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ORIGINAL ARTICLE

A VBM study demonstrating 'apparent' effects of a single dose of medication on T1-weighted MRIs

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Abstract Voxel-based morphometry (VBM) studies have interpreted longitudinal medication- or behaviorally induced changes observed on T1-weighted magnetic resonance images (MRIs) as changes in neuronal structure. Although neurogenesis or atrophy certainly occurs, the use of T1-weighted scans to identify change in brain structure in vivo in humans has vulnerability: the T1 relaxation time for arterial blood and gray matter are not clearly distinguishable and therefore, apparent reported structural findings might be at least partially related to changes in blood flow or other physiological signals. To examine the hypothesis that apparent structural modifications may reflect changes introduced by additional mechanisms irrespective of potential neuronal growth/atrophy, we acquired a high-resolution T1-weighted structural scan and a 5-min perfusion fMRI scan (a measurement of blood flow), before and after administration of an acute pharmacological manipulation. In a within-subject design, 15 subjects were either un-medicated or were administered a 20 mg dose of baclofen (an FDA-approved anti-spastic) approximately 110 min before acquiring a T1-weighted scan and a pseudo continuous arterial spin labeled perfusion fMRI scan. Using diffeomorphic anatomical registration through exponentiated lie algebra within SPM7, we observed macroscopic, and therefore implausible, baclofen-induced decreases in VBM 'gray matter' signal in the dorsal rostral anterior cingulate (family wise error corrected at $p < 0.04$, $T = 6.54$, extent: 1,460 voxels) that overlapped with changes in blood flow. Given that gray matter reductions are unlikely following a single dose of medication these findings suggest that changes in blood flow are masquerading as reductions in gray matter on the T1-weighted scan irrespective of the temporal interval between baseline measures and longitudinal manipulations. These results underscore the crucial and immediate need to develop in vivo neuroimaging biomarkers for humans that can uniquely capture changes in neuronal structure dissociable from those related to blood flow or other physiological signals.

Keywords Voxel-based morphometry · T1-weighted structural scan · Perfusion fMRI · Cerebral blood flow · Gray matter · Neurogenesis

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Introduction

A plethora of human studies, including our own, report volumetric and/or gray matter alterations induced by chronic medication, behavioral interventions or disease (Franklin et al. 2002; Draganski et al. 2004; Maguire et al. 2006; Yucel et al. 2007; Driemeyer et al. 2008; Foland et al. 2008; Tomelleri et al. 2009; Ebdrup et al. 2011). The majority of such longitudinal studies interpret the observed

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effects as neurogenesis or brain atrophy. Animal studies demonstrate that the conclusions drawn in these studies may very well be accurate or at least partially so. For example, Pereira and colleagues previously reported that MRI measurements of cerebral blood volume provide an imaging correlate of neurogenesis. They observed that chronic exercise in mice increased blood volume to the hippocampal formation that correlated with markers of neuronal cell growth. In parallel, they measured cerebral blood volume to the hippocampal formation in exercising humans that correlated with cognitive enhancement (Pereira et al. 2007), suggesting that neurogenesis occurred. Particularly relevant to this discussion is a study by Black et al. during which the investigators were able to dissociate neurogenesis from angiogenesis in 'acrobatic' versus 'active' versus 'inactive' rats. Acrobatic rats (learning a new behavior) had 25% more synapses in Purkinje cells in the cerebellum without increases in vascular density, while active rats had increased vascular density without greater numbers of synapses (Black et al. 1990). These are just a few examples from the animal literature supporting the construct of neuronal plasticity. Nevertheless, the current methodology used in human studies prohibits the inference that structural modifications are exclusively related to neuronal growth/atrophy.

In vivo human studies typically use the T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) neuroimaging technique to investigate neuronal change induced by disease progression, medication or behavior. However, the use of T1-weighted scans to identify change in brain structure has vulnerability: the T1 relaxation time for arterial blood and gray matter are not clearly distinguishable and therefore, apparent reported structural findings might be at least partially related to changes in blood flow or other physiological signals.

