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Arterial spin labeling versus BOLD in pharmacological fMRI

A carefully controlled study allowed us to compare the sensitivity of ASL (arterial spin labeling) and BOLD (blood oxygen level dependent) fMRI for detecting the effects of the adenosine A2a antagonist tozadenant in Parkinson disease . Only ASL detected the direct effect of tozadenant. BOLD was more sensitive to a cognitive task, which (unlike most drugs) allows on-off comparisons over short periods of time. Neither ASL nor BOLD could detect a cognitive-pharmacological interaction. These results are consistent with the known relative advantages of each fMRI method, and suggest that for drug development, directly imaging pharmacodynamic effects with ASL may have advantages over cognitive-pharmacological interaction BOLD, which has hitherto been the more common approach to pharmacological fMRI.

1 Arterial spin labeling versus BOLD in pharmacological fMRI

2 **Running Title:** ASL vs BOLD in pharmacological fMRI

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18 **Abstract**

19 A carefully controlled study allowed us to compare the sensitivity of ASL
20 (arterial spin labeling) and BOLD (blood oxygen level dependent) fMRI for
21 detecting the effects of the adenosine A2a antagonist tozadenant in
22 Parkinson disease. Only ASL detected the direct effect of tozadenant. BOLD
23 was more sensitive to a cognitive task, which (unlike most drugs) allows on-
24 off comparisons over short periods of time. Neither ASL nor BOLD could
25 detect a cognitive-pharmacological interaction. These results are consistent
26 with the known relative advantages of each fMRI method, and suggest that
27 for drug development, directly imaging pharmacodynamic effects with ASL
28 may have advantages over cognitive-pharmacological interaction BOLD,
29 which has hitherto been the more common approach to pharmacological
30 fMRI.

31 **Introduction**

32 Pharmacological magnetic resonance imaging (phMRI) uses fMRI to
33 determine drug-induced changes in brain activity and has multiple
34 applications for pharmaceutical development and efficacy testing. Before
35 the development of functional MRI (fMRI), pharmacological brain imaging
36 most often directly compared brain activity on drug to brain activity off drug
37 (Herscovitch, 2001; McCulloch, 1982). Generally, phMRI studies have
38 avoided this direct approach. Some used drugs with rapid onset and rapid
39 decay of action, and correlated brain BOLD (blood oxygen level dependent)
40 signal with noticeable transient physiological effects, *e.g.* repeated ratings
41 of cocaine-induced “high” (Breiter *et al.*, 1997). Other phMRI studies used
42 drugs with rapid uptake and rapid elimination, with sequential
43 measurements of plasma concentration, to detect brain changes with the
44 expected pharmacokinetics (Bloom *et al.*, 1999). Drug effects on functional
45 connectivity have also been examined (Schwarz *et al.*, 2007). The most
46 common phMRI approach examines the interactive effects of a drug on the

47 BOLD signal changes induced by a cognitive or sensory stimulus (Cole *et al.*,
48 2012; Moeller *et al.*; Wise *et al.*). All of these study designs were motivated
49 in part by limitations of BOLD fMRI, whose signal is nonquantitative and
50 fluctuates artifactually over space and time (Iannetti *et al.*, 2007).

51 By contrast, ASL (arterial spin labeling) is an fMRI method that produces a
52 temporally stable signal. Additionally, ASL images reflect regional cerebral
53 blood flow (rCBF) and thus allow relatively straightforward physiological
54 interpretation. These advantages have led some recent drug discovery
55 pHMRI studies to use ASL (Wang *et al.*, 2011; Zelaya *et al.*, 2014 [in press]).
56 These considerations, and our experience with pharmacological PET
57 (positron emission tomography) blood flow imaging (Black *et al.*, 1997;
58 Black *et al.*, 2000; Black *et al.*, 2005; Black *et al.*, 2002; Hershey *et al.*,
59 2003; Hershey *et al.*, 2000; Hershey *et al.*, 1998), led us to choose a pure
60 pharmacological challenge approach with perfusion fMRI for a
61 pharmacological challenge MRI study in Parkinson disease (Black *et al.*,
62 2010b). However, we designed the study so that we would also have data
63 from the more prevalent BOLD drug-task interaction design. The resulting
64 data set allows a fair comparison of these two methods, *i.e.* subjects
65 provided imaging data for both methods during the same imaging sessions,
66 with similar drug concentrations, the same task, and similar total MRI
67 acquisition times. Here we report the results of that comparison.

