from Sarrazin et al. Higher in vivo cortical intracellular volume fraction associated with lithium therapy in bipolar disorder: a multicenter NODDI study

Summary

of residuals from linear model after ComBat harmonization (left frontal region) (y-axis).

10. Supplementary figure 2A and 2B - Boxplot showing the mean ICVF of the eight regions of interest in patients depending on their current medication classes. In blue are depicted patients taking lithium and in red those not on lithium. In figure 2A are plotted patients from Creteil and in 2B those recruited at Grenoble.
11. Supplementary figure 3. Processing Pipeline.

Supplementary methods

MRI acquisitions. At Creteil site, patients were scanned using a diffusion-weighted MRI protocol over 3 different shells of the diffusion q-space optimized for Neurite Orientation Dispersion and Density Imaging (Siemens Prisma Fit 3T, PGSE EPI sequence, multiband factor 3, parallel acceleration factor 2, TR=7040ms, TE=67ms, b-values=200/1500/2700 s/mm², 30/60/60 directions, isotropic voxel resolution of 1.25mm, one b=0 per shell). In the Grenoble sample, patients were scanned with a different protocol and MR system (Philips Achieva 3T, PGSE EPI sequence, no multiband, parallel acceleration factor 2, TR=10800ms, TE=100ms, b-values of 600/1000/1400 s/mm² with a voxel resolution of 1.7x1.7x3mm, 30/30/30 directions, one b=0 per shell). In both samples, the protocol comprised a high-resolution millimetric T1 MRI acquisition (Philips Achieva 3T, voxel resolution of 0.8x0.8x0.8 mm, TR=1350ms, TE=6.4ms at Grenoble; Siemens Prisma Fit 3T, voxel resolution of 1x1x1mm, TR=2300ms and TE=2.98ms).

Mood scales. Patients were considered exhibiting depressive symptoms if having a MADRS above 8, and having manic symptoms if having a YMRS above 8.

Medication load. This approach was chosen in order to control for medications other than lithium despite variable prescription patterns between subjects due to the large number of medication approved for BD. It has the advantages that (1,2,3) (1) it does not interfere with illness severity, as unmedicated patients would be more likely to be less severe than medicated patients, (2) it allows for exploring a relationship between medications and imaging patterns even in a context of multiple classes, (3) it is usable in ecological contexts, as patients with bipolar disorder are usually prescribed with several medications. For antidepressants and mood stabilizers we used cut-offs

of daily dose as previously defined (4). Low dose (score=1) and high dose (score=2) corresponded to levels 1 or 2 and 3 or 4 respectively defined by (4). Regarding first and second generation antipsychotics, we converted the daily dose of each subject into its chlorpromazine equivalent (5) and subject's score was of zero if not taken, 1 or 2 if below or above the mean effective daily dose of chlorpromazine (6) respectively. Low or high dose score of benzodiazepines were defined by subject's dose being below or above the midpoint dose defined in the French Physician's Desk Reference (www.theriaque.org) respectively (2,7).

Statistical model choice and assumptions. To assess objectively which was the best model to use, we used Akaike's information criterion corrected for small samples (AICc) to retain the model with the lowest AICc among possible models (Supplementary table 3). Consequently, the retained model was ICVF ~ group + gender (fixed) + age|site (random slope of age and intercept per site). Preliminary checks of data revealed an interaction between age and site on ICVF ($t=-5.49, p<0.001$) but not between gender and site ($t=0.78, p=0.44$). Further visual inspection of data revealed a different slope between age and ICVF among the two sites, violating the assumption of equal slopes between sites (correlation between age and ICVF : At Créteil, $rho=0.53$, $p<0.001$ and at Grenoble $rho=-0.59$, $p=0.002$). Gender was therefore modeled as a fixed effect variable and age as a random effect variable with random slope. Site was included as a random effect variable with a random intercept. We checked statistical assumption for each model, including normality of residuals (qq plots and one-sample Kolmogorov-Smirnov test), linearity (component and residual plots), multicollinearity (variance inflation factor) and homoscedasticity (non-constant error variance test).

Supplementary results

Detailed demographics. Demographic data detailed per site are provided in supplementary table 1.

Effects of covariates. Effects of age, gender and site are reported in supplementary table 4.