Recently, a study conducted in our laboratory raised the concern that observed changes in brain structure may be open to more than one interpretation. We utilized a laboratory model that included a 3-week medication versus placebo regimen to examine drug-induced actions on brain circuitry. Specifically, we examined the effects of the medication baclofen, an FDA-approved anti-spastic with evidence for its use as a smoking cessation medication, on brain structure (unpublished) and function (Franklin et al. 2009). We observed medication-induced macroscopic (greater than 1,000 contiguous voxels) 'apparent' changes in VBM gray matter signal on the T1-weighted scan that seem implausible. Furthermore, these apparent changes overlapped considerably with measurements of cerebral blood flow (CBF). The similar functional and structural locus and the sizeable change in structure raised the concern that the structural observation was at least in part, a functional finding that reflected baclofen-induced changes in blood flow to the region. Consequently, the goal of the present study was to test the hypothesis that medication-induced alterations observed on T1-weighted MPRAGE scans may be partially related to additional parameters, such as CBF, irrespective of neurotrophic or neurolytic events.

To interrogate the hypothesis that apparent structural modifications may reflect changes introduced by additional mechanisms irrespective of potential neuronal growth/atrophy, we acquired T1-weighted MPRAGE scans and perfusion fMRI scans (a measure of cerebral blood flow) in a within-subject design, in subjects who were either unmedicated or who received one 20 mg dose of baclofen.

Methods

Subjects

The study was conducted at the Center for the Studies of Addictions, a University of Pennsylvania Perleman School of Medicine affiliated outpatient treatment center. All procedures were approved and monitored by the University of Pennsylvania Perelman School of Medicine Institutional Review Board, and adhered to the Declaration of Helsinki.

Subjects were screened, tested on study knowledge, and consented prior to study entry. Individuals with an abnormal structural MRI, a history of head trauma or other injury resulting in loss of consciousness lasting greater than 3 min or associated with skull fracture or intra-cranial bleeding, or who had magnetically active objects on or within their body were excluded.

The sample consisted of 15 individuals ($N = 9$ female) who were African-American (1), Caucasian (11) multiple ethnicity (2) and Asian. Subjects were between the ages of 18 and 53 (mean $\pm$ SEM $= 30.5 \pm 2.66$) and averaged 15.0 ± 0.65 years of education.

Design

Subjects participated in two separate identical scanning sessions. Approximately 110 min before the first session, subjects received a clinically relevant 20 mg dose of baclofen (peak plasma concentrations of baclofen are achieved within 2 h (NOVARTIS 2010). Subjects received no medication prior to the second session. High-resolution T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) structural MRI scans were acquired at both time points. A T1-weighted scan measures the relaxation time constant of different tissue types, such as gray and white matter and blood. In addition, at both time points, a 5-min *pseudo* continuous arterial spin labeled perfusion (*p*CASL) functional magnetic resonance imaging

(fMRI) scan was acquired in the brain in the resting state. Perfusion fMRI provides a measure of CBF through the capillaries in milliliters of blood per 100 g of tissue per minute providing oxygen and nutrients to regions of the brain that are being utilized (Detre and Alsop 1999; Aguirre et al. 2005). This quantitative feature facilitates the examination of medication- or behaviorally induced changes in CBF in the brain at rest or during tasks (Hermes et al. 2007; Khalili-Mahani et al. 2011). Simply described, the basic principle of perfusion fMRI is based on the perfusion-weighted difference between magnetically labeled and non-labeled (control) images. *p*CASL is a relatively novel ASL technique that provides improved labeling efficiency, improved sensitivity and is easier to implement in ASL protocols (Dai et al. 2008).

Imaging parameters

Data were acquired on a 3.0-T Trio whole-body scanner (Siemens AG, Erlangen, Germany), using a standard 8-channel receive array coil. A T1-weighted three-dimensional MPRAGE scan was acquired (FOV = 160 mm, TR/TE = 1,510/3 ms, 192 × 256 matrix, slice thickness = 1 mm. *p*CASL perfusion fMRI was used to acquire the 5-min resting baseline brain CBF (45 acquisitions). Interleaved images with and without labeling were obtained using a gradient echo-planar imaging sequence with a delay of 700 ms inserted between the end of the labeling pulse and image acquisition (FOV = 130 mm, matrix = 64 × 64, TR/TE = 3,400/17 ms, flip angle = 90°, 18 sequential slices with thickness of 6 mm with a 1.2-mm inter-slice gap).

