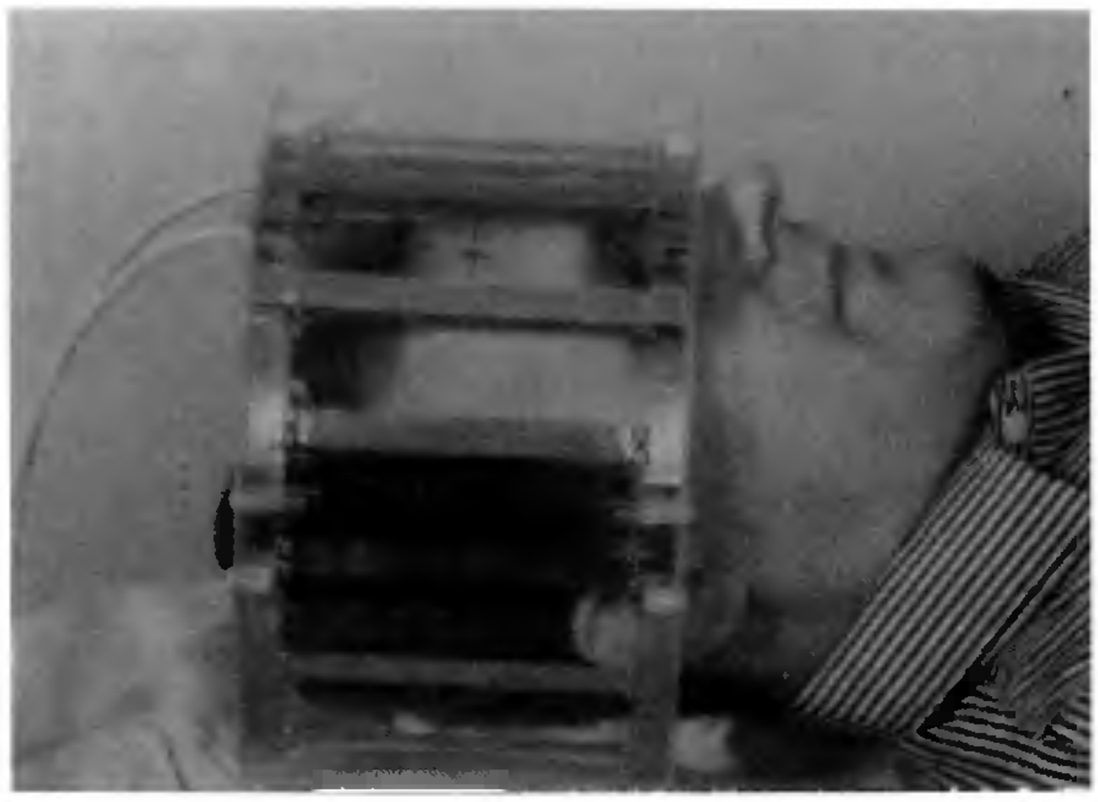
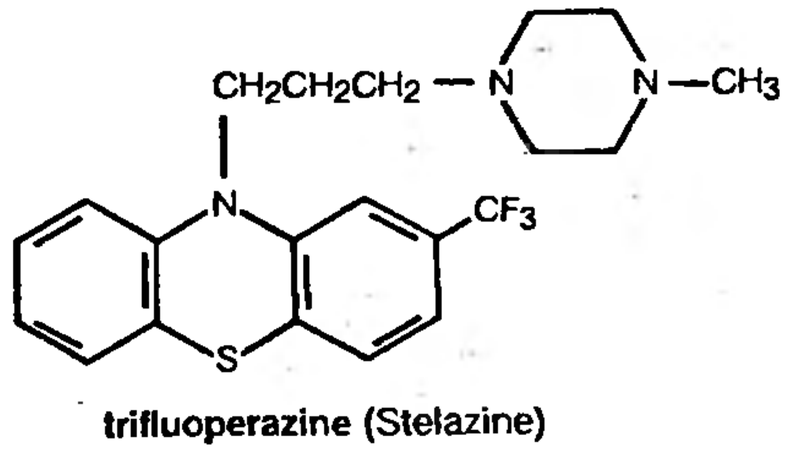