Sensitivity analyses. We performed sensitivity analyses to address whether the inclusion of patients from different sites with different imaging protocol may have interfered with the results (i.e. if there is a significant site effect in this sample of 40 patients with BD and 41 controls). We repeated the main result separately in each of the two sites of inclusion using linear models ICVF ~ Lithium status + gender + age. At Créteil, we found the main group effect ($F=4.34$, $p=0.018$) with a significant effect of age ($F=26.8$, $p<0.001$) but not of gender. Post-hoc analyses showed that patients with BD undergoing lithium treatment had higher ICVF values than patients not taking lithium ($\beta=0.019$, $t=2.94$, $p=0.005$). In the Grenoble sample, we found an expected lack of significant group effect due to the reduced sample size ($F=2.86$, $p=0.08$) with a significant effect of age ($F=10.13$, $p=0.005$) and we found the same pattern as in Créteil, i.e. higher ICVF in patients undergoing lithium compared to those without lithium ($\beta=0.024$, $t=2.38$, $p=0.027$). However there was no significant difference between controls and patients without lithium in both sample. Altogether, these results suggest that both sites are contributing to the results, despite methodological differences regarding acquisition of data.

Replication of results after data harmonization with ComBat.

To further check that the results were not primarily driven by site effect, we harmonized the data using the ComBat algorithm scripted for R (8) following the authors guidelines. Once harmonized, we again check our model using corrected AIC and choosing the lowest AICc (Supplementary table 5). Then the data harmonized for site were used to run linear models to test for an association between lithium status and ICVF using the best retained model.

We repeated the results from our main analysis. Firstly, we found a significant group effect on the ICVF values of the left frontal cortex (F=4.18,p=0.019). More precisely, patients taking lithium had higher ICVF values in the left frontal cortex than patient without lithium (t=2.884,p=0.005) and those without lithium had lower ICVF than control (t=2.12,p=0.037). Patients with lithium were not different from controls in terms of ICVF (t=1.16,p=0.25).

Lastly, we checked that the residuals of the linear mixed model without the lithium variable - that is the remaining variance to be explained by the lithium variable plus the error - (ICVF ~ gender + Age|site) were correlated with the residuals of the same model after harmonization with ComBat ($ICVF_{Harmonized}$ ~ gender + age - that is the remained variance to be explained by the lithium variable after harmonization plus the error*).* We found a very high correlation between the value of residuals from the two approaches (Pearson's rho=0.87,p<0.001, supplementary figure 1).

Analysis using ROI as a repeated measure. To explore whether the group effect was restricted to the left frontal ROI or might be widespread, we ran, on data harmonized for scanner, a linear mixed model using ROI as a repeated measure. We found a significant effect of lithium status (F(2,76)=4.24, p=0.018).

Other NODDI maps. Mean values of isotropic fraction and orientation dispersion for each region of interest are provided in supplementary table 2.

Supplementary references

1- Hafeman DM, Chang KD, Garrett AS, Sanders EM, Phillips ML. Effects of medication on neuroimaging findings in bipolar disorder: an updated review. Bipolar Disord. 2012 Jun;14(4):375-410.

2- Almeida JR, Akkal D, Hassel S, Travis MJ, Banihashemi L, Kerr N, Kupfer DJ, Phillips ML. Reduced gray matter volume in ventral prefrontal cortex but not amygdala in bipolar disorder: significant effects of gender and trait anxiety. Psychiatry Res. 2009 Jan 30;171(1):54-68.

3- Sarrazin S, Poupon C, Linke J, Wessa M, Phillips M, Delavest M, Versace A,Almeida J, Guevara P, Duclap D, Duchesnay E, Mangin JF, Le Dudal K, Daban C,Hamdani N, D'Albis MA, Leboyer M, Houenou J. A multicenter tractography study of deep white matter tracts in bipolar I disorder: psychotic features and interhemispheric disconnectivity. JAMA Psychiatry. 2014 Apr;71(4):388-96.

4 -Sackeim HA. The definition and meaning of treatment-resistant depression. J Clin Psychiatry. 2001;62 Suppl 16:10-7.

5 -Andreasen NC, Pressler M, Nopoulos P, Miller D, Ho BC. Antipsychotic dose equivalents and dose-years: a standardized method for comparing exposure to different drugs. Biol Psychiatry. 2010;67:255-262.

6- Davis JM, Chen N. Dose response and dose equivalence of antipsychotics. J Clin Psychopharmacol. 2004;24:192-208.

7 - Hassel S, Almeida JR, Kerr N, Nau S, Ladouceur CD, Fissell K, Kupfer DJ, Phillips ML. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. Bipolar Disord. 2008 Dec;10(8):916-27.