Data processing

Structural data processing

Prior to pre-processing, each subject's structural images were inspected for artifacts, and the origin was manually set at the anterior–posterior commissure line. Data were preprocessed using voxel-based morphometry-diffeomorphic anatomical registration through exponentiated lie algebra (VBM-DARTEL) within the SPM7 environment (Wellcome Department of Cognitive Neurology, London, UK) under the MATLAB platform (The Mathworks, Natick, MA, USA Version 7.1). Unified segmentation was applied to the structural images of each subject and the resulting tissue probability maps of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) were imported for DARTEL analysis. Images were spatially registered to a local template generated from all the subjects and subsequently to the MNI standard space. The DARTEL algorithm (Ashburner 2007) was applied to derive a local appearance template directly from the dataset along with transformations from the template to each image. SPM7 affine transformation was used to map the local template to the MNI space. The combination of the affine transform and the DARTEL transform was used to map the individual structural images into the MNI space.

Functional data processing

An SPM-based ASL data processing toolbox (Wang et al. 2008) was used for *p*CASL perfusion data analyses as described previously (Franklin et al. 2007). In brief, ASL image pairs were realigned to the mean of all control images and spatially smoothed with a 3D isotropic Gaussian kernel at 10-mm FWHM. Forty-five CBF image series were generated from the 45 label/control ASL image pairs using a simplified two-compartment model with the sinc interpolation method for CBF calculations (Aguirre et al. 2005). The mean control image of each subject's data was co-registered to the structural image using the mutual information based co-registration algorithm provided by SPM7. The same transformation parameters were applied to co-register the CBF map to each subject's anatomical image. Next, the structural image was spatially normalized to the MNI standard brain. The resulting transformation matrix was used to align the CBF images to MNI space. A binary brain mask was used to exclude the non-brain areas in the CBF maps. The final masked CBF map was used for calculating global CBF for each session. The whole brain CBF values were also calculated from each CBF map, resulting in a global CBF value time series with 45 time points.

Statistical analysis

For structural analysis, voxel-wise gray matter differences between the medicated and unmedicated conditions were examined using a paired *t* test. An absolute gray matter threshold of 0.2 was used to avoid GM and WM edge effects. FWE corrected clusters are reported.

For functional perfusion data, masked CBF maps of medicated versus unmedicated conditions were analyzed using a paired *t* test. In this exploratory analysis, *p* values were increased post hoc to observe whether there might be overlap between structural and functional findings. Clusters with voxels having a height threshold exceeding $p < 0.001$ (uncorrected), and an extent threshold of 100 contiguous voxels are reported.

To obtain Brodmann areas, the MNI coordinates from the above analysis reported in SPM were transformed to coordinates for reference to Talairach and Tournoux (1988) brain atlas using the icbm_spm2tal supplied with Java

program gingerALE available from http://www.brainmap.org/index.html (Lancaster et al. 2007). These coordinates were then referred to the Talairach Daemon Java Program (http://www.ric.uthscsa.edu/projects/talairachdaemon.html) to identify corresponding brain regions.

Results

Apparent acute effects of baclofen on brain morphometry

SPM7 analysis revealed a significant decrease in gray matter concentration in the right rostral dorsal anterior cingulate (BA 24b) in the acute baclofen condition compared with the unmedicated condition (FWE corrected $p < 0.04$, at the cluster level, $T = 6.54$, $Z = 4.36$, extent = 1,460 voxels). At this stringent threshold, it appeared as if baclofen's effects were lateralized to the right; however, a reduced threshold ($p < 0.001$ uncorrected) revealed that baclofen's effects spanned across the midline ($T = 4.71$, $Z = 3.59$, extent = 134) The anatomical location of this region is delineated in representative coronal, axial and sagittal slices taken from a high-resolution T1-weighted structural MRI in MNI space (see Fig. 1). No areas of increased gray matter were observed, and no differences between conditions in white matter concentration were observed. Data are displayed in neurological convention (left is left).