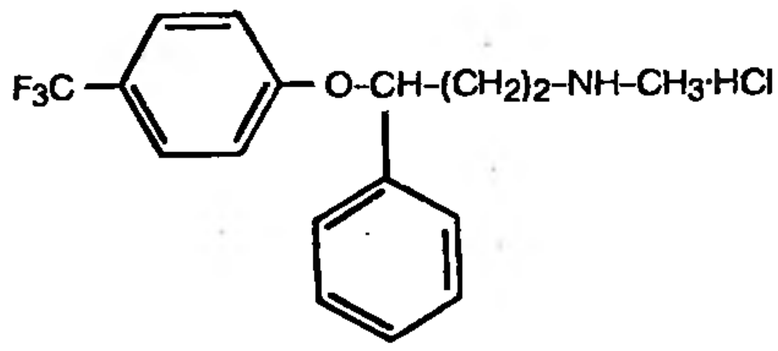
Figure 1. The ^{19}F quadrature, birdcage head coil used in some studies described here, and modeled by the author. The coil is tuned to 60.1 MHz for a General Electric Signa 1.5-T clinical MRI system.

structure. Applications of ^{19}F NMR *in vivo* to psychiatry have been reviewed[7,8]. In most cases, serum concentrations of psychoactive drugs used to treat mental illnesses provide little useful information concerning clinical response and side effects. Initial work centered on detection and quantitation of compounds containing trifluoromethyl groups, including the antipsychotic agents trifluoperazine (TFP) and fluphenazine, and the antidepressant fluoxetine, in either humans or animals[5,9–22]. The structures of two of these compounds are given in Figure 2.

Typically the *in vivo* spectrum will consist of a single peak from the fluorine-containing drug and metabolites in the tissue, and a second peak from a vial of standard compound, which is included in the active volume for calibration and quantitation. Figure 3 shows a typical ^{19}F *in vivo* spectrum from a patient on fluoxetine, a widely prescribed antidepressant in the United States. Based on data from 22 patients on fluoxetine[19], the brain concentration continued to increase long after the clinical effects were evident, and seemed to level off after a 6–8 month period. The drug accumulated to about 20 times the level in the plasma in these patients[18,19]. No correlation was seen with clinical response. The result in Figure 3 is interesting in that,



trifluoperazine (Stelazine)



fluoxetine (Prozac)

Figure 2. The molecular structures of the antipsychotic drug trifluoperazine and the antidepressant drug fluoxetine. The corresponding trade names are given in parentheses.