8- Fortin JP, Parker D, Tunç B, Watanabe T, Elliott MA, Ruparel K, Roalf DR, Satterthwaite TD, Gur RC, Gur RE, Schultz RT, Verma R, Shinohara RT. Harmonization of multi-site diffusion tensor imaging data. Neuroimage. 2017;161:149-170.

Supplementary tables and figures

Supplementary table 1 -Demographics at each site. **Abbreviations**: SD, standard deviation; F, female; M, male; n, number. ✣Linear score based on the French physician's reference desk for each medication class other than lithium, where low-dose equals 1, high dose equals 2. Medication classes included anticonvulsants, second-generation antipsychotics, first-generation antipsychotics, benzodiazepines and antidepressants. ¤Creteil sample only. *Data missing for 1 subject. **Data missing for 2 subjects. ***Data missing for 3 subjects. ****Data missing for 4 subjects. *****Data missing for 5 subjects.

	Patients with lithium carbonate > 2 months (n=26)		**Patients without lithium carbonate (n=15)**		**Healthy controls (n=40)**	
	Créteil	**Grenoble**	**Créteil**	**Grenoble**	**Créteil**	**Grenoble**
n	**18**	**8**	**10**	**5**	**28**	**12**
Mean age (SD)	32.0 (12.1)	48.4 (10.9)	38.2 (13.0)	50.4 (7.8)	32.5 (7.8)	44.7 (9.7)
Gender ratio (F:M)	7:11	4:4	7:3	1:4	13:15	8:4
Mean age at onset (SD)	21.8 (5.6)	28 (9.4)	22.3 (5.9)	32 (11.2)	-	-
Mean duration of illness (SD)	10.2 (11.5)	20.4 (12.7)	15.9 (8.9)	18.4 (4.8)	-	-
Alcohol abuse (n)	1	0	0	0	-	-
Cannabis use disorder (n)	1	0	1	0	-	-
BD I : BD II ratio	17:2	2:4**	5:5	1:2**	-	-
Mean lifetime number of manic or hypomanic episodes (SD)	4.3 (5.7)	5.1 (3.4)	2.4 (1.3)	3.5 (0.7)	-	-
Mean lifetime number of major depressive episodes (SD)	3.3 (6.0)	4.7 (4.3)*	12.5 (16.8)	6.0 (0)***	-	-

History of psychotic features (n)	13	3	3	2	-	-
Mood status (depressive symptoms: euthymic) (n)	4:10 ****	4:4	1:8*	2:3	-	-
Median delay after last lithium serum level (Créteil only,days, range)	38 (9-117)****	-	-	-	-	-
Median blood lithium level (Créteil only, mmol/L, range)	0.99 (0.6-1.2)****	-	-	-	-	-
Mean dose of lithium carbonate (mg) (SD)	982 (250)	-	-	-	-	-
Mean medication load score ⁑ (SD)	1.4 (1.2)	1.9 (1.5)	1.9 (1.1)	2.2 (1.6)	-	-
Mean NART score ¤ (SD)	108.7 (10.8)***	-	109.6 (7.4)***	-	-	-

Supplementary table 2 - Mean values of orientation dispersion and isotropic fraction for the three group (SD). Arbitrary units.

	Mean orientation dispersion (SD)			Mean isotropic fraction (SD)		
	Bipolar with lithium (n=26)	**Bipolar without lithium (n=15)**	**Healthy controls (n=47)**	**Bipolar with lithium (n=26)**	**Bipolar without lithium (n=15)**	**Healthy controls (n=47)**
Left frontal	0.51 (0.02)	0.51 (0.02)	0.51 (0.02)	0.38 (0.04)	0.39 (0.04)	0.37 (0.04)
Right frontal	0.51 (0.01)	0.51 (0.02)	0.51 (0.02)	0.37 (0.04)	0.38 (0.03)	0.37 (0.03)
Left parietal	0.51 (0.01)	0.51 (0.01)	0.52 (0.01)	0.37 (0.04)	0.38 (0.04)	0.36 (0.03)
Right parietal	0.51 (0.01)	0.51 (0.01)	0.52 (0.01)	0.36 (0.04)	0.37 (0.04)	0.35 (0.04)
Left temporal	0.53 (0.02)	0.52 (0.03)	0.52 (0.03)	0.34 (0.04)	0.35 (0.03)	0.33 (0.03)
Right temporal	0.53 (0.02)	0.52 (0.03)	0.52 (0.03)	0.32 (0.03)	0.34 (0.03)	0.32 (0.03)
Left ACC	0.52 (0.02)	0.51 (0.02)	0.52 (0.02)	0.36 (0.09)	0.38 (0.06)	0.35 (0.08)
Right ACC	0.52 (0.02)	0.50 (0.04)	0.52 (0.02)	0.37 (0.08)	0.38 (0.07)	0.37 (0.08)