Acute effects of baclofen on resting brain CBF

Baclofen-induced decreases in CBF were observed selectively in several regions including a dorsal rostral region of the anterior cingulate cortex that overlaps considerably with the structural finding. Table 1 lists the brain regions, coordinates of the peak voxel and t values modulated by acute baclofen administration in the brain at rest, independent of its global effects. Considerable overlap between the structural and functional findings was observed in subregions of both the posterior and anterior cingulate cortices and several other brain regions (Fig. 2). Baclofen-induced regional increases in CBF were not observed. An interactive visual display of all brain data in all three planes can be found at http://www.franklinbrainimaging.com.

Discussion

Herein, we report that an acute 20-mg dose of baclofen led to apparent reductions in VBM gray matter signal in the dorsal rostral anterior cingulate cortex. Further, these effects overlap with significant decreases in regional CBF. One acute dose of a medication is insufficient to alter brain structure to the level that it could be detected by the VBM method on a T1-weighted MPRAGE MR scan. Therefore, this study provides evidence that the most extensively used tool in the neuroimaging field for determining changes in gross gray matter structure, the T1-weighted MPRAGE, is impacted by nearly instant blood flow changes.

The impetus behind the investigation into the acute effects of baclofen on morphometry was the observation of apparent macroscopic changes in brain structure in individuals who were prescribed 3 weeks of baclofen to test its effects on brain circuitry in the resting brain. Using the same techniques used in the present study, resting baseline and T1-weighted scans were acquired before and after the medication regimen. Chronic baclofen as compared to

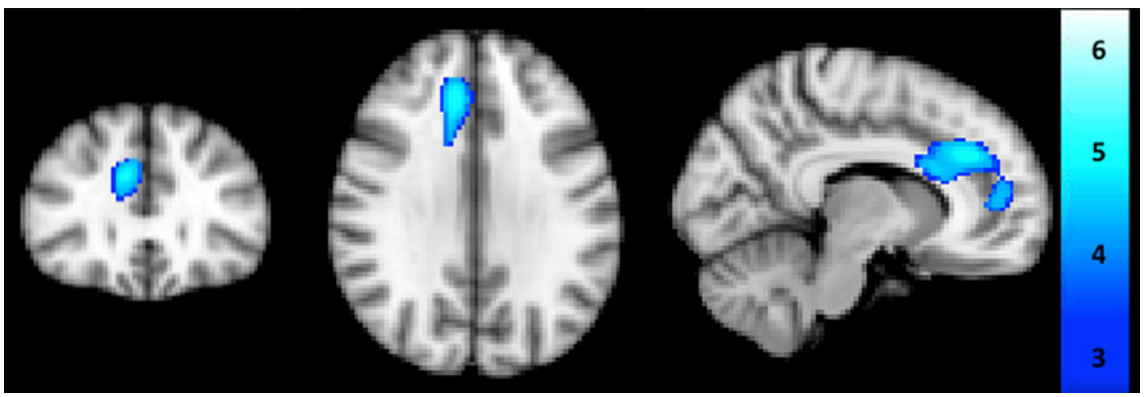

Fig. 1 Illustrated are representative T1-weighted high-resolution MPRAGE structural axial, coronal and sagittal images analyzed by the VBM-DARTEL method in SPM7 and overlain on the MNI brain. The *color bar* on the *right* represents t values. Shown is the anatomical location of a region in which perceived gray matter differences were observed between the medicated and unmedicated conditions. Significant differences were observed in the dorsal rostral anterior cingulate cortex ($T = 6.54$, FWE corrected at $p < 0.04$). Images are displayed neurologically (*left* is left). An interactive visual display of all brain data in all three planes can be found at http://www.franklinbrainimaging.com

Table 1 Table of regions showing CBF modulation by an acute dose of baclofen listed are the coordinates *x*, *y*, and *x* from the suprathreshold voxel within a cluster and the *t* value in regions wherein CBF was reduced by an acute dose of baclofen