68 **Materials & Methods**

69 ***Study participants***

70 Fourteen nondemented, nondepressed, ambulatory adults age 40–75 with
71 idiopathic Parkinson disease, treated with a stable dose of levodopa but no
72 dopamine agonists, participated in the clinical trial (registered at
73 <http://clinicaltrials.gov> with identifier NCT00605553). Detailed inclusion
74 and exclusion criteria were reported previously (Black *et al.*, 2010a). The
75 study was approved by the Washington University Human Research
76 Protection Office (IRB) approval # 08-0059, and all subjects provided

77 written documentation of informed consent prior to participation.

78 **Study protocol**

79 In this single-subject crossover study, subjects were randomly assigned to
80 one of two treatment groups: those assigned to group 1 took 60 mg of the
81 adenosine A2a antagonist tozadenant (SYN115) twice daily for one week,
82 waited for a one week washout period and then took a matching placebo
83 twice daily for one week; those assigned to group 2 participated in the
84 reverse order. The original report included additional subjects allocated to
85 20mg vs placebo, but for this report we focus only on the 60mg arms.

86 Subjects and investigators were blind to the group assignments.

87 Neuroimaging was performed on the last day of each treatment week. On
88 the morning of the scan day, they did not take their usual antiparkinsonian
89 medications, but did take the last dose of tozadenant or placebo at
90 approximately 6:00 AM. The timing of the fMRI assessments was planned to
91 approximately bracket the time to maximal plasma concentration of
92 tozadenant after chronic dosing. Subjects took 200 mg of carbidopa on
93 arrival to the imaging center and then underwent two sets of MRI
94 assessments, once before and once during an infusion of levodopa (LD). The
95 study design was optimized for tozadenant rather than levodopa, and the
96 dose of levodopa was relatively low by design, so analyses examining the
97 effect of levodopa were secondary (see Supplementary Materials).

98 **Subject behavior**

99 Each scanning session included two perfusion MRI (ASL) runs while the
100 subject performed the 2-back memory task, two control ASL runs while the
101 subject fixated on a crosshair, and two block-design BOLD runs, each with 3
102 fixation blocks bracketing 3 task blocks. In each session, scans were
103 obtained in the following order: fixation ASL, 2-back ASL, 2 BOLD runs,
104 fixation ASL, and 2-back ASL. Thus in each session the ASL scans bracketed
105 the BOLD runs. One subject was excluded from all analyses presented here
106 because his 2-back task performance was less than 80% accurate.

107 Tozadenant had no statistically significant effect on 2-back performance
108 (Campbell *et al.*, 2010).

109 **MR image acquisition**

110 Both BOLD and ASL MRI data were acquired on the Siemens 3T Tim Trio
111 with matrix head coil. BOLD-sensitive echo-planar images (EPI) were
112 obtained with flip angle 90° , echo time (TE) 27 ms, repetition time (TR)
113 2000ms, 36 planes with interleaved slice acquisition, field of view
114 256×256 mm, and voxel size $(4.0\text{mm})^3$. Over a period of 4.33 min for each
115 run, 130 volumes (frames) were acquired; the first 4 frames were discarded
116 to ensure steady-state magnetization.

117 ASL images were acquired with the commercial Siemens pASL sequence
118 (Wang *et al.*, 2003b). Fifteen echo-planar readout slices with center-to-
119 center slice distance 7.5 mm were acquired in the AC-PC plane with 64×64
120 $(3.4375\text{mm})^2$ voxels in each plane, TR 2600ms, TE 13.0 msec, and flip angle
121 90° . An M_0 image was followed by 31 tag-control pairs for a total acquisition
122 time for each ASL run of 2.73 min.