Supplementary table 3 - Model selection - linear mixed model

Tested models (frontal ICVF)	**AICc**
ICVF ~ Lithium (fixed) + Site (Random intercept)	-366.9
ICVF ~ Lithium (fixed) + Age (fixed) + Gender (fixed) + Site (Random intercept)	-346.7
ICVF ~ Lithium (fixed) + Age (random slope and intercept) + Gender (fixed) + Site (Random intercept)	-383.5

Supplementary table 4 - Effect of covariates - Mixed models

Region of interest	**Gender effect (fixed effect)**	**Age|Site effect (random effect)**
Left frontal	$F(1,75.2)=1.93$, $p=0.17$	$\chi2(df=2)=29.3, p<0.001$
Left parietal	$F(1,75.2)=1.05, p=0.31$	$\chi2(df=2)=14.7, p<0.001$
Left temporal	$F(1,75.6)=1.38, p=0.24$	$\chi2(df=2)=5.8, p=0.05$
Left ACC	$F(1,75.2)=1.52, p=0.22$	$\chi2(df=2)=24.4, p<0.001$
Right frontal	$F(1,75.2)=1.78, p=0.18$	$\chi2(df=2)=26.3, p<0.001$
Right parietal	$F(1,75.2)=1.84, p=0.18$	$\chi2(df=2)=12.9, p=0.002$
Right temporal	$F(1,75.4)=1.47, p=0.23$	$\chi2(df=2)=5.05, p=0.08$
Right ACC	$F(1,75.2)=0.11, p=0.74$	$\chi2(df=2)=16.3, p<0.001$

Supplementary table 5 - Model selection - linear model after harmonization with ComBat

Tested models (frontal ICVF)	AICc
ICVF ~ Lithium	-403.3
ICVF ~ Lithium + Gender	-404.5
ICVF ~ Lithium + Gender + Age	-406.2

Supplementary table 6 - Mean volume of the regions-of-interest for the three groups (SD). Linear mixed models.

	Mean volume (mm3) (SD)			
	Bipolar with lithium (n=26)	**Bipolar without lithium (n=15)**	**Healthy controls (n=47)**	**Group effect (Anova, F, p)**
Left frontal	8911.2 (1297.9)	8478.1 (901.3)	8813.9 (992.5)	F(2,70.3)=0.22,p=0.8
Right frontal	9019.7 (1305.1)	8558.9 (972.1)	8914.4 (1038.5)	F(2,66.3)=0.28,p=0.76
Left parietal	11047.5 (1504.7)	10092.0 (867.2)	10787.8 (1235.3)	F(2,74.4)=1.99,p=0.14
Right parietal	10786.9 (764.8)	10608.9 (666.1)	10888.3 (706.7)	F(2,85.1)=2.42,p=0.09
Left temporal	7102.5 (1011.4)	6676.7 (599.9)	7091.3 (853.3)	F(2,73.9)=1.35,p=0.26
Right temporal	8052.9 (1395.6)	7912.9 (1502.2)	8078.1 (1270.5)	F(2,78.2)=1.37,p=0.26
Left ACC	3276.6 (639.7)	3262.0 (531.4)	3297.3 (644.3)	F(2,76.8)=0.13,p=0.88
Right ACC	2384.3 (364.3)	2480.0 (441.4)	2424.9 (556.6)	F(2,76.5)=0.38,p=0.69

Supplementary table 7 - Details on medications classes per group and site.