Brain regions	Right					Left				
	Vol	*x*	*y*	*z*	*T* val	Vol	*x*	*y*	*z*	*T* val
Amygdala	3.8	24	−5	−13	5.5	1.7	−24	1	−17	4.5
Angular gyrus	0.6	36	−72	33	4.4	0.3	−44	−72	33	4
Cingulate										
Anterior (dorsal rostral peak voxel of overlap with structural data)	**0**				**n.s.**	**0.6**	**−2**	**36**	**20**	**3.4**
Posterior (peak 1)	3.5	8	−52	14	4.8	1.6	−2	−52	12	4.7
Posterior (peak 2)					n.s.	1	−2	−45	39	3.6
Cuneus	2.7	4	−93	10	4.2	3.5	−4	−97	12	4.9
Frontal cortex										
Ventral lateral orbital	4.2	22	13	−19	5.6	1.3	−36	27	−10	4.2
Medial PFC	7.4	8	43	38	5.1	2.2	−4	60	4	4.1
Middle PFC	8.9	28	35	39	5.3	2.8	−36	42	27	4.8
Superior	4.8	28	−8	63	4.3	3.2	−36	51	14	5.1
Superior (dorsal anterior cingulate)	2.6	14	8	47	4.8	0				n.s.
Fusiform gyrus	0.1	48	−49	−14	3.8	1	−46	−9	−26	4.9
Insula-dorsal posterior	0.8	46	−19	12	3.6	0.6	−48	−21	12	3.5
Insula-ventral anterior	2.8	44	6	−5	4	2	−40	−15	8	4.4
Lingual gyrus	0.5	12	−47	2	3.6	1.9	−10	−88	−9	4.2
Occipital gyrus										
Inferior	0.2	34	−88	−2	3.8	0.6	−32	−90	−6	4.4
Middle	1.9	50	−69	9	5.9	2.2	−42	−77	17	4
Superior	0				n.s.	0.4	−38	−74	28	4.4
Paracentral lobule	0.2	2	−15	47	3.4	0.4	−2	−15	47	3.4
Parietal lobule-inferior	4	53	−36	48	4.8	1.2	−63	−34	22	4.6
Parietal lobule-superior	0.4	28	−53	58	3.5	0.3	−18	−63	53	3.3
Postcentral gyrus	2.8	50	−21	51	4.1	1.4	−38	−32	57	3.7
Precentral gyrus	1.3	30	−30	62	3.7	0.9	−59	−10	34	5.7
Precuneus	3.7	14	−63	29	3.8	2.1	−12	−63	31	4.8
Subcallosal gyrus	0.2	14	19	−13	3.3	0.1	−22	3	−14	3.5
Supramarginal gyrus	1.5	61	−49	25	4.9	0.6	−57	−51	27	4.3
Temporal gyrus										
Inferior	2.1	53	−66	−2	5.1	1.5	−50	−7	−27	5.2
Middle	6.3	53	−66	9	5.7	6.1	−61	−39	−5	5.9
Superior	6.3	44	3	−12	4.8	6.6	−63	−34	18	6
Thalamus	2.2	8	−2	7	4.3	0.6	−4	−4	6	3.5
Ventral striatum	0.2	6	13	−6	4.1	0.1	−10	10	9	3.1
Cerebellum					n.s.	0				
	0.3	22	−62	−37	3.3	0.3	−42	−62	−41	3.2
	0.1	14	−83	−28	3.5	0.4	−22	−77	−30	3.8
	0.6	26	3	−19	4.6	0.4	−24	5	−19	4.6

A paired *t* test was used to compare the two conditions: on medication versus unmedicated. There were no regions wherein baclofen increased CBF. Clusters with voxels having a height threshold exceeding $p < 0.001$ (uncorrected), and an extent threshold of 100 contiguous voxels are reported