123 Brain structure was assessed from sagittal MP-RAGE acquisitions with voxel
124 size $(1.0\text{mm})^3$, TR = 2400 msec, TE = 3.08 msec, TI = 1000 msec, flip angle
125 = 8° . The structural images for each subject were inspected visually, images
126 of lower quality were rejected, and the remaining 1-4 MP-RAGE images for
127 each subject were mutually registered and averaged using a validated
128 method (Black *et al.*).

129 **Image preprocessing**

130 BOLD images from each subject were preprocessed to reduce artifacts,
131 including correction for intensity differences due to interleaved acquisition,
132 interpolation for slice time correction, correction for head movement, and
133 alignment to atlas space (Hershey *et al.*, 2004). Image intensity was
134 adjusted on a frame-by-frame basis so that each frame had a whole-brain
135 modal value of 1000 (Ojemann *et al.*, 1997). Frames were smoothed using a

136 6mm (FWHM) Gaussian filter and resampled to (3mm)³ cubic voxels. To
137 minimize motion-related artifact, frames were removed if framewise
138 displacement exceeded 0.9mm (Siegel *et al.*, 2014).

139 The 63 frames of the ASL run were smoothed using a 5.7mm (FWHM)
140 Gaussian filter (resolution chosen to best match the final smoothing
141 estimated from the BOLD images) and rigidly aligned using a validated
142 method (Black *et al.*, 2001a). Cerebral blood flow (CBF) was computed in
143 each voxel for each tag-control EPI pair as described (Wang *et al.*, 2003b).
144 The aligned EPI images were also summed to facilitate later alignment
145 steps, and the summed, aligned EPI images from each run were mutually
146 aligned within each subject and summed across runs. The resulting summed
147 EPI images from each subject were affine registered to a target image in
148 Talairach and Tournoux space made using validated methods from these
149 subjects' structural MR images (Hershey *et al.*, 2004). The products of the
150 registration matrix from this step and the matrices from the within-run
151 mutual registration step were used to resample the 31 tag-control pair CBF
152 images from each run into atlas space images with (3mm)³ cubic voxels in a
153 single resampling step. To minimize motion-related artifact we removed tag-
154 control pairs if framewise displacement in either EPI image exceeded
155 0.9mm (Siegel *et al.*, 2014). One subject's data was excluded from further
156 analysis because over half of his frame pairs were removed due to head
157 motion. The CBF images in atlas space from the remaining pairs were
158 averaged to create one atlas-registered CBF image for each ASL run. Each
159 CBF image was corrected to an idealized modal global (whole-brain) CBF of
160 50 mL/hg/min (Stewart *et al.*, 2014).

161 **Statistical analysis**

162 *Analysis strategy*

163 The analyses were designed so that each ASL-BOLD comparison included
164 the same scan sessions from the same group of subjects, and as nearly as
165 possible the same image smoothness. Furthermore, the images used to
166 compare the modalities were *t* images from the same sample, and hence

167 were commensurate. Statistical images were created for each imaging
168 modality to examine the 2-back task effect, the interaction of the 2-back task
169 with tozadenant, and a direct comparison of tozadenant versus placebo.

170 *Statistical images*

171 To identify regions of activation and deactivation, we used a mixed-effects
172 approach with partitioned variance (Penny *et al.*, 2007). First, for each study
173 subject, we used a voxelwise general linear model (GLM) that included main
174 effects of task (2-back vs. fixation), levodopa (during vs. before infusion) and
175 drug (tozadenant vs. placebo). For each effect analyzed (drug, 2-back task,
176 infusion and their interactions), SPM12b software
177 (www.fil.ion.ucl.ac.uk/spm/) generated a contrast image for each subject
178 from ASL data, and fIDL (<http://www.nil.wustl.edu/~fidl/>) did the same for
179 BOLD images (also correcting for linear drift within each run). Note for each
180 subject, every contrast image for ASL data was derived from the same set of
181 scans, and similarly for the BOLD data. These single-subject contrast images
182 were used as input to second-level SPM analyses based on a voxelwise
183 general linear model with a covariate for subject age and a factor for sex.
184 One-tailed one-sample t tests at each voxel tested whether the single-subject
185 contrast images at that voxel were significantly less than or greater than
186 zero, across subjects. After thresholding at the t value corresponding to
187 uncorrected $p=.001$, multiple comparisons correction was performed with
188 the cluster false discovery rate set at $p=.05$. Approximate anatomical
189 locations of peaks in the statistical images were provided by the Talairach
190 Daemon client (www.talairach.org) (Lancaster *et al.*, 1997; Lancaster *et al.*,
191 2000).