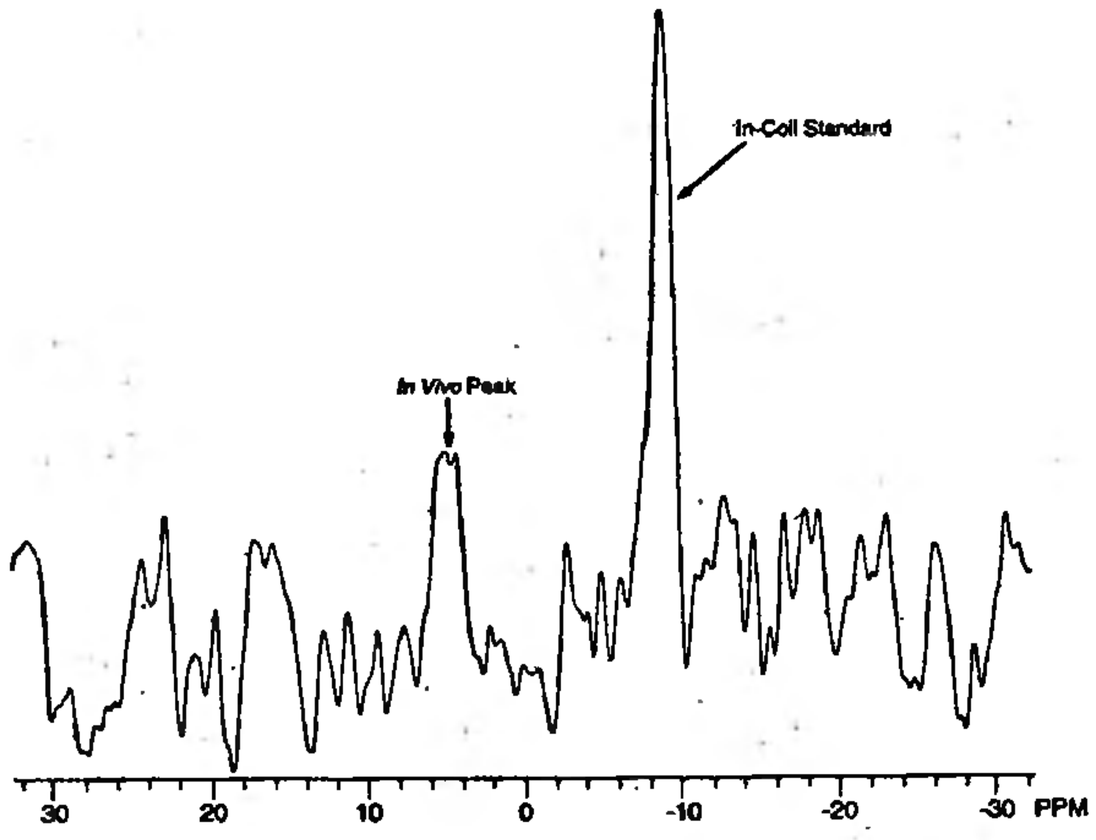


Figure 3. The *in vivo* ^{19}F NMR spectrum from the head of an 18-year-old patient who had received 20 mg/day of fluoxetine, which was discontinued 3.5 weeks before the scan. The scan was acquired with a conformal surface coil[16]. The in-coil standard was a vial of 12 mM 2,2,2-(trifluoroethyl)-*p*-toluene sulfonate in $CDCl_3$. The ppm scale shown is from the spectrum center. The chemical shift of the standard was taken as –74.16 ppm from CCl_3F.

even 3.5 weeks after ending the drug therapy, a significant fluoxetine signal can be observed in the brain, suggesting a very long washout time. In a more recent study, Miner *et al.*[22] saw a weak correlation of fluoxetine level with clinical response in patients with social phobia.

Fluoxetine metabolizes to the therapeutically active compound norfluoxetine in the brain. The ^{19}F chemical shift of norfluoxetine is very close to fluoxetine, and the two compounds are not resolved *in vivo*.[5,16] *In vitro* ^{19}F NMR studies of extracts of both human and rat brain confirmed that the *in vivo* signal arises roughly equally from fluoxetine and norfluoxetine[5].

Although given in comparable doses by weight, TFP does not visibly accumulate to the same extent as fluoxetine, and gives weaker ^{19}F NMR signals[14,16]. However, in unlocalized studies we observed signals for 6 clinically responding patients on TFP doses of 10–120 mg/day[16]. A good correlation was found between brain concentration and daily dose. Interestingly, despite repeated attempts, we could not observe a signal from a nonresponder who was on the very high dose of 120 mg/day of TFP. This result suggests that *in vivo* ^{19}F NMR may have a role in assessing nonresponse to antipsychotic medication. Current *in vivo* and *in vitro* work in our laboratory to identify TFP metabolites in rat brain suggests that the *in vivo* signal arises from the parent drug and 5–7 metabolites.

Recently, *in vivo* ^{19}F NMR has been used to study the trifluorinated drugs dexfenfluramine[23] and fluvoxamine[24], and the monofluorinated antipsychotic melperone[20].

^{7}Li NMR studies

Lithium (Li) is used to treat mania and manic-depressive illness[25]. The therapeutic serum concentration is about 0.5–1.2 meq/l, although 20–40% of the patients respond poorly or do not respond at all. Concentrations above 2 meq/l are often toxic, although neurotoxicity can be seen in the therapeutic range. Thus the Li concentration in the brain may be a better measure of efficacy or neurotoxicity than that in the serum[3,4]. Moreover, the spatial distribution in the brain may shed light on the still unknown mechanism of clinical action of Li[26].