n.s. not significant

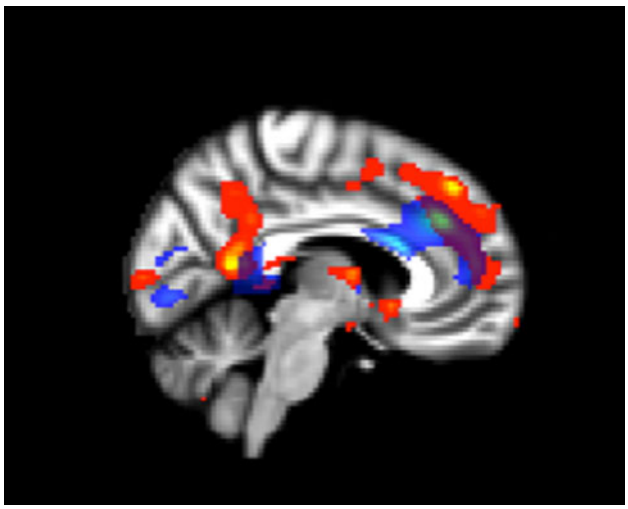

Fig. 2 A representative sagittal image, analyzed in SPM7 and overlain on the MNI brain showing the overlap between the regions of decreased CBF and the structural alterations in the medicated versus unmedicated conditions. Significant decreases in CBF were observed in several brain regions including the posterior and anterior cingulate cortices. *Red* perfusion differences, *blue* structural differences and *purple* regions of overlap. An interactive visual display of all brain data in all three planes can be found at http://www.franklinbrainimaging.com

placebo reduced CBF in the brain at rest in several regions (Franklin et al. 2011), while also apparently reducing gray matter density at a macroscopic level (greater than 1,000 contiguous voxels). As reported here, there was considerable overlap between the structural and functional findings, prompting further study.

Other investigators have analyzed high-resolution T1-weighted structural scans by the VBM method to examine brain changes that might occur in individuals learning a new behavior, to examine differences based on an individual's occupation, and to examine medication-induced brain alterations (Draganski et al. 2004; Maguire et al. 2006; Driemeyer et al. 2008) and interpreted the results as neuro- or atrophic. For example, in previous work in our laboratory, we utilized VBM to examine the structural MRIs of detoxified cocaine-dependent versus cocaine-naive individuals to assess differences between the two groups in gray and white matter concentration (Franklin et al. 2002). We observed apparent decreased gray matter density in prefrontal, insular, and temporal cortices of cocaine patients as compared to cocaine-naïve individuals. We interpreted the findings as gray matter deficiencies in cocaine patients that resulted from preexisting dysfunction and/or a result of the effects of chronic cocaine assault. Interestingly, in this same cohort of cocaine patients regional CBF, as measured by positron emission tomography, was increased during exposure to 25-min videos containing reminders of drug use (Childress et al. 1999) (exposure to drug cues has been shown to predict relapse; Janes et al. 2010). The overlain structural and functional images were in close alignment (Peoples 2002).

One method that has been used to imply pharmacological or behaviorally induced neurogenesis is to conduct concurrent animal and human studies. For example, using multiple probes, Periera and colleagues tested whether exercise resulted in neurogenesis in the dentate gyrus of the hippocampal formation (Pereira et al. 2007). The dentate gyrus is known to sustain neurogenesis in primates and other species (Kuhn et al. 1996; Gould et al. 1998; Tanapat et al. 2005). MRI cerebral blood volume was assessed in mice following 2, 4 and 6 weeks of daily exercise. Exercise had a robust effect on blood volume in the dentate gyrus following 2 weeks of exercise that correlated with post-mortem (at 6 weeks) measurements of neurogenesis. Parallel MRI volumetric experiments were conducted in exercising humans with similar findings. As in mice, exercise was found to have similar effects on dentate gyrus blood volume, and further, the blood flow changes were found to correlate with both cardiopulmonary and cognitive function. However, in the absence of a direct measurement, they were unable to confirm that neurogenesis is the principal factor that accounts for the observed relationship in humans.