192 **Results**

193 ***Cross-modality image comparison***

194 The final resolution of the $3 \times 3 \times 3$ mm ASL and BOLD images was similar
195 (Table 1). Total acquisition time was about 25% longer for ASL than BOLD,
196 but acquisition time for the data actually submitted to statistical analysis

197 was much more similar (Table 1), largely because each head movement lost
198 5.2sec of data in the ASL data versus 2.0sec in the BOLD data.

199 **Task activation**

200 The working memory task serves as a positive control, and significant
201 regional activations were identified. The analysis using the ASL data
202 identified one significant activation cluster (22 voxels = 0.6 ml, corrected
203 $p=0.030$, peak $t = 5.88$ at -32, -3, 57, left middle frontal gyrus, Brodmann
204 area [BA] 6). The analysis using the BOLD data identified 12 significant
205 clusters; the largest cluster also included -32, -3, 57 (515 voxels = 13.9 ml,
206 corrected $p<.001$, peak $t = 12.29$ at -40, 3, 33 (left precentral gyrus, BA6)
207 (see Suppl. Table 1). There were no significant deactivations in the ASL
208 data, while the analysis using the BOLD data identified 11 significant
209 deactivation clusters (the largest had volume 2142 voxels = 57.8 ml,
210 corrected $p<.001$, peak $t = 12.70$ at -4, -54, 12, left posterior cingulate,
211 BA29) (see Suppl. Table 2).

212 **Drug effect**

213 The task-drug interaction (tozadenant \times 2-back) showed no significant
214 results for ASL or BOLD. However, the same ASL data revealed significant
215 rCBF decreases on tozadenant in the thalamus bilaterally (Table 2, Suppl.
216 Figure 1). There were no significant clusters of increased rCBF. As
217 expected, the same contrast with the BOLD data found no significant
218 clusters of activation or deactivation. Table 3 summarizes all these
219 contrasts.

220 **Discussion**

221 Cognitive-pharmacological interaction is a common pHMRI approach.
222 However, in this study neither ASL nor BOLD analyses detected significant
223 clusters for the interaction of tozadenant with 2-back task activation,
224 whereas directly comparing rCBF on versus off drug using ASL did reveal
225 significant differences. The drug-induced rCBF decreases detected by ASL
226 are in the thalamus, consistent with animal studies suggesting that

227 adenosine A2a receptor antagonists inhibit neuronal activity in the indirect
228 pathway, including in pallidal afferents to thalamus (Black *et al.*, 2010b).

229 Positive controls built into the experiment confirm that the absence of
230 significant drug effects in the BOLD analysis cannot be comfortably
231 attributed to inadequate image quality or limited data: these same scans
232 were quite adequate to detect significant cognitive (2-back task) effects in a
233 pattern consistent with previous functional imaging studies on working
234 memory (Barch *et al.*, 2012; Bledowski *et al.*, 2010). BOLD is generally more
235 sensitive than ASL for comparisons like this one that can be made over very
236 brief time intervals (a minute or so) (Wang *et al.*, 2003a). However, noise in
237 BOLD data worsens as the time between activation and control acquisitions
238 increases (Aguirre *et al.*, 2002; Ollinger *et al.*, 2001), and this temporal
239 instability likely explains why the BOLD data could not detect direct drug
240 effects between sessions. By contrast, the temporal stability of ASL may suit
241 it better to measure the effects of medications, which after all often have
242 been optimized to require only a few doses a day, and hence have slow onset
243 and wearing off of action (Aguirre *et al.*, 2002; Wang *et al.*, 2011; Zelaya *et*
244 *al.*, 2014 [in press]).