	Patients with lithium carbonate > 2 months (n=26)		**Patients without lithium carbonate (n=15)**	
Site	**Créteil**	**Grenoble**	**Créteil**	**Grenoble**
n	**18**	**8**	**10**	**5**
Anticonvulsant	5 (27.7%)	0 (0%)	9 (90.0%)	5 (100%)
Antidepressant	1 (5.5%)	4 (50.0%)	4 (40.0%)	1 (20.0%)
First generation antipsychotic	3 (16.6%)	2 (25.0%)	0 (0%)	1 (20.0%)
Second generation antipsychotic	7 (38.9%)	2 (25.0%)	2 (20.0%)	1 (20.0%)
Benzodiazepines	2 (11.1%)	2 (25.0%)	2 (20.0%)	0 (0%)

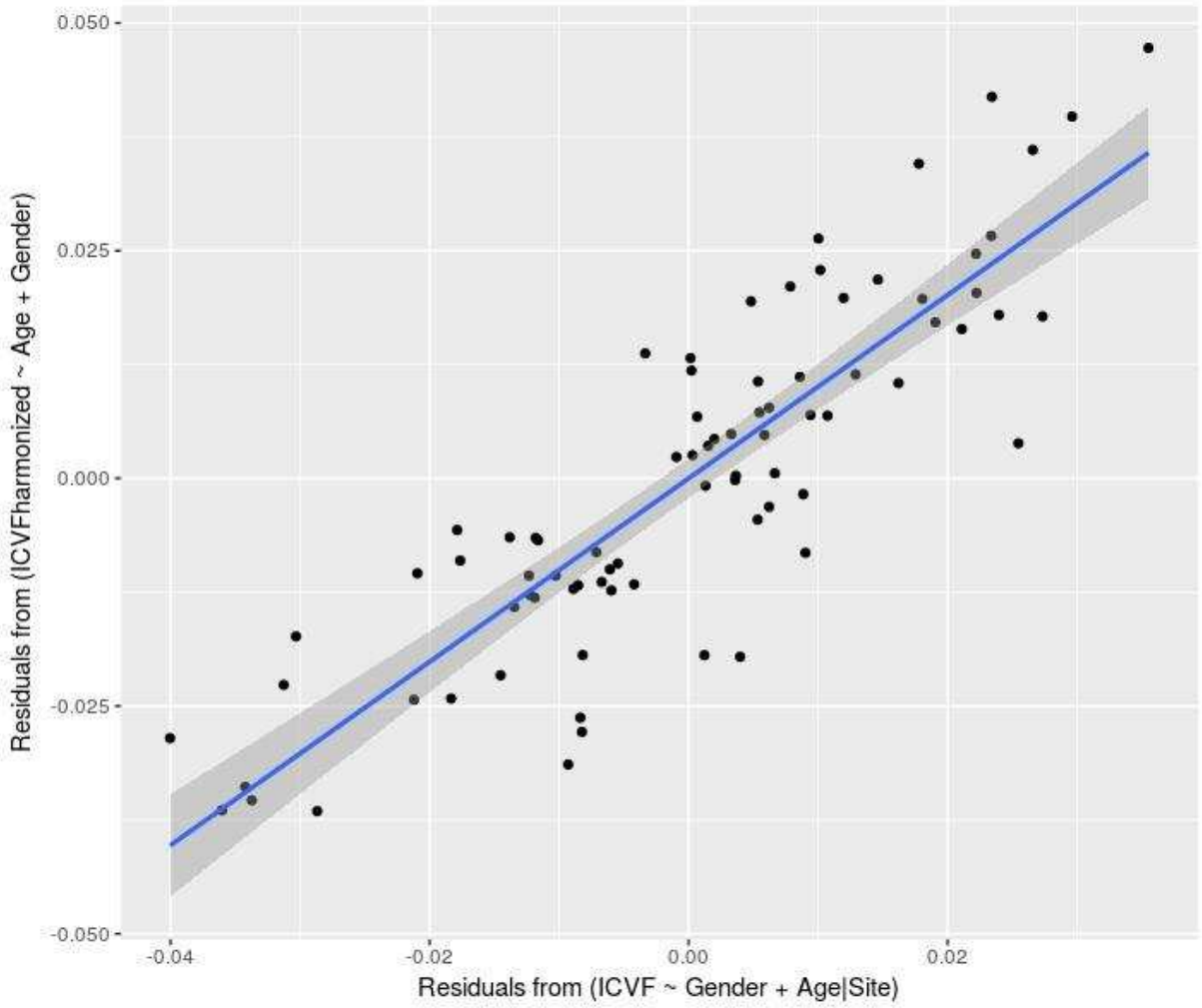
Supplementary table 8 - Details on mood stabilizing co-medications classes per site.