Lithium-7 is relatively favourable for *in vivo* NMR (Table 1), particularly because the tissue concentration is high enough to make *in vivo* imaging and localized spectroscopy possible. *In vivo* ^{7}Li NMR has been previously reviewed[27–31]. Analogous to the spectrum in Figure 3, an *in vivo* ^{7}Li spectrum of the brain typically consists of two resonances, the second from a vial of aqueous Li standard with position shifted from the *in vivo* resonance by a shift reagent. Reasonably good quality spectra can be obtained in about 20 minutes[32,33]. Usable unlocalized spectra can be obtained in 3 minutes[34]. Quantitation typically is relative to a separate Li phantom, with the small, shifted standard for calibration[33].

Early work in humans involved unlocalized *in vivo* ^{7}Li NMR spectroscopy with surface coils to follow Li pharmacokinetics in the human brain and muscle[32,33,35–37], and to correlate the brain concentrations with those in the serum. Further work involved improved quantitation, spatial localization, and measurement of spin relaxation times[6,34,38–41]. Gonzalez *et al.*[38] introduced substantial improvements in the technique, which have been reviewed[3]. Precision and accuracy varied from 5 to 9%, depending on the brain Li concentration. The brain/serum ratio of Li varied from 0.50 to 0.97. Patients with similar serum Li displayed substantial variation in the brain Li.

Recently, in a larger group of bipolar patients, the mean brain/serum ratio of Li was 0.80 ± 0.19 (ref. 41). Over a large serum concentration range, the correlation with brain concentration was weak but statistically significant. In the therapeutic range, the correlation was not significant. The recent work of Riedl *et al.*[34] also supports the weak correlation of the plasma and brain concentrations, confirming that the serum Li is less than ideal as a measure of Li therapy.

The pharmacokinetics of Li uptake and elimination from the brain can be readily studied[6,32,33,40]. Figure 4 shows uptake profiles of Li in the brain for a control and a bipolar patient upon initiation of Li therapy[33]. We showed that Li crosses the blood–brain barrier rapidly, and not over many days, as previously asserted. Plenge and coworkers[40] demonstrated that the brain Li follows the serum Li, in an attenuated form, over a daily cycle, with half-life of 28 h in the brain, and 16 h in the serum.