245 Comparing scans from different sequences was feasible here because both
246 BOLD and ASL data were acquired during the same scan sessions in the
247 same subjects, and because the images submitted to statistical analysis
248 were of similar spatial smoothness. Also, in each scan session, half of the
249 ASL scans came before and half after the two BOLD runs, so that any slowly
250 evolving effects of practice, fatigue or drug should be similar on average for
251 the two modalities. Limitations of this study include the imperfect matching
252 between ASL and BOLD of total acquisition time and original voxel size. The
253 different original voxel size is in part a technical limitation because ASL is
254 best suited to acquiring read-out planes in inferior-to-superior order,
255 whereas BOLD can be acquired with even and odd read-out planes
256 interleaved.

257 Decreased thalamic rCBF with tozadenant was also the most significant
258 result of the previously published analysis of ASL data from this study (Black
259 *et al.*, 2010b), but the present analysis detected fewer significant voxels.
260 This is probably because in order to match the BOLD data, the present
261 analysis excluded half the ASL data (acquired during additional behavior
262 states for which were no comparable BOLD data) and smoothed the data
263 less than in the published analysis. We now also excluded subjects with
264 excessive movement or poor 2-back task performance, censored frames for
265 head motion, and improved the correction for global CBF.

266 One additional advantage of this study comes from the following
267 consideration. A drug that produces symptomatic effects, for instance a
268 feeling of calm, may cause secondary effects on neuronal activity via the
269 effect on emotional state in addition to any direct neuronal effects (including
270 the neuronal effects that themselves produce the sense of calm). The same
271 reasoning applies to any placebo effect that may be heightened if the subject
272 notices any drug effect. In this study, most subjects were unable to
273 distinguish whether they were taking active drug or placebo, allowing more
274 straightforward interpretation of the drug's effects on neuronal activity.

275 **Conclusions**

276 In summary, these data offer direct, head-to-head evidence that phMRI using
277 ASL and pure pharmacologic activation may be more sensitive than task-
278 interaction BOLD phMRI.

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395 **Table 1: Comparison of BOLD and ASL images**

	BOLD	ASL
Total acquisition time per scanning session	8.7 min	10.9 min
Acquisition time per session, limited to frames retained after motion censoring (mean \pm SD)	8.5 \pm 0.1 min	9.2 \pm 1.1 min
FWHM (x \times y \times z) *	10.1 \times 10.5 \times 9.0 mm	9.4 \times 10.5 \times 11 mm

396 * Average of the FWHM estimates across SPM analyses.

397 **Table 2: Significant clusters of decreased rCBF on tozadenant**

	Significant clusters
cluster volume, voxels (cm ³)	25 (0.68)
p (FDR)	.004
peak t	5.67
atlas location	8, -15, 9
anatomical location of peak t	Right medial dorsal nucleus of thalamus
cluster volume, voxels (cm ³)	10 (0.27)
p (FDR)	.049
peak t	5.17
atlas location	-8, -21, 9
anatomical location of peak t	Left medial dorsal nucleus of thalamus

398 **Table includes all clusters with FDR-corrected p<.05.**

399 **Table 3: Summary of activation clusters for all contrasts**

Task Contrast	Number of Significant Clusters	
	ASL	BOLD
2-back activation	1	12
2-back deactivation	0	11
Tozadenant × 2-back activation	0	0
Tozadenant × 2-back deactivation	0	0
Tozadenant activation	0	0
Tozadenant deactivation	2	0

400 **Supplementary Material**

401 **Supplementary Table 1: Significant activations during 2-back task**
 402 **(BOLD)**