	Créteil (n=28)	Grenoble (n=13)
Lithium	6 (21.4%)	2 (15.4%)
Lithium + antipsychotic	7 (25.0%)	2 (15.4%)
Lithium + antidepressant	0 (0%)	3 (23.1%)
Lithium + anticonvulsant + antipsychotic	5 (17.8%)	0 (0%)
Lithium + antipsychotic + antidepressant	0 (0%)	1 (7.7%)
Anticonvulsant	3 (10.7%)	3 (23.1%)
Anticonvulsant + antipsychotic	2 (7.1%)	1 (7.7%)
Anticonvulsant + antidepressant	3 (10.7%)	0 (0%)
Antipsychotic + antidepressant	1 (3.6%)	1 (7.7%)
Unmedicated	1 (3.6%)	0 (0%)

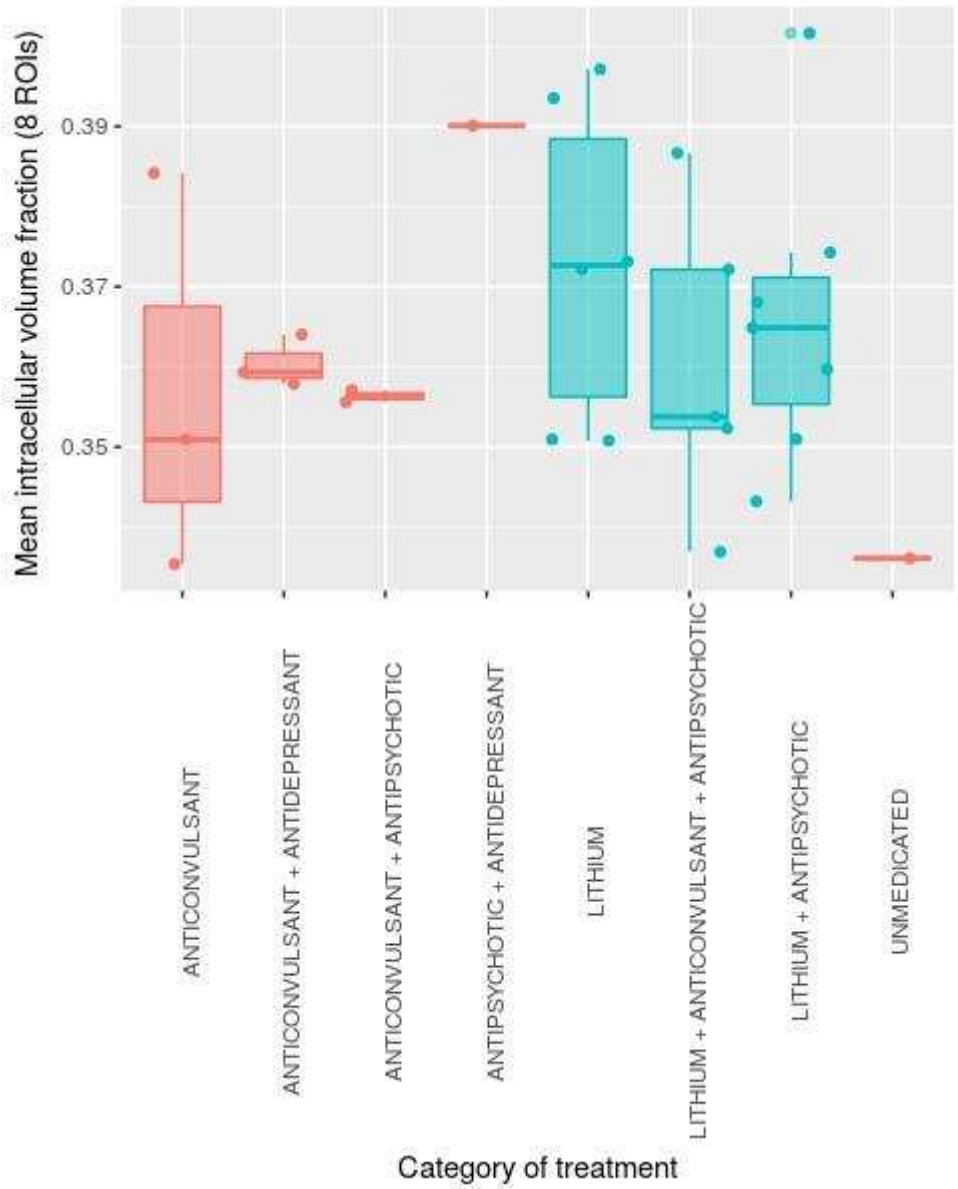
Supplementary table 9 - Co-medications duration in years (available at Créteil site only). * five missing, ** four missing.