As in our cocaine study described above, myriad VBM studies demonstrating associations between changes in structure, induced by medications, disease or behavior have not directly tested whether neuronal mechanisms are at the source of the observed structural findings. This is due to the simple fact that at the present time, and to our knowledge, there are no available biomarkers that can distinguish between blood flow changes or other physiological signals and neural growth/atrophy in vivo in human beings. However, evidence of neural plasticity, such as neuronal cell growth, glial proliferation and brain tissue atrophy is well characterized in animal and molecular studies and can occur rapidly on a microscopic level (Riadh et al. 2011; Black et al. 1990; Pang et al. 1993; Ferrario et al. 2005; Yan et al. 2007; Allagui et al. 2009). Postmortem studies in humans also demonstrate neural plasticity on a microscopic level. For example, Eriksson and colleagues examined whether progenitor cells reside in the adult hippocampus and whether new neurons are born. Bromodeoxyuridine (BrdU), a marker of newly born cells, was found in post-mortem neural cells in the dentate gyrus of cancer patients undergoing treatment. As mentioned previously, albeit these studies demonstrate neuronal plasticity in animals and microscopically in postmortem human tissue, to our knowledge, the technology necessary to directly test whether changes in CBF result in neuronal growth/atrophy in vivo in human beings, is nonexistent.

Regenold (2008) was the first to publically question the neurotrophic/atrophic explanation of drug-induced

morphometric alterations (Regenold 2008). Regenold's reservations were based on animal data wherein chronic administration of a psychotropic agent selectively increased brain tissue water. Phatak and colleagues fed rats regular food or regular food plus lithium chloride for 5 weeks, and assayed the dissected brains for tissue water (Phatak et al. 2006). In the food + lithium group, frontal cortex tissue water was significantly increased as compared to the food-only group. They also tested whether the drug-induced changes were a result of changes in inositol, which is reduced with lithium treatment, or changes in blood sodium concentration, and yielded that neither of these factors appeared to be involved in underlying mechanisms. These findings support our hypothesis of alternative explanations for the interpretation that longitudinal medication- or behaviorally induced changes observed on T1-weighted MRIs are exclusively related to changes in neuronal structure.

Non-neuroimaging physiological biomarkers can estimate some aspects of neuronal change. For example, using quantitative proton magnetic resonance spectroscopy, *N*-acetyl-aspartate (NAA) levels (a putative marker of neuronal viability and function) were investigated at baseline and following 4 weeks of treatment in lithium-treated bipolar patients as compared to healthy volunteers. NAA concentration in the lithium-treated group increased in all brain regions investigated, including the frontal, temporal, parietal, and occipital lobes (Moore et al. 2000). Although NAA concentration and other similar biomarkers can inform neurotrophic effects of a medication on neural change, they would not be advantageous in many instances (e.g., the examination of potential pre-existing dysfunction).

One interpretation of the data reported here is that blood flow is masquerading as changes in brain structure when measured by VBM analysis of T1-weighted structural scans. This may be related to the relatively similar relaxation time constants between gray matter and blood, thus they may be poorly distinguishable. Estimated T1 values of blood at 3.0 T are 1,664 ± 14 ms (arterial) and 1,584 ± 5 ms (venous) (Lu et al. 2004), while the T1 of gray matter at 3.0 T is 1,226 ± 97 (Wright et al. 2008). Conversely, T1 values of white matter (838 ±50) and cerebral spinal fluid (approximately equivalent to pure water at 2,200 ± 100) are significantly different from gray matter and blood and from each other, such that they are more easily distinguishable. Although the relatively similar relaxation time constants of blood and gray matter are acknowledged in the neuroimaging field, to our knowledge, this vulnerability has not been recognized when interpreting structural findings on T1-weighted scans as neural changes.

The difficulty in dissociating structural from functional brain change is that the two are potentially nested. Given that manipulations producing enduring changes in blood flow are associated with neurotrophic or neurolytic events in animals, theoretically the two should also be coupled in humans. However, the tools to examine the direct link between them are nonexistent irrespective of the temporal interval between baseline measures and longitudinal manipulations. The results reported here underscore the urgent and crucial need for the development of neuroimaging biomarkers in humans that can uniquely capture neuronal changes dissociable from those related to perfusion or other physiological signals.

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Conflict of interest Dr. J.A. Detre has received royalties for the commercial licensure of ASL perfusion fMRI. The following authors served as consultants within the past 2 years: CO (Abbott, Embera), AR (Abbott). None of the other authors have reported any potential conflicts of interest.

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