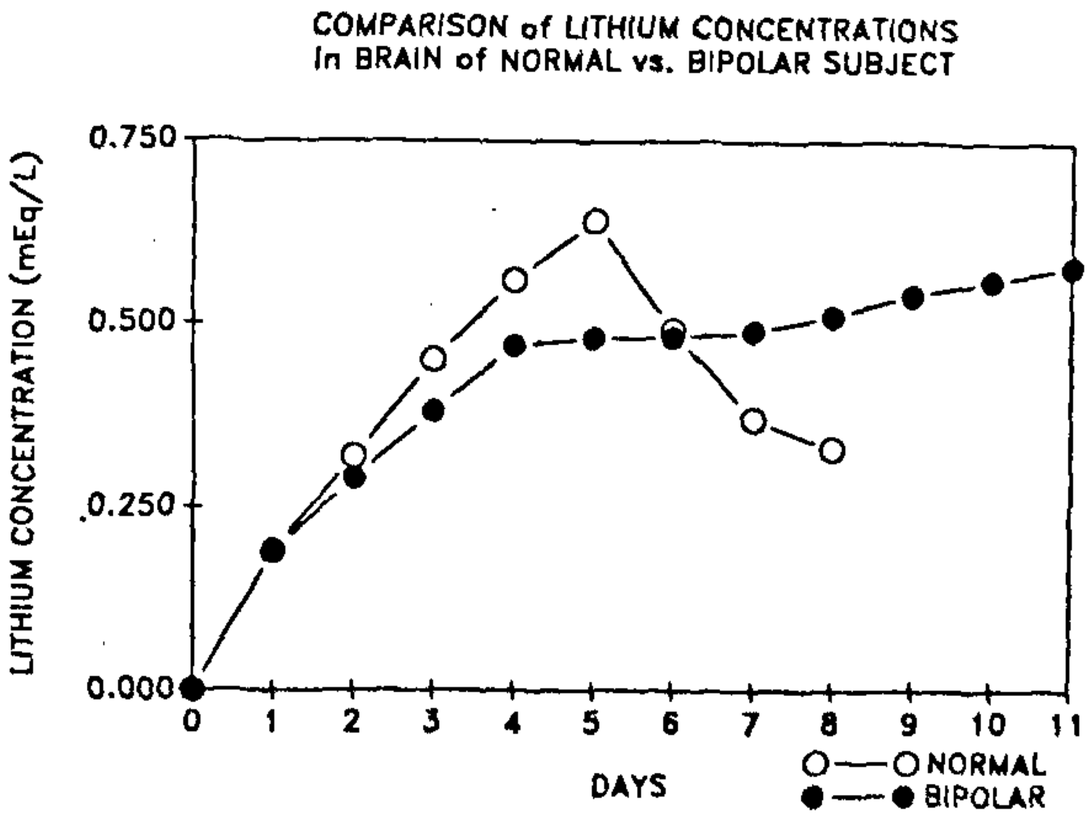


Figure 4. Plots of brain Li concentrations determined *in vivo* by NMR for a normal and a bipolar subject. Both subjects initiated Li treatment at day 0. The normal subject stopped treatment at day 5, and was not followed after day 8. (ref. 33).

While the muscle Li was not detected, the brain Li was detectable at 5–6 days after termination of therapy (for hand tremor) in one patient[6]. There was a gross correlation with remission of neurotoxic symptoms. Kato *et al.*[42] found that patients with hand tremor had higher brain Li than patients without tremor. However, they found no correlation of the brain Li level with a standard, general measure of side effects[42].

For one patient, the brain/serum Li ratio appeared to exceed one during the switch from the depressive to the manic state[36].

The earliest localization studies employed slice selection[33,38,41]. Preliminary ^{7}Li spectroscopic imaging work by us[6], and recently by Girard *et al.*[43], did not reveal large Li concentration differences among human brain regions *in vivo*.

Following the early work of Renshaw and coworkers[44,45], our laboratory pursued *in vivo* ^{7}Li MRI, localized spectroscopy, and diffusion in rats[28,46,47]. We recently completed detailed studies on the distribution of Li in the brain using *in vivo* ^{7}Li MRI and *in vitro* analysis[48–50]. Atomic absorption spectrophotometry (AA) and ^{7}Li NMR gave similar results for extracts of four brain regions and the muscle[49]. Figure 5 shows a comparison of the AA and *in vitro* NMR results for the midbrain[49].

Intensities from ^{7}Li NMR imaging in the neck muscle and the brain regions *in vivo* were obtained relative to the midbrain[48]. There was no difference between the average *in vivo* results and those for either *in vitro* method except for muscle, where the *in vivo* average was about 20% higher than the *in vitro* averages. The effect of the tissue region on Li concentration ratio was highly significant for all the techniques[48]. Actual Li concentrations were determined *in vivo* by ^{7}Li NMR imaging relative to a standard. The average, apparent concentrations were 10.1 mM for muscle and 4.2–5.3 mM for various brain regions under our dosing conditions[50]. Comparison of the apparent concentrations *in vivo* to those *in vitro* provided estimates of the ^{7}Li visibility[50]. For the muscle, the average visibility was slightly reduced. For the brain, the average visibility varied from 74 to 93% depending on the region and technique.

In vivo spin relaxation of ^{7}Li has been reviewed[28]. The ^{7}Li T_1 *in vivo* range from about 3 to 8 s, depending on conditions[28,32,33,39,46]. We do not expect a large range for the ^{7}Li T_1 in a single organ under constant conditions. Unless a long pulse delay is used, as by Gonzalez *et al.*[38], the acquired ^{7}Li signal will be saturated, and T_1 must be taken into account for quantitation. Few data are available for T_2, and none for humans. Previous T_2 measurements were unlocalized and hard to interpret[28,45,46]. Localized T_2 measurements may distinguish intra- and extracellular Li *in vivo*.