	Patients with lithium carbonate > 2 months (n=18)	Patients without lithium carbonate (n=8)	p
Lithium (mean duration, SD)	3.38 (6.9)	-	-
Anticonvulsants (mean duration, SD) **	8.9	3.7	0.42
Antipsychotics (mean duration, SD) *	2.7	0.8	0.32
Antidepressants (mean duration, SD)	-	0.6	-
Benzodiazepines (mean duration, SD)	0.21	0.21	0.97

Supplementary figure 1 - Plot showing the individual values of residuals (x-axis) from the linear mixed model approach and the individual values of residuals from linear model after ComBat harmonization (left frontal region) (y-axis).

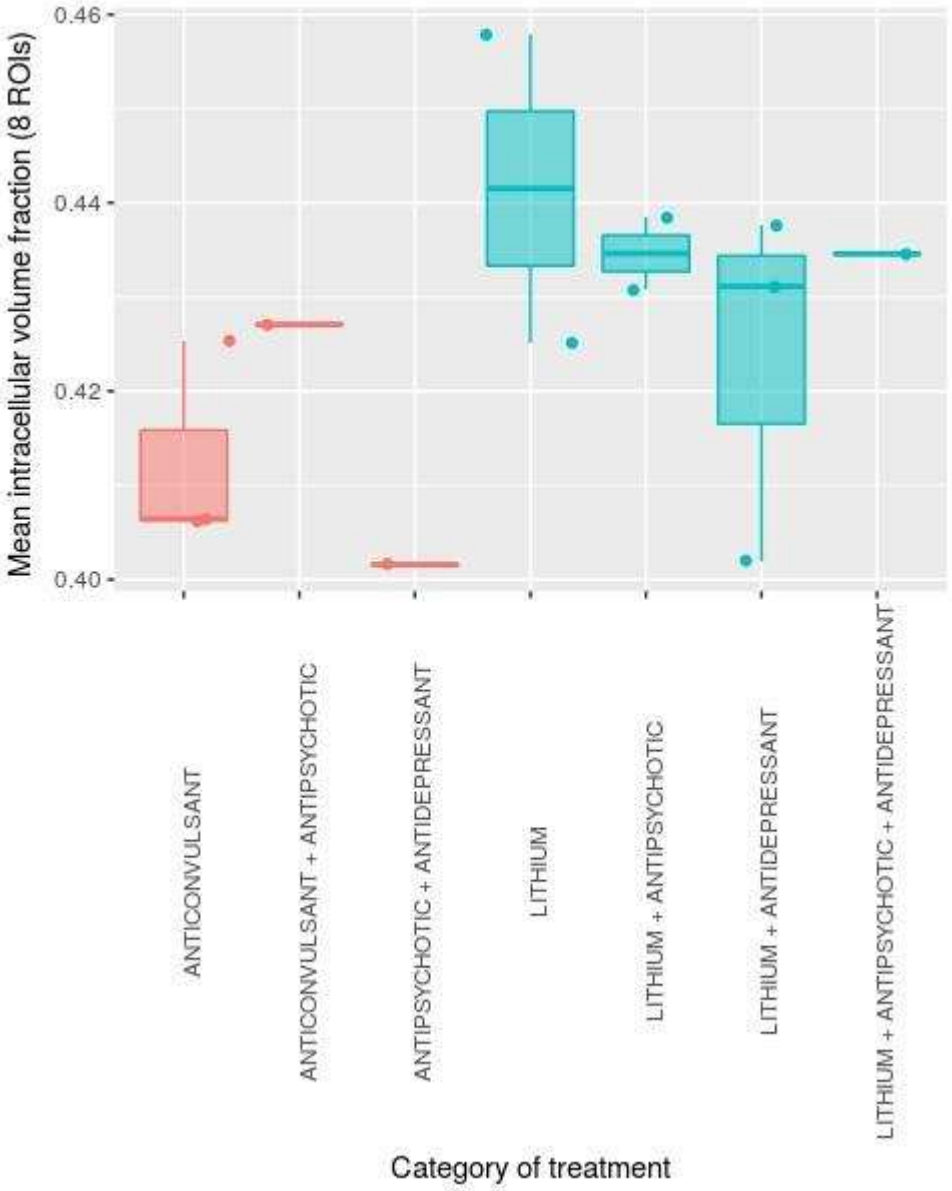


Supplementary figure 2A and 2B - Boxplot showing the mean ICVF of the eight regions of interest in patients depending on their current medication classes. In blue are depicted patients taking lithium and in red those not on lithium. In figure 2A are plotted patients from Creteil and in 2B those recruited at Grenoble.