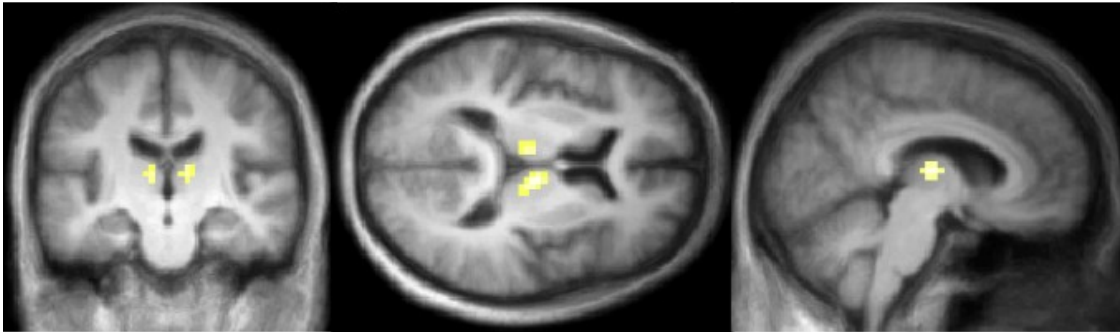
#	cluster volume, voxels	cluster volume, cm ³	p (FDR)	peak t	atlas location of peak t value	anatomical location *
1	515	13.9	<.001	12.29	-40 3 33	left precentral gyrus (BA 6)
2	471	12.7	<.001	9.80	4 12 48	right superior frontal gyrus (BA 6)
3	327	8.8	<.001	10.75	56 -54 -12	right inferior temporal gyrus (BA20)
4	224	6.0	<.001	9.40	-40 -63 -24	left posterior lobe
5	223	6.0	<.001	8.73	44 27 30	right middle frontal gyrus (BA9)
6	166	4.5	<.001	7.53	-10 -18 12	left caudate
7	163	4.4	<.001	6.38	44 -48 51	right postcentral gyrus (BA2)
8	142	3.8	<.001	13.42	32 21 6	right insula (BA 13)
9	127	3.4	<.001	12.94	-28 21 3	left claustrum
10	108	2.9	<.001	8.41	-2 -81 -27	left cerebellum
11	47	1.3	<.001	7.69	-28 -57 42	left superior parietal lobule (BA7)
12	22	0.6	.016	6.30	-38 48 18	left superior frontal gyrus (BA10)

403 * BA, Brodmann area

404 **Supplementary Table 2: Significant deactivations during 2-back task**
 405 **(BOLD)**

#	cluster volume, voxels	cluster volume, cm ³	p (FDR)	peak t	atlas location of peak t value	anatomical location *
1	2142	57.8	<.001	12.70	4 -54 12	right posterior cingulate (BA29)
2	507	13.7	<.001	8.03	4 12 0	right caudate
3	360	9.7	<.001	7.76	-38 -18 21	left insula (BA13)
4	132	3.6	<.001	8.78	-44 -75 30	left angular gyrus (BA39)
5	104	2.8	<.001	6.72	52 -75 21	right middle temporal gyrus (BA19)
6	65	1.8	<.001	6.81	-56 0 -15	left middle temporal gyrus (BA21)
7	59	1.6	<.001	7.57	26 6 -21	right uncus (BA28)
8	46	1.2	.001	9.74	10 -51 -42	right cerebellar tonsil
9	42	1.1	.001	6.50	32 -72 -33	right pyramis
10	40	1.1	.001	6.68	-34 -18 0	left lentiform nucleus
11	29	0.8	.006	7.18	14 39 54	right superior frontal gyrus (BA8)

406 * BA, Brodmann area



407 **Supplementary Figure 1:** Coronal, axial and sagittal sections showing the
408 significant CBF decreases on tozadenant 60mg twice daily. Colored voxels
409 indicate $p < .001$ uncorrected; the corrected p value is .004 for the cluster in
410 right thalamus and .049 for the left (see also Table 2).

411 **Supplementary Material (*continued*)**

412 ***Materials & Methods (secondary levodopa analyses)***

413 The data come from the same scans as reported in the main body of the
414 paper. The study design was optimized for tozadenant rather than levodopa
415 (LD), and the LD dose was relatively low, so analyses examining the effect of
416 levodopa were secondary.

417 The approach was identical to that reported for the task and tozadenant
418 analyses in the main body of the paper. To investigate the effects of LD we
419 created statistical images of the LD effect (comparing scans acquired during
420 the LD infusion to scans prior to infusion), of the interaction of the 2-back
421 task with LD, and of the 3-way interaction of the 2-back task, LD and
422 tozadenant.

423 ***Results (secondary LD analyses)***

424 There were no significant clusters for the pure LD effect, the task-LD
425 interaction, or the 3-way interaction in either the ASL or the BOLD images.