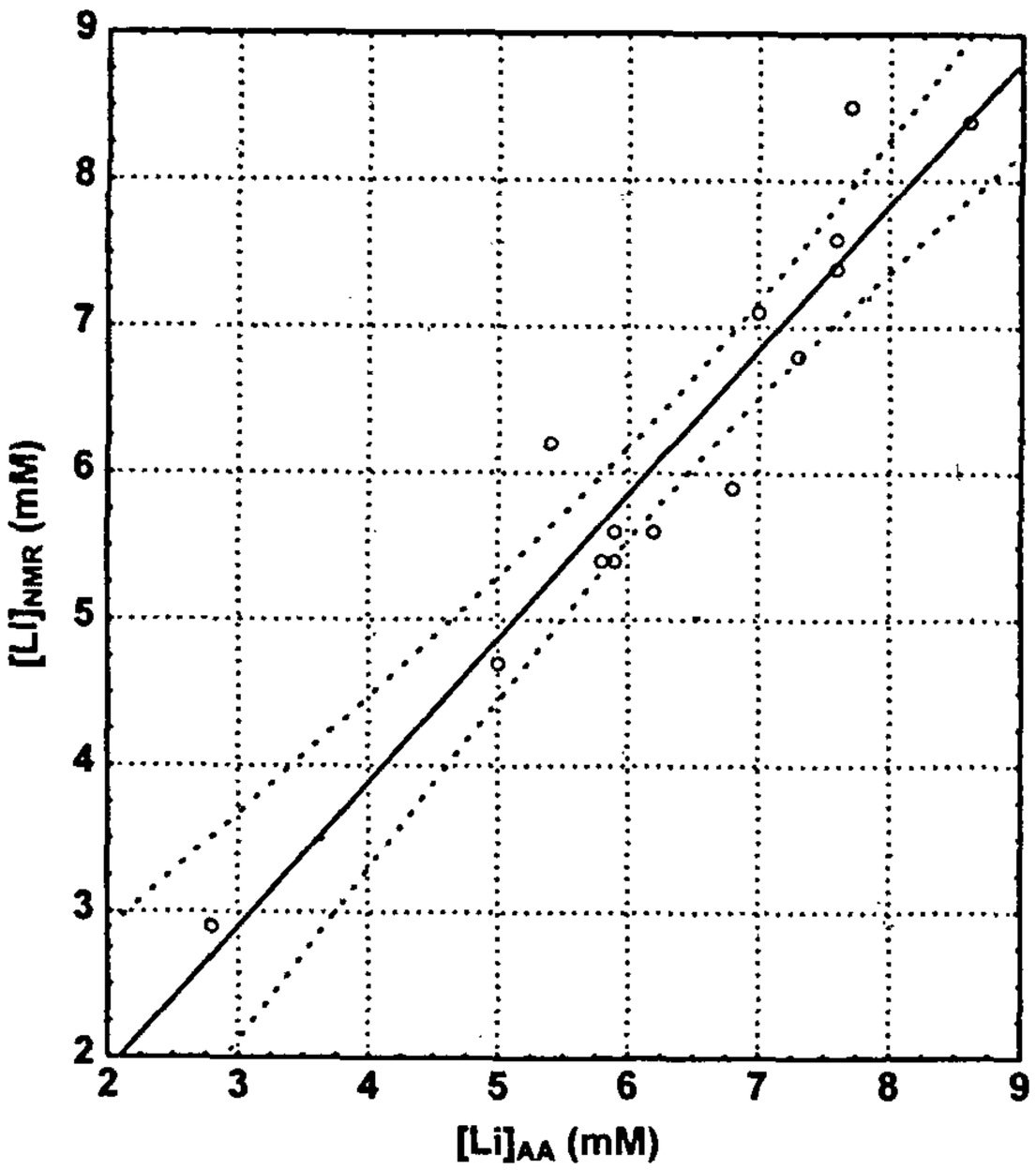


Figure 5. Plot of Li concentrations for rat midbrain determined *in vitro* by NMR versus atomic absorption (AA) spectrophotometry[49].

Conclusions

Although NMR techniques have limitations, their noninvasive character and unique information content insure that new applications and methodological improvements will continue to appear. Higher magnetic fields, more sensitive detection coils, and novel postprocessing methods should substantially reduce the minimum detectable drug concentration *in vivo* and/or improve spectral resolution. A potentially powerful feature of *in vivo* NMR is the ability to measure simultaneously the tissue concentration of a drug and related changes in the brain metabolism by ^{1}H or ^{31}P NMR.

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