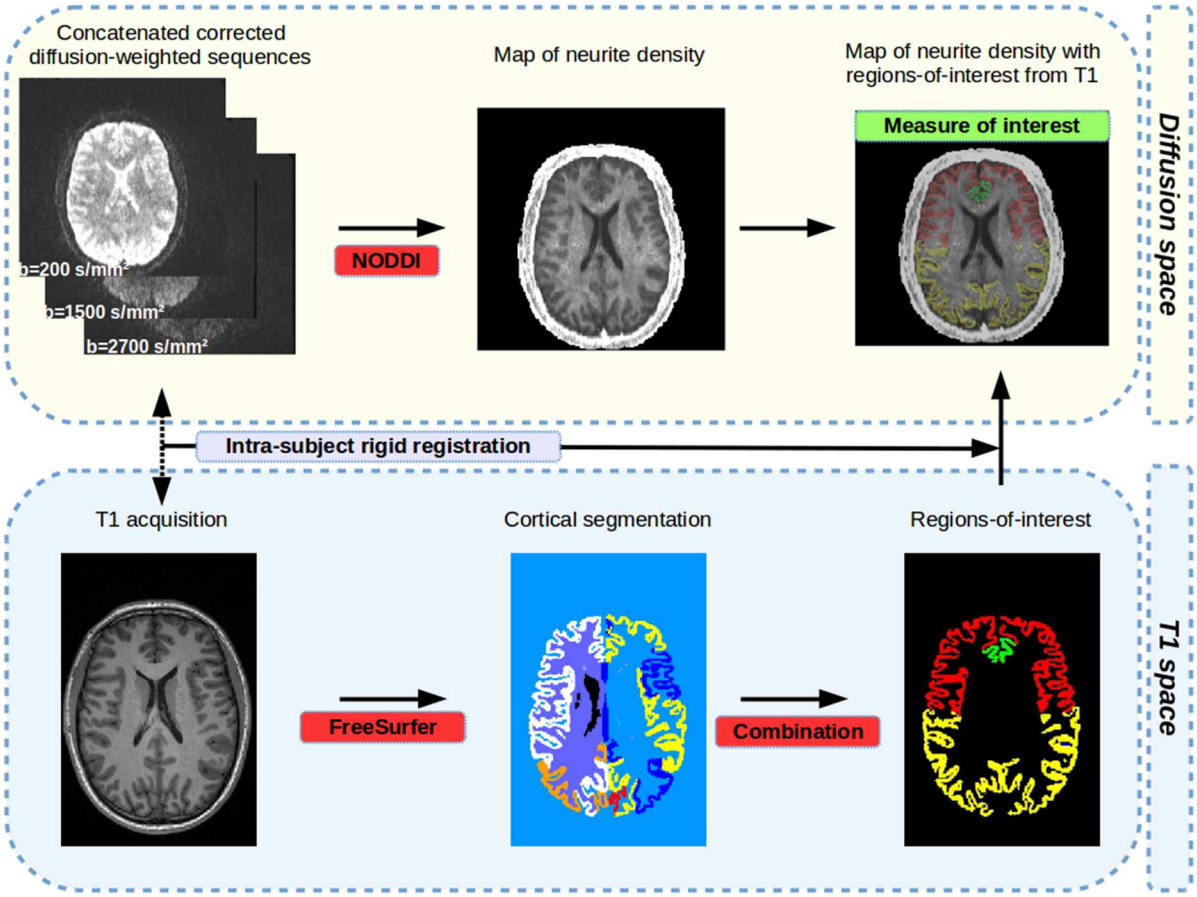


2A- Créteil

2B- Grenoble



Supplementary figure 3. Processing Pipeline.


Concatenated corrected diffusion-weighted sequences
b=200 s/mm²
b=1500 s/mm²
b=2700 s/mm²
NODDI
Map of neurite density
Map of neurite density with regions-of-interest from T1
Measure of interest
Diffusion space
Intra-subject rigid registration
T1 acquisition
FreeSurfer
Cortical segmentation
Combination
Regions-of-interest
T1 space